Chapter 15

Control of Growth and Development

KEY CONCEPTS

1. The development of a plant includes morphogenesis, the production of new organs of defined shapes, and differentiation, the creation of specific characteristics in a cell or tissue. Morphogenesis involves cell division and expansion; differentiation involves the controlled expression of genes at particular time and places.

2. Hormones are chemical signals that coordinate the development of an organ with what is happening in other parts of the plant and with environmental conditions. The well-characterized plant hormones include auxins, gibberellins, cytokinins, ethylene, and abscissic acid. Several additional hormones, including polypeptide hormones, have been discovered in the past few years.

3. Light influences plant development through different receptors that detect different colors of light. Phytochromes, which detect red and far-red light, influence the timing of seed germination, the greening of seedlings, and internode elongation in adult plants. They also are involved in the plant's mechanism for measuring day length.

4. Because changes in the relative lengths of day and night forecast changes in seasons, plants can control developmental processes in a way that adapts them to coming seasons by measuring the length of day and night.

15.1 PRINCIPLES OF PLANT DEVELOPMENT

A plant is a whole organism, not a collection of independent cells. As it grows and matures, tissues and organs develop in predictable patterns. Some depend only on internal conditions (governed by heredity, their position in the plant, or the age or size of the plant), and some respond to the external environment. The shapes of leaves and their arrangement on the mature stem, for instance, form a fairly constant set of inherited traits, which is similar among plants of a species no matter where they grow. The length of a stem and the direction in which it grows in contrast, may depend more on the light available in the environment than on heredity. For either hereditary or environmentally influenced traits, it is difficult to imagine that the patterns of growth and development could occur without signals--signals that somehow communicate to the constituent cells what is happening throughout the plant and outside it. This chapter describes some of the recent research on how these signals control growth and developmental processes.

What Are the Processes of Development?

The term development includes many events that occur during the life cycle of a plant. It includes growth--cell division and enlargement--and differentiation in both shoot and root. The formation of storage tissues and the events that lead to the mobilization of storage materials when they are needed--for example, when a seed germinates or when a tree's buds start to grow in the spring--also are considered to

be developmental processes. Development also includes the induced changes that allow cells to resist herbivory by insects or infection by pathogenic microorganisms, and it includes the distinctive growth patterns that distinguish juvenile, adult, and flowering shoots (Fig. 15.1)

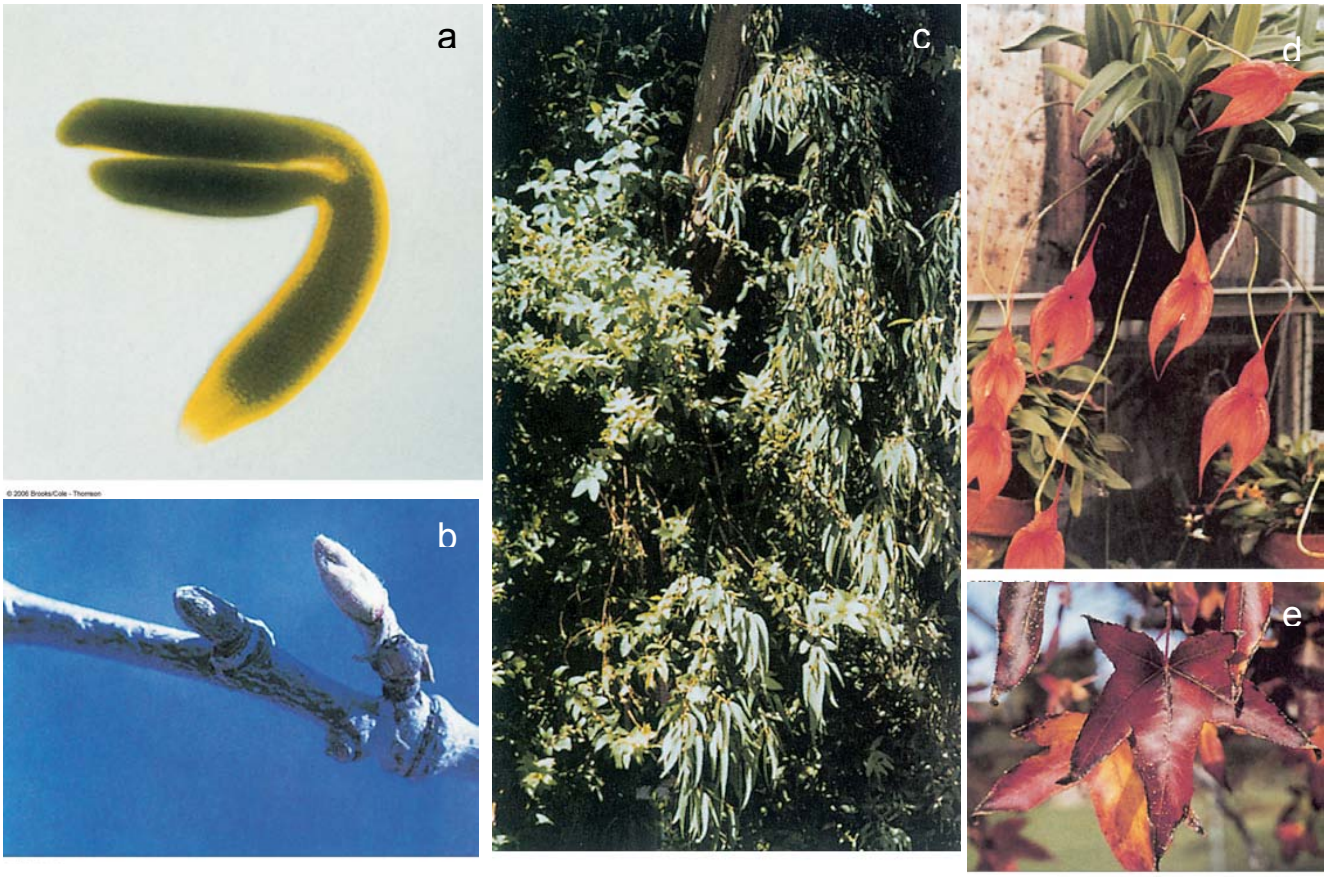


Figure 15.1. Events in the growth and development of plants. (a) An embryo dissected from a seed of mouse-eared cress (*Arabidopsis thaliana*). (b) Spring bud break in a London plane tree (*Platanus acerifolia*). (c) Juvenile and mature *Eucalyptus* shoots. The blunt, oval leaves (top left) mark a juvenile shoot; the long, sharp leaves (right) denote a mature shoot. (d) Induction of flower shoots in an orchid (*Masdevallia veitchiana*). (e) The brilliant color of a senescent *Liquidambar styraciflua* leaf in the fall. Each picture represents a new or changed phase of growth and development.

Some of these events reflect morphogenesis--developmental changes that lead to the formation of specific shapes such as the cylindrical shape of a shoot or root, the flat shape of a leaf (perhaps with a sculpted outline), or the specialized shape of flower petals. Because plant cells are attached to their neighbors by common cell walls, they cannot move around within the plant. This means that the shapes of new organs are determined by the directions in which cells divide and enlarge.

Other events represent differentiation--any process that makes cells functionally specialized and different from one another. Differentiation often occurs through the expression of genes. Gene expression includes all those steps by which genes direct the production of proteins. Many proteins, in one way or another, make a cell distinctive. For instance, the chlorophyll-binding proteins of

photosynthetic cells, enzymes that make pigments in the cells of flower petals, and carriers in root cell plasma membranes that take up mineral nutrients give their cells special traits.

Plants Have Indeterminate as Well as Determinate Growth Patterns

The pattern of growth in plants differs in a fundamental way from that in animals. In most animals, the pattern of growth is **determinate**, which means "having defined limits." In the context of developmental biology, it means that the cells formed from the zygote proceed through a predictable series of cell divisions, movements, and differentiation processes. At the end of the series, cell division and differentiation stop, and the result is the final, adult organism.

In plants, the growth of shoots and roots is **indeterminate**, that is, the shoots and roots will continue to grow until stopped by an environmental or internal signal. The ability to divide is maintained among the cells in the meristems and is not lost at any predictable point. The cells of the meristem divide continuously, producing new cells. Half of these stay with the meristem, and half become part of the plant body, dividing a few times, enlarging, and then differentiating into the various tissues. The meristem cells do eventually lose the ability to divide, but they do not have an inherent, limited number of divisions after which they must stop.

Not all plant growth is indeterminate. Dicot leaves and organs that are formed from modified leaves--such as bud scales, bracts, petals, sepals, stamens, and carpels--all show a limited, determinate growth pattern, which varies from species to species.

One important implication of the indeterminate growth pattern of plants is that much of development occurs by the repetition of a small number of "programs." A module of the shoot--which includes the leaf, lateral bud, and internode--might result from one of these programs. The instructions for forming leaves, starting branch shoots, and growing secondary tissues might be thought of as subroutines within the module program.

Determination and Competence Reveal Stages in Differentiation

Developmental biologists have demonstrated that cells go through a series of stages on their way to becoming mature, differentiated components of an adult organism. This is especially clear in the embryonic development of an animal. The first cell in the developmental pathway, the zygote, is **totipotent**. This means that it has the capability of making, through cell division and the other processes of development, all the cells in the future organism. The two cells resulting from the first division may also be totipotent, meaning that each one could make a complete organism if it were separated from the other and incubated under the appropriate conditions. However, after three or four division, the cells formed are not longer totipotent; separated from the others, they might form only a few tissues, or they might die. These cells are said to be **determined**, which means that their potential to differentiate is iimited. (The term determined should not be confused with *determinate* or *indeterminate*, as previous defined.)

A plant zygote, like an animal zygote, is totipotent. To a degree, plant cells may also become determined. During the formation of the embryo from the zygote,

certain cells become shoot cells, and others become root cells. The formation of a shoot or root meristem is a first step in determining the course of further development. It is unusual for cells in a shoot meristem to make rootlike structures, and vice versa; thus, plant cells are at least partially determined. Then during the primary growth of the shoot, the cells just below the apical meristem become parts of the three primary meristems: the protoderm, ground meristem, and procambium. Cells in these three primary meristems are even more determined, in that they have started to show characteristics that distinguish each from the other and that suggest they are not interconvertible.

The determination of plant cells often is reversible, however. For instance, adventitious roots form from shoot tissue (see Fig. 7.5) and shoots can form from roots, an indication that the determination of cells as shoot or root cells may be changed. Sometimes, protoderm cells divide so that the new cell wall is parallel to the surface of the apex (forming inner and outer cells). Then, the inner cells become part of the ground meristems and produce leaves (Fig. 15.2), an indication that the protoderm cells lose their determination as presumptive epidermal cells and

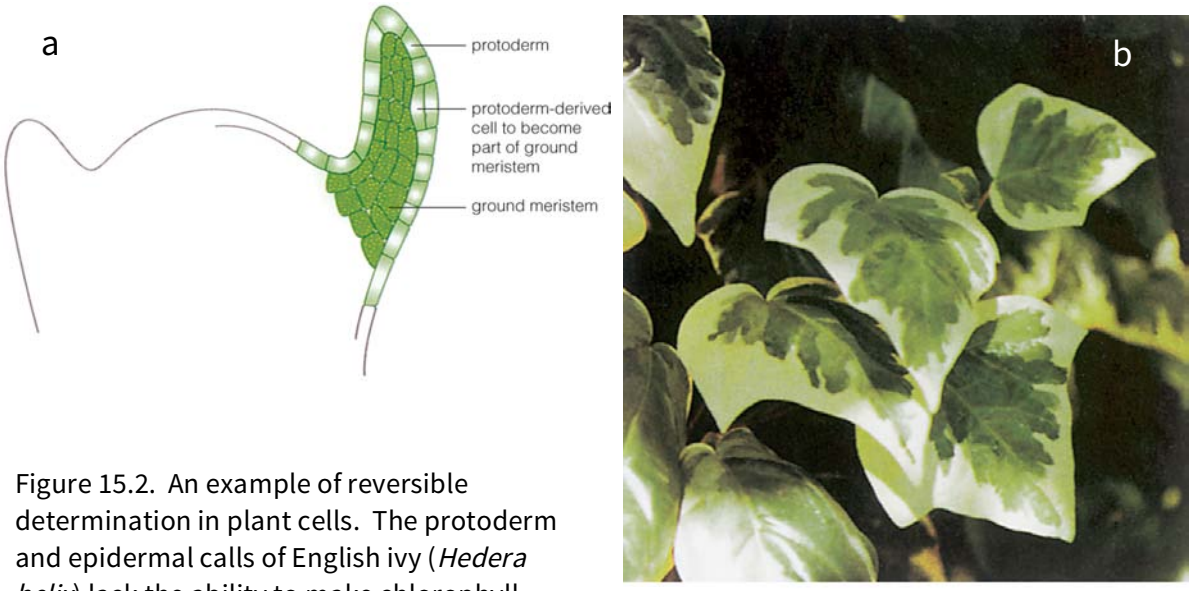


Figure 15.2. An example of reversible determination in plant cells. The protoderm and epidermal calls of English ivy (*Hedera helix*) lack the ability to make chlorophyll, but the ground meristem and its derivatives in a leaf produce chlorophyll normally. When a protoderm cell divides so that one of its resulting cells becomes part of the ground meristems (a), mesophyll in the section of leaf produced from that cell will lack chlorophyll and be light green or white (b). The cell that became part of the ground meristem lost its determination as protoderm.

form mesophyll cells. Also, removing pieces of tissue from shoots or roots and placing them in culture conditions can lead to the formation of whole plants (the conditions needed for this are discussed later in the chapter). This means that at least some cells within the mature shoot or root tissue--cells that have differentiated--can regain their totipotency.

Just as determination in a developmental program channels cells toward certain possible fates and away from others, other processes prepare cells for differentiation. This idea comes from the observation that some differentiation

processes occur after the cell has received a stimulus. For example, mesophyll cells produce chlorophyll only after being illuminated, and procambial cells produce secondary cell walls after being stimulated by sucrose. However, not all cells respond to stimuli in the same way. Procambial cells do not turn green when exposed to light, and palisade parenchyma cells do not make secondary cell walls in the presence of sucrose. The cells that do respond appropriately must have gone through preparatory steps that make them competent to respond to the stimulus.

Gene Expression Controls the Development of Traits

The processes of differentiation, by which cells gain properties that allow them to play specialized roles in the life of the organism, depend on the expression of genes. To explain how this works, we must describe how the information in genes is used to direct the synthesis of enzymes and other proteins. Recall that genetic information is encoded in the sequence of bases in DNA (see Chapter 2). Chromosomes containing the DNA are passed to each daughter cell by mitosis every time a cell divides (see Chapters 3 and 16).

There are several steps and many components to the process by which information locked in the sequence of bases in DNA produces the enzymes needed for a cell's life and growth. The process is amazing both for the simplicity of the basic principles behind it and the complexity of their implementation. An early step is to *transcribe* the genetic code of DNA onto an RNA molecule (Fig. 15.3). Transcription involves using the base sequence of a section of DNA as a template. The two strands of DNA separate, and an enzyme moves along one of the strands, assembling an RNA molecule with a base sequence that is complementary to that of the DNA strand. *Complementary* means that at each position the RNA base fits with the base on the DNA: A fits to T, C to G, G to C, and U to A. Thus, the base sequence of the DNA template specifies the base sequence of the RNA strand that is produced.

The RNA products of transcription in the nucleus are further processed. Pieces are removed. The ends are often trimmed, and, surprisingly, sections from the middle (called *introns*) are removed. When introns are removed, the pieces of RNA at the ends of the intron are re-attached ("spliced" together).

There are several types of RNA produced, each with its own base sequence specified by different sections of the template DNA (and modified by any cutting and splicing operations). One type is *ribosomal* RNA (rRNA). Three separate rRNAs, in combination with several proteins, form the basic machinery (ribosomes) for making proteins. A second type is *transfer* RNA (tRNA). Transfer RNA serves as a decoding molecule, translating a base sequence into an amino acid sequence. The RNA

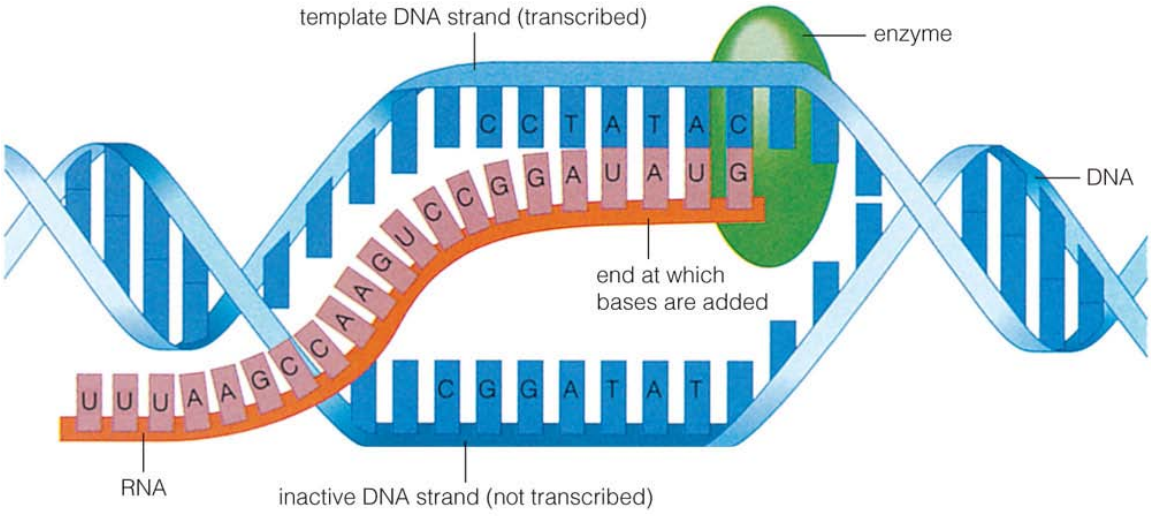


Figure 15.3. Transcription, the synthesis of RNA. One of the two DNA strands serves as a template to specify the order of bases in the growing RNA chain.

molecules that specify the amino acid sequences of particular proteins are called *messenger* RNAs (mRNAs). In plant cells, mRNAs carry the code (message) for the protein from the nucleus, where the genetic information is stored, to the cytoplasm, where the protein is synthesized.

The mRNA is translated to make a protein by interacting with ribosomes and tRNAs (Fig. 15.4). In translation, the ribosomes bind to the mRNA and then move along the mRNA three bases at a time while binding the appropriate tRNAs. A sequence of three mRNA bases is called *codon*. There are 64 different codons, each representing a particular amino acid, except for three codons that are "stop" signals. When the ribosome reaches a particular codon for an amino acid, it finds a tRNA with the complementary set of bases, known as the *anticodon*. This tRNA is carrying the amino acid specified by the codon on the mRNA. The ribosome connects the amino acid of the tRNA to the preceding amino acid with a peptide bond. More than one ribosome may work in this fashion on one mRNA, each ribosome forming one polypeptide chain. In some cases, the polypeptide chain automatically coils into the three-dimensional structure specified by its amino acid sequence. However, some polypeptide chains must be modified before they become active. Whatever the modifications, it is the DNA that provides the main information for producing each enzyme or other functional protein in the cell.

An example of the use of genetic information to make proteins is seen in the production of red and purple pigments in the petals of petunia (*Petunia* sp.) flowers. These pigments, called anthocyanins, are made in petal cells by a sequence of chemical reactions, each of which is catalyzed by an enzyme protein. The structure of each enzyme is specified by its own DNA template. The sequence of enzymes modifies the pigment molecules to make them absorb light more effectively. If a gene is mutated and an enzyme in the sequence is missing, the flower will have a less intense color (Fig. 15.5).

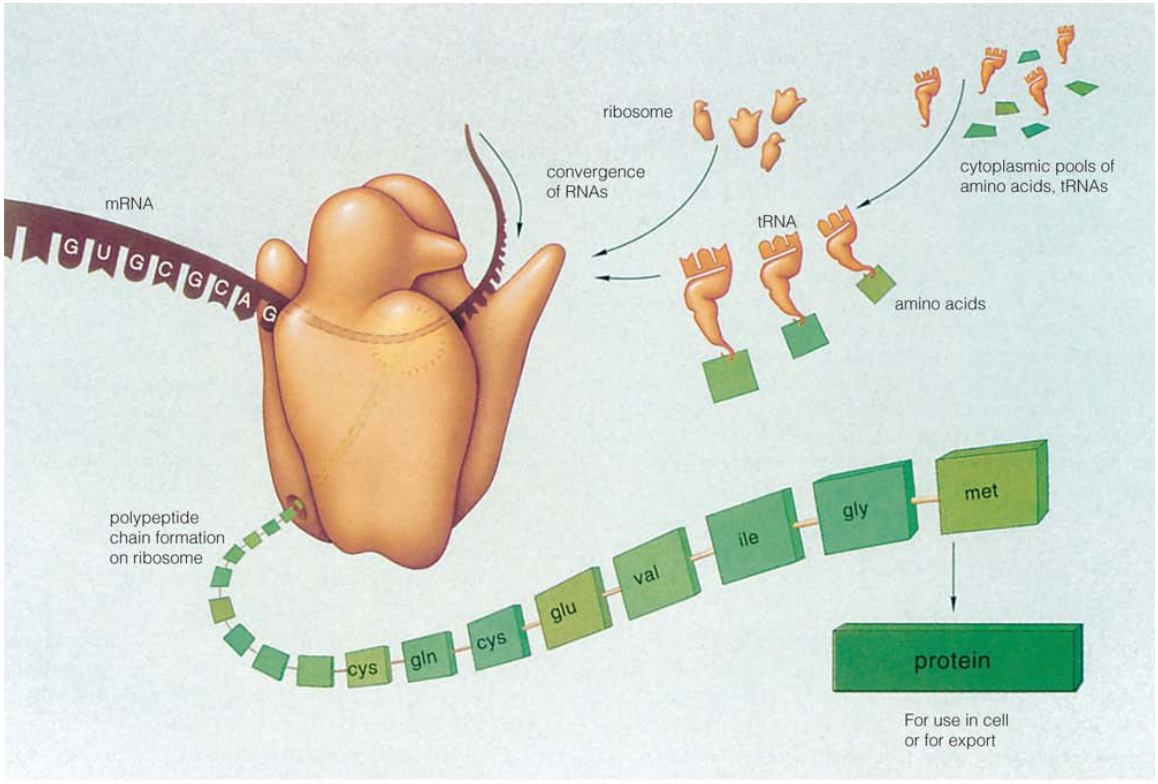


Figure 15.4. Translation, the synthesis of a protein. The ribosome serves as the central point where transfer RNA (tRNA) molecules match amino acids to the sequence of codons on the messenger RNA (mRNA). Through enzymatic action by the ribosome, the amino acids are joined to form a polypeptide chain.

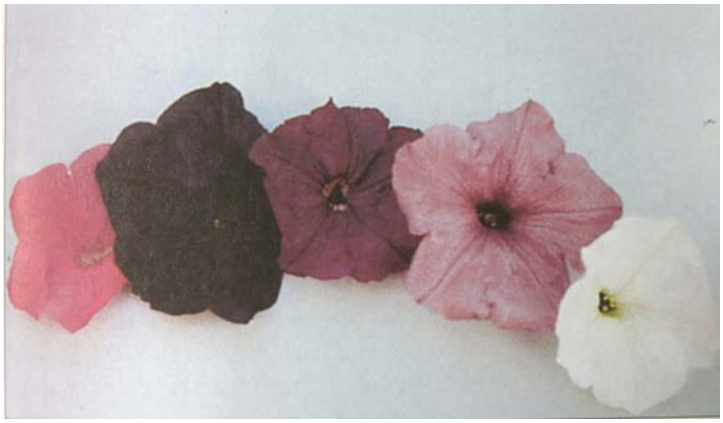


Figure 15.5. Differential effects of gene expression on the color of petunia petals. The dark purple flower (second from left) expresses all the genes needed to make the most complex anthocyanin pigment. The flowers third, fourth, and fifth from the left, by losing genes in the anthocyanin biosynthetic pathway, have progressively simpler pigments that are less effective in absorbing light. The pink flower on the left has all the genes in the anthocyanin biosynthetic pathway, but it also has a gene that changes the pH of the vacuole and thus the color of the pigment.

One particularly important enzyme early in the sequence is called *chalcone synthase*. Chalcone synthase is coded by the *Chs* gene, which is a segment of DNA on a chromosome in the nucleus of the cells. The expression of the *Chs* gene involves a long sequence of steps, the first of which is activating the DNA in the portion of the chromosome containing its gene. Inactive parts of chromosomes are

wound around proteins to form nucleosomes and more complex structures that fit into the nucleus. Activation may involve the removal of nucleosome proteins and unwinding of the DNA. The exact steps are still being worked out, but they may occur during the early development of the flower, rather than just before the synthesis of the enzyme. It is possible that the activation step is one of the processes leading to determination of the petal cells.

The next step is transcription of the *Chs* gene. As noted earlier, this involves the enzyme RNA polymerase and other proteins, which form a transcription complex, a structure that starts the process of RNA synthesis. Some of these proteins are always present in the cell; some may be synthesized specifically to start the transcription of a particular gene. For instance, certain genes must be expressed before the *Chs* gene itself can be expressed. It could be that they produce proteins needed for the transcription complex at the *Chs* gene. It is possible that the production of transcription complex proteins is one of the steps in a developmental program that makes a cell competent to respond to a stimulus.

The RNA molecule transcribed from the *Chs* DNA is called a nuclear RNA (nRNA). It is processed into mRNA and transported to the cytoplasm, where it is translated by ribosomes to make the protein. The protein may require further processing--for instance, the removal of a short sequence of amino acids--before being transported to the area in the cell where it functions. The *Chs* gene product, chalcone synthase, catalyzes a biochemical reaction in the cytoplasm. This reaction is the production of chalcone, which serves as a substrate (reactant) for the production of the red or purple pigments in the flower.

Each step in a gene's expression must occur for the gene to be expressed and the enzyme to be functional. The lack or inhibition of any one step would inhibit the synthesis of the enzyme and prevent the production of chalcone. Thus, turning off just one step has a major effect (for example, no color--that is, a white petal): turning it on reverses that effect. To learn how a gene's expression relates to development, it is necessary to learn what steps are turned off during some stages of development and what signals turn them on at the appropriate stages.

The Coordination of Development Requires a Series of Signals

Cells, tissues, and organs form in predictable patterns. The existence of patterns implies that the cells within a tissue or organ "sense" their position and develop accordingly. This in turn means that there must be short-range signals, presumably from adjacent cells, that identify their position within a tissue or organ. Furthermore, a plant generally has a characteristic shoot branching pattern, a pattern of root growth and function, and a pattern of flowering--all of which suggest coordination among the different organs. For such coordination to occur, long-range signals must exist that inform one part of the plant about conditions in another part. By the same reasoning, for plants to respond to the environment in appropriate ways, they must perceive signals from the environment. These signals include light, temperature, day length, water, nutrients such as calcium ions, and mechanical disturbances such as wind and wounding by herbivores.

Some signals may stimulate new patterns of gene expression; other signals may activate preexisting proteins or other cell components. In either case, the signal must be perceived by the target cells. That means that the cells must have a

receptor, a protein that is activated when it detects the presence of the signal. Although it is possible that the perception process may end there, recent studies of developmental control suggest that reception of the original signal generally starts a signal *cascade*--a series of events in which one signal leads to another and another (Fig. 15.6). Thus the original signal might be perceived by receptors on the plasma membrane (many are found there), yet, through a cascade system, trigger events at a distance, such as transcription in the nucleus. In addition, a single signal and receptor can through a branched cascade, affect several different developmental processes.

The original signals that coordinate the growth and development among cells are generally hormones, chemicals that diffuse or are transported from one part of the plant to another. Other possible signals are transient electrical impulses (that travel like nerve impulses from cell to cell along a stem or root) and hydraulic impulses (changes in the tension of the water column in the xylem). Environmental stimuli, such as light or temperature, also can serve as initial signals.

The signal receptors are proteins. Hormone receptors have binding sites with a three-dimensional shape into which the hormone fits. One common effect of activating a receptor is a transient increase of calcium in the cytoplasm. The normal calcium concentration in plant cell cytoplasm is low, but there is much calcium both outside the cell and inside, in the vacuole and in vesicles. Some stimulated receptors allow calcium to enter the cytoplasm, increasing the concentration by as much as 50 times. Because several enzymes are sensitive to calcium concentration, this change can have several effects. One possible effect is the activation of an enzyme that attaches a phosphate group onto other proteins, thereby either activating or inhibiting them. These proteins may lead to another link in the cascade of events. The cascade finally ends either in the activation (or inactivation) of a transcription factor--a protein that stimulates the reading of a particular gene--or an enzyme that produces the required differentiation of the cell.

Special Signals Regulate the Cell Cycle

One signal cascade is particular important for the control of growth. This is the signal that allows cells to pass checkpoints in the cell cycle, and thus to divide, forming new cells. Chapter 3 describes how cells fail to enter the S (DNA synthesis) or M (mitosis) phases of the cell cycle if they are starved. Researchers Lee Hartwell, Paul Nurse, and Timothy Hunt received the 2001 Nobel Prize for their discoveries of genes and proteins that control the cell cycles of yeast and animal cells. Plants have similar genes and proteins, although less is known about them.

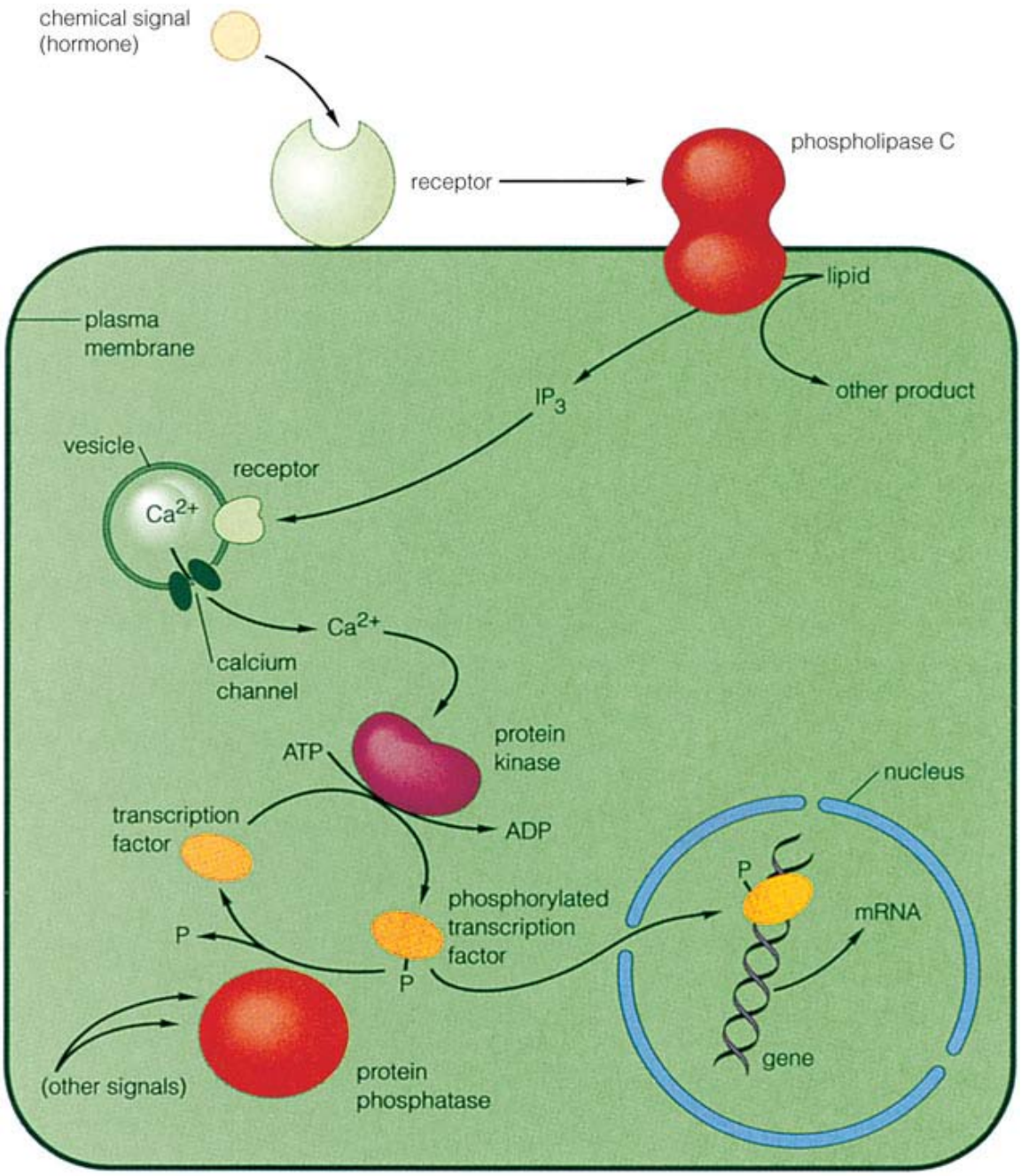


Figure 15.6. Hypothetical steps of a signal cascade in a plant cell. Arrows represent a series of activations or enzymatic reactions. The receptor is a protein in the plasma membrane that binds the hormone and is stimulated to activate phospholipase C. Phospholipase C is an enzyme in the plasma membrane that breaks down certain membrane lipids. Inositol 1,4,5-triphosphate (IP_3), a product of the reaction, stimulates a receptor on a vesicle, which releases calcium ions (Ca^{2+}) into the cytoplasm. The Ca^{2+} stimulates an enzyme, protein kinase, which adds a phosphate group from adenosine triphosphate (ATP) onto various proteins. One of the proteins may be a transcription factor, which stimulates the expression of a particular gene. A separate signal cascade could stimulate a protein phosphatase, which would inactivate the transcription factor.

One gene, the *cdc2* gene, is known to provide the genetic information for an enzyme protein involved in **phosphorylation** reactions. The enzyme is *cyclin-dependent protein kinase* (C-PK). This enzyme adds a phosphate functional group (-O-$PO_3H_2^-$) to another protein. Another kind of enzyme catalyzes **dephosphorylation** reactions. This removes phosphate from proteins. Proteins that have phosphate added or removed will change their three-dimensional shape and therefore their biological activity. In some cases, the phosphorylated form will be the active form of the protein, but in other cases, the dephosphorylated form will be the active form. This kind of phosphorylation/ dephosphorylation reaction seems to be the key to regulating the cell cycle.

C-PK works in conjunction with other proteins, call cyclins (Fig. 15.7). C-PK must itself be phosphorylated before it can combine with a cyclin. The initial combination is inactive. Then a phosphatase removes one phosphate, activating the C-PK-cyclin complex. The active form of the combined proteins acts as a protein kinase, and it is this enzyme that is the actual trigger to start the S or M phases. (Whether it starts an S or M phase depends on the type of cyclin.) After doing this, the complex is recycled. First, the complex is broken into its two parts. The C-PK can be reused by having new phosphate added to it. The cyclin protein is degraded into component amino acids; new cyclin must be resynthesized to restart the process. The need to regenerate C-PK and cyclin ensures that the cell must be healthy, not starved, to synthesize more DNA and divide again.

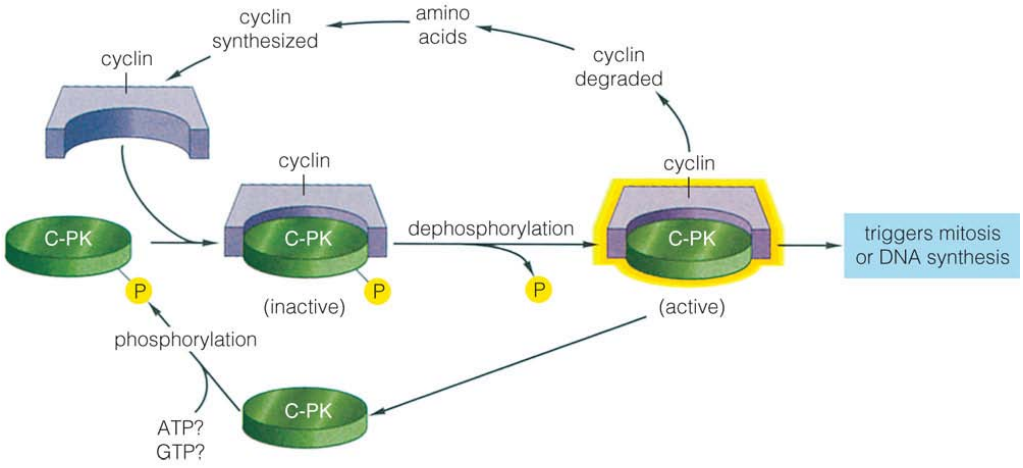


Figure 15.7. Diagram showing how the *cdc2* protein kinase (C-PK) and cyclin trigger cell cycle progression in cycling cells. At the left, cyclin has joined to C-PK in a phosphorylated form to make a new, but inactive, protein complex. An enzyme removes the phosphate (P) to activate this complex. This form of the protein complex acts as the trigger to switch on S phase and M phase. It is recycled by separating C-PK, which is phosphorylated again and recycled. The cyclin is broken down into amino acids and must be resynthesized.

15.2 HORMONES

Low concentrations of certain chemicals produced by a plant can promote or inhibit the growth or differentiation of various plant cells and coordinate development in different parts of the plant (Table 15.1). These chemicals have found many uses in agriculture (see "ECONOMIC BOTANY: using Plant Hormones" at the end of the chapter). By analogy with chemicals in animals that are secreted by glands into the bloodstream and influence development in different parts of the body, the chemical signals in plants have been called *hormones*. However, some scientists apply a rather strict definition to the term *hormone*: a chemical secreted from one organ in the body that influences another organ in specific ways. Although the chemicals in plants are diffusible, they often influence the same cells that produce them (as well as others); therefore, they are sometimes called growth regulators to distinguish them from the hormones that carry systemic or long-distance signals.

A variety of chemical compounds serve as hormones or growth regulators: auxins, gibberellins, cytokinins, abscisic acid, and ethylene are the five first studied.

Table 15.1 Coordination of Plant Development by Hormones

Hormone	Site of Synthesis	Site of Effect	Effect
Auxin	Stem apex, young leaves	Expanding tissues	Promotes cell elongation
		Roots	Initiates lateral roots
		Axillary buds	Inhibits growth (apical dominance)
		Cambium	Promotes xylem differentiation
		Leaves, fruits	Inhibits abscission
	Developing embryos	Ovary	Promotes fruit development
Gibberellins	Stem apex, young leaves	Stem internode	Promotes cell division, cell elongation
	Embryo	Seed	Promotes germination
	Embryo (grass)	Endosperm	Promotes starch hydrolysis
Cytokinin	Root apex	Stem apex, axillary buds	Promotes cell division (release of apical dominance)
		Leaves	Inhibits senescence
Abscisic acid	Leaves	Guard cells	Closes stomata
		Stem apex	Promotes dormant bud formation
	Ovule	Seed coat	Inhibits seed germination
Ethylene	Wounded tissues, aged tissues	Stem	Inhibits cell elongation
		Leaves	Promotes senescence
		Fruits	Promotes ripening

Plant Hormones Are Discovered by Studying Plant Developmental Processes

Auxin was discovered during studies of the growth of grass coleoptiles, a model system that has been used for investigation of growth since Charles Darwin and his son Francis worked of "The Power of Movement in Plants" in the 1880s. A coleoptile is a modified tubular leaf that surrounds the first true leaves and the stem of a germinating seedling of a member of the grass family (Poaceae). Moistened and incubated in the dark, a grass seed germinates, and its coleoptile grows rapidly straight up. By the time it is 1 to 2 cm in length, all cell divisions have ceased, and further elongation reflects only the expansion of the cells.

The Darwins' experiments demonstrated that the coleoptile tip was necessary for the elongation of the shaft (Fig. 15.8). A hypothesis attempting to explain this suggests that a substance produced at the tip is transported down the shaft, where it stimulates growth. The coleoptile also could be induced to bend toward a light, most effectively blue light, a movement called **phototropism**. If the coleoptile were placed on its side, it would bend upward, a movement called **gravitropism**. A reasonable explanation is that light and gravity cause a movement of the growth-promoting substance to one side of the shaft. The faster growth at that side causes the bending.

Experiments to test the idea of a diffusible growth-promoting substance were devised by N. Cholodny and Frits Went in the 1920s (Fig. 15.9). They cut off a coleoptile tip and placed it on a small block of agar (a gelatin-like substance), leaving it there long enough for diffusible substances to move into the agar. With the tip removed, growth of the shaft stopped. When they placed the agar on a decapitated coleoptile, the shaft resumed growth, showing that the agar had received chemicals from the tip that stimulated the elongation of the coleoptile (Fig. 15.9c). If the agar was placed on one side of the coleoptile, the growth was uneven, and the coleoptile bent away from the side that received the agar (Fig. 15.9d,e).

The growth of decapitated coleoptiles demonstrated the presence of a growth-promoting substance, which was named *auxin*. A substance that acted like an auxin was first purified from urine and identified as indoleacetic acid. (In our bodies, proteins are continually being broken down to their constituent amino acids, including one named tryptophan. A portion of the tryptophan is metabolized to indoleacetic acid and excreted in urine; it is simply a waste product.) Later, the same substance was found in plant extracts.

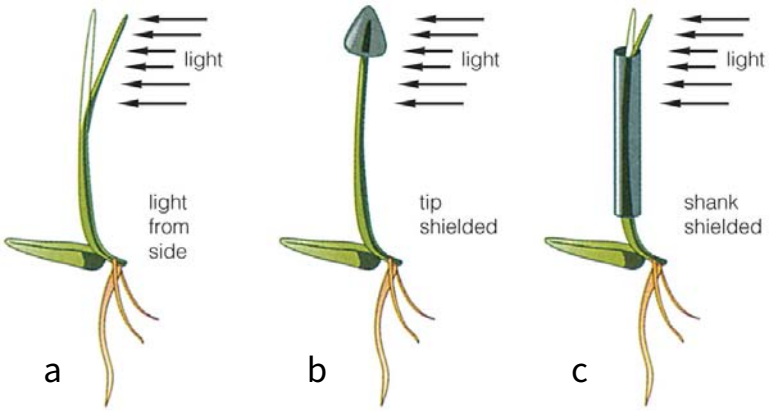


Figure 15.8. The Darwins' experiments on the dependence of grass coleoptile growth on light. The coleoptile is a modified hollow leaf. It grows upward in the dark but (a) bends toward a source of light. (b) If the tip is shielded from the light, there is no bending; (c) if the shaft is shielded but the tip is exposed, there is bending, showing that the perception of the light occurs at the tip.

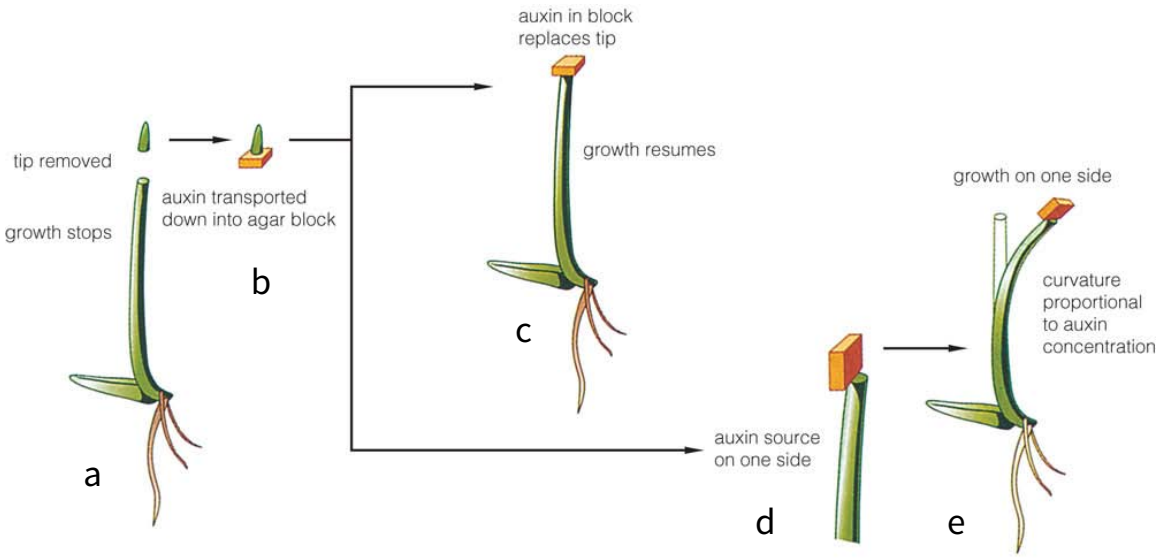


Figure 15.9. The dependence of coleoptile growth on auxin. (a) If the tip of the coleoptile is removed, growth ceases; thus, the tip produces some factor needed for growth. If (b) the tip is placed on an agar block; and later (c) the block is placed on the decapitated shaft, growth resumes. This shows that the growth factor is a diffusible chemical (auxin). If (d) the agar block is placed on one side of the shaft, the shaft bends as it grows, suggesting that the light-induced curvature (Figure 15.8) may be caused by a movement of auxin to the side away from the light.

Gibberellins are a group of similar plant hormones that stimulate the elongation of stem internodes. They were discovered in Japan by plant physiologists studying a disease of rice (*bakanae*, or *foolish seedling disease*) caused by a fungus named *Gibberella fujikuroi*. The infected rice seedlings grew much faster than uninfected ones, but they fell over and died before they could produce grain. The physiologists showed that chemicals produced by the fungus stimulated the early growth. Later, it was found that the same chemicals are normally made in low amounts in young leaves and transported throughout the plant in the phloem.

Cytokinin was discovered during experiments designed to define the conditions needed for culturing plant tissues. In the tissue culture procedure, pieces of stem, leaf, or root are removed from a plant. They are briefly sterilized on their surface to remove any microbes and then are placed in an enclosed flask contain a nutrient medium. The medium contains a carbon source such as sucrose or glucose, nutrient minerals, and certain vitamins. To induce the cells to divide and grow, hormones must also be added. Auxin is important, but most plant cells placed in a medium containing only auxin as a hormone enlarge without dividing. Early studies showed that a second hormone, found in solutions of boiled DNA and in coconut milk (a liquid endosperm), is also necessary. The active ingredient turned out to be cytokinin, a modified form of adenine, which is a component of DNA and RNA. In a medium containing nutrient components plus auxin and cytokinin, plant cells could divide and grow rapidly.

Further experiments showed that auxin and cytokinin influence the development, as well as the growth, of plant tissue cultures (Fig. 15.10). When there

is about 10 times more auxin than cytokinin added to the cell culture, growth is undifferentiated. The amorphous mass of cells, often loose and fluffy, is called a *callus*. When the auxin concentration is the medium is further increased, or when the nutrient concentration in the medium is reduced, the callus produces roots. But when the cytokinin concentration is increased, the callus becomes green and compact and produces shoots. These are general observations: in practice, the precise response of plant tissues to different auxin and cytokinin concentrations depends on the plant species and other growth conditions.

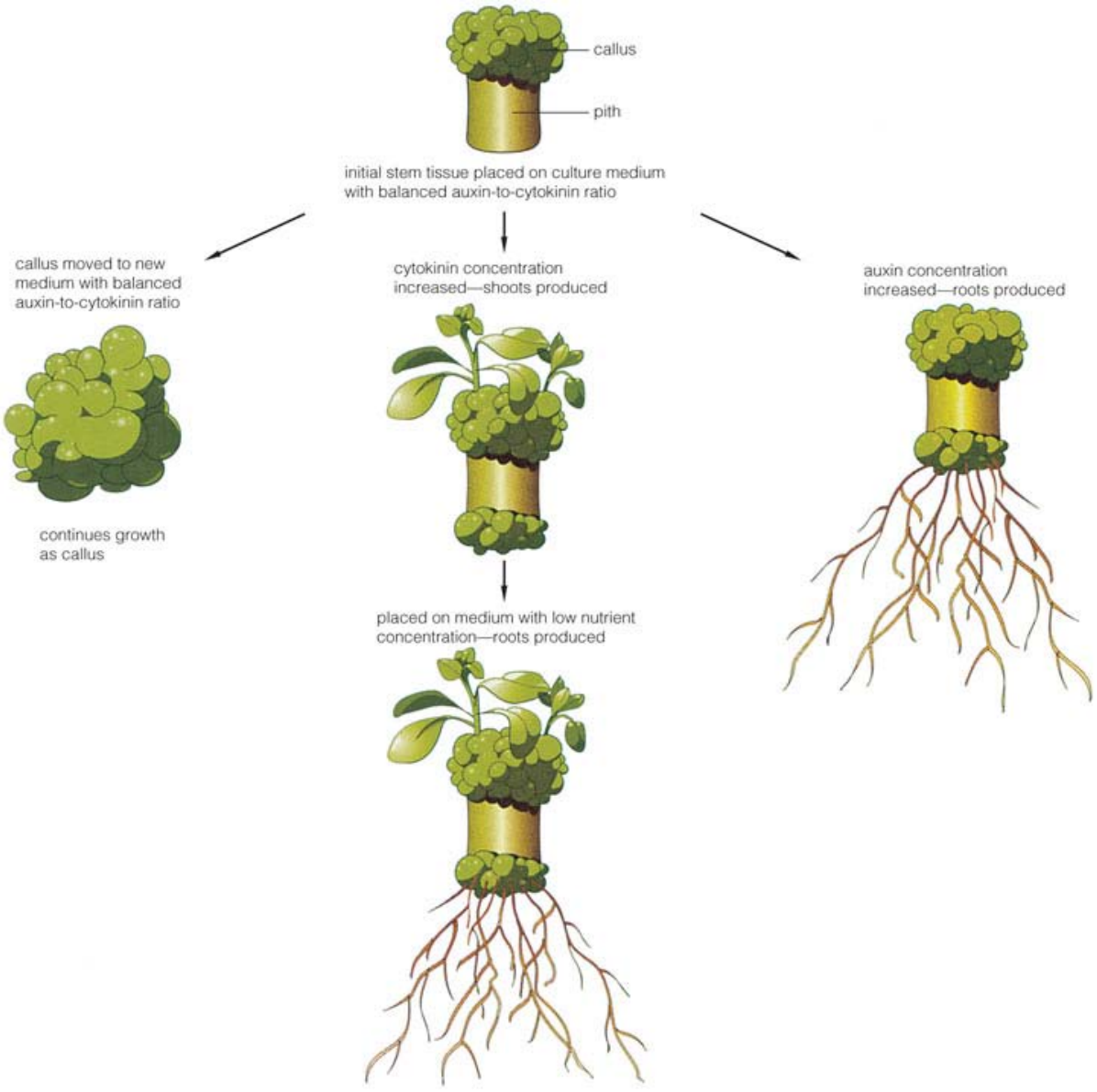


Figure 15.10. The interplay of auxin and cytokinin. Differences in the amounts of auxin and cytokinin can change the development of tobacco (*Nicotiana tabacum*) tissue in culture.

The hormone **abscisic acid** is more associated with the suspension of growth than with its stimulation. It was discovered at nearly the same time by two different groups of researchers. While at the University of California at Los Angeles, F.

Addicott discovered a compound that promoted the abscission (breaking off; see Chapter 6) of cotton cotyledon petioles from their stem. In Wales, P.F. Wareing and his coworkers found a compound that was associated with, and promoted, the dormancy of woody shoots in winter. These compounds turned out to be the same--absicic acid.

Ethylene is another hormone associated with the inhibition and modification of growth. Ethylene is a simple compound, with the composition C_2H_4. It is an unusual hormone because it is a gas at normal temperatures and pressures. The developmental effects of ethylene were discovered when the gas, produced by oil or kerosene heaters in greenhouses, stimulated the senescence of flowers and caused lemons and oranges to ripen (lose green color). Ethylene is produced by almost any wounded plant tissue and by unwounded tissues whose growth has been restricted, and it can move by diffusion to other nearby organs.

The following sections describe some phases of plant development that are initiated, accelerated, or inhibited by these compounds and by some newly discovered ones.

Signals from the Shoot Apex Promote Growth

In plants, auxin and gibberellins are normally produced by shoot apical meristems, young leaves, and developing fruits and seeds. From shoot apices and young leaves, they are actively transported down the stem toward the roots. They stimulate primary growth of the stem and have other effects.

The mechanism by which auxin stimulates the expansion of cells has inspired active research since the hormone was discovered. Cells enlarge when the internal pressure (turgor pressure) causes an irreversible stretching of the cell wall. It has been shown that the major effect of auxin is to increase the plasticity of the cell wall. This was accomplished by removing turgor pressure and exerting a known force on untreated and auxin-treated coleoptiles. The auxin-treated coleoptiles deformed more easily that the untreated coleoptiles and failed to snap back as far after the force was removed. We can assume that the same thing happens when the cell walls of auxin-treated cells are stretched normally by internal turgor pressure, and that this increased plasticity represents the reason for the increased growth rate induced by the auxin.

How does auxin stimulate the increase in plasticity? There are two hypotheses, and both may be correct. The first hypothesis, called the *acid-growth hypothesis,* suggests that the main effect of auxin is to cause cells to secrete acid (H^+ ions, rotons), and the acid stimulates the changes in plasticity. Experimental evidence to support this hypothesis comes from an increase in the rate of acid secretion by coleoptiles or stem tissue when they are treated with auxin. Auxin stimulates the activity of the plasma membrane proton pump, which results in the active secretion of H^+ ions. Also, coleoptile or stem sections that have been frozen and thawed several times (a treatment that kills their cells but leaves the components of their cell walls intact) become more plastic when they are soaked in acidic solutions. Notice that this hypothesis does not require that auxin treatment stimulate the expression of any genes.

The second hypothesis suggests that auxin works by inducing the expression of genes that make growth-promoting proteins. There also is evidence to support

this *induced gene expression hypothesis*. Translating mRNA is an essential step in gene expression. The application of chemicals that stop ribosomes from translating mRNA also prevents cells from increasing their growth rate in response to auxin. In addition, it has been possible to identify mRNAs that increase in concentration rapidly, within 10 to 20 minutes after stem sections have been treated with auxin. Soybean hypocotyls that bend under a gravitropic stimulus (lying on their side) show an asymmetric distribution of auxin-stimulated mRNAs, with more appearing on the lower side of the hypocotyls, before the bending starts. Although we still do not know what proteins all these mRNAs encode, these observations suggest that auxin-stimulated mRNAs, and the proteins for which they code, might be involved in the bending process, which is related to growth.

There is some evidence indicating the existence of special proteins that affect the cell wall's plasticity. Their activities may promote acidic conditions. For instance, one of the proteins induced by auxin stimulates the activity of the plasma membrane proton pump, which acidifies the cell wall. This is one way to explain how both of the hypotheses could be true. Other proteins may depend on acidic conditions. A protein called *expansin* has been extracted from apical regions of cucumber seedlings. When added to stretched, heated stem segments, it increases their plasticity, but only at pH values less than 6.0 (acidic conditions). Another protein has an enzymatic activity (*transglycosylation*) that fits what might be expected for an enzyme that promotes plasticity and growth. It breaks carbohydrate chains and re-forms them in new configurations that can result in a more extended wall (Fig. 15.11). It seems that the mechanisms of auxin-induced cell expansion are being unraveled.

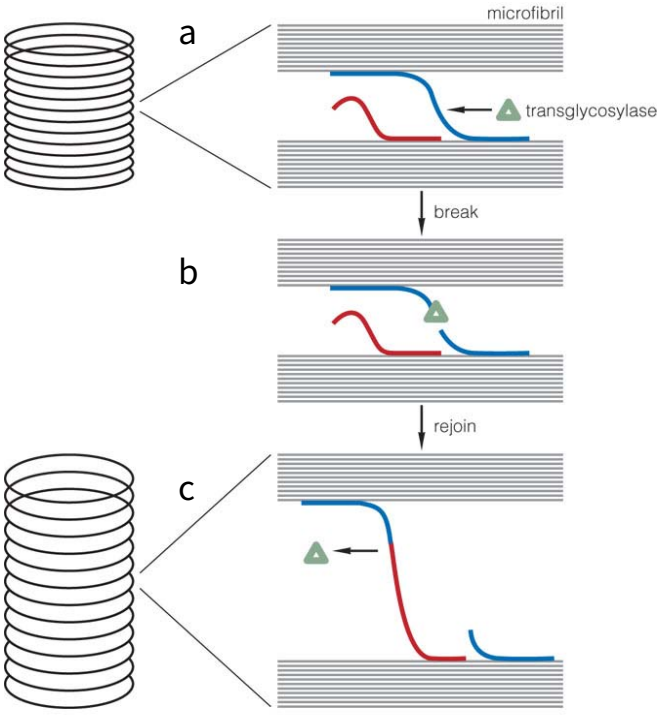


Figure 15.11. Model for the mechanism of cell wall elongation. (a) A cell is surrounded by cellulose microfibrils (gray), which are held together by other polysaccharides (red, blue). (b) A transglycosylation reaction breaks bonds between sugar molecules in cell wall polysaccharides and re-forms longer polysaccharides in a manner (c) that allows the microfibrils to separate and the cell to elongate. (Adapted from a model by S.C. Fry, University of Edinburgh, UK).

The elongation of stem internodes also depends on a supply of gibberellins. Evidence for that suggestion comes from experiments in which plants are sprayed with a chemical that inhibits gibberellin synthesis. Such plants develop shorter, stumpier stems with short internodes. The uninhibited gibberellin-stimulation of internode growth reflects both new cell division and increased cell elongation.

Auxin and gibberellins signal the presence and activity of the shoot apical meristems and help control further development of the shoot. Together with gibberellins, auxin stimulates cell division in the vascular cambium. Auxin promotes the formation of secondary xylem, whereas gibberellins stimulate formation of secondary phloem. Auxin also tends to inhibit the activity of axillary meristems near the apical meristems, restricting the formation of shoot branches, a phenomenon called apical dominance. When a plant is pruned, apical segments are removed, including the apical meristem and its auxin. With the inhibitory influence of the auxin gone, the axillary buds grow, producing a bushier plant. Finally, auxin stimulates the formation of new root apical meristems, both meristems forming lateral roots and meristems forming adventitious roots from the basal parts of shoots (Fig. 15.12). Thus, auxin helps coordinate root growth with shoot growth.

Figure 15.12. Holly (*Ilex opaca*) shoots form roots at their bases faster when the bases are treated with an auxin. The ends of these shoots were dipped for 5 seconds in solutions containing 50% ethanol and (from left to right) 0%, 0.1%, and 0.5% naphthalene acetic acid, a synthetic auxin. They were then rooted in moist vermiculite for 2 weeks.

Cytokinin Coordinates Shoot with Root Growth

Cytokinin is found in embryos and endosperm, where it promotes growth by stimulating the cell cycle, possibly by promoting cyclin synthesis. In mature plants, it has a similar role: it is produced in the roots and transported to the shoots in the xylem sap. A small amount of cytokinin applied to lateral buds of pea (*Pisum sativum*) shoots stimulates them to grow, even though their growth was previously inhibited by the apical dominance effects of auxin.

Cytokinin also delays senescence, the well-controlled process of deterioration that leads to the death of cells. The senescence of leaves, seen as they turn yellow, involves the breakdown of chlorophyll and proteins and the export of the products through the phloem to the meristems or other parts of the plant, where they can be recycled. Leaves detached from the stem and placed in water undergo this process, but adding cytokinin delays it by several days (Fig. 15.13). Because cytokinin is synthesized by the roots and moves in the xylem stream, the presence of cytokinin signals to the shoots the presence of healthy roots. Without roots, or when roots stop growing, the lack of cytokinin stops shoot growth and allows leaves to senesce.

Gibberellins, Abscisic Acid, and Ethylene Influence Shoot Growth in Response to Environmental Signals

A spectacular demonstration of the effect of gibberellins on internode growth is found in the bolting of plants with a rosette morphology (Fig. 15.14). Rosettes are plants with internodes so short that the leaves look almost as if they spring from a single node. Iceberg lettuce (*Lactuca sativa*), cabbage (*Brassica oleracea*), carrots (*Daucus carota*), and beets (*Beta vulgaris*) are all rosette plants. When these plants flower, the internodes of the stem elongate quickly. Flowers form on the new parts of the stem. Under natural conditions, bolting is triggered by environmental signals, such as a lengthening of the day or a period of cold temperature (Fig. 15.14c). Bolting also can be stimulated by spraying the plant with gibberellin (Fig 15.14b). Furthermore, it can be shown that the natural signal that stimulates bolting first induces the plant to synthesize gibberellin, Together , these two observations suggest that rosette plants are originally deficient in gibberellin and that they bolt when an environmental signal stimulates them to produce this hormone.

Figure 15.13. (above) The effect of cytokinin on senescence. Cytokinin applied to the right-hand primary leaf of this bean (*Phaseolus vulgaris*) seedling inhibits its senescence. The left-hand leaf was not treated with cytokinin.

Figure 15.14. (right) Gibberellin substitutes for a cold requirement in the bolting and flowering of a carrot plant (*Daucus carota*). (a) The rosette form of the carrot, maintained without a cold or gibberellin treatment. (b) A carrot plant that flowered in response to a treatment with gibberellin (no cold treatment). (c) A carrot plant that flowered in response to a treatment in the cold (no gibberellin treatment).

In the autumn, the shoot apices of most temperate region perennial plants go dormant. Dormancy is more complex than a simple inhibition of cell activity in the apical meristems. It involves growing protective bud scales (modified leaves that are small, nonphotosynthetic, dry, and tough) around the meristem before cell division stops. As days get shorter or temperatures decrease, abscisic acid accumulates in the shoots of perennial plants and stimulates the formation of a dormant bud at each shoot apical meristem.

Ethylene slows the growth of stem and roots (Fig. 15.15), when it is produced by wounded cells or by organs meeting a physical obstacle. A hypothesis has been advanced to explain this. In very small concentrations the gas disrupts the organization of microtubules close to the cell wall. These microtubules normally lie in parallel rows, and they are thought to control the direction of synthesis of the cellulose microfibrils so that the microfibrils lie parallel to the microtubules. Growth then occurs perpendicular to that direction. In the presence of ethylene, the microtubules are absent or disorganized, and the cellulose microfibrils lie in all directions. Growth, too, then occurs in all directions. Cells that develop that way are short and round, rather than long and thin. A stem or root growing under the influence of ethylene is short and stumpy because it is formed from short, round cells.

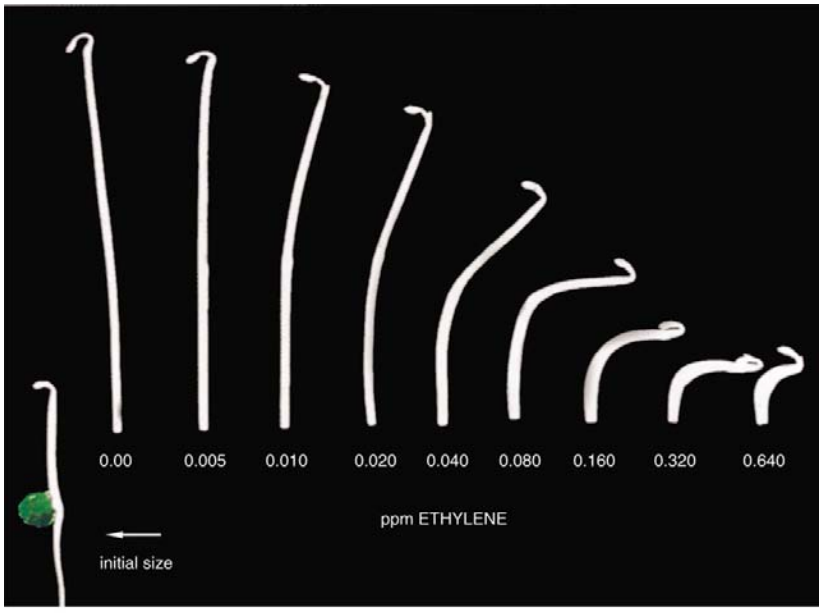


Figure 15.15. The effect of ethylene on growth. An increase in the concentration of ethylene makes dark-grown pea (*Pisum sativum*) seedlings shorter and thicker. Low concentrations of ethylene are effective. In this case, even the most strongly affected seedling has been treated with less than one part of ethylene per million parts of air. "ppm", parts per million.

Abscisic Acid and Gibberellins Control Seed Development and Germination

Abscisic acid plays a role in the formation of viable seeds. The presence of the hormone late in seed development induces the formation of large amounts of certain proteins by stimulating the transcription of their genes. These proteins are thought to store nitrogen, other elements, and energy for use by the embryo when it germinates.

Abscisic acid is also associated with dormancy of some seeds. It is accumulated in the seed coat during the seed's development. In the presence of this hormone, the embryo does not germinate, even if it is hydrated. Some of these seeds, for example, those of apple (*Malus sylvestris*) or cherry (*Prunus* sp.), require a long period under cool, wet conditions before they can germinate, conditions that stimulate the breakdown of the abscisic acid.

Initial experiments suggest that the abscisic acid receptor works through a signal cascade that includes phosphorylated proteins (Fig. 15.4). A mutant of mouse-eared cress (*Arabidopsis thaliana*) that lacks the ability to respond to abscisic acid lacks a protein phosphatase (an enzyme that removes the phosphate groups from various proteins). This suggests that removing phosphates is one step in the signal chain by which, in various tissues, abscisic acid stimulates storage protein synthesis or maintains dormancy.

Gibberellin promotes the germination of many types of seeds. In these systems, both cell division and cell elongation are stimulated. The mechanism of the stimulation remains obscure, although pea stems treated with gibberellin have an increased concentration of one of the cell wall-loosening enzymes described in the section on auxin-induced cell expansion. In addition, gibberellin apparently causes a reorientation of microtubules and, as a result, cellulose microfibrils, so that fewer microfibrils oppose the elongation of cells.

In at least one well-studied monocot system, barley (*Hordium vulgare*) seeds, gibberellin promotes the metabolic breakdown of storage materials (Fig. 15.16). Barley seeds have been studied for many years because the conversion of their starch to sugar is the key event in the malting process, the initial process in the brewing of beer. In malting, barley seeds are moistened with water, which begins the germination process. As the embryos start to grow, the seeds form enzymes (a-amylase, maltase) that convert the starch in the endosperm of the seed to the simple sugar glucose. The glucose would be used to nourish the growing seedling, except at this point the brewmaster bakes the malted seeds at high temperature to kill the seedlings and inactivate the enzymes. Caramelization of the sugars in the baking process gives the beer some of its flavor. The dry malt is ground up and then dissolved in water, ready for fermentation.

Without the embryos, moistened barley seeds soften, but they do not produce the enzymes that break down the starch. However, if the softened seeds are treated with low concentrations of gibberellins, a special layer of cells on the outer edge of the endosperm (the *aleurone* layer) synthesizes the enzymes and releases them into the starchy tissue. The gibberellins induce the transcription of the genes for a-amylase in that layer of cells. Newly made mRNA serves as a template for the synthesis of the enzyme. Under normal conditions, the gibberellins are made by the germinating embryo. Thus, they represent a signal from the embryo to the endosperm, announcing the need for nutrients.

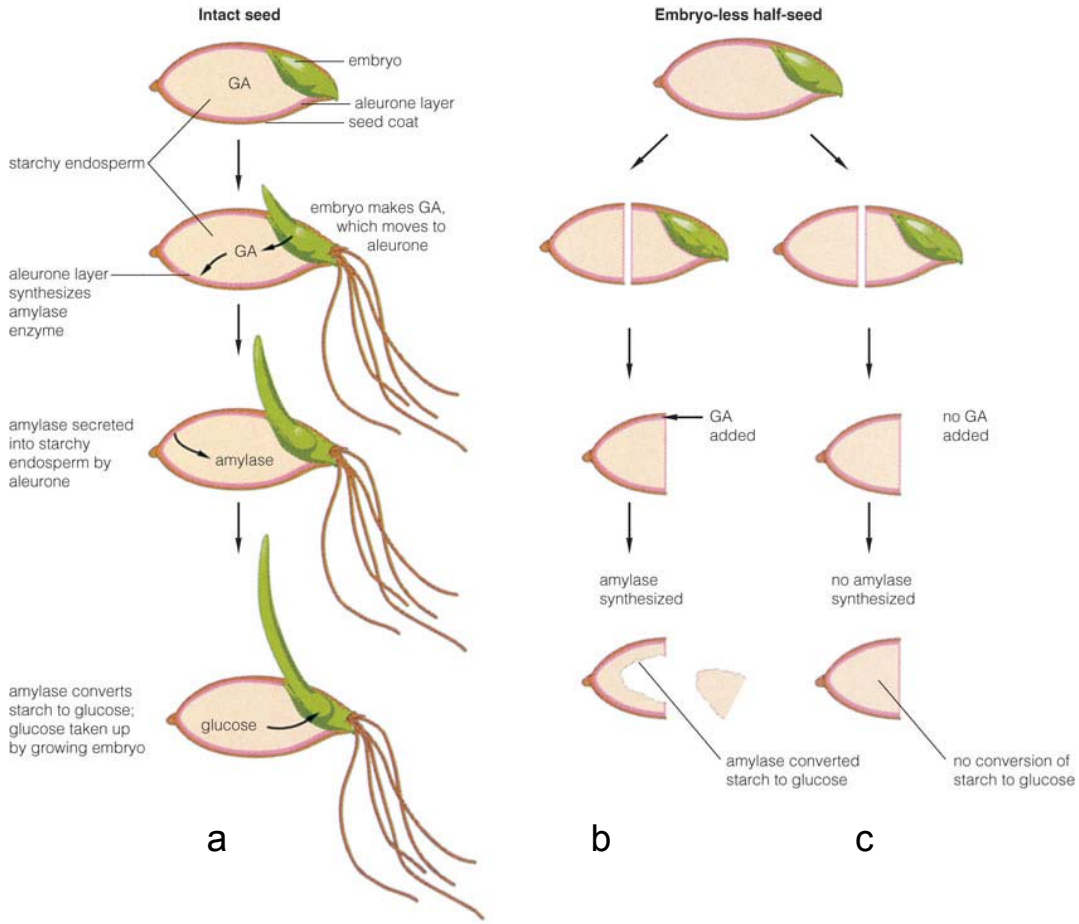


Figure 15.16. The effect of gibberellin (GA) on the germination of a barley (*Hordeum vulgare*) seed. (a) In an intact seed, GA from the embryo stimulates the synthesis of amylase, which breaks down starch to form the glucose that nourishes the embryo. (b,c) Removing the embryo removes the source of GA. If GA is added (b), amylase will be formed, but if not (c), no amylase will be synthesized and the endosperm starch will not break down.

Ethylene Stimulates Senescence

By far the most characteristic effect of ethylene is its stimulation of senescence. It takes special enzymes--chlorophyllases and proteases, by which chlorophyll and proteins are broken down--to start this process. Ethylene triggers the expression of genes leading to the synthesis of these enzymes. In many plants, senescence is associated with abscission, which is caused by enzymes that digest the cell walls in a localized region at the base of the petiole (the abscission zone). The petiole breaks from the stem at that point. The activities of the enzymes involved in both senescence and abscission are increased by ethylene. The mechanism by which ethylene induces the synthesis of these enzymes is not well understood, but a specific protein receptor that recognizes and binds to ethylene has been found. Many other pieces of the puzzle probably will be discovered in the next few years.

Figure 15.17. The effect of ethylene on the ripening of fruit. The box of tomatoes on the right was kept for 3 days in a room with an atmosphere containing 100 parts of ethylene per million parts of air. The fruit on the left had not yet been treated with ethylene.

The ripening of fruit is a variation on the process of senescence. Depending on the type of plant, it may involve the conversion of starch or organic acids to sugars and the softening of cell walls to form a fleshy fruit (Fig. 15.17), or the rupturing of the cell membrane with the resulting loss of cell fluid to form a dry fruit. In either case, ripening is stimulated by ethylene. There is an autocatalytic (self-promoting) aspect to this effect. Like wounded plant tissue, senescent plant tissue, including ripe fruit, forms ethylene. An overripe banana or apple is a potent source of ethylene. The ethylene that these fruits emit can stimulate senescence (ripening) in other adjacent fruits. This is the physiological truth behind the statement, "One bad apple spoils the barrel." By the same token, the ripening of fruit can be controlled by reducing the concentration of ethylene in the atmosphere. Picked applies, for instance, often are stored for months, without ripening, in an atmosphere containing scrupulously reduced concentrations of ethylene and high concentrations of CO_2, which inhibits the effect of ethylene, possibly by binding to its receptor.

A Variety of Compounds Serve as Stress Signals

Abscisic acid has another role, which is not concerned with development, but rather with the control of the photosynthetic system under stress. When water becomes so scarce that leaves wilt, it is important that the stomata close to prevent any further water loss. This is true even though closing stomata will cut off the supply of CO_2 and shut down the photosynthetic system. Abscisic acid is a signal of this emergency situation. Under drought conditions, wilted mesophyll cells of a leaf rapidly synthesize and excrete abscisic acid. This abscisic acid diffuses to the guard cells, where an abscisic acid receptor recognizes the presence of the hormone and acts to release potassium ion (K^+), chloride ion (Cl^-), and H_2O, closing the stomata.

As the attention of plant scientists has shifted to include developmental responses to biotic stress and infection, new signaling compounds have been discovered. One type of stress experienced by plants is attack by fungi and fungus-like parasitic protists. Once they detect a fungal infection or the threat of an infection, plants may develop various resistance mechanisms near the site of infection. They may, for instance, produce hydrogen peroxide (H_2O_2), which is thought to act as an antibiotic, killing the infectious agent. They may produce

enzymes that break down fungal cell walls. The plant cells surrounding the infection site may die, producing tannins (organic polymers related to lignin) in the process. This is effective because food and water are cut off them the site--and therefore from the fungus--and the tannins are hard to the fungus to digest. Thus the fungus has difficulty spreading through the dead area to new, live cells. Plants recognize the presence of a fungus from oligosaccharides, short chains of sugar molecules released from the fungal cell wall or from the plant cell wall under fungal attack. There are many kinds of oligosaccharides, but only the few kinds released from fungal cell walls signal the presence of fungus. It is possible that other oligosaccharides may influence developmental processes of healthy plants. For instance, certain oligosaccharides stimulate the formation of flowers from section of leaves grown in tissue culture.

The resistance induced by the perception of an infection may spread throughout a plant. That is, a plant that has been infected by a virus, bacterium, or fungus and survived may become less susceptible to a later invasion into other part of its body by the same pathogen (and some other pathogens, too). This phenomenon is known as *systemic acquired resistance* (Fig. 15.18). The mechanism is not fully understood, but it involves the transmission of a signal from the infected organ to new leaves, probably through the phloem, and the induction of several types of antibiotic proteins in the new leaves. (It does not involve antibodies of the type that appear in an immunized animal.)

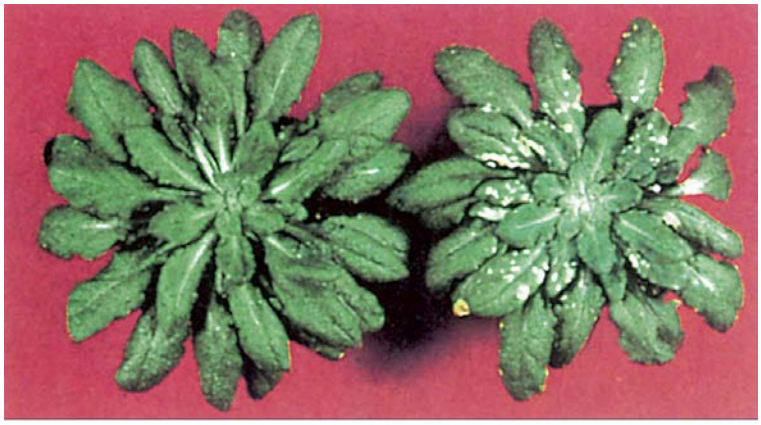


Figure 15.18. Systemic acquired resistance. Both mouse-eared cress (*Arabidopsis thaliana*) plants were inoculated with the pathogenic bacterium, *Pseudomonas syringae*. To stimulate its resistance mechanisms, the plant on the left had been treated earlier with a synthetic compound thought to mimic one of the natural signals that induces systemic acquired resistance. The plant on the right did not receive this treatment.

Recent research has focused on identifying the signals that induce resistance in leaves distant from the primary site of infection. Several compounds have been tentatively identified as being involved. Salicylic acid, a compound related to aspirin, is formed in infected plants, and it induces systemic resistance when it is applied to a plant. Both jasmonic acid, which is formed by the oxidation of a membrane lipid molecule during infections, and hydrogen peroxide can stimulate resistance

responses when applied to leaves. Perhaps the most interesting compound is an 18-amino-acid polypeptide chain named *systemin*, which is produced in response to infections and is required for establishing systemic resistance. If systemin is the signal that moves form the infected leaf to induce resistance in new leaves, it is the first polypeptide hormone to be discovered in plants.

Even more recently, physiologists have identified a set of compounds that may function to alleviate nutrient stress. These compounds, *strigolactones*, inhibit shoot branching, which may allow more focused growth in height. But more importantly, they are exuded from the roots, and serve to attract ascomycete fungi to form mycorrhizal associations with the roots. The fungi increase the ability of the roots to take up phosphate, an essential mineral (See Chapter 7, *IN DEPTH: How Do Roots Advertise their Presence?*).

15.3 LIGHT AND PLANT DEVELOPMENT

Plant development is strongly influenced by environmental factors, as well as by internally produced chemicals. Light is the most important of these factors. The form, growth rate, and reproduction of plants often are influenced by the light in the environment. Recognizing and responding to light is a major way in which plants adapt to their surroundings and match their activities to the time of day and season of the year. These responses result from mechanisms in addition to photosynthesis, which is itself a major light-influenced process. Plants "sense" three colors of light, which correspond to three (or more) distinct light receptors. Red light activates receptors known as phytochromes; blue and near-ultraviolet (black) light stimulate different receptors, called cryptochromes; intermediate-wavelength ultraviolet light (the ultraviolet B [UVB] radiation from the sun that causes sunburn) is detected by a third, currently unnamed, receptor.

The Red/Far-Red Response Acts Like an On/Off Switch

Some small seeds do not germinate in darkness. But after a brief soaking, a 1-minute exposure to red light will induce them to germinate. Surprisingly, the effect of the red light can be canceled if, immediately after the red light, the seeds are exposed to 5 to 10 minutes of far-red light. Far-red light includes those wavelengths of the spectrum that are longer than visible red light but shorter than the infrared radiation commonly thought of as heat radiation. A second exposure to red light, given after the far-red light, will stimulate germination again, showing that the far-red light reverses the first red signal, but does not inhibit the seeds in any irreversible way. It is as though an on/off switch were controlled by the two wavelength of light.

The red/far-red response governs many aspects of plant development, including the growth of seedlings. A pea seedling grown in the dark is long and light yellow, with unexpanded leaves and a tight hook just below the apical meristems (Fig. 15.19). This syndrome is called etiolation. Its purpose is to allow the shoot apex of these seeds, which may germinate several centimeters underground, to reach the

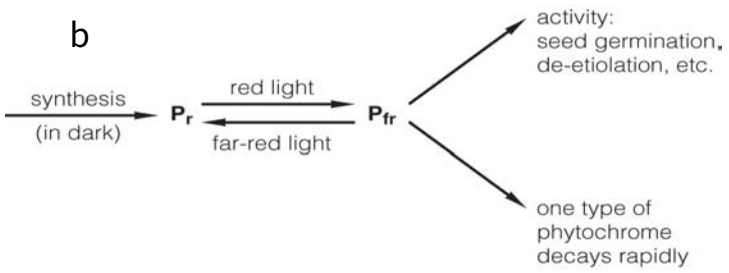


Figure 15.19. The red/far-red response. (a) Pea (*Pisum sativum*) seedlings grown in the light (left) and in the dark (right). (b) Light changes the form of phytochromes.

surface as rapidly as possible. Exposure of the seedlings to red light starts a process of de-etiolation: the hook open, the leaves expand and the growth of the stem slows. However, the de-etiolation process is retarded if the red signal is followed immediately by far-red light.

The red/far-red reversibility reflects the structure and function of **phytochromes**. Phytochromes are a type of protein containing a pigment molecule related to heme, the oxygen-carrying molecule in animal blood cells. When phytochrome is synthesized in the dark, its pigment can initially absorb red light. This form of phytochrome is called P_r (r for red-absorbing); it is thought to be inactive. If this phytochrome is irradiated with red light, it changes its form, and its pigment becomes capable of absorbing far-red light. This is the active form of the phytochrome, called P_{fr}, the form that stimulates seed germination and de-etiolation. When P_{fr} is exposed to far-red light, most of the phytochrome returns to its original form. Because P_r is inactive, whereas P_{fr} is active, irradiating phytochrome with red and then far-red light is equivalent to turning a switch on and then off. Scientists have not identified with certainty what a phytochrome actually does, but one possibility is that is attaches phosphates to other proteins.

In at least some plants, there are actually three or more types of phytochromes, each encoded by a different gene. All types are present in young, dark-grown seedlings. One type, present in greater concentrations that the others, is degraded by light. That is, in the light its P_{fr} form is rapidly broken down, and the gene that codes the protein is turned off. In adult plants growing in the light, only the more stable types of phytochromes are present. These, however, are adequate to make sure that adult plants show some red/far-red responses. For instance, the internodes of many plants are longer in the shade of a leafy canopy and shorter in bright light, a response that helps shoots grow out of darker areas and into the sun. This response occurs because chlorophyll in the canopy leaves absorbs red light more than far-red light. Thus the ratio of far-red light to red light is higher under the canopy than in unshaded areas. This means there will be more of the P_r form of phytochrome, which allows (does not inhibit) internode growth. Out from under the canopy, there is a higher ratio of red to far-red light; therefore, more P_{fr} is present, which inhibits internode growth.

Photoperiodic Responses Are Controlled by a Biological Clock

Over most of the earth's surface, there are pronounced seasonal differences in temperature, water availability, and illumination. Many plants show developmental changes that prepare them for the coming of both harsh and mild seasons. Before the winter, dormant buds are formed at the shoot apical meristems. The leaves of deciduous plants become senescent, turning colors as their chlorophyll and proteins are broken down. As spring comes, buds break open and start to grow, and the plant may flower. Of all the earth's seasonal variables, the most reliable and the most useful for anticipating changes in climate is the annual cycling of day and night lengths (Fig. 15.20). Plants have a system that measures the lengths of the days and nights. The control of development by this system is called **photoperiodism**.

Many plants use photoperiodism to time their flowering. These plants fall into two major groups, which traditionally are called long-day plants and short-day plants (Fig. 15.21). As you will see, these names are somewhat misleading, but they are too firmly embedded in the language of physiologists to be changed. (Many other plants time their flowering without reference to the length of the day, and these are called day-neutral plants.) In the northern hemisphere, long-day plants begin flowering sometime between January and June, when the days are getting longer. Each variety has a characteristic day length and begins flowering when the days are longer than that day length. Similarly, in the northern hemisphere short-day plants begin their flowering between July and December, when the days are getting shorter. Each variety of short-day plant begins flowering when the days become shorter than its characteristic day length.

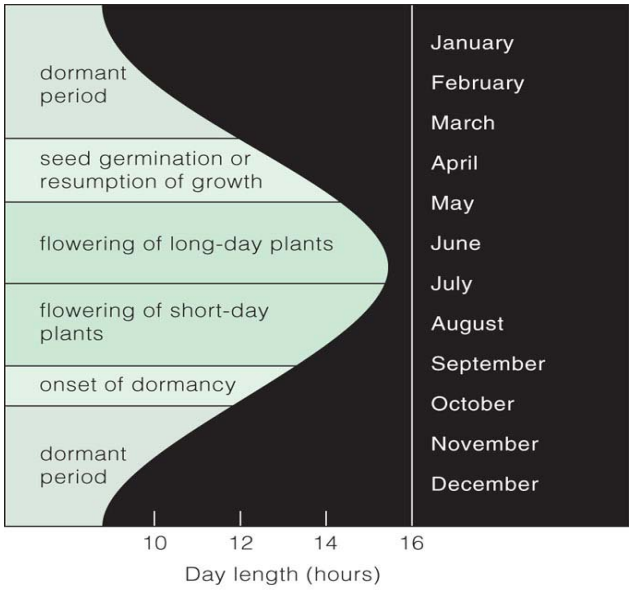


Figure 15.20. Effect of day length on flowering and other plant activities in temperate regions of the northern hemisphere.

A simple experiment demonstrates that the plants actually measure the length of the night, rather than of the day (Fig. 15.22a-c). Short-day plants are grown to maturity under conditions of long days (light periods) and short nights (dark periods). Then they are placed in a growth room in which the days are shorter than the characteristic length and the night are correspondingly longer. Under these conditions, the plants will flower. However, if a plant receives a pulse of light in the middle of the night, it does not flower. A pulse of darkness during the day has no effect. Because a light pulse at night blocks the signal to start flowering, it can be

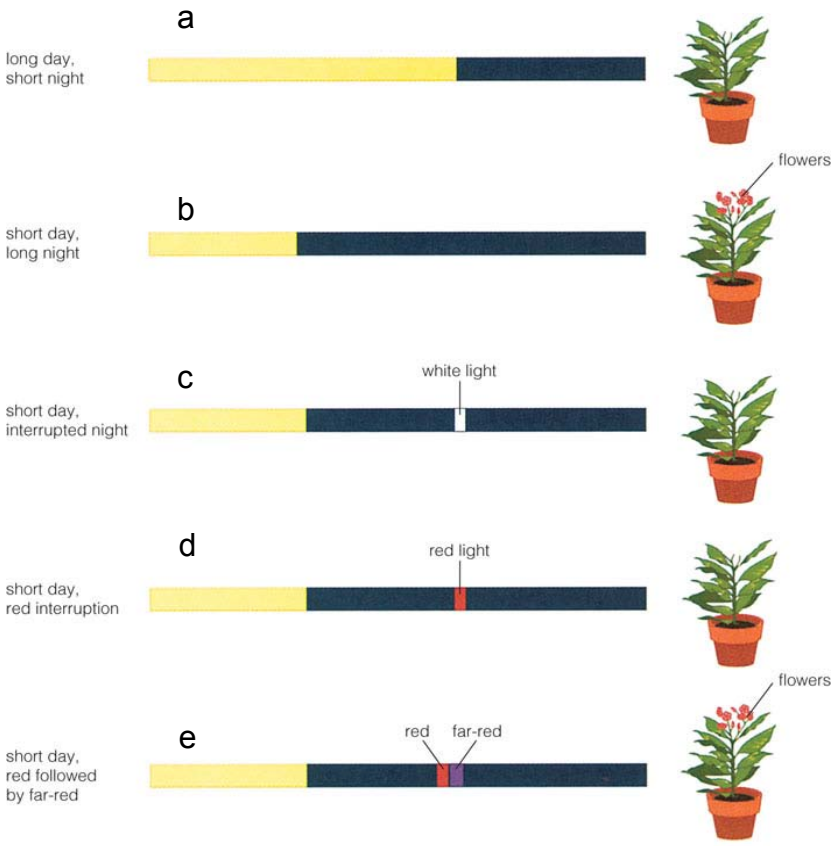


Figure 15.21. (left) Flowering in response to day (night) length. (a) Spinach (*Spinacia oleracia*), a long-day plant, initiates flowers and elongates its stem when the days are 14 hours or longer (nights are 10 hours or shorter). The plant on the right was kept in a growth chamber in 14-hour days; the one on the left was kept in 12-hour days. (b) *Chrysanthemum*, a short-day plant, flowers in the autumn, when the nights are long. Both plants were kept in short days, but the plant at the right received 1 hour of light near the middle of each night and did not flower.

Figure 15.22. (right) Effect of night length on short-day plants. (a-c) Experiments demonstrate that the length of night, not day, is the critical signal that stimulates flowering. (d,e) Far-red reversibility shows that phytochrome is the receptor by which the plant perceives an interruption of night by red light.

concluded that it is the length of the uninterrupted night that is the important part of the signal that stimulates flowering.

The same type of experiment can be used to show that phytochrome is the receptor by which the short-day plant perceives light (Fig. 15.22d,e). If different wavelengths (colors) of light are used to interrupt the long night period, it is red light that inhibits flowering, and only a short pulse of red light is needed. However, if a

short pulse of red light is followed by 10 minutes of far-red light, flowering proceeds as though the interruption never happened. The red/far-red reversibility is a characteristic of phytochrome.

Finally, this experiment can be modified to show that a biological clock is involved in measuring the length of the night. Biological clocks are known in all eukaryotic organisms. They are seen, for example, as daily (diurnal) rhythms in the internal temperature of animals and in the pattern of spore formation in fungi. The leaflets of some plants, such as *Mimosa,* open during the day and close at night. These **nyctinastic movements**, which are caused by the transport of ions and the resulting changes in the turgor pressures of cells on opposite sides of the petiole, provide clear evidence for the operation of a clock. Biological clocks are generally reset everyday by exposure to light; but because they continue to operate even in continuous darkness, they are called *endogenous*--that is, coming from within. They probably represent a basic biochemical or physical oscillation within cells, although the nature of this oscillation remains a mystery.

In one experiment, mature short-day plants are given their short day and then placed in the dark for 48 hours. Different plants are given pulses of light at different times after the start of the dark period. The effect of the light depends on the time it is given, with a periodicity that cycles about every 24 hours (Fig. 15.23a). Because the effect of light on the flowering responses of the experimental plants changes periodically, the responses of thought to be controlled by interaction between light and the endogenous biological clock. In this experiment, the action of the biological clock is made visible by how the plants respond to light, as if the clock turned on and off some aspect of the light-reception process (Fig. 15.23b).

The signal pathway that initiates flowering may be complex. The perception of day or night length occurs in the leaves, but flower development occurs at the apical or axillary meristems. Stimulating a single leaf by exposing it to a critical night length can be enough to channel development of the meristems toward flowering; in fact, grafting a stimulated leaf onto an unstimulated plant can induce the plant to flower. These observations have led to the hypothesis that a chemical compound, named florigen, is produced in the leaf and transported to the meristems. However, the chemical nature of florigen has not been determined, and even its existence is questioned. Gibberellins stimulate flowering in some plants, especially long-day plants and plants that require a period of cold temperatures (Fig. 15.14), but gibberellins do not work on many of the plants thought to respond to florigen. Auxin transport is needed for flower formation, but auxins are present in the meristems even when they are not flowering. Neither gibberellins nor auxins appear to be florigen. It is possible that florigen is a compound, such as an oligosaccharide or a protein, that is difficult to preserve once it has been extracted from a plant because enzymes destroy it in the extract; or perhaps it is difficult to detect because it cannot normally be taken up into plant tissues once it has been extracted.

Other responses that are controlled by photoperiodism, probably through the interaction between light and the endogenous clock, include dormancy and senescence in the fall and the resumption of growth in the spring.

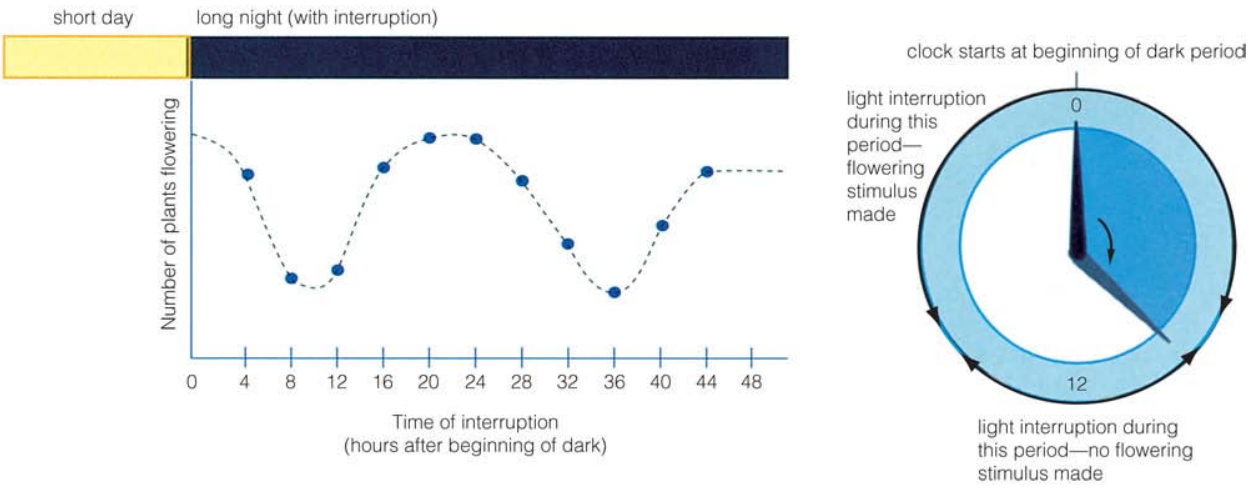


Figure 15.23. Evidence that a biological clock regulates flowering in short-day plants. (left) Each point on the graph represent a set of plants that was placed in the dark but was given a light pulse (interruption of the dark) at the time shown on the x-axis. Plants interrupted at different times showed different degrees of flowering, revealing a sensitivity to the light pulse that varied with a 24-hour period. (right) An interpretation of the night interruption experiment. An internal biological clock starts running when the lights are turned off. When the lights are turned on, the clock is reset. The effect of light depends on the position of the clock when it is reset.

Plants Respond to Light in Many Ways

Many responses of plants to light involve the blue part of the spectrum. These include such responses as bending toward light---phototropism (Fig. 15.8). They also include the induction of enzymes that synthesize the red pigments in the skins of such fruits as apples and plums. Actually, the latter example is a good way of demonstrating the complexity of light responses. The red pigments are anthocyanins, water-soluble compounds often seen in the lower epidermis of leaves and the petals of flowers. The induction of one of the enzymes involved, chalcone synthase, was described earlier in this chapter. In some seedlings, these pigments are formed after irradiation with red light, with phytochrome as the receptor. In seedlings of other species, these pigments are formed only after blue light irradiation. Still other plants require both red and blue light for a full response, although either red or blue light will stimulate the formation of some pigment. It seems that induction of the enzymes synthesizing these compounds may be connected to one or both light-signaling pathways.

Chlorophyll synthesis is another process that requires more than one color of light. Although bean seedlings that are grown in the dark will start the process of de-etiolation after a short red-light exposure, their leaves will not turn green if that is the only light they receive. The green color is chlorophyll, and the synthesis of chlorophyll requires a longer exposure to red or blue light. The receptor,

protochlorophyll, is a precursor to chlorophyll, and it must be excited by light photons before it can be converted to chlorophyll.

Thus the response of plants to light is complex, both at the molecular level and at the level of the whole plant. This should not be surprising, given the importance of light to the survival of a photosynthetic organism.

KEY TERMS

abscisic acid
auxin
cytokinin
dephosphorylation
determinate
determined
ethylene
gibberellins
gravitropism
indeterminate
nyctinastic movements
phosphorylation
photoperiodism
phototropism
phytochromes
totipotent

SUMMARY

1. The development of plants depends on both morphogenesis and differentiation. Morphogenesis is the creation of shape, determined by the frequency and direction of cell division and cell elongation. Differentiation is the acquisition of properties, determined by differential gene expression, that distinguish a cell, tissue, or organ from other cells, tissues, or organs.

2. Shoots and roots have indeterminate growth patterns: they may continue to grow without an obvious stopping point so long as conditions are favorable. An indeterminate growth pattern depends on the existence of a meristem. Some plant organs, such as flowers and dicot leaves, have a determinate growth pattern, which means that their growth stops at a predetermined size or age.

3. A *totipotent* cell can give rise to any cell type in a plant. Once a cell has become *determined*, however, it and its progeny have a limited number of possible differentiated states. A *competent* cell is primed to respond to a signal by differentiating in a predetermined manner.

4. The expression of a gene includes all of the following steps: activation of chromosomal DNA, transcription, translation, processing of the translation product to form an enzyme, transport of the enzyme to its site of action, activation of the enzyme, and formation of the product of the enzymatic reaction.

5. Chemical signals (hormones) coordinate development among different parts of a plant. Signals are perceived by receptors in a cell; perception often starts a chain of

events (a cascade) that results in the activation of specific genes, resulting in growth or differentiation. The *cdc2* gene in yeast, and other genes in plant cells, code for enzymes that are essential for allowing cells to start DNA synthesis or mitosis and thus to divide to form new cells.

6. The hormone auxin stimulates growth through cell enlargement in leaves, stems, and roots. It also stimulates cell division and differentiation in the vascular cambium, promotes the formation of root meristems, and inhibits stem branching (through apical dominance).

7. The stimulation of cell enlargement by auxin reflects an increase in cell wall plasticity. Two hypotheses have been advanced to explain this. The acid-growth hypothesis states that auxin works by stimulating the excretion of protons from the cell; the induced gene expression hypothesis states that auxin works by inducing the synthesis of cell wall proteins.

8. The hormone gibberellin stimulates the growth of stems, the germination of seeds, and the resumption of growth in winter-dormant buds. Gibberellin from the embryo triggers the metabolism of stored starch in germinating monocot (barley) seeds.

9. The hormone cytokinin stimulates cell division and inhibits senescence. Different ratios of cytokinin to auxin concentrations lead to the induction of different types of organs (callus, shoots, or roots) in plant tissue cultures.

10. The hormone abscisic acid is responsible for inducing dormancy in the shoot buds of perennial plants and for maintaining dormancy in seeds. It stimulates abscission of petioles from cotton seedlings and induces the synthesis of certain storage proteins during seed development. It is also a signal of drought stress in leaves, stimulating the closure of stomata.

11. Ethylene, a gaseous plant hormone produced by senescent plant tissues, stimulates senescence of leaves and fruits. It also inhibits stem and root growth by disrupting the organization of cellulose microfibrils in the cell wall.

12. Several chemicals with hormone-like signaling activity are involved in the induction of resistance to disease. These include oligosaccharides (parts of fungal cell walls), salicylic acid, hydrogen peroxide, jasmonic acid, and a polypeptide chain called systemin.

13. Plants have several light-receptive systems that influence their development: phytochromes respond to red and far-red light; cryptochrome(s) respond ot blue and near-ultraviolet light; and a separate system responds to intermediate-wavelength ultraviolet (UVB) radiation.

14. Phytochromes are proteins that have two interconvertible forms: P_r, which absorbs red light, and P_{fr}, which absorbs far-red light. P_r is converted to P_{fr} by absorbing red light; P_{fr} is converted to P_r by absorbing far-red light. Seedlings that develop in darkness have only the P_r form. They have long stems, poorly developed leaves, undeveloped chloroplasts, and are said to be etiolated. The conversion of P_r to P_{fr} by red light de-etiolates the seedlings by inhibiting stem growth, stimulating leaf growth, and starting maturation of the chloroplasts.

15. Plants use night length to control developmental processes in a way that adapts them to coming seasons. Plants that flower in response to short nights are called long-day plants, and those that flower in response to long nights are called short-day plants. In perennials, night length also influences the establishment of winter dormancy and the regrowth of buds in the spring.

16. The perception of day length by plants involves phytochrome and an endogenous oscillator (the biological clock). Plants seemingly measure the period of darkness between two light events; therefore, a light pulse that forms P_{fr} at a critical time during a dark period will stimulate flowering in a long-day plant and inhibit flowering in a short-day plant.

Questions

1. Which of the following organs show indeterminate growth: leaf, vegetative shoot, flowering shoot, carpel, root, stolon?

2. Describe how each of the following concepts applies to plant development: the formation of determined cells; the appearance of competent cells; the preservation of totipotent cells; indeterminate growth and modules.

3. If pieces of stem tissue are placed in the appropriate tissue culture medium, they will regenerate a whole plant. Discuss possible reasons why this can be done with plants and not with humans.

4. The expression of genetic information in the production of a protein involves several sequential steps. Place the following steps in the correct order: translation; activation of chromosomal DNA; transcription; activation of the enzyme; processing of nuclear RNA; processing and transport of the translation product; enzyme activity; editing of the transcript.

5. Criticize the following statement: Each hormone affects plant development by turning on the expression of a specific gene.

6. Using the concept of a signal cascade, describe how it is possible for both auxin and gibberellin to stimulate the elongation of young internode cells.

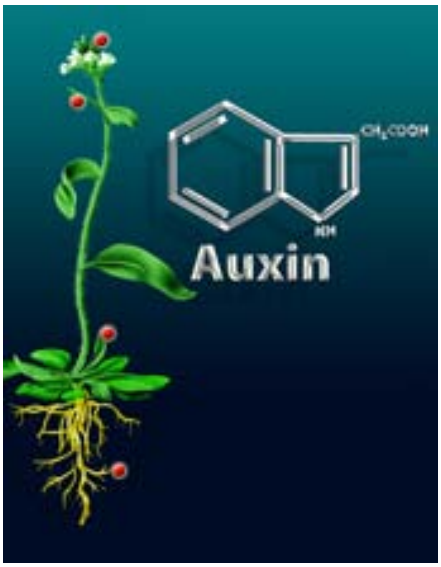
7. Name the hormone that is probably involved in the following responses:
   a. In early summer, your cabbage plants bolt and flower.
   b. In the fall, your ash tree stops growing and develops protective bud scales over its shoot apexes.
   c. The *Ficus* tree in your apartment is leaning toward the window.
   d. The last of your peaches is getting soft.
   e. You spray your *Ficus* tree with a new chemical, which causes lateral buds on the tree to start growing brach shoots.

8. Four oat coleoptiles have been cut from their seed. The first (a) has been slit vertically; the second (b) has its lower section covered by a light-tight shield; the third (c) has its upper section covered by a light-tight shield; the fourth (d) has had its tip removed. The coleoptiles have been placed in a support with light coming from one side. Which coleoptiles will bend toward the light? Assume no coleoptile shades another coleoptile.

9. Mature plants become leggy--that is, they have longer than normal internodes--when they grow under a think canopy of leaves. Chlorophyll in the canopy absorbs more red than far-red from sunlight. It is hypothesized that one form of phytochrome inhibits stem growth more than the other. In this hypothesis, which form , P_r or P_{fr}, inhibits stem growth more? Explain your answer.

10. A scientist has suggested that the synthesis of phytochrome is controlled by phytochrome itself because the protein is synthesized less rapidly in the light than in the dark. Which of the observations below would most convincingly support this suggestion? Discuss your answer.
   a. The mRNA for phytochrome is present only in the light.
   b. High intensities of light are necessary for the plant to make normal amounts of phytochrome.
   c. The rate of synthesis of phytochrome is greater in plants treated with a pulse of red light followed by a pulse of far-red light than in plants treated with only red light.
   d. Phytochrome is synthesized only in red light.

ECONOMIC BOTANY: ***Using Plant Hormones***



Plant hormones are used extensively to manage plant growth and development in home gardens and in agriculture. The first hormone to be discovered, auxin, also is the one most used by home gardeners. However it is generally not easy to tell when an auxin is present by inspecting the labels of products in a garden shop. The word auxin is seldom mentioned on labels, and the chemicals are generally synthetic analogs of the natural auxin (indoleacetic acid). Concentrations of auxins greater than the optimal inhibit plant growth and kill meristems. Dicots are particularly sensitive, but monocots are much less sensitive. Therefore, auxins such as 2,4-dichlorophenoxyacetic acid (2,4-D) are used as weed killers to remove dicot weeds from monocot grass lawns. Auxins stimulate fruit growth; therefore, a preparation of chlorophenoxyacetic acid is available for spraying on tomato blooms to increase fruit set. Because auxins stimulate the formation of roots, indole-3-butyric acid is sold as a "root stimulator and starter." Following are just a few of the uses of hormones in commercial horticulture and agriculture:

Auxins suppress dicot weeds in fields of corn and other monocots; stimulate root growth in cuttings of roses and other nursery plants; and delay fruit and leaf drop.

Gibberellins stimulate germination of seeds (many species); promote the growth of grape inflorescence internodes, leading to more open berry clusters that are less susceptible to fungal infections; promote berry growth in grapes; increase malt production for brewers; stimulate sugarcane elongation in winter, leading to an increase in yield of 0.7 metric tons per hectare (2 tons per acre); and stimulate early cone formation in conifers, leading to faster breeding programs.

Cytokinins, in a mixture with gibberellins, promote the growth and change the shape of apple fruits. Together with auxin, cytokinins are used in tissue culture propagation of, for instance, orchids.

Ethylene, applied as a solution of 2-chloroethane phosphonic acid, known as ethephon or by the trade name Ethrel, which releases ethylene when taken up by plant cells, stimulates and synchronizes flowering of pineapples; thins (causes a partial drop of) the fruit of cotton, cherries, and walnuts; and induces the ripening of apples, tomatoes, and other fruits. Reducing the amount of ethylene or inhibiting ethylene action is an important method of delaying fruit ripening, allowing fruits to be stored longer and shipped to markets farther away. Apples, for example, can be

stored for long periods under high CO_2 concentration and low atmospheric pressure, which inhibits ethylene action.

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