Chapter 39

Plant Responses to Internal and External Signals

Lecture Outline

Overview: Stimuli and a Stationary Life

- Some plants open and close their flowers at particular times of the day; these times are presumably when their insect pollinators are most active.
- The passage of time is only one of many environmental factors that a plant must sense in order to survive and reproduce successfully.
- Plants are far from inanimate objects. A plant's morphology and physiology are tuned to its surroundings by complex interactions between environmental stimuli and internal signals.
- At the organismal level, plants and animals respond to environmental stimuli by very different means.
  - Animals, being mobile, respond mainly by behavioral mechanisms, moving toward positive stimuli and away from negative stimuli.
  - Rooted in one location for life, a plant generally responds to environmental cues by adjusting its pattern of growth and development.
  - As a result, plants of the same species vary in body form much more than do animals.
- Before plants can initiate growth responses to environmental signals, they must detect the change in their environment.
- At the cellular level, the processes by which plants and animals perceive environmental changes are equally complex and often homologous.

Concept 39.1 Signal transduction pathways link signal reception to response.

- All organisms, including plants, have the ability to receive specific environmental signals and respond to them in ways that enhance their survival and reproductive success.
  - Like animals, plants have cellular receptors that they use to detect important changes in their environment.
  - These changes may be an increase in the concentration of a growth hormone, an injury from a caterpillar munching on leaves, or a decrease in day length as winter approaches.
- For a stimulus to elicit a response, certain cells in the organism must possess an appropriate receptor, a molecule that is affected by the specific stimulus.
- Upon receiving a stimulus, a receptor initiates a specific series of biochemical steps, a signal transduction pathway, that couples reception of the stimulus with response of the organism.
- Plants are sensitive to a wide range of stimuli, each initiating a specific signal transduction pathway.

- Plant growth patterns vary dramatically in response to the presence versus the absence of light.
- For example, a potato (a modified underground stem) can sprout shoots from its "eyes" (axillary buds).
  - These shoots are ghostly pale and have long, thin stems; unexpanded leaves; and reduced roots.
- Morphological adaptations for growing in darkness, called **etiolation**, also occur in seedlings germinated in the dark and make sense for plants that sprout underground.
  - Expanded leaves would hinder soil penetration and be damaged as the shoot pushes upward.
  - Because little water is lost in transpiration, an extensive root system is not required.
  - The production of chlorophyll is unnecessary in the absence of light.
  - A plant growing in the dark allocates as much energy as possible to the elongation of stems in order to break ground before the nutrient reserves in the tuber are exhausted.
- Once a shoot reaches the sunlight, its morphology and biochemistry undergo profound changes, collectively called **de-etiolation**, or greening.
  - The elongation rate of the stems slows, the leaves expand, the roots start to elongate, and the shoot produces chlorophyll.
- The de-etiolation response is an example of how a plant receives a signal—in this case, light—and how this reception is transduced into a response (greening).
- Studies of mutants have provided valuable insights into the roles that various molecules play in the three stages of cell-signal processing: reception, transduction, and response.
- Signals are first detected by receptors, proteins that change shape in response to a specific stimulus.
- The receptor for de-etiolation in plants is called a *phytochrome.*
  - Unlike many receptors, which are in the plasma membrane, this phytochrome is in the cytoplasm.
  - The importance of this phytochrome was confirmed through investigations of a tomato mutant, called *aurea*, that greens less than wild-type tomatoes when exposed to light.
  - Injecting additional phytochrome from other plants into *aurea* leaf cells and exposing them to light produced a normal de-etiolation response.
  - These experiments indicate that phytochrome functions in light detection during de-etiolation.
- Receptors such as phytochrome are sensitive to very weak environmental and chemical signals.
  - For example, exposure to only a few seconds of moonlight slows stem elongation in dark-grown oak seedlings.
- These weak signals are amplified by **second messengers**—small, internally produced chemicals that transfer and amplify the signal from the receptor to proteins that cause the specific response.
  - In the de-etiolation response, each activated phytochrome may give rise to hundreds of molecules of a second messenger, each of which may lead to the activation of hundreds of molecules of a specific enzyme.
- Light causes phytochrome to undergo a conformational change that leads to increases in the levels of the second messengers' cyclic GMP (cGMP) and Ca^{2+}.
- Changes in cGMP levels can lead to ionic changes within the cell by influencing the properties of ion channels.

- Cyclic GMP also activates specific protein kinases, enzymes that phosphorylate and activate other enzymes.
- Microinjection of cyclic GMP into *aurea* tomato leaf cells induces a partial de-etiolation response, even without the addition of phytochrome.

- Changes in cytosolic Ca^{2+} levels also play an important role in phytochrome signal transduction.
  - The concentration of Ca^{2+} is generally very low in the cytosol (about 10^{-7} *M*).
  - Phytochrome activation can open Ca^{2+} channels and lead to transient 100-fold increases in cytosolic Ca^{2+}.
- Ultimately, a signal transduction pathway leads to the regulation of one or more cellular activities.
- These responses to stimulation usually involve the increased activity of certain enzymes through two mechanisms: by transcriptional regulation (modifying the transcription of mRNA) or by post-translational modification (activating existing enzyme molecules).
- In transcriptional regulation, transcription factors bind directly to specific regions of DNA and control the transcription of specific genes.
  - In the case of phytochrome-induced de-etiolation, several transcription factors are activated by phosphorylation, some through the cyclic GMP pathway, while the activation of others requires Ca^{2+}.
  - The mechanism by which a signal promotes a new developmental course may depend on the activation of positive transcription factors (proteins that *increase* transcription of specific genes) or negative transcription factors (proteins that *decrease* transcription).
  - Some *Arabidopsis* mutants have a light-grown morphology (expanded leaves and short, sturdy stems) when grown in the dark.
  - These mutants are not green because the final step in chlorophyll production requires light.
  - The mutants have defects in a negative transcription factor that inhibits the expression of other genes normally activated by light.
  - When the negative factor is eliminated by mutation, the blocked pathway becomes activated.
  - Hence, these mutants, except for their pale color, appear to have been grown in the light.
- During post-translational modifications of proteins, the activities of existing proteins are modified by the phosphorylation of specific amino acids, altering the hydrophobicity and activity of the protein.
  - Many second messengers, such as cyclic GMP, and some receptors, including some phytochromes, activate protein kinases directly.
  - One protein kinase can phosphorylate other protein kinases, creating a kinase cascade, finally leading to the phosphorylation of transcription factors and influencing gene expression.
  - Thus, they regulate the synthesis of new proteins, usually by turning specific genes on and off.
- Signal pathways must also have a means for turning off when the initial signal is no longer present.
- Protein phosphatases, enzymes that dephosphorylate specific proteins, are involved in these "switch-off" processes.
- At any given moment, the activities of a cell depend on the balance of activities of many types of protein kinases and protein phosphatases.
- During the de-etiolation response, a variety of proteins are either synthesized or activated.
- These proteins include enzymes that function in photosynthesis directly or that supply the chemical precursors for chlorophyll production.
- Other proteins affect the levels of plant hormones that regulate growth.

- For example, the levels of two hormones (auxins and brassinosteroids) that enhance stem elongation decrease following phytochrome activation—hence, the reduction in stem elongation that accompanies de-etiolation.

Concept 39.2 Plant hormones help coordinate growth, development, and responses to stimuli.

- **Hormones** are chemical signals that are produced in minute amounts in one part of the body, are transported to other parts of the body, bind to specific receptors, and trigger responses in target cells and tissues.
- Plants and animals differ in their responses to hormones.
  - Plants don't have blood or a circulatory system to transport hormone-like signal molecules.
  - Some plant hormones act only locally.
  - Some signal molecules in plants, such as sucrose, typically occur at concentrations that are hundreds of thousands times higher than the concentration of a typical hormone.
  - Nevertheless, these signal molecules are transported through plants and activate signal transduction pathways that greatly alter the functioning of plants in a manner similar to a hormone.
- Many plant biologists prefer to use the broader term *plant growth regulator* instead of *hormone* to describe organic compounds, either natural or synthetic, that modify or control one or more specific physiological processes within a plant.
- For historical continuity, we shall use the term *plant hormone* and adhere to the criterion that plant hormones are active at very low concentrations.
- Virtually every aspect of plant growth and development is under hormonal control to some degree.
- A single hormone can regulate a diverse array of cellular and developmental processes.
- Conversely, multiple hormones may influence a single process.

Research on how plants grow toward light led to the discovery of plant hormones.

- Plants grow toward light; if you rotate a plant, it will reorient its growth until its leaves again face the light.
- Any growth response that results in curvature of whole plant organs toward or away from stimuli is called a **tropism**.
- The growth of a shoot toward light is called positive **phototropism**; growth away from light is negative phototropism.
- Much of what is known about phototropism has been learned from studies of grass seedlings, particularly oats.
- The shoot of a grass seedling is enclosed in a sheath called the coleoptile, which grows straight upward if kept in the dark or illuminated uniformly from all sides.
- If the shoot is illuminated from one side, it curves toward the light as a result of differential growth of cells on opposite sides of the coleoptile.
  - The cells on the darker side elongate faster than the cells on the brighter side.
- In the late 1800s, Charles Darwin and his son Francis observed that a grass seedling bent toward light only if the tip of the coleoptile was present.

- This response stopped if the tip was removed or covered with an opaque cap (but not a transparent cap or an opaque shield below the coleoptile tip).
  - Although the tip was responsible for sensing light, the actual growth response occurred some distance below the tip, leading the Darwins to postulate that some signal was transmitted from the tip downward.
- A few decades later, Peter Boysen-Jensen demonstrated that the signal was a mobile chemical substance.
  - Boysen-Jensen separated the tip from the remainder of the coleoptile by a block of gelatin, thus preventing cellular contact but allowing chemicals to pass.
  - These seedlings responded normally, bending toward light.
  - If the tip was separated from the lower coleoptile by an impermeable barrier, however, no phototropic response occurred.
- In 1926, Frits Went extracted the chemical messenger for phototropism, naming it *auxin.*
  - Modifying the Boysen-Jensen experiment, Went placed excised coleoptile tips on agar blocks and collected the substance that diffused into the agar.
  - If an agar block with this substance was centered on a coleoptile without a tip, the plant grew straight upward.
  - If the block was placed on one side, the plant began to bend away from the agar block.
  - Went concluded that the agar block contained a chemical produced in the coleoptile tip, that this chemical stimulated growth as it passed down the coleoptile, and that a coleoptile curved toward light because of a higher concentration of the growth-promoting chemical on the darker side of the coleoptile.
- Auxin was later purified by Kenneth Thimann, at the California Institute of Technology, and identified as indoleacetic acid (IAA).
- The classical hypothesis for what causes grass coleoptiles to grow toward light, based on the research described above, is that an asymmetrical distribution of auxin moving down from the coleoptile tip causes cells on the darker side to elongate faster than cells on the brighter side.
- However, studies of phototropism by organs other than grass coleoptiles provide little support for this idea.
  - There is no evidence that illumination from one side causes an asymmetrical distribution of auxin in the stems of sunflowers or other eudicots.
  - There *is* an asymmetrical distribution of certain substances that may act as growth *inhibitors*, with these substances more concentrated on the lighter side of a stem.

Plant hormones help coordinate growth, development, and responses to environmental stimuli.

- Some of the major classes of plant hormones are auxins, cytokinins, gibberellins, brassinosteroids, abscisic acid, and ethylene.
  - Many molecules that function in plant defenses against pathogens are probably plant hormones as well.
- Plant hormones tend to be relatively small molecules that are transported from cell to cell across cell walls, a pathway that blocks the movement of large molecules.
- Plant hormones are produced at very low concentrations.
- Signal transduction pathways amplify the hormonal signal many-fold and connect it to a cell's specific responses.

- These responses include altering the expression of genes, affecting the activity of existing enzymes, and changing the properties of membranes.
- Each modification redirects the metabolism and development of a cell responding to a hormone.

- In general, plant hormones control plant growth and development by affecting the division, elongation, and differentiation of cells.
- Some hormones also mediate shorter-term physiological responses of plants to environmental stimuli.
- Each hormone has multiple effects, depending on its site of action, its concentration, and the developmental stage of the plant.
- Response to a hormone usually depends not so much on its absolute concentration as on its relative concentration compared to other hormones.
- It is hormonal balance, rather than hormones acting in isolation, that control the growth and development of plants.
- The term **auxin** is used for any chemical substance that promotes the elongation of coleoptiles, although auxins actually have multiple functions in angiosperms.
- The natural auxin occurring in plants is indoleacetic acid (IAA), but several other compounds also have auxin activity.
- In growing shoots, auxin is transported unidirectionally, from the shoot apex down to the base.
    - The speed at which auxin is transported down the stem from the shoot apex is about 10 mm/hr, a rate that is too fast for diffusion but slower than translocation in the phloem.
    - Auxin seems to be transported directly through parenchyma tissue, from one cell to the next.
    - This unidirectional transport of auxin is called polar transport and has nothing to do with gravity.
    - Auxin travels upward if a stem or coleoptile is placed upside down.
    - The polarity of auxin transport is due to the polar distribution of auxin transport protein in the cells.
    - Concentrated at the basal end of the cells, auxin transporters move the hormone out of the cell and into the apical end of the neighboring cell.
- Although auxin affects several aspects of plant development, one of its chief functions is to stimulate the elongation of cells in young shoots.
- The apical meristem of a shoot is a major site of auxin synthesis.
- As auxin moves from the apex down to the region of cell elongation, the hormone stimulates cell growth, binding to a receptor in the plasma membrane.
    - Auxin stimulates cell growth over only a certain concentration range—from about 10^{-8} to 10^{-4} *M*.
    - At higher concentrations, auxins may inhibit cell elongation, probably by inducing the production of ethylene, a hormone that generally acts as an inhibitor of elongation.
- According to the acid growth hypothesis, in a shoot's region of elongation, auxin stimulates the plasma membrane's proton pumps, increasing the voltage across the membrane and lowering the pH in the cell wall.
    - Lowering the pH activates **expansin** enzymes that break the cross-links between cellulose microfibrils and other cell wall constituents, thus loosening the wall.
    - Increasing the membrane potential enhances ion uptake into the cell, which causes the osmotic uptake of water, increasing turgor and elongating the loose-walled cell.

- Auxin rapidly alters gene expression, causing cells in the region of elongation to produce new proteins within minutes.
- Some of these proteins are short-lived transcription factors that repress or activate the expression of other genes.
- Auxin stimulates a sustained growth response of making the additional cytoplasm and wall material required by elongation.
- Auxin is used commercially in the vegetative propagation of plants by cuttings.
  - Treating a detached leaf or stem with rooting powder containing auxin often causes adventitious roots to form near the cut surface.
- Auxin is also involved in the branching of roots.
  - One *Arabidopsis* mutant that exhibits extreme proliferation of lateral roots has an auxin concentration 17-fold higher than normal.
- Synthetic auxins, such as 2,4-dinitrophenol (2,4-D), are widely used as selective herbicides.
  - Monocots, such as maize or turfgrass, can rapidly inactivate these synthetic auxins.
  - Dicots cannot activate the synthetic auxins, however, and they die from a hormonal overdose.
  - Thus, spraying cereal fields or turf with 2,4-D eliminates dicot (broadleaf) weeds such as dandelions.
- Auxin also affects secondary growth by increasing cambial activity and influencing the differentiation of cambial initials.
- Developing seeds synthesize auxin, which promotes the growth of fruit.
  - Synthetic auxins sprayed on tomato vines induce the development of seedless tomatoes without pollination.
- **Cytokinins** stimulate cytokinesis, or cell division.
- Cytokinins were originally discovered in the 1940s by Johannes van Overbeek, who found that he could stimulate the growth of plant embryos by adding coconut milk to his culture medium.
- A decade later, Folke Skoog and Carlos O. Miller induced cultured tobacco cells to divide by adding degraded samples of DNA.
- The active ingredients in both cases were modified forms of adenine, one of the components of nucleic acids.
- These growth regulators were named cytokinins because they stimulate cytokinesis.
- The most common naturally occurring cytokinin is zeatin, named for the maize *(Zea mays)* in which it was found.
- Much remains to be learned about cytokinin synthesis and signal transduction, but the effects of cytokinins on cell division and differentiation, apical dominance, and aging are well known.
- Cytokinins are produced in actively growing tissues, particularly in roots, embryos, and fruits.
  - Cytokinins produced in the root reach their target tissues by moving up the plant in the xylem sap.
- Cytokinins interact with auxins to stimulate cell division and influence differentiation.
  - If a piece of parenchyma tissue is grown in tissue culture without cytokinins, the cells grow large but do not divide.
  - If cytokinins and auxins are added to the tissue, the cells divide, whereas cytokinins alone have no effect.
- The ratio of cytokinins to auxins controls cell differentiation.

- If the ratio of cytokinins to auxins is at a particular level, then the mass of growing cells, called a callus, remains undifferentiated.
- If cytokinin levels are raised, shoot buds develop from the callus.
- If auxin levels are raised, roots form.

- Cytokinins, auxins, and other factors interact in the control of apical dominance, the ability of the apical bud to suppress the development of axillary buds.
- Until recently, the leading hypothesis for the role of hormones in apical dominance—the direct inhibition hypothesis—proposed that auxins and cytokinins act antagonistically in regulating axillary bud growth.
  - Auxins transported down the shoot from the apical bud would inhibit axillary bud growth, causing the shoot to lengthen without branching.
  - Cytokinins entering the shoot system from the roots would signal axillary buds to grow.
- Many observations are consistent with the direct inhibition hypothesis.
  - If the apical bud, the primary source of auxins, is removed, the inhibition of axillary buds is removed and the plant becomes bushier.
  - This action can be inhibited by adding auxins to the cut surface.
  - Mutants that overproduce cytokinins or plants treated with cytokinins are bushy.
- The direct inhibition hypothesis predicts that decapitation, removing the primary source of auxins, should lead to a decrease in auxin levels in the axillary buds.
- However, auxin levels actually *increase* in the axillary buds of decapitated plants, so further research is necessary to connect all the pieces of this puzzle.
- Cytokinins retard the aging of some plant organs.
- Cytokinins inhibit protein breakdown by stimulating RNA and protein synthesis and by mobilizing nutrients from surrounding tissues.
  - Leaves removed from a plant and dipped in a cytokinin solution stay green much longer than otherwise.
  - Cytokinins also slow the deterioration of leaves on intact plants.
  - Florists use cytokinin sprays to keep cut flowers fresh.
- A century ago, farmers in Asia noticed that some rice seedlings grew so tall and spindly that they toppled over before they could mature and flower.
- In 1926, E. Kurosawa discovered that a fungus in the genus *Gibberella* causes this "foolish seedling disease."
- The fungus produced hyperelongation of rice stems by secreting a chemical, which was given the name **gibberellin**.
- In the 1950s, researchers discovered that plants also make gibberellins.
- Researchers have identified more than 100 different natural gibberellins.
  - Typically each plant produces a much smaller number.
  - "Foolish rice" seedlings from an overdose of growth regulators normally found in lower concentrations.
- Gibberellins have a variety of effects, such as stem elongation, fruit growth, and seed germination.
- Roots and young leaves are the major sites of gibberellin production.
- Gibberellins stimulate growth in both leaves and stems but have little effect on root growth.

- In stems, gibberellins stimulate cell elongation *and* cell division.
  - One hypothesis proposes that gibberellins stimulate cell wall–loosening enzymes, facilitating the entry of expansin proteins.
  - In a growing stem, auxins, by acidifying the cell wall and activating expansins, and gibberellins, by facilitating the penetration of expansins, act in concert to promote elongation.
- The effects of gibberellins in enhancing stem elongation are evident when dwarf varieties of plants are treated with gibberellins.
  - After treatment with gibberellins, some dwarf pea plants grow to normal height.
  - If gibberellins are applied to wild-type plants, however, there is often no response, perhaps because these plants are already producing the optimal dose of the hormone.
- The most dramatic example of gibberellin-induced stem elongation is bolting, the rapid formation of the floral stalk.
- In many plants, both auxins and gibberellins must be present for fruit to set.
- If Thompson seedless grapes are sprayed with gibberellin during development, the internodes of the grape bunch elongate, allowing more space for each grape.
  - The extra space promotes air circulation between the grapes and makes it harder for yeast and other microorganisms to infect the fruits.
- The embryo of a seed is a rich source of gibberellins.
  - After water is imbibed, the release of gibberellins from the embryo signals the seed to break dormancy and germinate.
  - Some seeds that require special environmental conditions to germinate, such as exposure to light or low temperatures, break dormancy when they are treated with gibberellins.
  - Gibberellins support the growth of cereal seedlings by stimulating the synthesis of digestive enzymes that mobilize stored nutrients.
- **Brassinosteroids** are steroids that are chemically similar to cholesterol and the sex hormones of animals.
- Brassinosteroids induce cell elongation and division in stem segments and seedlings at concentrations as low as 10^{-12} *M*.
- They also retard leaf abscission and promote xylem differentiation.
- Their effects are so qualitatively similar to those of auxins that it took several years for plant physiologists to recognize that brassinosteroids were not types of auxins.
- Joann Chory and her colleagues provided evidence from molecular biology that brassinosteroids are plant hormones.
  - A brassinosteroid-deficient *Arabidopsis* mutant has morphological features similar to those of light-grown plants even when grown in the dark.
  - The mutation affects a gene that normally codes for an enzyme similar to one involved in steroid synthesis in mammalian cells.
  - The mutant was restored to normal by the experimental application of brassinosteroids.
- **Abscisic acid (ABA)** was discovered independently in the 1960s, when one research group studying bud dormancy and another investigating leaf abscission isolated ABA.
- Ironically, ABA is no longer thought to play a primary role in either bud dormancy or leaf abscission, but it is an important plant hormone with a variety of functions.
- ABA generally *slows* growth.

- Often ABA antagonizes the actions of the growth hormones—auxins, cytokinins, gibberellins, and brassinosteroids.
- It is the ratio of ABA to one or more growth hormones that determines the final physiological outcome.

- One major effect of ABA on plants is seed dormancy.
  - Seed dormancy has great survival value because it ensures that seeds germinate only when there are optimal conditions of light, temperature, and moisture.
- Levels of ABA may increase 100-fold during seed maturation, leading to inhibition of germination and inducing the production of special proteins that help seeds withstand the extreme dehydration that accompanies maturation.
- Many types of dormant seeds germinate when ABA is removed or inactivated.
  - For example, the seeds of some desert plants break dormancy only when heavy rains wash ABA out of them.
  - Other seeds require light or prolonged exposure to cold to inactivate ABA.
- The ratio of ABA to gibberellins determines whether the seed remains dormant or germinates.
  - The addition of ABA to seeds that are about to germinate makes them dormant again.
  - Inactivated ABA or low levels of ABA can lead to precocious (early) germination.
  - A maize mutant whose seeds germinate while still on the cob lacks a functional transcription factor required for ABA to induce expression of certain genes.
- ABA is the primary internal signal that enables plants to withstand drought.
  - When a plant begins to wilt, ABA accumulates in leaves and causes stomata to close rapidly, reducing transpiration and preventing further water loss.
  - ABA causes an increase in the opening of potassium channels in the plasma membrane of guard cells, leading to a massive loss of potassium from the cells.
  - The accompanying osmotic loss of water leads to a reduction in guard cell turgor, and the stomatal pores close.
  - In some cases, water shortages can stress the root system early, leading to the transport of ABA from roots to leaves and functioning as an "early warning system."
  - Mutants that are prone to wilting are often deficient in ABA production.
- In 1901, Dimitry Neljubow demonstrated that the gas **ethylene** was the active factor that caused leaves to drop prematurely from trees near leaking gas mains.
- Plants produce ethylene in response to stresses such as drought, flooding, mechanical pressure, injury, and infection.
- Ethylene production also occurs during fruit ripening, during programmed cell death, and in response to high concentrations of externally applied auxins.
- Ethylene instigates a seedling to perform a growth maneuver called the **triple response,** which enables a seedling to circumvent an obstacle such as a stone as it grows through soil.
- Ethylene production is induced by mechanical stress on the stem tip.
- In the triple response, stem elongation slows, the stem thickens, and curvature causes the stem to start growing horizontally.
- As the effects of the initial ethylene pulse lessen, the stem resumes vertical growth.
  - If the stem again detects a solid object above, another pulse of ethylene is generated, and the stem continues its horizontal progress.

- If upward probes detect no solid object, then ethylene production decreases, and the stem resumes normal upward growth.

- It is ethylene, not the physical obstruction, that induces the stem to grow horizontally.
  - Normal seedlings that grow free of all physical impediments undergo the triple response if ethylene is applied.
- *Arabidopsis* mutants with abnormal triple responses have been used to investigate the signal transduction pathways leading to this response.
- Ethylene-insensitive *(ein)* mutants fail to undergo the triple response after exposure to ethylene.
  - Some lack a functional ethylene receptor.
- Other mutants undergo the triple response in the absence of physical obstacles.
  - Such ethylene-overproducing *(eto)* mutants may produce ethylene at 20 times the normal rate.
  - These mutants can be restored to wild type by treatment with inhibitors of ethylene synthesis.
- Other mutants, called constitutive triple-response *(ctr)* mutants, undergo the triple response in air but do not respond to inhibitors of ethylene synthesis.
  - Ethylene signal transduction is permanently turned on, even when no ethylene is present.
- The affected gene in *ctr* mutants codes for a protein kinase.
  - Because this mutation *activates* the ethylene response, this suggests that the normal kinase product of the wild-type allele is a *negative* regulator of ethylene signal transduction.
  - One hypothesis proposes that binding of the hormone ethylene to a receptor leads to inactivation of the kinase and inactivation of this negative regulator allows synthesis of the proteins required for the triple response.
- **Senescence** is the programmed death of certain cells or organs or the entire plant.
- Cells, organs, and plants that are genetically programmed to die on schedule do not simply shut down their cellular machinery and await death.
- During programmed cell death, or **apoptosis**, there is active expression of new genes, which produce enzymes that break down many chemical components, including chlorophyll, DNA, RNA, proteins, and membrane lipids.
- A burst of ethylene production is associated with apoptosis.
- The loss of leaves each autumn is an adaptation that keeps deciduous trees from desiccating during winter, when their roots cannot absorb water from the frozen ground.
- Before leaves abscise, many essential elements are salvaged from the dying leaves and stored in stem parenchyma cells.
- These nutrients are recycled back to developing leaves the following spring.
- When a leaf falls in autumn, the breaking point is an abscission layer near the base of the petiole.
  - The parenchyma cells here have very thin walls, and there are no fiber cells around the vascular tissue.
  - The abscission layer is further weakened when enzymes hydrolyze polysaccharides in the cell walls.
  - The weight of the leaf, with the help of the wind, causes a separation within the abscission layer.
  - Before the leaf falls, a layer of cork forms a protective scar on the twig side of the abscission layer, preventing pathogens from invading the plant.
- A change in the balance of ethylene and auxin controls abscission.

- An aging leaf produces less and less auxin, and this makes the cells of the abscission layer more sensitive to ethylene.
- As the influence of ethylene prevails, the cells in the abscission layer produce enzymes that digest the cellulose and other components of cell walls.

- Ethylene plays a role in the consumption of ripe fruits by animals, which help disperse the seeds of flowering plants.
  - Immature fruits are tart, hard, and green but become edible at the time of seed maturation, triggered by a burst of ethylene production.
  - Enzymatic breakdown of cell wall components softens the fruit, and the conversion of starches and acids to sugars makes the fruit sweet.
  - The production of new scents and colors helps advertise fruits' ripeness to animals, which eat the fruits and disperse the seeds.
- A chain reaction occurs during ripening: Ethylene triggers ripening, and ripening triggers more ethylene production—a rare example of positive feedback on physiology.
  - Because ethylene is a gas, the signal to ripen spreads from fruit to fruit.
  - Fruits can be ripened quickly by storing them in a plastic bag, where they accumulate ethylene gas, or by increasing ethylene levels in commercial production.
  - Alternatively, to prevent premature ripening, apples may be stored in bins flushed with carbon dioxide, which prevents ethylene from accumulating and inhibits the synthesis of new ethylene.
- Genetic engineering of ethylene signal transduction pathways has potentially important commercial applications after harvest.
  - For example, molecular biologists have blocked the transcription of one of the genes required for ethylene synthesis in tomato plants.
  - These tomato fruits are picked while green and are induced to ripen on demand by the addition of ethylene gas.
- Plant responses often involve the interactions of many hormones and their signal transduction pathways.
- The study of hormone interactions can be a complex problem.
  - For example, flooding deepwater rice leads to a 50-fold increase in internal ethylene levels and a rapid increase in stem elongation.
  - Flooding also leads to an increase in sensitivity to gibberellin, which is mediated by a decrease in ABA levels.
  - Thus, stem elongation is the result of interaction among three hormones and their signal transduction chains.
- Imagine that you are a molecular biologist genetically engineering a rice plant that will grow faster when submerged.
- What is the best molecular target for genetic manipulation? Is it an enzyme that inactivates ABA, an ethylene receptor, or an enzyme that produces more gibberellins?
- Many plant biologists promote a systems-based approach, which attempts to discover and understand biological properties emerging from the interactions of many system elements.
- Using genomic techniques, biologists can identify all the genes in a plant.
  - Three plants are already sequenced: the research plant *Arabidopsis,* rice *(Oryza sativa)*, and cottonwood *(Populus trichocarpa).*

- Using microassay and proteomic techniques, scientists can determine which genes are inactivated or activated in response to an environmental change.
- Identifying the genes and proteins in a plant provides a catalog of components, but it does not reflect the complexity of the interactions underlying the integrated system.
- A systems-based approach may greatly alter how plant biology is done.
  - Laboratories equipped with fast-moving (high-throughput) robotic scanners will record which genes in a plant's genome are activated in which cells and under what conditions.
  - New hypotheses and avenues of research will emerge from analysis of these comprehensive data sets.
- One goal of systems biology is to model a living plant predictably.
- The ability to model a living plant will enable scientists to predict the result of a genetic manipulation without even setting foot in the laboratory.

Concept 39.3 Responses to light are critical for plant success.

- Light is an especially important environmental factor in the lives of plants.
- Light is required for photosynthesis, and it cues many key events in plant growth and development.
- The effects of light on plant morphology are what plant biologists call **photomorphogenesis**.
- Light reception also allows plants to measure the passage of days and seasons.
- Plants detect the presence, direction, intensity, and wavelength of light.
- A graph called an **action spectrum** depicts the relative effectiveness of different wavelengths of radiation in driving a particular process.
  - The action spectrum of photosynthesis has two peaks, one in the red and one in the blue, matching the absorption peaks of chlorophyll.
- Action spectra can be useful in the study of *any* process that depends on light.
  - A close correspondence between the action spectrum of a plant response and the absorption spectrum of a purified pigment suggests that the pigment may be the photoreceptor involved in mediating the response.
- Action spectra reveal that red and blue light are the most important in regulating a plant's photomorphogenesis.
- Researchers identified two major classes of light receptors: **blue-light photoreceptors** and **phytochromes** that absorb mostly red light.
- The action spectra of many plant processes demonstrate that blue light is effective in initiating diverse responses.
- The biochemical identity of the blue-light photoreceptor was so elusive that it was called cryptochrome.
- In the 1990s, molecular biologists analyzing *Arabidopsis* mutants found three completely different types of pigments that detect blue light.
  - *Cryptochromes* are molecular relatives of DNA repair enzymes, involved in the inhibition of stem elongation.
  - *Phototropin* is a protein kinase involved in mediating phototropic curvature and chloroplast movements in response to light.

- There is debate about whether phototropin or a carotenoid-based photoreceptor called *zeaxanthin* is the major blue-light photoreceptor involved in stomatal opening.

Phytochromes regulate many plant responses to light.

- Phytochromes were discovered in studies of seed germination.
- Because of limited food resources, small seeds, such as lettuce, sprout successfully only when they germinate under near-optimal conditions, especially light conditions.
  - Such seeds often remain dormant for many years until light conditions change.
  - For example, the death of a shading tree or the plowing of a field may create a favorable light environment.
- In the 1930s, scientists at the U.S. Department of Agriculture determined the action spectrum for light-induced germination of lettuce seeds.
- The scientists exposed water-swollen seeds to a few minutes of monochromatic light of various wavelengths, stored the seeds in the dark for two days, and then recorded the number of seeds that had germinated under each light regimen.
- Red light (660 nm) increased germination, but far-red light (730 nm) *inhibited* it.
- The response depended on the *last* flash of light; the effects of red and far-red light are reversible.
- The photoreceptor responsible for these opposing effects of red and far-red light is a phytochrome.
- A phytochrome consists of a protein covalently bonded to a nonprotein part that functions as a chromophore, the light-absorbing part of the molecule.
  - So far, researchers have identified five phytochromes in *Arabidopsis,* each with a slightly different protein component.
- The chromophore of a phytochrome is photoreversible and reverts back and forth between two isomeric forms.
  - P_r absorbs red light maximally and converts to P_{fr}.
  - P_{fr} absorbs far-red light maximally and converts to P_r.
- This interconversion between isomers is a switching mechanism that controls various light-induced events in the life of the plant.
  - The P_{fr} form triggers many of the plant's developmental responses to light.
  - For example, P_r in lettuce exposed to red light is converted to P_{fr}, stimulating the cellular responses that lead to germination.
- Plants synthesize phytochrome as P_r, and if seeds are kept in the dark, the pigment remains almost entirely in the P_r form.
- If the seeds are illuminated with sunlight, the phytochrome is exposed to red light (along with other wavelengths), and much of the P_r is converted to P_{fr}, triggering germination.
- The phytochrome system also provides plants with information about the *quality* of light.
- During the day, sunlight includes both red and far-red radiation, and the $P_r \leftrightarrow P_{fr}$ photoreversion reaches a dynamic equilibrium.
- Plants can use the ratio of these two forms to monitor and adapt to changes in light conditions.
  - For example, a tree that requires high light intensity might use changes in this equilibrium to assess appropriate growth strategies.
  - If other trees shade this tree, its phytochrome ratio will shift in favor of P_r because the canopy screens out more red light than far-red light.

- The tree could use this information to indicate that it should allocate resources to growing taller.
- If the target tree is in direct sunlight, then the proportion of P_{fr} will increase, which stimulates branching and inhibits vertical growth.

Biological clocks control circadian rhythms in plants and other eukaryotes.

- Many plant processes, such as transpiration and synthesis of certain enzymes, oscillate during the day in response to changes in light levels, temperature, and relative humidity that accompany the 24-hour cycle of day and night.
- Even under constant conditions in a growth chamber, many physiological processes in plants, such as the opening and closing of stomata and the production of photosynthetic enzymes, continue to oscillate with a frequency of about 24 hours.
  - For example, many legumes lower their leaves in the evening and raise them in the morning.
  - These movements continue even if the plants are kept in constant light or constant darkness.
- Such physiological cycles with a frequency of about 24 hours that are not directly paced by any known environmental variable are called **circadian rhythms**.
  - Circadian rhythms are common to all eukaryotic life.
- Because organisms continue their rhythms even when placed in mine shafts or orbited in satellites, the rhythms do not appear to be triggered by any subtle but pervasive environmental signal.
- All research thus far indicates that the oscillator for circadian rhythms is internal.
- This internal clock, however, is entrained (set) to a period of precisely 24 hours by daily signals from the environment.
- If an organism is kept in a constant environment, its circadian rhythms deviate from a 24-hour period to free-running periods ranging from 21 to 27 hours.
  - Deviations of the free-running period from 24 hours do not mean that the biological clocks drift erratically but that they are not synchronized with the outside world.
- In considering biological clocks, we need to distinguish between the oscillator (clock) and the rhythmic processes it controls.
  - For example, if we restrain the leaves of a bean plant so that they cannot move, the leaves will return to the appropriate position for that time of day when we release them.
  - We can interfere with a biological rhythm, but the clockwork goes right on ticking off the time.
- A leading hypothesis for the molecular mechanism underlying biological timekeeping is that it depends on the synthesis of a protein that regulates its own production through feedback control.
  - This protein may be a transcription factor that inhibits transcription of the gene that encodes for the transcription factor itself.
  - The concentration of this transcription factor may accumulate during the first half of the circadian cycle and then decline during the second half due to self-inhibition of its own production.
- Researchers have recently used a novel technique to identify clock mutants in *Arabidopsis*.
  - Molecular biologists spliced the gene for luciferase (the enzyme responsible for bioluminescence in fireflies) to the promoter of certain photosynthesis-related genes that show circadian rhythms in transcription.

- When the biological clock turned on the promoter of the photosynthesis genes in *Arabidopsis*, it also stimulated the production of luciferase, and the plant glowed.
- This enabled researchers to screen plants for clock mutations, several of which are defects in proteins that normally bind photoreceptors.
- These mutations may disrupt a light-dependent mechanism that sets the biological clock.

Light entrains the biological clock.

- Because the free-running period of many circadian rhythms is longer or shorter than the 24-hour daily cycle, the rhythms eventually become desynchronized with the natural environment when denied environmental cues.
  - Humans experience this type of desynchronization when we cross several times zones in an airplane, leading to the phenomenon called jet lag.
  - Eventually, our circadian rhythms become resynchronized with the external environment.
- Plants are capable of reestablishing (entraining) their circadian synchronization.
- Both phytochrome and blue-light photoreceptors can entrain the circadian rhythms of plants.
- The phytochrome system involves turning cellular responses off and on by means of the $P_r \leftrightarrow P_{fr}$ switch.
  - In darkness, the phytochrome ratio shifts gradually in favor of the P_r form, in part from the synthesis of new P_r molecules and, in some species, by the slow biochemical conversion of P_{fr} to P_r.
  - When the sun rises, the P_{fr} level suddenly increases by rapid photoconversion of P_r.
  - This sudden increase in P_{fr} each day at dawn resets the biological clock.
- Interactions between phytochrome and the biological clock enable plants to measure the passage of night and day.
- The relative lengths of night and day change over the course of the year, except at the equator.
- Plants use this change to adjust their activities in synchrony with the seasons.

Photoperiodism synchronizes many plant responses to changes of season.

- The appropriate appearance of seasonal events, such as seed germination, flowering, and the onset and breaking of bud dormancy, is of critical importance in the life cycles of most plants.
- The environmental stimulus that plants use most often to identify the time of year is the photoperiod, the relative lengths of night and day.
- A physiological response to the photoperiod, such as flowering, is called **photoperiodism**.
- One of the earliest clues to how plants detect the progress of the seasons came from a mutant variety of tobacco studied by W. W. Garner and H. A. Allard in 1920.
- This variety, Maryland Mammoth, does not flower in summer as normal tobacco plants do, but in winter.
- In light-regulated chambers, the Maryland Mammoth flowered only if the day length was 14 hours or shorter, which explained why it did not flower during the longer days of the summer.
- Garner and Allard the Maryland Mammoth a **short-day plant** because it required a light period *shorter* than a critical length to flower.
  - Other examples of short-day plants are chrysanthemums, poinsettias, and some soybean varieties.
- **Long-day plants** flower only when the light period is *longer* than a critical number of hours.
  - Examples include spinach, radish, lettuce, iris, and many cereals.

- **Day-neutral plants** flower when they reach a certain stage of maturity, regardless of the day length.
  - Examples include tomatoes, rice, and dandelions.
- In the 1940s, researchers discovered that it is actually night length, not day length, that controls flowering and other responses to photoperiod.
  - Research demonstrated that the cocklebur, a short-day plant, flowered if the daytime period was broken by brief exposures to darkness, but not if the nighttime period was broken by a few minutes of dim light.
- Short-day plants are actually long-night plants, requiring a minimum length of uninterrupted darkness.
  - Cocklebur is actually unresponsive to *day* length, but it requires at least 8 hours of *continuous darkness* to flower.
- Similarly, long-day plans are actually short-night plants.
  - A long-day plant grown in photoperiods of long nights that would not normally induce flowering will flower if the period of continuous darkness is interrupted by a few minutes of light.
- Although the critical factor is night length, the terms *long-day* and *short-day* are embedded firmly in the jargon of plant physiology.
- Long-day and short-day plants are distinguished *not* by an absolute night length but by whether the critical night length sets a maximum (long-day plants) or minimum (short-day plants) number of hours of darkness required for flowering.
  - In both cases, the actual number of hours in the critical night length is specific to each species of plant.
- Red light is the most effective color in interrupting the nighttime portion of the photoperiod.
- Action spectra and photoreversibility experiments show that phytochrome is the active pigment.
- If a flash of red light during the dark period is followed immediately by a flash of far-red light, the plant detects no interruption of night length, thus demonstrating red/far-red photoreversibility.
- Plants measure night length very accurately.
  - Some short-day plants will not flower if night is even one minute shorter than the critical length.
  - Some plants species always flower on the same day each year.
- Humans can exploit the photoperiodic control of flowering to produce flowers "out of season."
  - By punctuating each long night with a flash of light, the floriculture industry can induce chrysanthemums, normally a short-day plant that blooms in fall, to delay their blooming until Mother's Day in May.
  - The plants interpret this time as not one long night but two short nights.
- Although some plants require only a single exposure to the appropriate photoperiod to begin flowering, others require several successive days of the appropriate photoperiod.
- Some plants respond to photoperiod only if pretreated by another environmental stimulus.
  - For example, winter wheat will not flower unless it has been exposed to several weeks of temperatures below 10^0C (called **vernalization**) before exposure to the appropriate photoperiod.

Leaves detect photoperiod and trigger flowering.

- Although buds produce flowers, it is the leaves that detect photoperiod and trigger flowering.
  - If even a single leaf receives the appropriate photoperiod, all buds on a plant can be induced to flower, even if they have not experienced this signal.
  - Plants that lack leaves will not flower even if exposed to the appropriate photoperiod.
- The floral stimulus can move across across a graft from an induced plant to a non-induced plant and trigger flowering in the latter.
- The stimulus appears to be the same for short-day and long-day plants, despite differing photoperiod conditions required for leaves to send this signal.
- The flowering signal, called **florigen**, resisted identification for 70 years.
- Large macromolecules, such as mRNA and proteins, can move symplastically via plasmodesmata and regulate plant development.
- Is florigen a macromolecule? This may be the case.
- *Arabidopsis* is a long-day plant that requires a functional *CONSTANS* gene to flower in long days.
- Brian Ayre and Robert Turgeon, of Cornell University, noted that *Arabidopsis* plants flowered when *CONSTANS* was expressed only in the leaf, a finding consistent with the observation that florigen is made only in leaves.
- Ayre and Turgeon provided further evidence for a role for *CONSTANS* in floral signaling when they grafted *Arabidopsis* plants that contained no CONSTANS protein onto plants that synthesized CONSTANS in their leaves.
- This elegant experiment showed that CONSTANS, or another factor that it interacts with, can move through the graft junction to signal flowering in the parts of the plant that had been devoid of the protein.
- More recent evidence suggests that CONSTANS upregulates another gene called *FLOWERING LOCUS T (FT)* in the leaf and that the FT protein travels to the shoot apical meristem and initiates flowering.
- Whatever combination of environmental cues (such as photoperiod or vernalization) and internal signals (such as hormones) is necessary for flowering to occur, the outcome is the transition of a bud's meristem from a vegetative state to a flowering state.
  - This requires that meristem identity genes that induce the bud to form a flower must be switched on.
  - Then organ identity genes that specify the spatial organization of floral organs—sepals, petals, stamens, and carpels—are activated in the appropriate regions of the meristem.
- Identification of the signal transduction pathways that link external cues to the gene changes required for flowering is an active area of research.

~~**Concept 39.4 Plants respond to a wide variety of stimuli other than light.**~~

Concept 39.5 Plants respond to attacks by herbivores and pathogens.

- Plants do not exist in isolation but interact with many other species in their communities.
- Some of these interspecies interactions—for example, associations with fungi in mycorrhizae or with insect pollinators—are mutually beneficial.
- Most interspecies interactions are not beneficial to the plant.

- As primary producers, plants are at the base of most food webs and are subject to attack by a wide variety of plant-eating (herbivorous) animals.
- Plants are also subject to attacks by pathogenic viruses, bacteria, and fungi.

- Plants counter these threats with defense systems that deter herbivory and prevent infection or combat pathogens that infect the plant.

Plants deter herbivores with both physical and chemical defenses.

- Herbivory—animals eating plants—is a stress for plants in any ecosystem.
- Plants resist herbivory with both physical defenses, such as thorns, and chemical defenses, such as the production of distasteful or toxic compounds.
- For example, some plants produce an unusual amino acid, *canavanine,* that resembles arginine.
- If an insect eats a plant containing canavanine, canavanine is incorporated into the insect's proteins in place of arginine.
- Because canavanine is different enough from arginine to adversely affect the conformation and, hence, the function of the proteins, the insect dies.
- Some plants "recruit" predatory animals that help defend them against specific herbivores.
- For example, a leaf damaged by caterpillars releases volatile compounds that attract parasitoid wasps, thus hastening the destruction of the caterpillars.
  - Parasitoid wasps inject their eggs into their prey, including herbivorous caterpillars.
  - The eggs hatch within the caterpillars, and the larvae eat through their organic containers from the inside out.
  - The stimulus for the leaf's response is a combination of physical damage to the leaf caused by the munching caterpillar and a specific compound in the caterpillar's saliva.
- These volatile molecules can also function as an "early warning system" for nearby plants of the same species.
  - Lima bean plants infested with spider mites release volatile chemicals that signal "news" of the attack to neighboring, noninfested lima bean plants.
  - The leaves of the noninfested plant activate defense genes whose expression patterns are similar to that produced by exposure to **jasmonic acid**, an important plant defense molecule.
  - As a result, noninfested neighbors become less susceptible to spider mites and more attractive to mites that prey on spider mites.
- Iris Kappers and her colleagues, at Wageningen University in the Netherlands, transgenically engineered *Arabidopsis* plants to produce two volatile chemicals that have been found to attract carnivorous predatory mites in other plants.
- The predatory mites were attracted to the genetically modified *Arabidopsis*, a finding with implications for the genetic engineering of insect resistance in crop plants.

Plants use multiple lines of defense against pathogens.

- A plant's first line of defense against infection is the physical barrier of the epidermis of the primary plant body and the periderm of the secondary plant body.
- However, viruses, bacteria, and the spores and hyphae of fungi can enter the plant through injuries or through natural openings in the epidermis, such as stomata.
- Once a pathogen invades, the plant mounts a chemical attack as a second line of defense that destroys the pathogens and prevents their spread from the site of infection.

- Plants have an innate ability to recognize invading pathogens and to mount successful defenses.
- Successful pathogens cause disease because they are able to evade recognition or suppress host defense mechanisms.
  - Those pathogens against which a plant has little specific defense are said to be **virulent**.
  - **Avirulent** pathogens gain enough access to their host to perpetuate themselves without severely damaging or killing the plant.
- **Gene-for-gene recognition** is a widespread form of plant disease resistance that involves the recognition of pathogen-derived molecules by the protein products of specific plant disease resistance *(R)* genes.
  - There are many pathogens, and plants have many *R* genes; *Arabidopsis* has several hundred.
  - An R protein usually recognizes only a single corresponding pathogen molecule that is encoded by an avirulence *(Avr)* gene.
  - Many Avr proteins play an active role in pathogenesis and are thought to redirect host metabolism to the advantage of the pathogen.
- The recognition of pathogen-derived molecules, called *elicitors,* by R proteins triggers signal transduction pathways that lead to the activation of defense responses, including the **hypersensitive response**, the genetically programmed death of infected cells, tissue reinforcement, and antibiotic production at the infection site.
- Pathogen invasion can also trigger **systemic acquired resistance**, a long-lasting systemic response that primes the plant for resisting a broad spectrum of pathogens.
- Local and systemic responses to pathogens require extensive genetic reprogramming and commitment of cellular resources.
- Plants activate their defenses only after they detect an invading pathogen.
- The hypersensitive response (HR) is a complex early defense response that causes cell and tissue death near the infection site and restricts the spread of a pathogen.
  - After cells at the infection site mount a chemical defense and seal off the area, they destroy themselves.
- The HR is initiated when pathogen elicitors bind to R proteins, altering the selective permeability of the plasma membrane and stimulating the production of *phytoalexins*, toxic compounds with fungicidal and bactericidal properties.
- The HR also induces the production of *PR proteins* (pathogenesis-related proteins), which hydrolyze components in the cell walls of the pathogens.
- Infection also stimulates cross-linking of molecules in the cell wall and deposition of lignins, which sets up a local barricade that slows the spread of the pathogen to other parts of the plant.
- The HR is localized and specific, a containment response based on gene-for-gene *(R-Avr)* recognition between host and pathogen.
- Pathogen invasions can also produce chemical signals that “sound the alarm” of infection to the whole plant.
- The resulting systemic acquired resistance (SAR) is associated with the systemic expression of some defense genes, including those that encode for PR proteins.
- SAR is nonspecific, providing protection against many pathogens for days.
- The most likely candidate for the signal mobilized from the infection site to elicit SAR is **salicylic acid (SA)**.
  - SA is synthesized at high levels around the infection site.

- SA may then be carried by the phloem, which accumulates throughout the plant at lower levels.
- External application of SA induces the production of PR proteins and resistance to pathogens.

- A modified form of SA, acetylsalicylic acid, is the active ingredient in aspirin.
  - Centuries before aspirin was sold as a pain reliever, some cultures learned that chewing the bark of a willow tree *(Salix)* lessened the pain of toothache or headache.
  - In plants, salicylic acid also appears to have medicinal value, but only through the stimulation of SAR.
- Plant biologists investigating disease resistance and other evolutionary adaptations of plants are learning how a plant responds to both internal and external signals.