F1 Features of growth and development

Key Notes

Growth and development

Growth involves cell division followed by cell enlargement. Primary meristems produce files of cells in concentric rings, which form the major tissues of the plant. Development occurs when cells and tissues change form and function to give the organs and structures required during the life cycle of a plant. Growth originates with new cells formed by meristems.

Cell growth

Cell growth occurs when the cell wall is made plastic by enzymes. The driving force for cell expansion is turgor pressure, which pushes the plasma membrane out against the cell wall. The direction of growth is governed by the orientation of cellulose fibers in the wall.

Embryogenesis

The fertilized ovule first divides to give an apical and a basal cell. The basal cell forms the suspensor and the root cap; the apical cell gives the root, shoot and cotyledons of the seedling. Cell lineages can be traced from the seedling through the various stages of cell division, the octant stage, the dermatogen stage and the heart-shaped embryo.

Development of tissues

The cells laid down in the meristem form all the tissues of the plant. The first stage of development is determination, in which the cell becomes established on a pathway of change. The cell then becomes differentiated to its new function. Determination and differentiation involve altered gene expression.

Tissue culture and totipotency

In tissue culture, tissue explants are de-differentiated to form a callus and then redifferentiated by varying hormone or other growth conditions. Single cells in culture can be shown to be totipotent as they can regenerate to form an entire plant.

Cell-to-cell communication

Cell-to-cell communication occurs through plasmodesmata connecting rows or blocks of cells symplastically.

Plant and animal development compared

Cell walls prevent cell movements that are characteristic of animal development. Plant embryonic tissue is maintained through the life of the plant, whereas animals have a distinct embryonic stage. This gives greater plasticity of plant development. Plant cells show totipotency, the ability for single cells to regenerate an entire organism. Cell to cell communication in plants is limited to plasmodesmata.

Related topics

Meristems and primary tissues (C1)
Biochemistry of growth regulation (F2)
Molecular action of hormones and intracellular messengers (F3)

Growth and development

Growth occurs when new cells and tissues are formed by **cell division** (Topic B6) followed by **cell enlargement**. **Development** is the process whereby those cells change form and function to form the specialized tissues, organs and structures required during the life cycle of a plant. It commences with the first cell division after fertilization of the ovule and continues through seed development, seed germination, the development of the seedling to the mature plant, flowering and production of the next generation of ovules. It also includes the processes of cell death and plant senescence.

Plant growth is **accretionary**, new cells being constantly added in meristems (Topic C1), regions that essentially remain **embryonic** throughout the life of the plant. Plant growth begins with **cell division**. Cell enlargement and change in form and function follows subsequently. In the meristem, dividing cells surround the **quiescent center** where no cell divisions occur. As the new cells are surrounded by a cell wall, migration of cells to new locations is impossible. Therefore, the rows (or files) of cells formed (usually in concentric rings) predict the future tissues of the root or shoot. As primary growth occurs by the formation of new tissues at growing tips, different cells and tissues in the same plant are of different ages. The growth of a herbaceous dicotyledon may be considered to occur as successive **phytomeres** consisting of stem, bud and a leaf (*Fig. 1*).

Cell growth

Cell growth in plants can only occur when the cell wall (Topic B2) is made **plastic** by the action of enzymes that break the cellulose cross-linkages. The direction of cell expansion is governed by the orientation of the major fibers

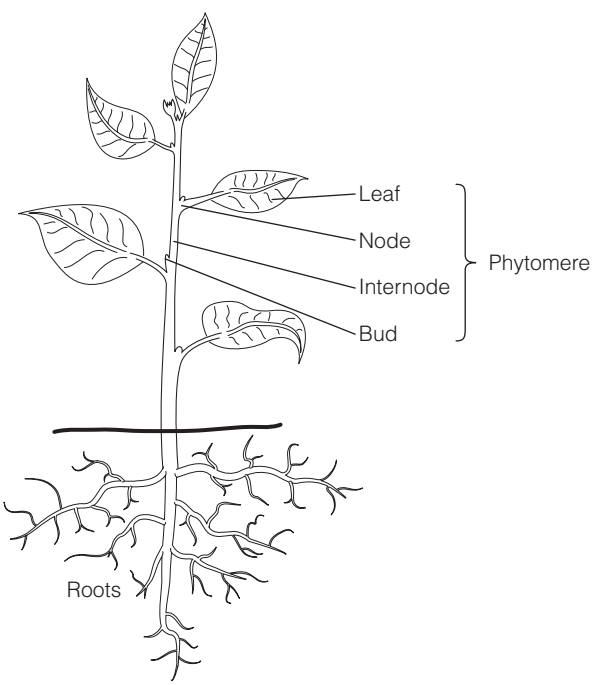


Fig. 1. The plant may be considered as a number of repeating units (phytomeres) which in this dicotyledon comprises a leaf or leaves, a node, an internode and a bud.

(cellulose fibrils) within the wall. The driving force for cell expansion is **turgor pressure**, which pushes the plasma membrane out against the cell wall. Cell growth largely occurs separately from cell division.

Embryogenesis

The basic plan of the plant is established soon after the ovule is fertilized, in the early stages of the development of the embryo (**embryogenesis**) in the formation of the seed (Topic D3). Embryogenesis in arabidopsis (Topic E1), a dicot, is shown in *Fig. 2*. The fertilized ovule divides to give two cells: the **apical cell** and the **basal cell**. The basal cell forms the **suspensor**, connecting the embryo to maternal tissue and also the **root cap meristem**. The apical cell undergoes many cell divisions. The first stage is the **octant stage** (named from the eight cells in two tiers formed). This is followed by the **dermatogen stage** (where tangential cell divisions have occurred creating tissue layers). Finally the **heart-shaped embryo** is formed. This contains the origins of all the major structures of the seedling. The lobes of the heart shape are the cotyledons; between them lies the shoot meristem. The center of the heart forms the hypocotyl and the lower layers form the root. As plant cells cannot migrate during development, it is possible to trace **cell lineages** back to the dermatogen and octant stages. These lineages are illustrated in *Fig. 2*.

Development of tissues

The concentric rings of cells laid down in meristems are initially similar in form: non vacuolate, isodiametric (roughly cuboid) and with thin cell walls. Subsequently they form all the tissues of the plant. This involves stages during which major changes in gene expression occur. The initial stage of this process is **determination**, in which the cell becomes established on a pathway of change but physical changes are not yet detectable. At this stage, the cell is **committed** to a pathway of development. The cell then becomes **differentiated** to its new function, losing some characteristics and gaining others. In plants, both determination and differentiation are frequently reversible given suitable treatments. A major question about development and differentiation is: what causes the altered gene expression that results in ordered patterns of differentiated tissue?

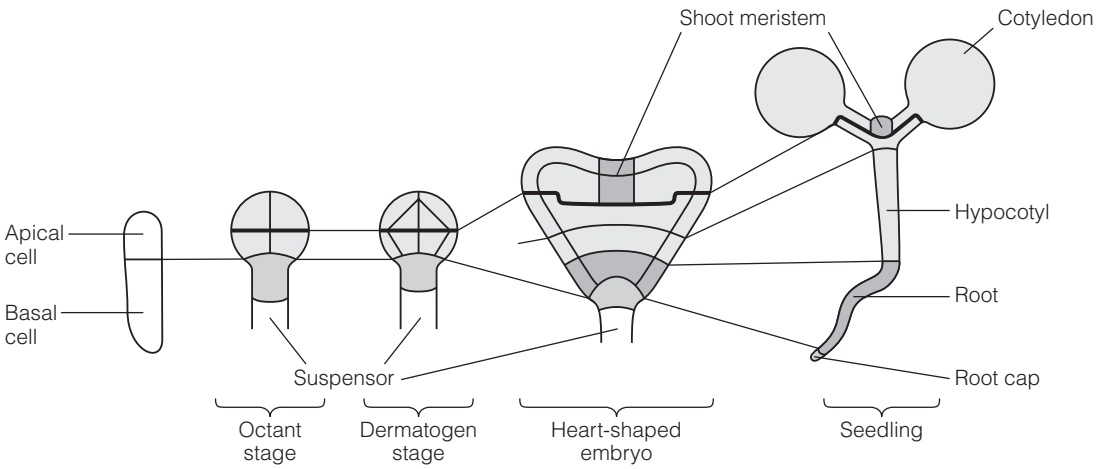


Fig. 2. Embryogenesis in a typical dicotyledon. (Redrawn from T. Laux and G. Jurgens. Embryogenesis, a new start in life. Plant Cell *1997; 9: 989–1000. American Society of Plant Physiologists.)*

The position of the cell is likely to be important and endogenous chemical signals within the tissue are involved (Topics F2 and F3).

Tissue culture and totipotency

Plant tissue may be cultured in either liquid or solid media containing an energy source (sucrose), plant hormones (auxins and cytokinins; Topic F2) and a range of other minor components. In tissue culture, tissue explants (small pieces of plant) are first **de-differentiated** to form **callus**, an amorphous mass of cells, and then **re-differentiated** to form roots, shoots and other organs by varying hormone or other growth conditions. In suspension cultures, it has been shown that single cells can regenerate to form an entire plant, going through all the normal stages of embryo development. As the parent cell did not originate from reproductive cells it demonstrated that plants show **totipotency** – the ability for a differentiated cell to retain all the genetic material in a form required to form an entire organism.

Cell-to-cell communication

While plant plasma membranes are separated by the presence of the cell wall, cell-to-cell contact is made by **plasmodesmata** (Topic B2). Rows or blocks of cells are therefore connected as if in colonies. Macromolecules such as RNA and smaller signalling molecules can move between cells.

Plant and animal development compared

- Plant cells are not mobile during development due to a cell wall.
- In animals, determination and differentiation occurs in the embryo. In plants, cells in the meristems keep dividing and the newly formed cells keep differentiating throughout the life of the plant. This means that different parts of the same plant are of different ages.
- Determination and differentiation of plant cells is much more **plastic** than animals. Application of hormones, wounding or other treatments result in plant cells altering pathways of development to form different tissues and organs.
- Plant cells are **totipotent** (in other words a single, non germ-line cell can be induced to regenerate to a whole organism), a property not generally seen in animals. This implies that the entire genome of that cell is intact and functional and that it can be brought back to an embryonic state.
- In spite of the cellulose cell wall, plant cells may communicate via plasmodesmata.

F2 Biochemistry of growth regulation

Key Notes

Hormones in plants

Plant hormones or 'growth substances' are compounds that act specifically to regulate growth and development at low concentrations. Each plant hormone regulates a variety of processes; the range of concentrations over which they act is broad and there is frequently no clear separation between point of synthesis and point of action.

Auxins

Auxins have a variety of effects including elongation growth, cell division and differentiation, and apical dominance. They frequently work with other hormones, principally cytokinins. Plants show polar (directional) auxin transport. Non polar transport also occurs in phloem.

Ethylene

Ethylene (ethene) is a gaseous hormone first identified as a regulator of fruit ripening. It also stimulates senescence and abscission. In seedlings, it initiates the triple response: epinasty, lateral growth and inhibition of elongation. It is synthesized from S-adenosyl methionine (SAM) via 1-aminocyclopropane-1-carboxylic acid (ACC).

Gibberellins

Gibberellins are a large group of compounds formed from isoprene units. Gibberellins stimulate elongation in dwarf plants and mediate the transition from rosette form to flowering in response to temperature or daylength. They also modulate processes involved in seed and bud dormancy.

Cytokinins

Cytokinins promote cell differentiation and division, often acting in association with auxin. They delay senescence, and promote chloroplast maturation in etiolated seedlings. Their synthesis, based on isoprene units, occurs in various tissues and organs. They are transported in the xylem as cytokinin conjugates and inactivated by oxidation.

Abscisic acid

Abscisic acid is a regulator of dormancy and germination of seeds and of plant responses to stress. It is synthesized in roots and shoots, at high rates in stressed tissue. It is transported in xylem and phloem.

Polyamines

Polyamines, like putrescine and spermidine, are compounds with more than one amine group, synthesized from lysine and arginine. They have effects in cell growth and development and in stress responses.

Brassinosteroids

Brassinosteroids are derived from the sterol campesterol. They are present in plants at low levels but stimulate cell division and elongation.

Oligosaccharides

Oligosaccharides, carbohydrate fragments released from plant cell walls, have been shown to elicit plant defenses against fungal attack and may be regulators of development.

Related topics

- Features of growth and development (F1)
- Methods in experimental plant science (E2)
- Molecular action of hormones and intracellular messengers (F3)

Hormones in plants

A **hormone** is defined as a naturally occurring, organic substance that, at low concentration, exerts a profound influence on a physiological process and is not a part of a major metabolic pathway. The plant hormones coordinate processes as diverse as development of the embryo and response to stress. As plant development shows some major differences from animal development (Topic F1), it is not surprising that plant hormones have many differences in mode of action and nature from mammalian hormones. To avoid this confusion, other terms have been used, such as **plant growth substance** (**PGS**) and **phytohormone**. A summary of the key features of plant hormones, and how they differ from animal, is presented in *Table 1*.

Table 1. Differences and similarities between plant and animal hormones

	Animal	Plant
Naturally occurring organic molecule exerting profound effect on physiological process	Yes	Yes
Active at low concentrations	Yes (10× range usual between inactive and fully active)	Yes (may be a 1000× range between inactive and active)
Synthesized in a discrete organ or tissue remote from point of action	Yes	Not necessarily; synthesis may be diffuse through the plant or at, or near, point of action
Transported in a circulatory system	Yes	No circulatory system; transport in a specific direction (e.g. in xylem, or cell to cell) may occur
Has one, or a few, functions	Yes	Often multiple responses, depending on tissue, age and other factors
Require specific receptors in the cell to function	Yes	Yes. In view of the multiple effects of plant hormones, the presence of specific receptor proteins is essential in determining the final response

Auxins

The major plant auxin is **indole-3-acetic acid** (**IAA**). A number of other compounds with auxin activity include **phenoxyacetic acid** and **indole 3-butyric acid** (*Fig. 1*).

Auxin effects

Elongation growth. The primary effect of auxin is to regulate stem growth. It does this by stimulating the growth of cells in the direction of elongation. Shoot growth is stimulated by 10^{-6}–10^{-7}M auxin. Root elongation, on the other hand, is

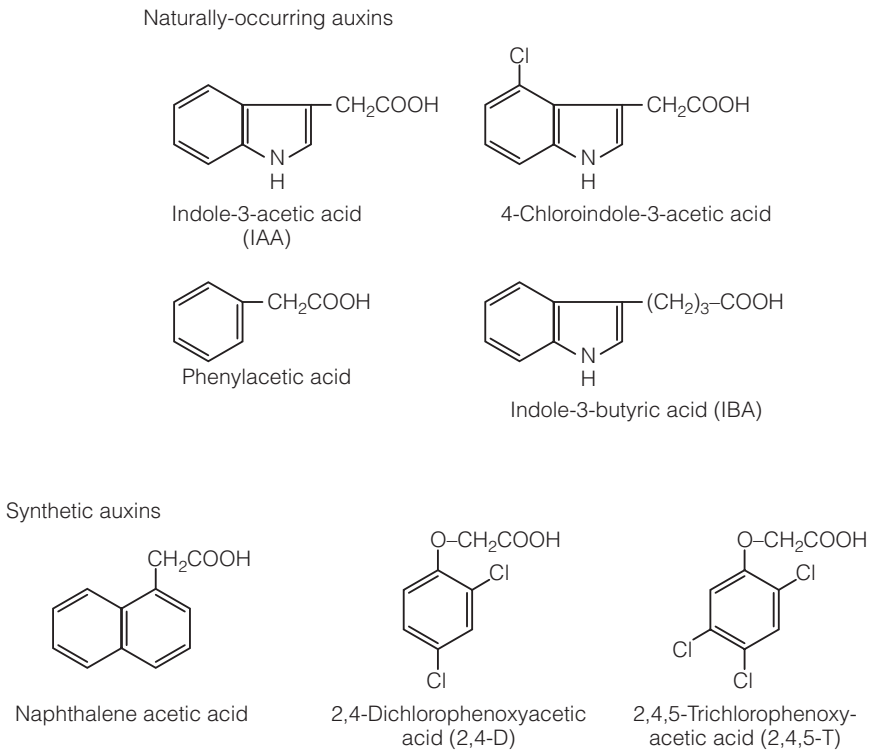


Fig. 1. Chemical structures of auxins. Indole-3-acetic acid (IAA) is the major active auxin found in plants. Naphthalene acetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) are all synthetic auxins with commercial applications.

much more sensitive, with maximum stimulation of growth at 10^{-9}–10^{-10}M auxin, and inhibition at higher concentrations.

Cell division and differentiation. When **callus**, an amorphous mass of undifferentiated cells (Topic F1) is grown on an agar plate containing nutrients, the degree of cell division and differentiation to form roots and shoots can be varied by altering the **auxin:cytokinin ratio**. Both hormones are required; *Figure 2* summarizes the results of such an experiment. These effects are also found in plants where auxins induce lateral root formation in stem cuttings.

Apical dominance. A characteristic of the growth of many plants is the dominant growth of the apical bud. When this bud is removed, growth of axillary buds formed a little way behind the apex is stimulated, until one of them becomes dominant and the growth of the others is suppressed. Replacement of the apical bud with auxin inhibits the axillary buds, suggesting that high auxin concentrations generated at the apex inhibit axillary buds. Application of cytokinin to axilliary buds releases them from inhibition and therefore auxin–cytokinin interactions are responsible for the phenomenon.

Other auxin effects. Auxin has a range of other effects, either alone or with other hormones, including fruit development. Some plants, e.g. strawberry, tomato, cucumber, pumpkin, citrus fruits, produce **parthenocarpic** (seedless)

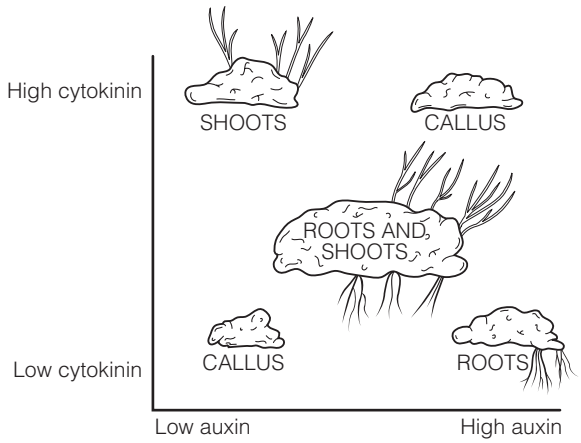


Fig. 2. Regulation of growth and development by auxin:cytokinin ratio. Explants grown on a nutrient-containing agar may be induced to form amorphous callus, or roots, shoots, leaves and buds by varying the auxin:cytokinin ratio.

fruits if they are treated with auxin. Senescence and abscission of mature leaves, fruits and flowers is inhibited by auxin; however, abscission of young fruits is enhanced by auxin treatment.

Commercial applications

Synthetic auxins find widespread application in agriculture and horticulture. At high concentrations, **2,4-dichlorophenoxyacetic acid (2,4-D**) and **2,4,5-trichlorophenoxyacetic acid** (**2,4,5-T**) (*Fig. 1*) are used as **herbicides**, particularly on broad-leaved plants, which are much more sensitive to them than monocots. Naphthalene acetic acid (NAA) is used to **stimulate rooting** of cuttings ('hormone rooting powder'), while other synthetic auxins are used to reduce fruit number early in the season in apples and to promote fruiting in tomatoes and citrus fruits.

Synthesis

Auxins are mostly synthesized from the amino acid **tryptophan**, predominantly in young leaves, shoot meristems and developing fruits, wherever cells are dividing rapidly. IAA is also made by bacteria (see *Agrobacterium tumifaciens*, Topic P3) and several pathways exist, including one in which IAA is synthesized from indole or indole-3-glycerol phosphate rather than tryptophan.

Auxin transport

Auxin shows **polar transport** (unidirectional). It moves **basipetally** (from the apex to the base) in isolated coleoptiles (the sheath encasing the primary leaf in a grass) and stems. Small amounts of auxins produced at the root apex may also move basipetally (in this case from the root tip up the root) but this is limited in comparison with that from the shoot. Polar transport in stems occurs in the parenchyma surrounding the vascular tissue involving specific **auxin transport proteins**. Its transport can be inhibited by **auxin transport inhibitors** such as **1-N-naphthylphthalamic acid** (**NPA**). Auxin synthesized in the leaves is also transported in a non-polar fashion in the phloem; this process is about 10 times faster than polar transport. Studies in *Arabidopsis* have revealed a gene, ***AUX1***,

expressed in the root apex, which encodes an auxin transport protein which is involved in directional elongation, for instance in gravitropism (Topic G2).

Auxin conjugation and degradation

The amount of auxin available in a cell or tissue depends on three processes. The rate of auxin biosynthesis or import from other cells, the rate of auxin degradation and the amount that is conjugated (chemically bound) to other molecules. Conjugated auxin is not biologically active. Most auxin within a plant is covalently bonded to organic compounds (e.g. esters of myo-inositol and glucose, and high-molecular weight compounds such as glycoproteins) and is inactive. Transport of IAA in the phloem is predominantly in the form of these complexes, and their breakdown to release IAA supplies it to tissues like the coleoptile tip. There are several pathways for IAA breakdown, involving peroxidation of IAA to 3-methyleneoxindole and non-decarboxylation to oxindole-3-acetic acid.

Ethylene

Ethylene (ethene) was discovered in the early 1900s as a gas that regulated **fruit ripening**. It had been realized that the close proximity of ripe fruit, such as oranges or apples, speeded up the ripening of other fruits, such as tomatoes and bananas. Regulating ripening, and therefore ethylene, has become an important part of the storage, transport and marketing of fruit worldwide. Ethylene has a variety of other roles in plants, including senescence of leaves and fruit, elongation of roots, and responses to waterlogging and other stresses. Although a simple molecule, its effects are highly specific.

Ethylene effects

Fruit ripening. Many ripening fruits show a rise in ethylene production that precedes the onset of ripening. Fruits that produce and respond to ethylene in ripening are the **climacteric fruits** (apples, tomatoes and bananas); the climacteric is a characteristic burst of respiration that occurs just before the final stages of ripening take place. Ethylene production in climacteric fruit is **autocatalytic**, i.e. ethylene stimulates its own production, the rapidly rising ethylene concentration then triggering the rapid burst of respiration.

The triple response. Ethylene-treated shoots (e.g. pea seedlings) show three characteristic growth responses simultaneously: **epinasty** (downward curvature of the leaves); **decreased elongation** and **lateral cell expansion** (i.e. increase in stem width) and **loss of gravity response** to give horizontal growth. Ethylene-induced epinasty gives the apex of young dicot seedlings a 'hook' like appearance.

Ethylene and waterlogging responses. Whereas ethylene normally inhibits elongation growth, in some wet-land species, including rice, ethylene induces rapid elongation growth, allowing the plant to reach air. The formation of **aerenchyma** (air spaces in the root cortex, formed by programmed cell death; Topic C1) is also induced by ethylene, which is synthesized in response to low oxygen and accumulates in waterlogged roots.

Other roles of ethylene. High concentrations of ethylene (>10 $\mu l\ l^{-1}$) induce adventitious rooting and root hair formation. In leaf abscission, ethylene accelerates the synthesis of cell-wall degrading enzymes in the abscission layer, a specialized layer of cells at the leaf pulvinus which separate from adjacent cells permitting the leaf to fall from the plant.

Synthesis and degradation

Ethylene is produced from the amino acid **methionine**, via **S-adenosyl methionine** (**SAM**) and **1-aminocyclopropane-1-carboxylic acid (ACC**). The enzyme producing ACC is key in regulating ethylene production; it has a very short half-life and the expression of its gene is stimulated by factors known to induce ethylene responses. Ethylene is inactivated by oxidation (e.g. to ethylene oxide or CO_2) or it can diffuse from the plant. Rates of ethylene production rise rapidly in tissues subject to stress or wounding and subsequently decline to normal levels. Ethylene is active at very low concentrations (around 1 ppm or 1 μl l^{-1}).

Gibberellins

Gibberellins were discovered in the 1930s by Japanese scientists investigating a disease of rice caused by the fungus *Gibberella fujikuroi*, that results in tall, seedless plants. Nearly 100 gibberellins have been identified in plants though many do not have biological activity. The most studied, and probably most significant gibberellin is $\mathbf{GA_3}$. In common with the other gibberellins, it has a structure based on *ent*-gibberellane (*Fig. 3*).

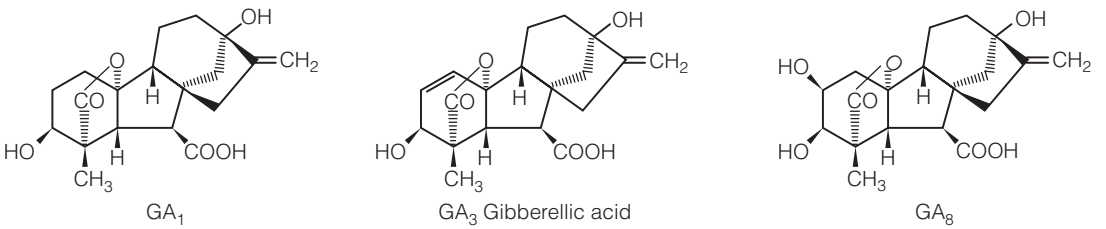


Fig. 3. Some active and inactive gibberellins. They are all based on a structure known as ent-*gibberellane.*

Gibberellin effects

Environmental responses. Many species remain as **rosette plants** until they have been exposed to either low temperatures (**vernalization**) or a number of **long days**. Spinach, for instance, retains a short, squat form until day-length increase, when it begins to grow upwards and flower. Gibberellin levels are low in rosette plants, but increase dramatically in response to the changed environment and initiate the growth response. $\mathbf{GA_1}$ is most significant in elongation responses and $\mathbf{GA_9}$ in flowering.

Seed germination

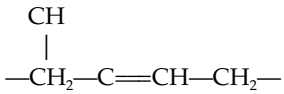
In some seeds which show dormancy, gibberellin application will break dormancy. In other seeds, gibberellins are essential in coordinating the processes of germination, increasing in activity upon rehydration of the seed and initiating the activity of the hydrolases which mobilize seed storage reserves. The role of gibberellins in germinating barley is of economic importance in the malting process, part of beer brewing.

Other effects of gibberellins

Gibberellins are involved in regulating the transition from **juvenile** to **mature** growth form in some perennial species such as ivy (*Hedera helix*); in **initiating flowering** and promoting **fruit formation**.

Synthesis

Gibberellins are **diterpene acids** synthesized by the **terpenoid pathway**. Terpenoids are compounds built of repeating **isoprene units**:



The location of the early stages of gibberellin synthesis is the **plastid**, where isoprene synthesis occurs from glyceraldehyde-3-phosphate and pyruvate. Later stages occur in plastids in meristems and in enzymes of the endoplasmic reticulum (ER) and cytoplasm.

Transport

Highest levels of active gibberellins in plants are found in young rapidly growing tissues like young leaves and buds, and developing seeds and fruits. Transport of gibberellins in the plant occurs predominantly in the **phloem** and is **non-polar**.

Cytokinins

Cytokinins were described as compounds that regulate cell division in plants (Topic B6) in experiments in which compounds were screened for their effects on tissue cultures. From these experiments came two compounds: the first, **kinetin**, was isolated as the active ingredient in herring sperm DNA that caused massive cell proliferation in plant cell culture. **Zeatin** was the first natural plant cytokinin, isolated from the **liquid endosperm** of the coconut. The cytokinins constitute a small number of compounds, which are derivatives of adenine or amino purine.

Cytokinin effects

Cytokinin effects are generally associated with promotion of growth and development and delay of senescence. Cytokinins applied to leaves will delay senescence; they speed up chloroplast maturation in etiolated (dark-grown) tissue and promote cell expansion in young leaves. Cytokinins applied to lateral buds in plants showing strong apical dominance will overcome growth inhibition by auxin, causing the bud to grow out. Cytokinins, with auxins, are involved in plant tumor formation and in morphogenesis, the development of roots and shoots. *Figure 2* illustrates the effects of varying the ratio of auxin to cytokinin on the growth and morphogenesis of plant material in tissue culture.

Cytokinin synthesis

Like gibberellins, cytokinins contain isoprene subunits. The first stage involves the reaction of isopentenyl pyrophosphate with adenosine monophosphate (AMP), catalyzed by the enzyme **cytokinin synthase** to yield isopentenyl adenine ribotide. From this compound, cytokinin ribotides, ribosides and cytokinins are formed. Cytokinins are also synthesized by gene products resulting from the insertion of bacterial genes from ***Agrobacterium tumifaciens*** (Topic P3) The **TI** (**tumor- inducing**) **plasmid** from *A. tumifaciens* introduces the gene for **isopentenyltransferase**. This enzyme generates **isopentenyl adenine**, which is converted to ***trans*-zeatin** and **dihydrozeatin** in the plant. These hormones, together with auxin produced by another TI-plasmid gene product, cause a tumor or crown gall to form at the site of infection.

Cytokinin transport

Cytokinins are synthesized in various tissues and organs, though the root apical meristem (Topic C2) is a major site of its production. They have been identified in the xylem flow from cut roots, suggesting that this may be a route for long-distance transport of cytokinins through the plant. Cytokinins in the root xylem are predominantly **zeatin ribosides**, which are rapidly converted to **free cytokinin** in leaves. Cytokinin inactivation occurs when they are oxidized to adenine by the enzyme **cytokinin oxidase**.

Abscisic acid

Abscisic acid (**ABA**) is found in all higher plants and mosses. ABA regulates **dormancy** and is central to plant responses to stress. The nature of the molecule means that it can exist in several forms. First, the carboxyl group at the end of the side chain may be ***cis*** or ***trans*** (*Fig. 4*), while the C at position one of the ring is asymmetric and gives either (+) or (–) (*S* or *R*) **enantiomers.** The **active form** of ABA is (+) ***cis* ABA**; (+)-2-*trans*-ABA also exists in plants and is active in some long-term ABA responses; it may be converted to the (+) *cis* form in tissues.

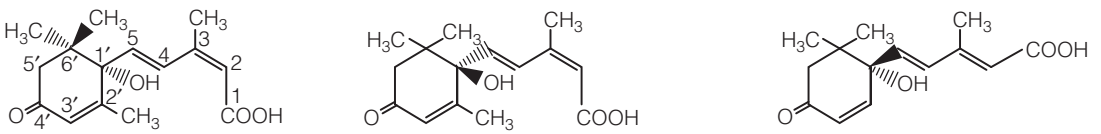


Fig. 4. Chemical structures of (+) and (–) forms of ABA. (+) cis *ABA is active; (–)* cis *ABA is active in slow ABA responses, but not rapid ones like stomatal closure. (+)* trans *ABA is inactive, but may be converted to (+)* cis *ABA.*

Abscisic acid effects

ABA has a variety of effects related (i) to **seed dormancy** and (ii) to **stress responses**. ABA levels rise initially during **embryo development** within the seed and then decline. ABA regulates the expression of genes for proteins in the embryo that prepare it for the final stages of seed development in which the seed desiccates and becomes dormant; it also activates genes for seed storage proteins (Topic H4). ABA also keeps some seeds dormant until the environment becomes suitable for growth. Controlling dormancy is very important in temperate climates since precocious germination may lead to the death of the seedling. ABA also accumulates in the dormant buds of woody species, although control of dormancy here is likely to be the result of the action of several hormones.

ABA also regulates several plant stress responses. Rising ABA levels in water stress initially cause stomatal closure (Topic I2) and subsequently increases the ability of root tissue to carry water; it also promotes root growth and inhibits shoot growth.

Abscisic acid synthesis

ABA biosynthesis begins in chloroplasts and amyloplasts. Synthesis, like that of gibberellins and cytokinins, involves the isoprene subunit in isopentenyl pyrophosphate, which is used to produce an oxygenated carotenoid compound, **zeaxanthin**. Zeaxanthin is modified in a multi-stage process to **neoxanthin**, which is cleaved to the C15 compound **xanthoxin**; xanthoxin is then modified in

two stages to produce ABA. ABA is degraded, either by oxidation or by conjugation, to form **ABA-glucosyl ester**.

Abscisic acid transport

ABA is synthesized in roots and shoots, and at much higher levels in tissues undergoing stress. Water-stressed roots, for instance, produce up to 1000 times more ABA that is transported through the xylem to the shoot. ABA is also transported from shoot to root in the phloem.

Polyamines

Polyamines are compounds containing two or more amine groups. Typical examples with biological activity are:

- Putrescine: $H_2N\text{-}(CH_2)_4\text{-}NH_2$
- Spermidine: $H_2N\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_3\text{-}NH_2$
- Spermine: $H_2N\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_3\text{-}NH_2$

Biosynthesis originates from the amino acids lysine and arginine. Levels of polyamines increase where rapid cell division is occurring; putrescine levels increase in response to some forms of stress and they may be involved in some aspects of embryo and fruit development.

Brassinosteroids

Brassinosteroids (or **brassins**) are a recently discovered, complex group of lipids synthesized from the sterol **campesterol**. They are present at low levels, but have strong growth promoting effects, stimulating both **cell division** and **cell elongation**. The structure of one brassinosteroid is shown in *Fig. 5*. It appears that brassinosteroids act with the other plant hormones to regulate growth and differentiation. Mutants of *Arabidopsis* and pea which are deficient in brassinosteroid biosynthesis are dwarf; application of brassinosteroid restores them to a normal phenotype, indicating that they are essential for cell elongation in normal plants.

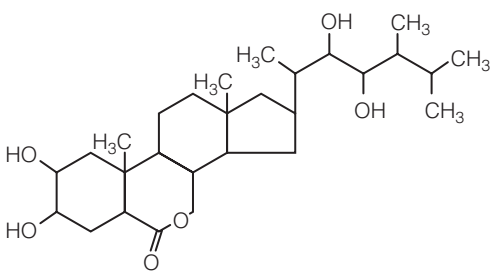


Fig. 5. Brassinolide, a brassinosteroid.

Oligosaccharides

The complex polysaccharide cell wall of plants is a dynamic structure, which may be modified by both endogenous and exogenous enzymes. Wall changes lead to the release of fragments of long-chain polysaccharides, **oligosaccharides**, into the apoplast, some of which have been shown to have effects on development in plant tissue cultures and others of which are released during **fungal pathogen** attacks and elicit the **defense responses** of the plant (Topics G5 and M4).

F3 Molecular action of hormones and intracellular messengers

Key Notes

How do plant hormones act?

Plant hormones act in a variety of types of responses that may be long term, such as growth and development, or rapid. Hormones alter the activity of enzymes or other cytoplasmic components directly, alter gene expression and the production of new cellular components, or both.

Receptors and target tissues

In order for a hormone response to occur, the presence of the hormone must first be perceived by a receptor protein. Receptors for ethylene (ETR1) and auxin (ABP1) have been identified and characterized. Target tissues respond to a given hormone because they possess the necessary receptors and pathways for a response.

Hormones and gene expression control

Many genes are regulated by plant hormones. Some respond very rapidly, in a matter of minutes; others require hours to days. Genes that respond to hormones have a region termed a response element in the promoter region that is regulated by a protein (transcription factor) which in turn is regulated by the hormone.

Intracellular messengers

Intracellular messengers alter in response to a stimulus causing a coordinated response within the cell. $Ca^{2+}$and inositol trisphosphate (IP_3) are examples. IP_3 is produced when phospholipase C is activated and releases Ca^{2+} from intracellular stores. Intracellular messengers frequently activate protein kinases which phosphorylate other proteins thereby producing the cellular response.

Related topics

Features of growth and development (F1)
Biochemistry of growth regulation (F2)
Tropisms (G2)

How do plant hormones act?

Plant hormones influence both long-term processes, such as growth and development, and short-term responses such as the closure of stomata or curvature in unilateral light or gravity. These effects may involve altered gene expression and altered activity of cellular components such as enzymes or the cytoskeleton. To do this, there must be a cellular component called a **receptor** that alters in function or property in response to the presence of the hormone. Between the receptor and point of action there may be an inter-linked series of chemical or ionic signals, termed **intracellular messengers**, within the cell that transmits and amplifies the initial signal. Plant hormones act in a variety of different

ways, with the same hormone having several different effects using several different mechanisms in the same plant, and even in the same tissue. *Figure 1* illustrates some of the potential pathways involved, while *Table 1* details some examples of plant hormone actions and their mechanisms.

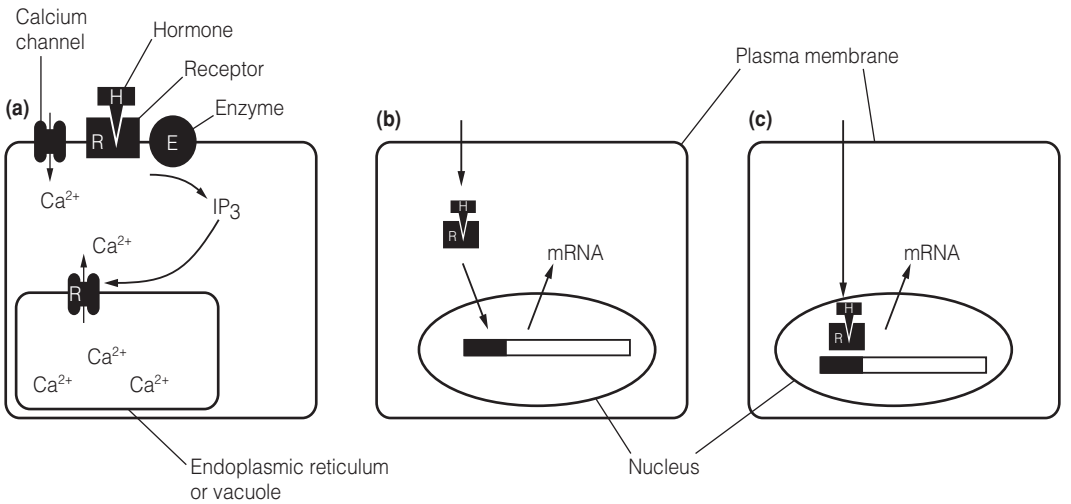


Fig. 1. Pathways for hormone action in plants. In pathway (a), the hormone (H) interacts with a receptor protein (R) in the plasma membrane. This activates an ion channel, giving an influx of Ca^{2+}, or activates enzymes in the plasma membrane producing intracellular messengers such as inositol trisphosphate (IP_3). This initiates subsequent events in the cell. In (b) and (c), the hormone (H) influences gene expression, either by interaction with a cytoplasmic receptor (b) or a nucleoplasmic receptor (c).

Receptors and target tissues

A **receptor** is a protein which binds the hormone. Binding has high affinity and specificity, but is reversible. In other words, a hormone is bound at low concentrations, but can be readily released, 'switching' off the response. Similar compounds not effective as hormones are not bound. Binding of the hormone produces a change in the receptor that results in it being able to activate other processes. Receptors may be located in membranes or may be in the cytoplasm.

Only a few plant hormone receptors have been identified with certainty. **ABP1** is a soluble **auxin binding protein** that normally resides in the lumen of the endoplasmic reticulum (ER). It binds auxin **reversibly**, with **high specificity** and **affinity** and is a strong candidate as an **auxin receptor**. An **ethylene receptor**, **ETR1**, has been cloned in *Arabidopsis* and other plants, and has a high affinity and specificity for ethylene. The receptor is a **protein kinase** containing a copper atom as part of the ethylene-binding site. It passes on the signal by **phosphorylating** other proteins in a **signal transduction chain** (see below). While other proteins have been suggested to be receptors for hormones, none have yet been characterized in detail.

It is evident that some tissues respond to a hormone while others do not. Such hormone-responsive tissues are known as target tissues because they contain the receptors and signal transduction machinery necessary to respond in a particular way.

Table 1. Examples of evidence for mechanisms of hormone action

Hormone	Effect	Mechanism
Auxin	Cell elongation	Activates the plasma membrane proton pump; evidence for a soluble auxin binding protein (ABP) that may result in activation of the pump and increased expression of the ABP gene
Auxin	Altered protein synthesis	A wide range of auxin-responsive genes have been identified in many species
Abscisic acid	Stomatal closure	Plasma membrane ABA receptor; regulation of ion channels and altered cell turgor; mechanism involves intracellular messengers including Ca^{2+} and IP_3
Abscisic acid	Dormancy and stress	ABA-response elements which are transcription factors have been identified which regulate gene expression; a GA-activated, ABA-repressed gene has been identified in seeds which regulates one of the enzymes (α-amylase) involved in germination
Ethylene	Abscission and ripening	A number of ethylene-responsive genes have been identified and a putative ethylene receptor protein identified
Cytokinins	Delay of senescence; chloroplast maturation; development	A possible cytokinin receptor has been identified and cytokinins have been shown to alter mRNA abundance for key proteins, possibly post-transcription; cytokinin action may also involve intracellular messengers
Gibberellins	Seed germination; development	Strong evidence for intracellular messenger involvement; gibberellin causes altered gene expression (e.g. of α-amylase in germinating seeds); gibberellin-specific gene promoters and a transcription factor stimulated by gibberellin have been identified

ABA, abscisic acid; GA, gibberellic acid; IP_3, inositol triphosphate.

Hormones and gene expression control

Altered gene expression is an essential component of many hormone-regulated processes. Altered gene expression results in the production of new proteins involved in the changes in cell function that hormones regulate (*Fig. 2*). This may occur in addition to hormone action via intracellular messengers (see below) or by directly modulating cell processes by interaction with proteins already present. In this section, two examples will be given of gene regulation by plant hormones: **auxin and elongation growth** and **ethylene and fruit ripening**. Hormone-responsive genes have identifiable sequences in the **promoter region** (Topic B5) termed **response elements** that interact with **protein modulators** (**transcription factors**) which in turn are regulated by the hormone.

Auxin and elongation growth

The mechanism of the stimulation of **elongation growth** by auxin has been the subject of scientific controversy for many years and is still not fully understood for all plants. When oat coleoptiles are exposed to auxin, their rate of elongation increases as a result of **cell elongation** (Topic B2). The effect of auxin can be mimicked by weak acid, and it can be shown that when auxin is added to the coleoptile, the cell wall space becomes acidified. This acidification results from

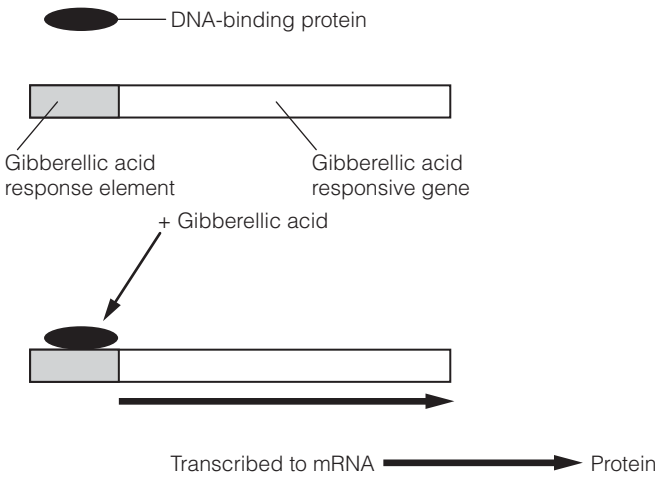
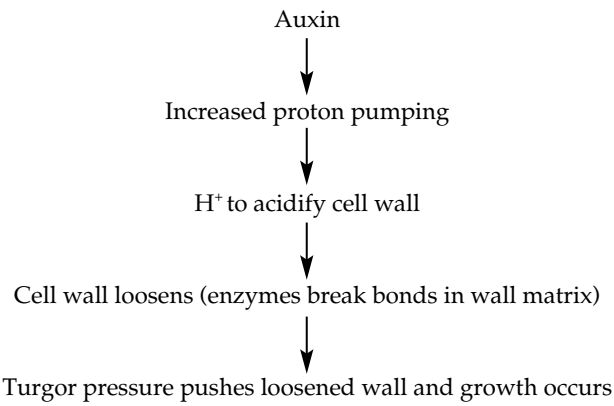


Fig. 2. Model of regulation of a gene by a hormone (gibberellic acid). The grey hatching is the gibberellic acid response element (GARE), regulated by a gibberellic acid-responsive DNA-binding protein.

increased pumping of protons (H^+) into the cell wall by the plasma membrane **proton ATPase** (**proton pump**; Topic I3). The **acid growth theory** states that the following stages occur:



Proteins (**expansins**) present in the cell wall catalyze pH-dependent wall extension growth, by loosening bonds between components of the wall matrix. How does auxin increase the acidification of the cell wall? Two possibilities exist: the first is the activation of proton ATPase already present at the plasma membrane; the second is that auxin causes an increase in the amount of proton ATPase present by upregulating gene expression. In addition to the effect of auxin on the proton pump, other **auxin-responsive mRNAs** have been found, some of which also increase during elongation. *Table 2* summarizes some of these and their possible functions.

Table 2. Examples of auxin-responsive mRNAs

Messenger RNAs identified that are up- or down-regulated by auxin	Time taken to respond	Notes
Auxin/IAA family	15 min–1 h	A family of proteins, many of which are abundant in elongating tissue; range in size from 20–35 kDa; present in low amounts, several targeted to the nucleus
GST family	15 min–3 h	A family of enzymes which conjugate glutathione to many substrates; believed to be involved in detoxification of xenobiotics and cytokinins
SAUR family	2.5–5 min	Abundant in elongating coleoptiles incubated in the presence of auxin
ACS family	20 min–20 h	ACC-synthases, which are an essential component of the ethylene biosynthetic pathway
Others	30 min–48 h	A wide range of other mRNAs are either increased or decreased by auxin

IAA, indole-3-acetic acid; GST, gluthathione-S-transferase; SAUR, soybean auxin upregulated; ACS, ACC synthase; ACC, 1-aminocyclopropane-1-carboxylic acid.

Ethylene and fruit ripening

Tomato **fruit ripening** follows a well defined series of events typical of **climacteric** fruits (fruits which show a burst of respiration during ripening; *Fig. 3*). Ripening involves softening of the cell walls, increase in sugar content and color changes which ultimately result in an attractive red fruit. Studies of the proteins involved in ripening reveal that a well-regulated series of changes in **gene expression** occurs. **Ethylene** synthesis increases rapidly before the climacteric as genes for the enzyme **ACC oxidase** (Topic F2) are expressed, and declines after it. The ethylene signal is then sensed by the ethylene receptor (ETR1) and transcription of a range of mRNAs for proteins involved in ripening is enhanced. The level of transcription of the gene for ETR1 also increases greatly during ripening. These ethylene regulated genes encode mRNAs for cell wall softening and pigment synthesis. One well studied gene, which is transcribed in response to ethylene, encodes the wall-loosening enzyme **polygalacturonase** (PG). Tomatoes in which expression of PG has been reduced by antisense technology show enhanced storage as they ripen more slowly than conventional tomatoes. In this technique, a single strand of DNA is present that binds specifically with the PG mRNA strand, thereby inactivating it. Such fruits also have better qualities for processing and suffer fewer losses in harvest and transport to the consumer or processing plant.

Intracellular messengers

Hormones generally convey signals from cell-to-cell and tissue-to-tissue. **Intracellular messengers** carry out signaling within cells. They may respond to a signal from a hormone first perceived at the plasma membrane, or they may respond to a stimulus (such as light or temperature) first perceived inside the cell. A number of intracellular signaling molecules have been found in plants; two, **Ca^{2+}** and the **inositol trisphosphate** (**IP_3**) pathway will be described here.

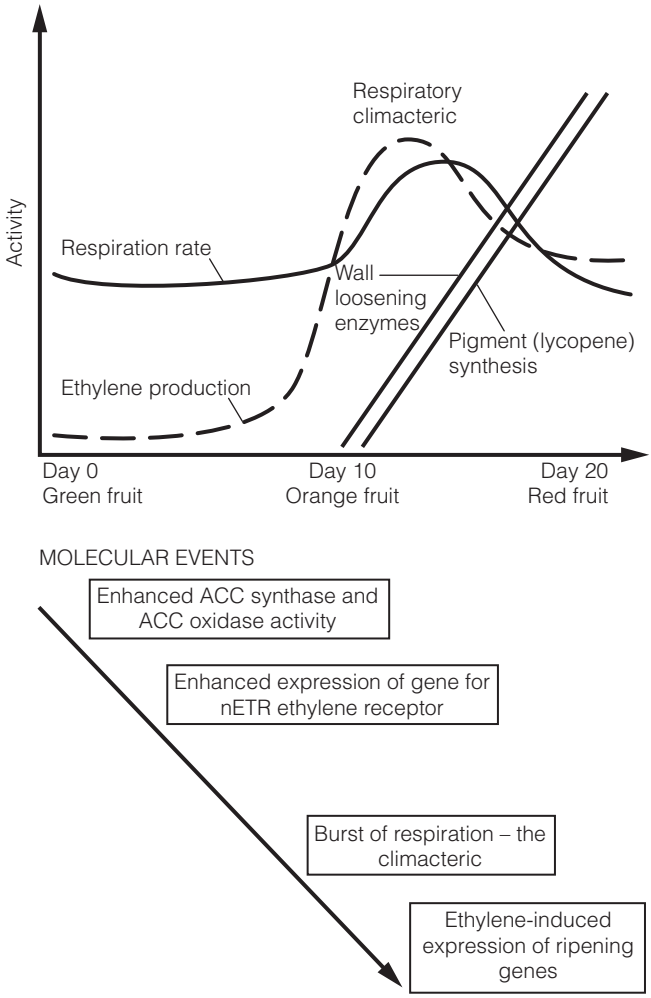


Fig. 3. Regulation of the ripening of a tomato fruit by ethylene.

Calcium as an intracellular messenger

Cells maintain a very low concentration of Ca^{2+} in the cytoplasm (<1 μM), by pumping it out across the plasma membrane to the apoplast using **Ca^{2+} ATPases**. They also pump Ca^{2+} into **intracellular stores** such as the vacuole and endoplasmic reticulum. This results in a very steep Ca^{2+} gradient across several cell membranes. When **calcium channels** (Topic I3) in these membranes open, Ca^{2+} floods into the cytoplasm, giving a **Ca^{2+} 'wave'**. Channel opening may be regulated by a hormone receptor or by some other stimulus. This amplifies the signal (one molecule of hormone bound can keep a channel open long enough to permit tens of Ca^{2+} ions to enter) and can integrate (coordinate) several signals. The Ca^{2+} wave is then perceived by receptor proteins. The best described of these is **calmodulin**, a protein with four Ca^{2+} binding sites. When calmodulin binds Ca^{2+}, it changes conformation and activates a range of proteins, including **Ca^{2+}-calmodulin-dependent protein kinases** (**CaMPKs**).

Plants also have a range of **Ca^{2+}-dependent** (but calmodulin independent) **protein kinases**. Protein kinases are important in signal transduction pathways as they phosphorylate other proteins at specific sites, altering their activity.

Inositol trisphosphate

The **inositol triphosphate** (**IP_3**) pathway begins with the conversion of a plasma membrane lipid, **phosphatidyl inositol** into **phosphatidyl inositol bisphosphate** (**PIP_2**) by **kinase** enzymes in the plasma membrane. PIP_2 is then hydrolyzed by **phospholipase C** (**PLC**) to give **IP_3** and **diacylglycerol** (**DAG**). PLC activity is regulated by a signal transduction pathway initiated by the binding of a hormone to a receptor, so IP_3 and DAG levels respond to stimuli. IP_3 causes Ca^{2+} channels to open at the tonoplast (vacuole) and endoplasmic reticulum, resulting in a Ca^{2+} signal being initiated (see above). DAG activates **protein kinase C** that regulates other processes. *Figure 4* summarizes the Ca^{2+} and inositol trisphosphate signaling pathways.

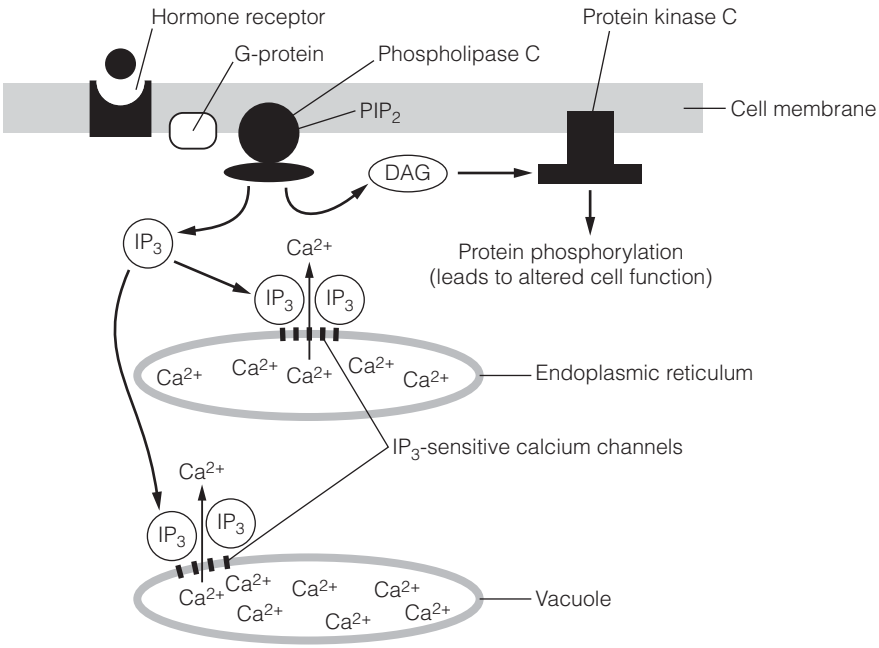


Fig. 4. Intracellular messengers. Release of Ca^{2+} from the endoplasmic reticulum (ER) and vacuole results in a transient rise in cytoplasmic Ca^{2+} concentration. The Ca^{2+} binds with proteins like calmodulin- and Ca^{2+}-dependent protein kinases to alter cell function.