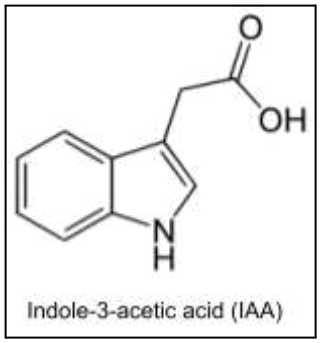
4.1 Cell Communication (Left Side Processing)

The term auxin is derived from the Greek word *auxein*, which means "to grow." Auxins are the main hormones responsible for cell elongation in phototropism and gravitropism. They also control the differentiation of meristem into vascular tissue, and promote leaf development and arrangement. Commercial use of auxins is widespread in plant nurseries and for crop production. IAA is used as a rooting hormone to promote growth of adventitious roots on cuttings and detached leaves.



Indole-3-acetic acid (IAA)

The distribution of IAA in plant bodies occurs over both short and long distances (e.g., between adjacent cells as well as between the shoot and sites of lateral root initiation, respectively). Auxin molecules can move over long distances through the vascular system (through the phloem from leaves to the roots) by mass flow.

However, there is also another system for auxin translocation—over both short and long distances. This system involves a cell-to-cell mechanism and it is mostly polar. The key to understanding how auxin(s) can move across the plasma membrane (PM) lies in the physical–chemical nature of auxin molecules. Because all auxins are weak acids, their molecular form and their ability to penetrate through the membrane, depends on pH ($-\log[H^+]$). Although the hydrophobic nature of the indole group in IAA allows association of IAA with the PM surface, the negative charge of a proton dissociated carboxyl group (COO^-) will prevent it from crossing the membrane. Therefore only the proton-associated IAA molecules (COOH) can enter the cell by diffusion across the plasma membrane without assistance of a carrier protein.

Credit: Plant Sensory Systems and Responses, Auxin Transporters—Why So Many?, Indole-3-acetic acid (text edited for length)

Mark the text and figure (concepts, points, vocabulary etc.) and then answer the following questions.

1) **Explain** how auxins can be involved in long distance communication in the plant body.

2) **Describe** how auxins can be involved in short distance communication.

3) **Make a prediction** about the reason why plant cuttings may be rubbed with the gel from an aloe plant to stimulate the process of new root formation.

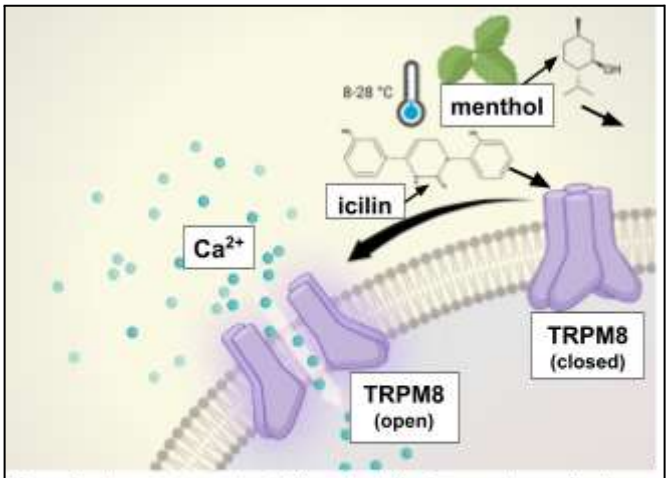
4) **Draw** a model explaining structural reasons why the proton (H^+) dissociated version of IAA cannot diffuse through the membrane while proton-associated IAA can diffuse across the membrane without the need of a carrier protein. Include appropriate labels with your drawing.

4.2 Introduction to Signal Transduction (Left Side Processing)

Our sensory systems can detect subtle changes in ambient temperature, due to the efforts of thermosensory neurons. At the level of primary afferent nerves, the site at which thermal stimuli are converted into neuronal activity, temperature-sensitive members of the TRP channel family are found. Many of these channels are receptors for ligands that elicit psychophysical sensations, such as the heat associated with capsaicin and the cold of menthol.

Most cold-sensitive neurons are also sensitive to the cooling compound menthol, a cyclic terpene alcohol found in mint leaves. It is known that moderate concentrations of menthol induce a cooling sensation, common examples include menthol-containing candy and vapo-rubs. However, higher doses of menthol can be noxious, causing burning, irritation, and pain. In studies conducted in the 1950s, menthol elicited its "cool" sensation by increasing the threshold temperature for activation of cold receptors. Indeed, the researchers hypothesized that menthol exerted its actions on "an enzyme" that was involved in the activation of these nerves. Surprisingly, it took more than 50 years for this hypothesis to be validated.

Credit: TRPM8: The Cold and Menthol Receptor, TRPM8 from ResearchFate



Transient receptor potential melastatin 8 receptor activation. TRPM8 is a protein activated by cold temperatures (8-28°C), menthol, and icilin. TRPM8 mediates normal thermosensation and has a role in both cooling-mediated analgesia and cold hypersensitivity.

Mark the text and figure (concepts, points, vocabulary etc.) and then answer the following questions.

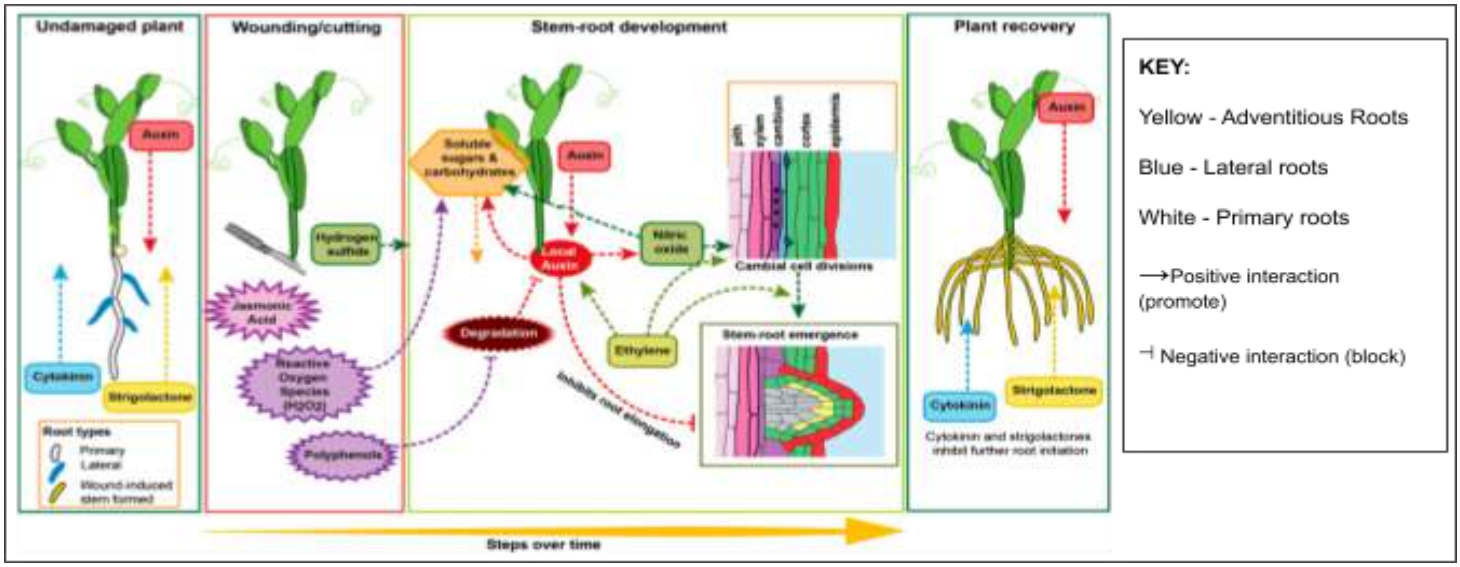
1) **Identify** the ligand involved in the cooling sensation caused by menthol.

2) **Identify** the receptor involved in the cooling sensation caused by menthol.

3) **Make a claim** about possible impacts of a genetic mutation that alter the amino acid sequence of TRPM8.

4) Neurons transmit signals by a process called an action potential which causes changes in electric potential inside and outside the neuron cell membrane. **Make a claim** about why chemicals like icilin or menthol can lead to a signal sent by an afferent nerve to the brain that is interpreted as a cooling sensation.

4.3 Signal Transduction (Left Side Processing)



In intact plants, cytokinin is predominantly produced in the root, while auxin is predominantly produced in the shoot. On wounding, jasmonic acid peaks within 30 min and is required for successful root development. Reactive oxygen species, polyphenols, and hydrogen sulfide also increase and promote adventitious rooting. Polyphenols do this via reducing auxin degradation. Auxin builds up in the base of the cutting, acting upstream of nitric oxide to promote adventitious root initiation. Auxin, nitric oxide, and hydrogen peroxide (H_2O_2) increase soluble sugars, which can be used for root development. Furthermore, levels of root initiation inhibitors (cytokinin and strigolactone) are reduced with the removal of the original root system. At later stages, auxin inhibits primordial cells elongation while ethylene promotes adventitious root emergence. As the new root system establishes, the production of cytokinin and strigolactones is restored.

Credit: The Physiology of Adventitious Roots

Mark the text and figure (concepts, points, vocabulary etc.) and then answer the following questions.

1) **Describe** how the wounding/cutting process will create lower levels of cytokinin in the plant cutting.

2) **Describe** how the cellular response to cutting leads to altering phenotypes of some cells of the plant stem.

3) **Provide reasoning to justify a claim** that using commercial rooting products that have a high auxin content and low cytokinin content will encourage the formation of adventitious roots.

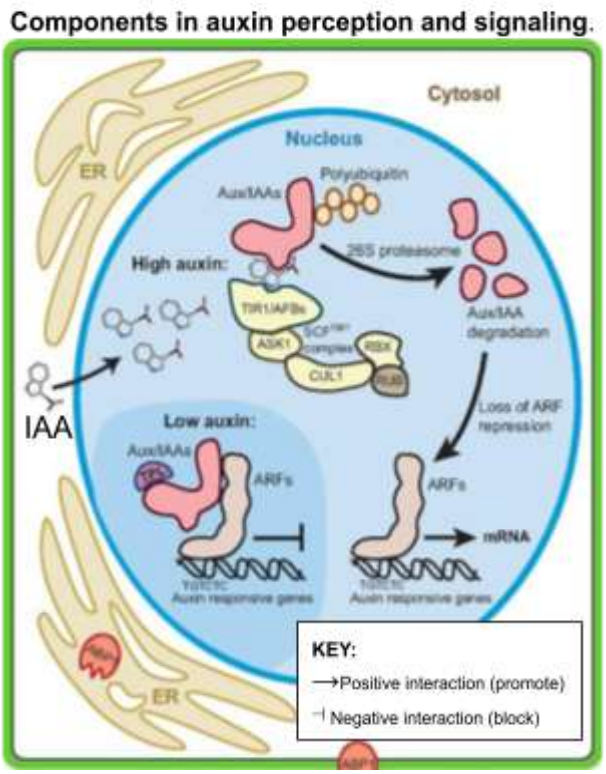
4.4 Changes in Signal Transduction Pathways (Left Side Processing)

In the nucleus, the auxin hormone indole-3-acetic acid (IAA) binds to its receptors, the *transport inhibitor response 1/auxin signaling F-box proteins* (TIR1/AFBs) and to the *auxin/indole-3-acetic acid* (Aux/IAA) proteins. The TIR1/AFB and Aux/IAA proteins function as co-receptors for IAA, binding IAA with high affinity.

When IAA levels are low (darker blue), the Aux/IAA proteins form heterodimers with *auxin response factors* (ARFs) to repress gene transcription. However, when IAA levels are high, the binding of IAA to its co-receptors targets the Aux/IAA proteins for degradation by the 26S proteasome, which leads to derepression of ARF transcriptional regulation and the expression of auxin responsive genes.

AUXIN BINDING PROTEIN 1 (ABP1), which is located in the endoplasmic reticulum (ER) or at the plasma membrane and/or in the apoplast (extracellular space), is also believed to function as an IAA receptor, mediating rapid auxin responses such as cell wall loosening, cytoskeleton rearrangement and regulation of endocytosis, leading to cell expansion.

Credit: Auxin metabolism and homeostasis during plant development



Mark the text and figure (concepts, points, vocabulary etc.) and then answer the following questions.

1) **Describe** the impacts on auxin responsive genes when auxin levels are high in the cell.

2) An important step of protein synthesis occurs when transcription produces mRNA from a DNA template molecule. **Describe** how the Aux/IAA molecule can turn protein synthesis of auxin responsive genes on.

3) **Make a prediction** about likely impacts of a mutation which causes Aux/IAA to lose the ability to bind to indole-3-acetic acid (IAA) on auxin responsive genes. **Explain** your prediction.

4.5 Feedback (Left Side Processing)

Adventitious root formation is defined as the formation of new roots on above-ground plant parts and is considered crucial for the survival of a plant under harsh environmental conditions (i.e., flooding, salt stress, and other abiotic stresses) as well as in the nursery industry. Clonal propagation is based on the ability of a plant part to grow and generate a completely new plant, genetically identical to the mother plant, where the plant part came from. Nurseries exploit this potential by multiplying millions of new plants. Most nurseries use cuttings to achieve that, through the induction of adventitious root formation. Many factors have been implicated in the capacity of a cutting to root, with the major role being played by auxins. During the last few decades, intense interest has emerged in the role of other potential rooting co-factors, such as carbohydrates, phenolics, polyamines, and other plant growth regulators, as well as signal molecules, such as reactive oxygen species (ROS) and nitrogen species. Among the latter, hydrogen peroxide (H_2O_2) and nitric oxide have been found to play significant roles in adventitious root formation.

ROS in general, and more specifically H_2O_2, are closely connected to the wound response of cuttings, as it is produced through the action of diamine oxidases. It has been found that its concentration increases within the first 12 h, reaching a maximum within 36 h after cutting severance from the mother plant, and this has been connected with the external application of auxin, suggesting the existence of feedback loops between auxin and peroxide. This is further supported by the decreased activities of both peroxidase and ascorbate peroxidase after auxin application, two enzymes responsible for the inactivation of H_2O_2.

Credit: Adventitious Root Formation in Plants: The Implication of Hydrogen Peroxide and Nitric Oxide

Mark the text and figure (concepts, points, vocabulary etc.) and then answer the following questions.

1) **Describe** reasons why a feedback mechanism would be important in the process of clonal propagation achieved by wounding a plant to encourage the development of adventitious roots.

2) **Make a claim** about whether the possible feedback between auxin and peroxide is positive or negative.

3) **Defend your claim** by providing evidence and explaining how that evidence supports your claim.

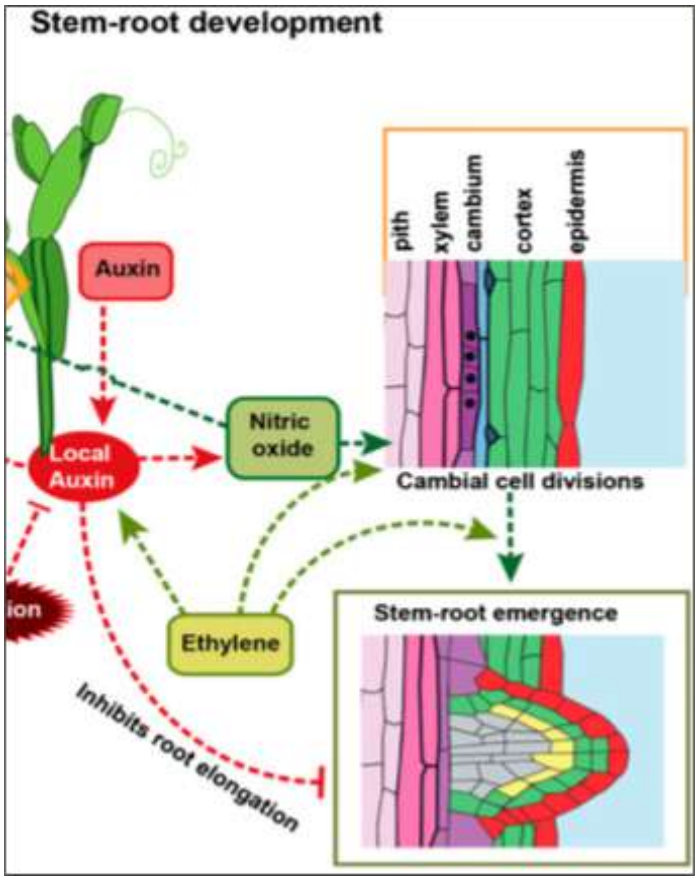
4) **Predict** the effect on the formation of adventitious roots if altered forms of peroxidase and ascorbate peroxidase whose activity are not influenced by auxin levels are present in a wounded plant.

4.6 Cell Cycle (Left Side Processing)

Most plants continue to grow throughout their lives. Like other multicellular organisms, plants grow through a combination of cell growth and cell division. Cell growth increases cell size, while cell division (mitosis) increases the number of cells. As plant cells grow, they also become specialized into different cell types through cellular differentiation. Once cells differentiate, they can no longer divide. How do plants grow or replace damaged cells after that? Adventitious roots generally form by the following process.

Induction (dedifferentiation phase) begins after taking the cuttings and lasts approximately 24 h. Cambium cells turn meristematic (undifferentiated) and become sensitive to auxin-inducing root formation. The action of auxin and ethylene affects enzymatic activities involved in cell dedifferentiation and division. ***Initiation*** is the second phase. The cells are able to respond to auxins (naturally occurring or applied externally) that induce cell division, resulting in development of preformed roots. ***Formation of root primordia*** occurs after placing microcuttings on the rooting medium. ***Root elongation*** is apparent as root tips emerge through the tissue at the base of the microcuttings. In this phase externally applied auxins might act in an inhibitory way.

Credit: Plant Growth, Adventitious Root, Physiology of Adventitious Roots



Mark the text and figure (concepts, points, vocabulary etc.) and then answer the following questions.

1) **Explain** the importance of mitosis in the development of adventitious roots.

2) **Justify** a claim made by a plant scientist who states that the action of auxin and ethylene to cause cambium cells to dedifferentiate is critical for the process of adventitious root formation.

3) **Represent** the process of cell division by creating a labeled drawing of cells in the phases of the cell cycle.

4.7 Regulation of Cell Cycle (Left Side Processing)

The formation of adventitious roots (ARs) is an ecologically and economically important developmental process in plants. The evolution of AR systems is an important way for plants to cope with various environmental stresses. This review focuses on identified genes that have been known to regulate the induction and initiation of ARs and offers an analysis of this process at the molecular level. The critical genes involved in adventitious rooting are the auxin signaling-responsive genes, including the AUXIN RESPONSE FACTOR (ARF) and the LATERAL ORGAN BOUNDARIES-DOMAIN (LOB) gene families, and genes associated with auxin transport and homeostasis, and the root apical meristem (RAM) initiation. Two steps are required for cell fate transition to RAM formation. Firstly, regeneration-competent cells dedifferentiate to become root founder cells. Secondly, the root founder cells transform into root primordium cells and initiate mitosis to form undifferentiated RAM capable of forming new roots.

GRAS Family Transcription Factors (TFs)

In Arabidopsis, the SCARECROW(SCR) gene interacts with SHORT-ROOT(SHR) gene to activate downstream target genes, thereby regulating RAM. SHR regulates the expression of direct target genes, including the D-type cyclin gene CYCD6;1 and the cyclin-dependent kinase genes CDKB2;1 and CDK2;2, and induces endodermal cell identity and the expression of SCR. SCR controls asymmetrical cell divisions and limits the movement of SHR. These results indicate that SCR/SHR complex activates D-type CYCLIN genes and is involved in initiation of cell dedifferentiation in Arabidopsis.

Credit: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7876083/

Mark the text and figure (concepts, points, vocabulary etc.) and then answer the following questions.

1) **Identify** the cyclin and cyclin-dependent kinase genes that have been shown to play roles in the development of adventitious roots.

2) **Describe** how the SCR/SHR complex helps to regulate the cell cycle processes that lead to the development of adventitious roots.

3) **Predict** the outcome of an experiment examining formation of adventitious roots which utilizes a chemical agent to block the activity of the SCR/SHR complex.