Introduction to Phytohormones
What are phytohormones?

“........characterized by the property of serving as chemical messengers, by which the activity of certain organs is coordinated with that of others”.

-Frits Went and Kenneth Thimann, 1937
Phytohormones regulate cellular activities (division, elongation and differentiation), pattern formation, organogenesis, reproduction, sex determination, and responses to abiotic and biotic stress.
Phytohormones – old timers and newcomers

- Auxin
- Cytokinin
- Gibberellins
- Abscisic Acid
- Ethylene
- Brassinosteroids
- Salicylates
- Strigolactones
- Jasmonates
Phytohormones regulate all stages of the plant life cycle:

- Fertilization and fruit formation
- Embryogenesis
- Seed dormancy
- Germination
- Growth and branching
- Flower development
- Fruit ripening
- Growth and branching
Hormones also help plants cope with stress throughout their life.
Most hormones affect most stages of the plant life cycle

We will examine each hormones within the context of one of its roles.

Remember that these are merely examples; most hormones affect most processes in one way or another.
Lecture outline

How hormones work
Hormonal control of vegetative development
  Auxin
  Cytokinins
  Strigolactones
  Gibberellins
  brassinosteroids

Hormonal control of reproduction
  Ethylene
  Abscisic Acid

Hormonal responses to stress
  Salicylates
  jasmonates

Cross-regulation of hormonal effects
Hormones: Synthesis, transport, perception, signaling and responses

Production of active hormone

Transport

Binding to receptor

Signal transduction

Downstream effects

Downstream effects
Many tightly regulated biochemical pathways contribute to active hormone accumulation. Conjugation can temporarily store a hormone in an inert form, lead to catabolic breakdown, or be the means for producing the active hormone.
Transport and perception

Several hormone receptors have recently been identified. They can be membrane bound or soluble.

Hormones can move:
- through the xylem or phloem
- across cellular membranes
- through regulated transport proteins

Transport

Production of active hormone

Binding to receptor
Hormonal signals are transduced in diverse ways. Common methods are reversible protein phosphorylation and targeted proteolysis.

Signal transduction

Protein phosphorylation

Protein dephosphorylation

Proteolysis

Signal transduction

H
Downstream effects can involve changes in gene transcription and changes in other cellular activities like ion transport.
Hormones: Synthesis, transport, perception, signaling and responses

- **Synthesis**
  - Production of active hormone
  - Binding to receptor

- **Transport**
  - Conjugation
  - De-conjugation

- **Breakdown**
  - Degradation

- **Downstream effects**
  - Transcription

- **Non-genomic effects**
  - (e.g. Ion channel regulation)

- **Signal transduction**
  - Protein phosphorylation
  - Protein dephosphorylation
  - Proteolysis
Receptors can be membrane-bound

Hormone binding initiates an information relay
Soluble receptors can facilitate interactions between proteins

Hormones can act like “molecular glue”
Some receptors initiate protein proteolysis

The hormones (red) bind to receptors (green), initiating proteolysis of repressors (yellow) to activate a transcriptional regulator (blue)

Auxin  Gibberellin  Jasmonate

Proteolytic targets are covalently linked to ubiquitin.
Ubiquitin ligase complexes ubiquitinate target proteins

The auxin and jasmonate receptors are F-box proteins, part of an SCF ubiquitin ligase complex

Ubiquitin is ligated to the target
Ubiquitinated proteins are targeted for proteolysis
Hormones affect vegetative growth: elongation, branching and organogenesis

Elongation in the shoot and root of a germinating soybean

Germinated seedling

Growth by elongation

Organogenesis

Growth by branching

Photo courtesy of Shawn Conley
Disrupting hormone synthesis or response interferes with elongation

<table>
<thead>
<tr>
<th>GA</th>
<th>Auxin</th>
<th>Brassinosteroid</th>
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<tbody>
<tr>
<td>Pea</td>
<td>Arabidopsis</td>
<td>Arabidopsis</td>
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<tr>
<td>Wild type</td>
<td>Wild type</td>
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<tr>
<td>Gibberellin biosynthesis mutant</td>
<td>Auxin response mutant</td>
<td>Brassinosteroid biosynthesis mutants</td>
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Auxin

- Growth
- Phototropism and gravitropism
- Branching
- Embryonic patterning
- Stem cell maintenance
- Organ initiation

Indole-3-acetic acid (IAA), the most abundant natural auxin
Auxin controls growth

Charles Darwin studied the way seedlings bend towards light, a direct effect of auxin action.

Site of signal perception

Site of response

Darwin concluded that a signal moves through the plant controlling growth

“We must therefore conclude that when seedlings are freely exposed to a lateral light some influence is transmitted from the upper to the lower part, causing the latter to bend.”

Differential cell growth is a result of auxin movement to the shaded side

Auxin concentration on shaded side stimulates elongation and bending.

Auxin moves in part by a chemiosmotic mechanism

Auxin is a charged anion (IAA-) in the cytoplasm (pH 7).

In the more acidic cell wall (pH 5.5) some is uncharged (IAAH). The uncharged form crosses the plasma membrane into the cell where it is deprotonated and unable to exit other than through specific transporters.

Auxin transport out of cells is controlled by three families of transport proteins that collectively control the directionality of auxin movement. Asymmetric distribution of the transporters controls polar auxin transport.

Net flow of auxin

IAA is produced from tryptophan (Trp) via several semi-independent pathways and one Trp-independent pathway.

Environmental and developmental control of the genes controlling auxin biosynthesis, conjugation and degradation maintain auxin homeostasis.

Auxin regulates plant development

- Lateral organ initiation at the shoot apical meristem
- Inhibit branching in the shoot
- Patterning and vascular development
- Maintain stem cell fate at the root apical meristem
- Promote branching in the root

Many of auxin’s effects are mediated by changes in gene expression.
The auxin signaling pathway

1. Auxin binds to SCF$^{TIR1}$ and Aux/IAA
The auxin signaling pathway

1. Auxin binds to SCF\(^{TIR1}\) and Aux/IAA

2. Aux/IAA ubiquitinated and degraded by 26S proteasome
The auxin signaling pathway

1. Auxin binds to SCF\textsuperscript{TIR1} and Aux/IAA

2. Aux/IAA ubiquitinated and degraded by 26S proteasome

3. Degradation of repressor permits transcriptional activation by ARF transcription factors
Cytokinins

- Cell division
- Control of leaf senescence
- Control of nutrient allocation
- Root nodule development
- Stem cell maintenance
- Regulate auxin action

trans-zeatin, a cytokinin
Cytokinins are a family of related adenine-like compounds

Cytokinin (CK) biosynthesis

Regulated by CK and nitrogen

Tissue specific; auxin, CK and ABA sensitive

Meristem specific

CK biosynthesis and inactivation are strongly regulated by CK, other hormones and exogenous factors.

Inactive form

Upregulated by CK and ABA

Cytokinins act antagonistically to auxins

**CK**
- Promote stem cell fate at the shoot apical meristem
- Promote differentiation at the root apical meristem

**Auxin**
- Promote lateral organ initiation at the shoot apical meristem
- Maintain stem cell fate at the root apical meristem
- Promote branching in the root

**Inhibit branching in the shoot**

**Promote branching in the shoot**
Auxin and cytokinin regulate each other’s function at the root apex

Through effects on each other’s synthesis, transport and response, auxin and cytokinin establish two mutually exclusive domains that coordinate cellular activities at the root apex.
Auxin, cytokinin and strigolactones control branching

Shoot branches are promoted by CK and inhibited by auxin and strigolactones

Root branches, called lateral roots, are promoted by auxin and inhibited by CK

Branching controls every aspect of plant productivity from nutrient uptake to crop yields.

Cytokinin signaling is mediated by a two-component system

A two-component system is a short signaling pathway that moves information from an input to an output. In bacteria it usually consists of two proteins, a histidine kinase (HK) with a conserved histidine residue (H) and a response regulator (RR) with a conserved aspartate residue (D).
Information and phosphoryl groups are relayed between the components

Stimulation of the input domain activates the kinase activity, phosphorylating the transmitter domain. The phosphoryl group is subsequently relayed to the response regulator.
Two component signaling in plants

Most plant HKs are hybrid histidine kinase, which transfer the phosphoryl group to a histidine phosphotransferase. The HPt transfers it to a response regulator.
Cytokinin control of gene expression in *Arabidopsis*

Type B *Arabidopsis* response regulators (ARRs) are transcription factors. Type A or C ARRs interfere with CK signaling through as yet unknown means.
Cytokininins affect grain production and drought tolerance

Rice plants that accumulate more CK can produce more grain per plant because of changes in inflorescence architecture.

Tobacco plants that produce more CK are more drought tolerant because of the delay in leaf senescence conferred by CK.

Strigolactones, synthesized from carotenoids, are produced in plant roots. They attract mycorrhizal fungi and promote the germination of parasitic plants of the genus *Striga*.
Strigolactones inhibit branch outgrowth

• Growth
• Seed germination
• Promote flowering
• Promote sex determination in some species
• Promote fruit growth

A Gibberellin (GA₄)
Gibberellins are a family of compounds

Only some GAs are biologically active. The major bioactive gibberellins are shown here.

GA$_4$ is the major active GA in Arabidopsis.

Major GA biosynthetic and catabolic pathways in higher plants

Gibberellins regulate growth

The pea mutant *le*, studied by Mendel, encodes GA₃ oxidase, which produces active GA. Loss of function of *le* reduces active GA levels and makes plants dwarfed.

Genes controlling GA synthesis are important “green revolution” genes

Tremendous increases in crop yields (the Green Revolution) during the 20th century occurred because of increased use of fertilizer and the introduction of semidwarf varieties of grains.

The semidwarf varieties put more energy into seed production than stem growth, and are sturdier and less likely to fall over.

Distinguished plant breeder and Nobel Laureate Norman Borlaug 1914-2009

Photos courtesy of S. Harrison, LSU Ag center and The World Food Prize.
Several of the green revolution genes affect GA biosynthesis

Semidwarf rice varieties underproduce GA because of a mutation in a the GA20 oxidase biosynthetic gene.
DELLA proteins inhibit growth, in part through blocking transcription. GA triggers DELLA protein proteolysis.

A gene affecting DELLA protein stability is a “green revolution” gene.

The wheat *Rht1* locus encodes a DELLA protein. The dwarf allele lacks the DELLA domain and resists proteolysis.

Brassinosteroids

- Cell elongation
- Pollen tube growth
- Seed germination
- Differentiation of vascular tissues and root hairs
- Stress tolerance
Brassinosteroid (BR) mutants are dwarfed

BRs promote cell elongation in part by loosening cell walls

Lowered resistance to internal turgor pressure; cell expansion
Reducing BR signaling produces dwarf barley

The *uzu* plants have a missense mutation in the BR receptor, making them less sensitive to BR. This is the first dwarf grain produced through modification of BR signaling.

Without BR, the receptor (BRI1) is bound to an inhibitor (BKI1). The active BIN2 kinase phosphorylates and inactivates transcription factors.
BR signaling

BR-binding causes BAK1 and the BRI1 receptor to phosphorylate each other and BSKs. BSKs phosphorylate and active BSU1 phosphatase, which inactivates BIN2. When BIN2 is inactive, its target transcription factors are dephosphorylated and active.
Summary – hormonal control of vegetative growth

Plant hormones have diverse effects on plant growth.

Auxin, gibberellins and brassinosteroids contribute to elongation growth.

Auxin, cytokinins and strigolactones regulate branching patterns.

Growth and branching profoundly affect crop yields.
Hormonal control of reproductive development

In angiosperms:

- transition from vegetative to reproductive growth
- flower development,
- fruit development and ripening
- seed development, maturation and germination

Photo courtesy of Tom Donald
Transition to flowering

The decision to reproduce is tightly controlled by environmental and hormonal factors. For many plants day-length is critical in this transition, but other plants are day-length neutral. Similarly, some plants absolutely require specific hormonal signals which have little or no effects on other plants.
GA’s role in initiating flowering varies by species and growth-habit

Malus domestica
Perennial
Yes

Lolium temulentum
Annual temperate grass
Yes

Beta vulgaris
Biennial
Yes

Arabidopsis thaliana
Annual
Short Days
Yes

Beta vulgaris
Biennial
Yes

Malus domestica
Perennial
No

Arabidopsis thaliana
Annual
Long Days
No

Photos courtesy of Plate 271 from Anne Pratt's Flowering Plants, Grasses, Sedges and Ferns of Great Britain c.1878, by permission of Shrewsbury Museums Service; David Kuykendall ARS; Vincent Martinez; Takato Imaizumi.
Ethylene promotes flowering in pineapples and other bromeliads

A pineapple is a fruit produced from pineapple flowers. Commercial growers treat the plants with ethylene to synchronize flowering.

Images courtesy of Dave McShaffrey, Marietta College, ©2009, used by permission.
Hormones contribute to flower development in many ways:

- Patterning of the floral meristem
- Outgrowth of organs
- Development of the male and female gametophytes
- Cell elongation
Ethylene and gibberellins are involved in sex determination.
Fruit development and ripening are under hormonal control.

Pollination initiates petal senescence, cell division and expansion in the ovary to produce a fruit, and fruit ripening.
Auxin and GA promote cell division and growth of the fruit

Seedless varieties of grapes and other fruits require exogenous application of GA for fruit development. Strawberry receptacles respond to auxin.

Photo credits: Grape flowers by Bruce Reisch; Strawberry flower by Shizhao
Fruit ripening is induced by ethylene

Ethylene is a gaseous hormone that promotes fruit softening and flavor and color development.
Ethylene

- Control of fruit ripening
- Control of leaf and petal senescence
- Control of cell division and cell elongation
- Sex determination in some plants
- Control of root growth
- Stress responses

Ethylene induces the triple response:
- reduced elongation,
- hypocotyl swelling,
- apical hook exaggeration.
Ethylene promotes senescence of leaves and petals.

In gas-lit houses, plants were harmed by the ethylene produced from burning gas. **Aspidistra** is ethylene-resistant and so became popular houseplant.

Ethylene shortens the longevity of cut flowers and fruits

Ethylene levels can be managed to maintain fruit freshness, commercially and at home.

**Strategies to limit ethylene effects**

**Limit production** - high CO\(_2\) or low O\(_2\)

**Removal from the air** - KMnO\(_4\) reaction, zeolite absorption

**Interfere with ethylene binding to receptor** - sodium thiosulfate (STS), diazocyclopentadiene (DACP), others

Molecular genetic approaches can limit ethylene synthesis

S-adenosyl methionine

\[ \text{ACC synthase} \rightarrow \text{ACC} \]

(1-aminocyclopropane-1-carboxylic acid)

\[ \text{ACC oxidase} \rightarrow \text{Ethylene} \]

Introduction of antisense constructs to interfere with expression of biosynthesis enzymes is an effective way to control ethylene production.

Ethylene-regulated gene expression is negatively regulated

In the absence of ethylene, CTR binds the receptor and prevents transcription.

Ethylene binding to the receptor releases CTR, permitting transcription.

Several mutations that affect ethylene perception and signaling interfere with fruit ripening.

Abscisic acid

• Seed maturation and dormancy
• Desiccation tolerance
• Stress response
• Control of stomatal aperture
ABA accumulates in maturing seeds

Seed maturation requires ABA synthesis and accumulation of specific proteins to confer desiccation tolerance to the seed.
ABA synthesis and signaling is required for seed dormancy.

Loss of function of ABA signaling (protein kinase or transcription factor function) interferes with ABA-induced dormancy and causes precocious germination.


Once dormant and dry, seeds can remain viable for very long times. These date palm seeds are nearly 2000 years old, but still viable and capable of germination. Five-hundred year old lotus seeds have also been successfully germinated. Having a thick seed coat may help these super seeds retain viability.

GA is required for seed germination

Seed germination requires elimination of ABA and production of GA to promote growth and breakdown of seed storage products.
GA is used by brewers to promote barley germination.

Breakdown of starch in the endosperm is initiated by GA produced by the endosperm or added during the malting process.
GA and ethylene promote flowering in some plants.

Fruit growth, maturation and ripening are regulated by auxin, GA and ethylene.

Seed maturation and germination are regulated by ABA and GA.

Understanding the roles of hormones in plant reproduction is important for food production, because most of our caloric intake is derived from seeds.
Hormonal responses to abiotic stress

- Photooxidative stress
- High temperature stress
- Water deficit, drought
- Soil salinity
- Air pollution
- Wounding and mechanical damage
- Cold and freezing stress

Plants’ lives are very stressful.....

ABA and ethylene help plants respond to stress.

ABA biosynthesis is strongly regulated.

ABA levels are tightly controlled. Critical steps in ABA biosynthesis (circled in red) are encoded by multiple tightly regulated genes to ensure rapid and precise control.

ABA synthesis is strongly induced in response to stress

ABA levels rise during drought stress due in part to increased biosynthesis

ABA induces stress-responsive genes

Osmoprotectants (sugars, proline, glycine betaine)

Membrane and protein stabilization (HSPs, LEAs)

Oxidative stress responses – peroxidase, superoxide dismutase

Movement of water and ions (aquaporins, ion channels)
ABA binding to an intracellular receptor initiates transcriptional responses

PYL1 is an ABA receptor. When PYL1 binds ABA, it also binds the protein phosphatase PP2C, inhibiting its function.

ABA signal transduction affects gene expression

When ABA is present, inactivation of the PP2C phosphatase permits a protein kinase (e.g. SnRK) to phosphorylate and activate ABA-inducible TFs, promoting transcription of ABA-inducible genes.
ABA regulates stomatal aperture by changing the volume of guard cells

Pairs of guard cells surround the openings of plant pores called stomata.

Guard cells control the opening and closing of stomata to regulate gas exchange: a fine balance is required to allow CO₂ in for photosynthesis and prevent excessive water loss.
ABA controls stomatal aperture by changing the volume of guard cells.

When stomata are open, plants lose water through transpiration. ABA induced by drought causes the guard cells to close and prevents their reopening, conserving water.

ABA-induced stomatal closure is extremely rapid and involves changes in ion channel activities.

ABA triggers an increase in cytosolic calcium (Ca$^{2+}$), which activates anion channels (A$^{-}$) allowing Cl$^{-}$ to leave the cell. ABA activates channels that move potassium out of the cell ($K^{+}_{\text{out}}$) and inhibits channels that move potassium into the cell ($K^{+}_{\text{in}}$). The net result is a large movement of ions out of the cell.

As ions leave the cell, so does water (by osmosis), causing the cells to lose volume and close over the pore.

Hormonal responses to biotic stress

Bacteria, fungi, viruses – Biotrophic organisms

Salicylic Acid

Jasmonates

Herbivores – insects, other animals, fungi – Necrotrophic organisms

Photo credits: A. Collmer, Cornell University; Salzbrot.
Jasmonates

• Response to necrotrophic pathogens
• Induction of anti-herbivory responses
• Production of herbivore-induced volatiles to prime other tissues and attract predatory insects
JA biosynthesis

Jasmonate signaling contributes to defense against herbivory

When exposed to hungry fly larvae, plants unable to produce JA have low rates of survival.

Jasmonates induce the expression of anti-herbivory chemicals

Wound-induced signals
Insect oral secretions

Protease inhibitors
Feeding deterants
Jasmonates contribute to systemic defense responses

Defense responses are activated in distant tissues
Jasmonates stimulate production of volatile signaling compounds

Herbivore-induced volatiles prime other tissues (and other plants) for attack making them unpalatable (indicated in red).

Herbivore-induced volatiles are recognized by carnivorous and parasitoid insects
JA-induced changes in gene expression

**Low JA-Ile**
- JA-responsive transcription factor
- No transcription
- F-box protein receptor (COI1)

**High JA-Ile**
- JA-Ile
- Proteolysis
- Transcription
- Defense genes
Salicylic Acid – plant hormone and painkiller

- Response to biotrophic pathogens
- Induced defense response
- Systemic acquired resistance

Salicylic acid is named for the willow *Salix* whose analgesic properties were known long before the chemical was isolated.

Acetylsalicylic Acid - aspirin
Salicylate synthesis is induced upon pathogen attack

Isochorismate synthase (ICS) is induced by pathogen infection

SA accumulation induces PATHOGENESIS RELATED (PR) and other defense genes

Salicylates contribute to systemic acquired resistance

SA is necessary in systemic tissue for SAR, but the nature of the mobile signal(s) is still up in the air.

It is likely that multiple signals contribute to SAR.
Plants recognize PAMPs (pathogen-associated molecular patterns)

PAMP-triggered immunity

Flagellin is a conserved bacterial protein recognized by plants, also known as a pathogen-associated molecular pattern (PAMP).

Recognition of PAMPs by a plant cell triggers a set of immune responses that are mediated by salicylic acid.

Some pathogens elicit a stronger defense response

Many plants express resistance genes that recognize the effects of bacterial proteins effector proteins.

The interaction of an R protein with an effector protein promotes a stronger immune response, including the hypersensitive response.

Effector-triggered immunity

The hypersensitive response involves cell death

Pathogen Response (PR) genes
Antimicrobial compounds
Strengthening of plant cell walls
Programmed cell death
Hypersensitive response (HR)

The hypersensitive response seals the pathogen in a tomb of dead cells

The HR kills the infected cells and cells surrounding them and prevents the pathogen from spreading.

Without a hypersensitive response, the pathogen can multiply.

Other hormones affect defense response signaling

As part of their immune responses, plants modulate synthesis and response to other hormones. Some pathogens exploit the connections between growth hormones and pathogen-response hormones to their own advantage, by producing “phytohormones” or interfering with hormone signaling.

Hormonal signaling is critical for plant defenses against abiotic and biotic stresses.

ABA and ethylene are produced in stressed plants and critical for activating their defense pathways.

JA and SA contribute to local and systemic defenses against pathogens.

Understanding plant hormonal responses to stress is needed to improving agricultural yields. Abiotic and biotic stresses are major causes of crop losses and reduced yields and which must be minimized.
Crosstalk between hormone signaling pathways

Crosstalk (or cross-regulation) occurs when two pathways are not independent. It can be positive and additive or synergistic, or negative.
Crosstalk between hormone signaling pathways

Crosstalk (or cross-regulation) occurs when two pathways are not independent. It can be positive and additive or synergistic, or negative.

Crosstalk can affect the synthesis, transport or signaling pathway of another hormone.
Synergistic requirement for JA and ET signaling in defense response

JA and ET signaling are both required for high-level expression of ERF1, a TF that induces defense gene expression.

Negative interaction between JA and SA in defense responses

In defense signaling, the JA and SA pathways are mutually antagonistic (locally), and both are antagonized by ABA.

Why does ABA reduce SA and JA signaling? Perhaps a plant that is already stressed and producing high levels of ABA may be better off temporarily restricting its responses to pathogens.

GA and DELLAs interact extensively with other signaling pathways

Just about every stage of the plants life is coordinated by GA and its effects on the other hormone signaling pathways.

Although it is clear that GA’s effects on DELLA proteins are very important, we don’t yet understand what these proteins do.

GA and DELLAs interact extensively with other signaling pathways.

Ongoing research

Hormones coordinate plant growth and defense
Many aspects of hormone synthesis, homeostasis and signaling are still being discovered
Knowledge of these processes provides tremendous opportunities for agricultural improvements including the development of stress-resistant and pathogen-resistant plants, plants with greater abilities to take up nutrients, foods that stay fresh longer, and increased crop yields
The field of plant hormones is blossoming and fruitful