Balance of Hormones regulate growth and development
Environmental factors regulate hormone levels
- light - e.g. phototropism
- gravity - e.g. gravitropism
- temperature

Mode of action of each hormone
1. Signal **perception** by a receptor on or in target cells.
2. Signal **transduction** and amplification via
   a. R \(\rightarrow\) \(\rightarrow\) 2 messengers \(\rightarrow\) modify/activate protein
   b. R \(\rightarrow\) \(\rightarrow\) gene expression
3. **Response**: changes in protein activities and/or gene expression
   \(\rightarrow\) \(\rightarrow\) growth/development response

Balance of hormones regulate growth and development
Balance of IAA: cytokinin determines root or shoot development.
e.g. in pith tissue
Hormones are used to regenerate transgenic plants.

23. Shoot and root development depend on ratio of IAA: CK
   
   ![Diagram of IAA and CK in transgenic plants](image)

21-4 Taiz. Tumour induction by cytokinin.
   
   ![Diagram of Agrobacterium-mediated transformation introducing new gene into plants](image)

Box 21-1 Taiz. Regenerating whole plants from transgenic cells or tissues.

Balance of hormones regulate growth and development
1. Shoot and root development depend on ratio of Auxin IAA: cytokinin
2. Leaf abscission depends on auxin and ABA
3. Seed development and germination depends on relative amount of ABA and GA

Effect depends on
- **Type of hormones**
- **conc**
- **balance of hormones**
19-27. Light causes redistribution of auxin to shaded side in phototropism.

**Environmental signals regulate hormone levels. How?**

- Monitor auxin levels using a reporter gene GUS or GFP.
  - A promoter that is activated by auxin is fused to GUS or GFP.
    - DR5::GUS (enzyme gives blue color)
    - DR5::GFP (green fluorescence protein)
  - Plant is transformed with a or b. construct.
  - Gene is incorporated into genome and inherited. Progeny plants carry a or b.

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**Auxin level changes in response to light and to gravity are due to auxin transport.**

![Diagram of auxin transport](image)

**Evidence auxin transport is critical for phototropism**

**Genetic evidence:** Mutants of auxin transport respond poorly to light and to gravity

- b, c. pin3 hypocotyls are defective in gravitropic (b) as well as phototropic (c) responses.
- d. pin3 mutants are defective in root gravitropism

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**Auxin levels can be changed by auxin efflux carriers**

- **PIN1**: Basipetal auxin transport in the stem
- **PIN2**: Basipetal auxin transport
- **PIN3**: Lateral auxin efflux in gravitropic responses

- PIN1-GFP is localized to base of cell
- PIN3-GFP is localized to lateral side of cell

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19-33 Taiz. Why do roots placed horizontally bend downwards?

**Gravitropism depends on distribution of hormones**

![Diagram of gravitropism](image)
Gravity changes auxin distribution

Before gravistimulation
After 3 h

DR5::GFP signal reflect auxin cone

19.15 In Arabidopsis, PIN transport proteins direct the flow of auxin (Part 2)

19-30. Taiz. Root cap cells are important for sensing gravity

Mode of Action of Hormones
1. Signal perception by a receptor in/on target cell
2. Signal transduction and amplification
3. Responses: a. molecular
   - early gene expression
   - late gene expression
   proteins activated
   repressor removed
b. Cellular & Physiological response

Auxin

Stimulate cell elongation
How? Two modes of action
a. Stimulates H⁺ efflux (Membrane protein trafficking)
b. Cause changes in gene expression
   Removal of repressors
15-21. Taiz. What causes cell elongation?
Acid pH causes wall extension.

15-22. Wall protein expansins are required for acid growth.

19-23. Auxin induces H\(^+\) extrusion and increases cell extension

How does auxin stimulate cell elongation?
Acid growth theory
• Auxin induce H\(^+\) extrusion
• Ion uptake \(\rightarrow\) osmotic water uptake
• Turgor pressure builds up
• Wall loosens, allowing cell wall to expand

How does auxin stimulate H\(^+\) extrusion?
Hypotheses:
• Activate the PM H\(^+\)-pumping ATPase
• Increase synthesis of new PM H\(^+\) pumps

19-18. Model of auxin-induced H\(^+\) extrusion and cell expansion

19-31. Auxin also induced expression of specific mRNAs
Many SAUR (small auxin upregulated RNA) are transcription factors.

**Auxin induces expression of early and late genes**

**Early genes** [primary response genes]
- expressed in 5-60 min after adding auxin
- include transcription factors
- have roles in intercellular communication
- stress adaptation

**Late Genes** [secondary response genes]
- target genes

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Auxin receptor identified in 2005.
TIR1 receptor

Auxin binds to TIR1 complex.
Auxin activates TIR1 (F-box protein)

TIR1 complex targets the degradation of IAA repressor proteins.
Removal of repressor activates transcription of early genes.

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**Model for auxin binding to TIR auxin receptor**

**Removal of repressor protein by degradation**

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**Binding of TIR1 to IAA7 depends on auxin conc**

- Plant (expressing TIR1-myc)
- extract + GST-IAA7 (repressor) + 0 - 50 auxin

- GST-IAA7 will pull down X if it binds to IAA7
- Bound protein identified by SDS-PAGE & Immunoblot with anti-myc is TIR1

- 3H-IAA interacts with TIR1 complex
- Crude extracts (TIR1-myc) + GST-IAA7 + 3H-IAA

- pull-down & count cpm (a)
- Competed by IAA, 2,4-D, NAA (b)

Dharmasiri et al 2005 Nature
Where is TIR1 localized?

- GFP-TIR1 DNA is expressed in tobacco leaf cells. Located to nucleus.
- 4 related F-box proteins in Arabidopsis are auxin receptors. So quadruple mutants were made to knock out all 4 proteins.

Auxin-responsive gene expression is severely reduced in tir1 mutant

Seedling growth affected in tir1 (4 genes) mutants

Root tip in Wt (col, Ws), and in mutants

TIR1 and AFB genes act collectively

Specificity in IAAs and ARFs

- 23 ARFs, and 29 Aux/IAA proteins
  - E.g.
    - Root & Hypocotyl growth, ARF2, 7, 8, 19
    - Tropism, ARF 2, 7, 19
    - Embryogenesis, ARF5
    - flower

Summary: Auxin action

- 1. Increase H+ pumping
- 2. Change gene expression
  Auxin binding to TIR receptor stimulated repressor breakdown. ARF can then bind regulatory region to enhance expression of early response genes
GA
Gibberellin

Chap. 20

20-33. GA and barley seed germination
• Promote germination
• Activate vegetative growth
• Mobilize stored food
• Q? How does GA stimulate amylase activity?
• What is the signal transduction pathway?

20-34. GA-Myb is an early response gene that regulates expression of α-amylase

Model: GA signals degradation of repressors

GA binding to receptor targets repressor for degradation
Rice GA receptor gid1-1 mutant is stunted

Other mutants:
slr1-1 mutant is longer than Wt.

SLR protein is high in gid1 mutant

3H-GA binding to GST-Gid1

Gid1 binding to SLR1 is GA-dependent
Gid2 mutant is insensitive to GA.
GID2 is an F-box protein
(a subunit of the ubiquination complex)

**Immunoblot to test protein**
Extract from Wt & gid2-1 mutant.
SDS-PAGE to separate protein.
Test for SLR1 with anti-SLR1.
SLR1 is high in gid2-1 mutant

Sakai et al. 2003 Science

Gid2-1 mutant is small

Model of GA-induced amylase synthesis