Introduction

- **Hormones** are the principle means of **intracellular communication** in plants.
- They are **signal molecules** that act individually or **cooperatively** to carry information between cells. They are essential to **coordinate growth and development**.
- These few slides will try to explain:
  - Hormone biochemistry – What kind of molecules these hormones are.
  - Understanding how hormones work via mutant studies.
  - Hormone metabolism and biosynthesis.

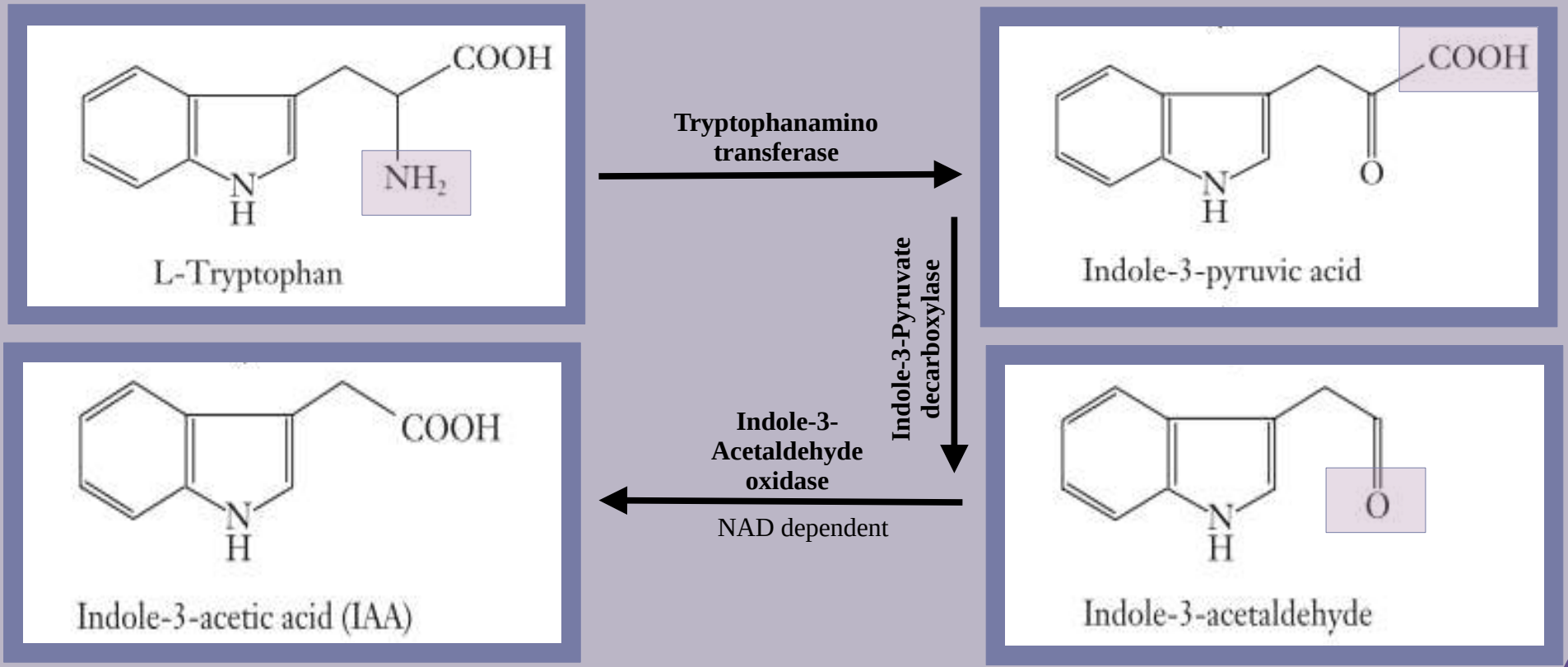
The Hormone Concept in Plants

- **What are hormones?**
  - These are **naturally occurring organic molecules** present at **low concentrations** that influence the physiological processes.
  - They are (according to the animal physiologists):
    - **Synthesized by discrete organ or tissue.**
    - **Transported in the bloodstream to a specific target tissue.**
    - **Control the physiological response in a concentration dependent manner.**
  - Unlike animal hormones, the **site of synthesis and mode of transport of plant hormones are not always clearly localized.** The synthesis of plant hormones appears to be much more **diffuse manner** and **cannot be localized to discrete organs**.
  - Hormones are **short lived** and serve a regulatory role. They display a **rate of metabolic turnover that is rapid relative to the response it control** (it simply means that a longer response will have a hormone with longer metabolic turnover and vice versa).

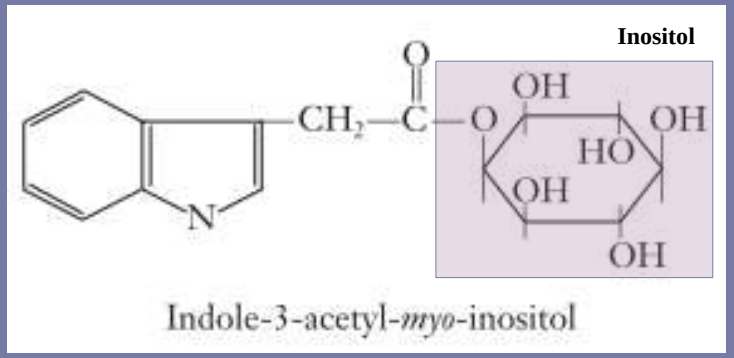
Discovery of Auxin

- The beginnings of this research can be traced back to **Darwin** in his book *The Power of Movements in Plants*.
- One of the movements he studied was **the tendency of canary grass (*Phalaris canariensis*) seedlings to bend towards light** coming from the window. This phenomenon we now know as **phototropism**.
  - The **primary leaves** of grass seedlings are **enclosed in a hollow sheath like structure** called **coleoptile**. This structure protect the leaves as they grown through the soil.
  - Darwin observed that coleoptile, like stem **grow towards the light source.** The growth would happen in a slight curvature, bending towards the light source. However this curvature did not happen if **the tip of the coleoptile was removed or covered to exclude light**.
  - Darwin concluded that **the phototropic signal was perceived by the tip**.

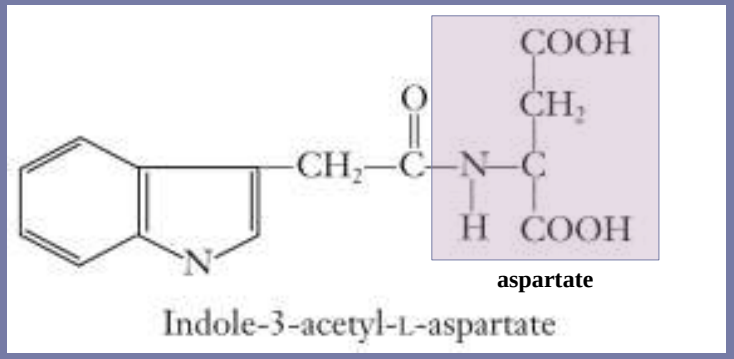
IAA is Synthesized from the Amino Acid L-Tryptophan



IAA may be Stored as Inactive Conjugates



Reversible conjugates



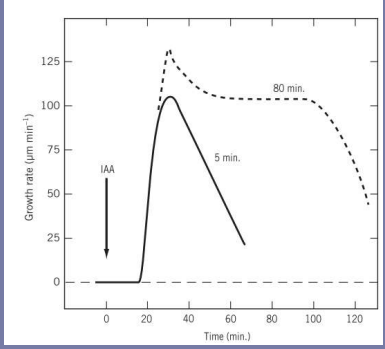
Irreversible conjugates

R. Cleland and D. Rayle on Auxin Promoted Growth

- **What was the theory provided by R. Cleland and D. Rayle for auxin stimulated growth?**
  - They explained that **auxin caused acidification of cell walls** by stimulating excretion of protons and this stimulated the increase in cell wall extensibility.
  - This lower pH will activate the cell wall loosening enzymes and help in cell wall expansion.
- At the same time **A. Hager**, furthered this theory by adding that auxin caused release of protons by **activating plasma membrane bound ATPase proton pumps.** The resultant increase in cell-wall extensibility allows for turgor induced cell expansion.
- This combined Cleland-Hager proposal is known as **acid-growth hypothesis.**
- Auxin does not bind to ATPase proton pumps but does enhance it's activity. Therefore, there must be an auxin receptor that initiates a signal transduction pathway. A putative **auxin receptor has been isolated from *Zea mays*** but the details of the whole pathway are still obscure.

Maintenance of Auxin Induced Growth and Other Auxin Effects Require Gene Activation

- The growth based on acid-growth and proton secretion (for example by Fusicoccin) does not portray the complete picture. The **growth associated with effectiveness after first 30 to 60 minutes**. However, **auxin induced growth kinetics show initial rapid increase in growth rate and reaches maximum at 30 to 60 minutes.** It then steadily decreases over next 16 hours.
- This shows that the growth after that rapid initial burst must be because of additional growth factors.

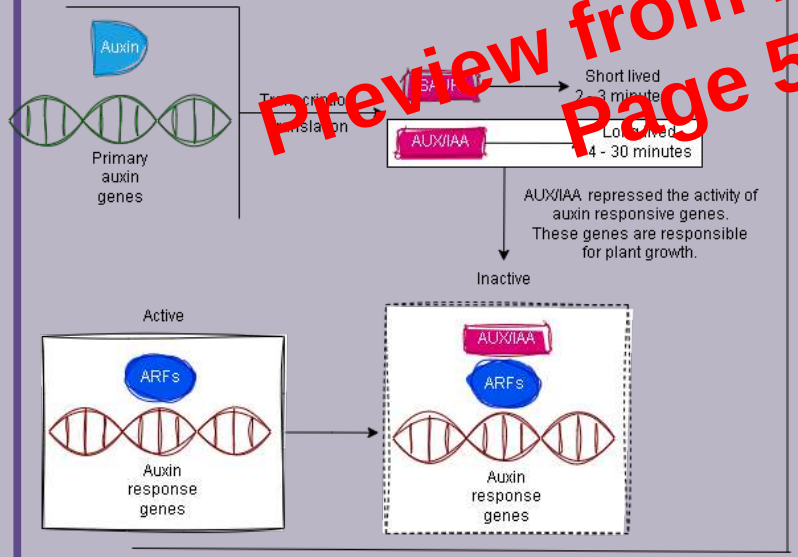


The continuous line shows the growth rate when auxin is removed really early **(t = 5 minutes)**. This curve shows the growth that is associated with **acid growth and proton secretion.** The growth rate sustains itself for **30 – 60 minutes.**

The second dotted line shows growth rate when auxin is removed really late **(t = 80 minutes).** This shows extensive sustained growth rate even after 60 minutes and growth being there for around **100 minutes.**

Primary response genes

These are activated by auxins and regulate gene expression by repressing ARFs.
ARFs bind to auxin response genes and activate them.

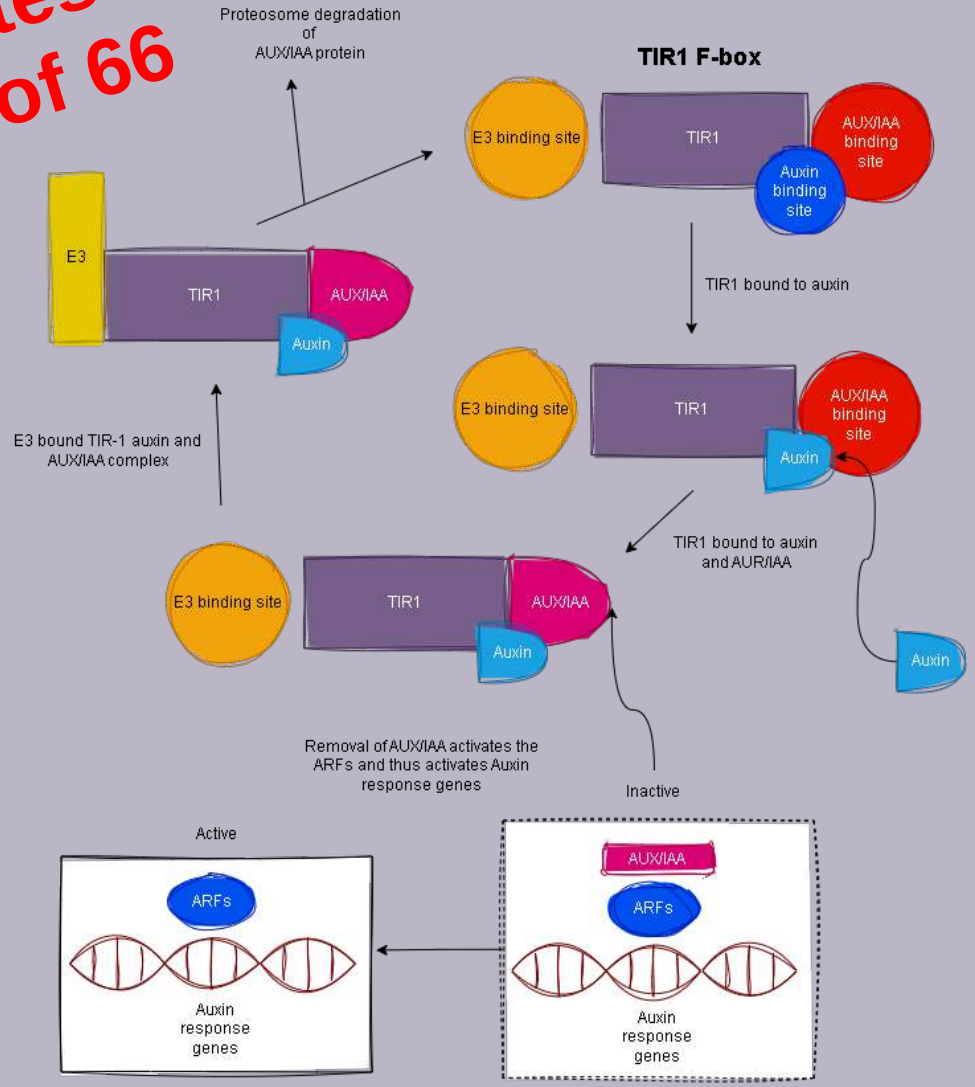


De-repression of auxin responsive genes

To reactivate these auxin responsive genes. these AUX/IAA repressors are degraded.
They are degraded via ubiquitin mediated proteosomal degradation.

TIR1 F - box

TIR1 F - box has two bonding regions. One binds to AUX/IAA and other binds to E3 ubiquitin ligase.
The AUX/IAA binding region also has a binding site for auxin which enhances TIR1-AUX/IAA binding.



Many Aspects of Plant Development are Linked to the Polar Transport of Auxin

- **Chemiosmotic model of auxin transport.**
  - This theory was provided by **P. H. Rubery, A. R. Sheldrake** and **J. A. Raven.** It has three essential features:
    - PH gradient or a PMF (Proton Motive Force) is required as a driving force for IAA uptake.
    - An **influx carrier protein** is required for the entry.
    - An **efflux carrier** is required at the base of auxin transporting cells.
- **The process of transport.**
  - IAA is a **weakly acidic lipophilic molecule.** Depending on pH it may exist in a protonated form (IAAH) or in unprotonated form (IAA^-). The **cell wall** space is **moderately acidic with a pH of about 5.5.** At that pH, approximately **20 percent of the IAA will be protonated** (IAAH).

Many Aspects of Plant Development are Linked to the Polar Transport of Auxin

- **The putative efflux and influx carriers of auxins.**
  - Two studies on *Arabidopsis* mutants were carried out and the influx and efflux carriers were identified.
  - The putative influx carrier is the protein **AUX1.** The mutations in AUX1 gene locus causes auxin resistant root growth, reduced lateral root initiation and reduced response to root gravity. This is consistent with the reduced capacity to take up IAA. The sequence matches with the known **amino acid permeases**. These membrane proteins function as amino acid/proton symport carriers. This (and the similarity of IAA to the amino acid tryptophan) suggests that the **AUX1 is a auxin/proton symport carrier.**
  - Furthermore, **synthetic auxin NAA restores the gravitropic response to mutant** (aux1) seedlings.
    - *NAA uptake by cells is not carrier-mediated, so the loss of AUX1 does not interfere with the response.*

Many Aspects of Plant Development are Linked to the Polar Transport of Auxin

- **Other roles of PIN proteins.**
  - PIN proteins are responsible for establishing **apical-basal axis** in young established embryos.
    - Immediately after the first division of zygote, **PIN proteins are located acropetally in the basal cell, directing the flow of auxin to the apical cell.** This establishes the apical cell as the profounder of the embryo.
    - As apical cells further divide, they start to form auxin themselves. **The PIN proteins shift to basipetal position and the flow of auxin is reversed.** This develops the root pole.