

BOTANY 512: Supplemental information to your texts and literature references.

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GENERAL PRINCIPALS OF HORMONE ACTION.

Regulation of levels of active hormone is controlled at multiple stages of synthesis, transport, catabolism, and compartmentation. The mode of regulation depends on the developmental and physiological context of the plant.

The ability of a cell to respond to a hormone depends on the activity of the receptor and signal transduction components. This provides an additional set of controls. The response of a cell to a hormone thus depends on the developmental and physiological context of the cells exposed to the hormone.

Negative feedback loops modulate metabolism and signal transduction.

*Regulatory signals can be transmitted via a ramified chain of reactions. In different tissues, functioning of various branches of this chain can be independent or interrelated. (*paraphrased from Ladyzhenskaya's comments on ABA signal transduction, Membrane Cell Biol 2001;14:699-713)

Tools to investigate hormone metabolism and function:

In vivo application of radiolabeled or isotopically labeled precursors

benefits

- Natural levels of cofactors, substrates, enzyme in natural concentrations
- enzyme activities not destroyed

problems:

compound may not get in.

compound may be metabolized in an atypical way before it reaches the site

(subcellular, cellular, or organ) where it would normally be metabolized.

In vitro incubation with radiolabeled or isotopically labeled (putative) precursors (using a "cell-free extract" or an isolated enzyme)

benefits

- compartmentalization/permeability/translocation problems eliminated
- substrate/cofactors can be manipulated
- intermediates present in trace quantities in vivo can be obtained on a preparative scale for analysis and for utilization for other experiments.
- first step in enzyme characterization/gene cloning.

problems:

unnatural-may get reaction that wouldn't occur in vivo, due to mixing of cofactors, altered pH, etc., or to mixing of enzymes from different cell types or

compartments.

may lose endogenous activity during preparation of cell-free extract

quantitation of endogenous metabolites

benefits

-determine real concentrations of molecules in the plant

problems:

-no idea as to subcellular or cellular location of molecules unless these are separated before analysis

-no idea as to dynamics, i.e., what is converted to what

mutants of metabolic genes

benefits

-powerful tool to elucidate hormone metabolism

problems

-must obtain mutant and identify location of mutation (being done systematically by the community for Arabidopsis)

- results difficult to interpret if there are multiple pathways leading to the same hormone, or if there are pleiotropic effects.

functional genomics (microarray, proteomics, metabolomics, etc)

benefits

may be able to get an idea of the genes involved in a pathway because they co-express.

problems

-technology needs improvement to detect low concentrations of molecules

-expensive

-computational technologies in their infancy

AUXINS

IAA is implicated in

stimulation of cell division and enlargement

stimulation of shoot growth

induction of root growth

control of vascular tissue development

inhibition of somatic embryo development

apical dominance

delay of senescence

promotion of flowering and fruit set and ripening

Natural and synthetic auxins

Predominant natural auxin

Indole acetic acid (IAA)

Other natural auxins that may have activity:

4-chloro-3 indole acetic acid

more active than IAA, but only found in immature seeds of peas and beans

indole butyric acid

synthesized in roots, less active than IAA

phenylacetic acid

not as active as IAA, but present in higher concentrations various tissues of tomato, tobacco, sunflower, pea, barley, corn. Precursor = phenylalanine

synthetic auxin

2,4-D

HISTORY

Charles and Francis Darwin, 1880

Noted that a grass coleoptile bends towards the light: PHOTOTROPISM

If a coleoptile tip was shaded from the light, a phototropic response (i.e. the bending of the coleoptile tip towards light) was inhibited.

“When seedlings are freely exposed to a lateral light some influence is transmitted from the upper part of the coleoptile that acts on the lower part of the coleoptile”

“The power of movement” 1880. Darwin

Boysen-Jensen 1913

If the coleoptile tip is cut off, and a piece of gelatin is inserted between the coleoptile tip and the rest of the coleoptile, phototropism still occurs

Fritz Went 1928

After light is given unilaterally to a coleoptile with an inserted agar block, the agar block can be removed, reoriented on another decapitated coleoptile, and will induce the coleoptile to bend in the appropriate direction

* THERE IS A DIFFUSABLE PLANT GROWTH SUBSTANCE CAUSING THE PHOTOTROPIC RESPONSE

* A QUANTITATIVE **BIOASSAY** FOR AUXIN WAS ESTABLISHED

Kogl et al., 1934

What is the chemical nature of **auxin** ??

IAA was extracted from **urine** and identified as a compound with auxin-like activity.

Haagen-Smit et al., 1946

IAA was isolated from corn kernels

IAA is a primary auxin of plants

IAA present in plants at 1-100 ug/Kgfw (5-500 nmolar concentrations)

AUXIN METABOLISM

Particular problems in study of IAA metabolism:

High level of precursors: endogenous tryptophan vs IAA (over 1000 x more)

Drying tryptophan results in about 30% being converted to IAA non-enzymatically (Epstein, Cohen, Bandurski, 1980).

Microbial contamination results in conversion of tryptophan to IAA, confuses data.

Feeding studies must quantitate the level of conversion of metabolites.

Lack of IAA biosynthetic mutants

Low activity of enzymes involved

Much effort concentrated on IAA biosynthesis in microorganisms (*Agrobacterium tumefaciens*; *Pseudomonas savastanoi*), which may not be via same pathways.

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-IAA SYNTHESIS

Pathways for IAA biosynthesis still not resolved.

Several proposed pathways for synthesis of IAA in higher plants.

Tryptophan-dependant pathways

Evidence of trp as a precursor in developing maize kernels by retrobiosynthetic analysis (this means feeding radiolabeled precursors, and looking at which positions the label is incorporated into in the product (IAA)).

Glawischnig et al., 2000. Plant Physiol 123:1109.

Indole-3 pyruvic acid pathway-

indole-3 pyruvic acid formed enzymatically in plant extracts, from trp (tryptophan aminotransferase; acts on tyrosine, phenylalanine, too) (tobacco callus); *indole-3 pyruvic acid* unstable, present in low quantities, but found in plants (peas, mung bean seedling, lime fruit, barley and tomato shoots, wheat leaves, tomato shoot extracts, pea stem sections).

indole-3-acetaldehyde is derived via *indole-3-pyruvic acid* (Schnieder and

Wightman, 1978; Phytohormones and related compounds, a comprehensive treatise, p 29-71, Letham et al, eds vol 1). (This is shown by lack of retention of 3H on amino carboxyl group) indole-3-pyruvic acid decarboxylase activity found in mung bean, tomato endogenous *indole-3-acetaldehyde* isolated (pea, mung bean seedling, tomato shoots, sunflower, cucumber shoots)

indole-3-acetaldehyde is converted to IAA. Cell free systems do this, also radiofeeding studies show conversion of indole-3-acetaldehyde to IAA. Attempts to purify the enzyme responsible for this catalysis have shown that enzymes with a wide range of characteristics achieve this goal: metalloflavoprotein which doesn't require added cofactors found in cucumber (Bower et al, 1978); soluble NAD-dependant oxidase in *Avena*, tobacco, requires oxygen (Liu et al, 1978).

interconversion of indole-3-acetaldehyde to the ethanol. this cpd is endogenous; the rxn occurs in cell free systems, plant tissues and indole-3-ethanol has activity in an auxin bioassay. Radiofeeding studies show indole-3-ethanol is derived from trp, via tryptamine, in cucumber and pea seedling (Magnus, et al, 1983). Radiolabeled indole-3-acetaldehyde is converted to IAA and to indole-3-ethanol.

indole-3-ethanol oxidase, a reversible reaction using molecular oxygen, is a flavoprotein, inhibited by IAA and indole-3-acetaldehyde and present in plant extracts. In cucumber seedling, three distinct enzymes have been isolated that catalyze this rxn. All are called "indole-3-acetaldehyde reductase": (1) NADH dependent (microsomal), (2) NADH dependent (cytosolic) (Brown and Purves, 1978) (3) NADP(H) dependant, (microsomal), inhibited by NADPH, subject to feedback regulation by IAA. The subcellular compartmentation of these enzymes may have significance for metabolic function.

Tryptamine pathway

tryptamine and trp derivatives (N-coumaryl; N-ferulyl) present in plants (Schnieder and Wightman, 1978).

Conversion of tryptamine to N-hydroxy-tryptamine by YUCCA

(Zhao et al., 2001. Science)

YUCCA codes for a flavin-dependant monooxygenase cloned by enhancer-trapping.

Increased expression causes phenotype of IAA over-accumulation or auxin treatment.

YUCCA enzyme catalyzes hydroxylation of tryptamine to form N-hydroxy-tryptamine

N-hydroxy-tryptamine could be converted to IAA oxime

IAA oxime could then be converted to IAA via indole acetaldehyde or indole acetonitrile.

Null mutants in YUCCA appear wild-type, consistent with: 1) alternative pathways supplying IAA (redundancy), or 2) with the YUCCA gene not having a physiological involvement in IAA synthesis.

l-tryptophan decarboxylase converts trp to tryptamine.; this enzyme activity is present in plants (cuc. hypocotyls, tomato shoots, barley shoots, tobacco shoots) and in these species, ¹⁴C trp is converted to ¹⁴C tryptamine and ¹⁴C IAA. amino oxidases convert tryptamine to IAA, although these enzymes aren't specialized for using tryptamine as a substrate.

Tryptamine, indole-3-ethanol, indole 3 acetaldehyde are present in plants.

Bacterial pathway: *oxidative decarboxylation of trp to indole-3 acetamide* followed by the hydrolysis to yield ammonia and IAA is a demonstrated bacterial rxn (eg *Rhizopus*, *Agrobacterium*). Genes for these two enzymes are present in *A. tumefaciens* and are responsible for the tumor formation and other abnormal morphology induced by this organism.

Indole-3-acetaldoxime pathway

conversion of trp to indole-3-acetaldoxime

Cyp P450s CYP79B2ox and CYP79B3 both catalyze the conversion of trp to indole-3-acetaldoxime. (Zhao et al, 2002)

Overexpression of CYP79B2 (driven by 35-S promoter) results in IAA-overproduction phenotypes, induction of IAA-induced genes, increased accumulation of IAA and IAN. *cyp79b2cyp79b3* double mutants have no indole glucosinolates, reduced levels of IAA, and elongated hypocotyls.

from aldoxime to the acetonitrile and to indole acetic acid. May proceed via glucosinolates. Problem: **Nitrilase activity present only in some species, these species also contain glucosinolates (Brassica)**. 4 nitrilase genes in Arabidopsis, three of the genes encode proteins have activity using indole-acetonitrile as a substrate. All four nitrilase mutants have normal phenotype (a “quadruple knockout” is in the works). Nitrilase has other functions (i.e., in glucosinolate catabolism).

from aldoxime to indole-3-acetaldehyde and to IAA

Tryptophan-independent pathway(s).

IAA appears also to be generated via a trp precursor by an undetermined pathway that does not require trp, but may use some of the same steps in the trp-IAA biosynthetic pathways. This trp-independent pathway is prevalent in some developmental stages, such as somatic embryo formation in carrot.

See Michalczuk et al., 1992, *Plant Physiol* 100:1346

Normanly, 1997 *Auxin metabolism. Plant Physiol* 100:431

CONJUGATION OF IAA

Hypothesized to be controlling point of metabolically active IAA.

Studied by:

Assays of cell free systems for catabolic enzymes

Radiolabeled tracer studies using IAA and metabolites analyzed

Endogenous IAA metabolites characterized

Genetics/mutant screens

Functional genomics

Types of conjugates:

IAA can be conjugated (typically at the carboxyl-group) to amino acids, sugars, carbohydrates, depending on the species and developmental state.

Some IAA conjugates are used as to sequester free IAA for later use, other conjugates, especially IAA-asp, tend to target the IAA for catabolism.

50-90% of IAA in maize is conjugated (Cohen and Bandurski, 1982. An Rev Plant Phys 33:403-430) maize conjugates include:

- high molecular weight esters (beta 1-4 glucan),
- esters of IAA and myoinositol, myoinositol arabinose, myoinositol galactose
- IAA conjugated to glycoprotein or peptide
 - beta 1-4,1-3 glucoprotein (maize kernels)

specific conjugates from other species/organs include:

- IAA-aspartate (pea leaf)
- IAA-1-aspartate (soybean seed)
- IAA-glutamate (legume)

METABOLISM OF CONJUGATES

IAA-myoinositol synthesis in maize:

IAA + UDP-glucose --- IAA-glucose + UDP

IAA-glucose + myo-inositol --- IAA-myoinositol + glucose

IAA-myoinositol + UDP galactose ---- IAA-myoinositol-galactose + UDP

IAA overaccumulators:

Sur1/rooty – amino transferase- may make IAA conjugate

Sur2 - cyt P450 oxygenase- involved in synthesis of glucosinolates from trp

Both accumulate high levels of conjugated IAAs

IAA-conjugate hydrolysis:

Wild-type Arabidopsis seedlings, respond to applied IAA conjugates and to applied IAA by decreased root elongations and increased hypocotyle elongation; mutants in IAA hydrolases such as *IAR3* (IAA-ala hydrolase activity) and *IRL1* (IAA-leu hydrolase activity) have been identified as lines that do not respond to specific IAA conjugates, but do respond to free IAA. Overexpression of *IAR3* and *IRL1* results in increased sensitivity to specific IAA conjugates. These mutants themselves appear normal in the absence of added IAA conjugates, indicating either a redundancy is built in, or possibly they do not have a physiological role in IAA conjugate hydrolysis.

Arabidopsis may have 6 IAA hydrolases. The pattern of expression of different hydrolases varies with the organ and developmental stage.

The IAR1 gene of Arabidopsis is required for sensitivity to IAA conjugates, but does not seem to be a hydrolase and their mechanism of action are not known.

IAA CATABOLISM

Hypothesized to be controlling point of metabolically active IAA.

Two types of IAA oxidation proposed:

- **DECARBOXYLATION** is first step of catabolism (called “IAA Oxidase”) via peroxidase (Tang and Bonner, 1947)
 - horseradish peroxidase best studied, but many other peroxidases present in many cell types.
 - Monitor rxn by $^{14}\text{C}\text{O}_2$ evolution from 1- ^{14}C IAA
 - Converts IAA to 3-hydroxymethyloxindole, or indole-3-aldehyde.
 - Many plant enzymes catalyze similar reactions; they have not been fully characterized by studying the purified enzymes. (The specificities of these enzymes are unclear because they haven't been fully purified)
 - require Mn (most)
 - require monohydric phenols (eg paracoumaric acid)
 - compartmentation not known(??)
 - endogenous cpds rarely demonstrated to be endogenous
 - ...may be an artifact**
- **NON-DECARBOXYLATION**: formation of a ketone at carbon is the first step of catabolism (decarboxylation is second step). Formation of the ketone can occur to free IAA or IAA-asp conjugate...
 - Good evidence in many species (eg., Zea mays, Vicia faba, rice)
 - Many in vitro assays of catabolism don't distinguish between non-decarboxylation and decarboxylation (i.e, O_2 uptake. or reduction in 280 nm absorbance (indole decrease) together with increase in 247 nm absorbance (oxindole increase)).
 - $^{14}\text{C}\text{O}_2$ evolution does distinguish between non-decarboxylation and decarboxylation.
 - Quantitative feeding studies, and determination of endogenous levels of IAA catabolites show pathway with NON-DECARBOXYLATION as first step is main degradation route in maize endosperm, broad bean.**

