

GIBBERELLINS

- 1809 “BAKANAE” foolish seedling disease of rice, Japan
Up to 40% of the yield of rice was lost due to this disease
- 1898 Hori
Bakanae caused by an imperfect (non-conjugating) fungus “*Fusarium moniliforme*”
- 1926 Kurusawa
The perfect fungus, ***Gibberella fujikuroi*** was the same as *Fusarium moniliforme*.
Cell-free media filtered extract added to rice could mimic the effect of the bakanae disease
Autoclaving didn’t alter potency, indicating the substance was probably not an enzyme
- 1938 Yabuta, Sumiki
Crystallized growth-promoting substances. Chemically identified gibberellin ring structure as a selenium catalyzed dehydrogenation product, a fluorescent derivative.
- 1950s Stodola, Caltech; ICI scientists, Great Britain became aware of the Japanese work, and began to try to identify GA
- 1954 Cross et al, ICI
GA3 purified from strain of *G. fujikuroi*. Work was made easier by Brain with the selection of a *G. fujikuroi* strain and culture conditions to get a high production of mostly a single GA compound.
- 1955 Stodola
GA3, GA1 purified
Complete structural identifications of GA1 and GA3 took over 6 years.
- 1954-7 Radely, Phinney
Suggested that GA might be an endogenous PGR
Showed that dwarf mutants of peas (Brian and Hemming, 1955) and single gene dwarf mutants of maize (Phinney, 1956) grew normally following application of GA. The maize dwarfs responded to 0.001 ug/plant !!
SO: *GA MW=320 about... then 0.003 nmol/ng (Avogadro = 10 to the minus 26) or 10 to the 17 molecule.*
If a plant has tens of millions of cells, still a billion or so molecules per cell.)
- 1958 Macmillan and Sutter
GA1 chemically identified from immature seeds of *Phaseolus coccineus*

GAs endogenous to plants

Terminology is a bear!!! perhaps the worst you will ever experience

Two groups of GAs

C20- have 20 carbons

C19- have 19 carbons

ent-gibberellane skeleton is basis for GAs (*ent*= enantiomer. The original skeleton designated to be “gibberellane” was exact mirror image of the naturally occurring GAs)

This causes a problem in terminology. Chiral centers normally designated as alpha becomes beta, and visas versa, because the “*ent*” designation implies the reverse.

GAs are named by number, in the order of the identification of their structures.

(GA₁, GA₂, etc..)

Therefore....

GA₁₉ has 20 carbons

GA₂₀ has 19 carbons

GA₂₀ vs GA₂₀-GA

Enzyme names- metabolic enzymes that use GAs as substrates are referred to by the carbon that they oxidize or hydroxylate.

- **GA₂₀ oxidase** (also, and even less fortunately, called GA₂₀ oxidase)

Might be expected to be an enzyme that oxidizes GA₂₀.

NOPE it is an enzyme that oxidizes *carbon* 20. When carbon 20 is fully oxidized it is typically lost, creating a C19 GA. (which happens to be called GA₂₀).

GA₃-beta hydroxylase (also called GA 3-oxidase or GA_{ox}) hydroxylates at carbon 3.

1978 Corey et al,

Chemical synthesis of GA₃: 38 reactions needed for synthesis

GA conjugates --usually glycosides

Immature seeds contain up to 16 mg/Kg fw GA

Other tissues have about 0.1 mg/Kg fw GA (10⁻⁷)

GAS are implicated in a variety of physiological and developmental processes

Stem growth (stimulation of cell elongation and sometimes cell division, in young tissues, bolting under long days in LDP)

Male flower formation in dioecious plant

Germination of seeds (induction and de novo synthesis of enzymes)

Fruit set and growth

Embryo development

Sites of synthesis

Seeds, growing shoots, other.

Transport

mechanism not known, evidence for transport as GA₂₀

Biosynthesis

From isopentenyl pyrophosphate (IPP) (the isoprenoid pathway) via the cyclic diterpenoid *ent*-kaurene

Sites of biosynthesis:

IPP to <i>ent</i>-kaurene	plastids
<i>ent</i>-kaurene to GA₁₂ aldehyde	ER
GA₁₂ aldehyde to GAs	ER, soluble

Endogenous derivatives and related compounds

GA₁ typically major active form (GA₄ active in *Arabidopsis*, Cucurbits)

C₂₀ and C₁₉ GAs identified to date include 125+ derivatives, differing primarily by site(s) of hydroxylation and degree of oxidation of carbon 20

Stereochemistry critical for activity

SYNTHESIS OF GAS

The isoprenoid (=terpenoid) pathway

this pathway is central to the production of three classes of plant growth regulators and a variety of secondary metabolites.

also specific enzymes regulate the flow of metabolites through the pathway as a whole, and the formation of the hormones, specifically.

Isoprenoid compound formation based on the "isoprene rule"; number of carbon atoms (multiple of five; derived via isopentenyl pyrophosphate)

ABA, GA and the isoprenoid side chain of cytokinin. The five carbon subunit is recognizable in many secondary metabolites.

1887 Wallach

isoprene rule:

naturally occurring cpds are built up of five carbon isoprene carbon skeletons, therefore arise via head to tail isoprene polymerization

1952 Ruzica

biogenetic isoprene rule:

all terpenoids synthesized from five carbon "active isoprene" precursor

"active isoprene" later identified as IPP (Lynen, 1950s)

Two pathways for IPP formation:

MVA to IPP occurs in cytosol only

How is MVA formed??

acetoacetyl-CoA + acetyl-CoA ->->HMG-CoA

also

leucine (valine) ->-> HMG-CoA

HMGCoA + NADPH → MVA+NADP

HMGCoA reductase irreversible rxn

MVA ->MVA 5P -> MVA5-PP

MVA has 5 prochiral/chiral centers. All rxns here on procede stereospecifically- only cpds with correct chirality are enzymatically converted.

MVA kinase-cofactor isATP Mg/Mn

MVAP kinase-ATP Mg/Mn

MVAPP + ATP -> IPP + ADP + CO₂

MVA 5 pyrophosphate decarboxylase

Rohmer pathway to IPP

Pathway first identified in bacteria, 1990s

In plastid stroma

3-glycerophosphate plus pyruvate are converted to IPP: soluble enzymes in stroma
Since GA and ABA made in plastid, this **may** be main source of IPP for their synthesis.
Caveate: Although MVA is probably not permeable to plastids, however, IPP may be at some developmental stages. Thus cytosolic IPP might be incorporated into GA, ABA, and plastidic IPP may be translocated to cytosol. In fact, and Hedden has evidence that applied MVA is converted rapidly into GAs in Cucurbit seeds in vivo. Isolated plastids but not chloroplasts are permeable to IPP; osmotic stress increases permeability of isolated chloroplasts to IPP.

GENERAL INFO ON ISOPRENOIDS

Isoprene/hemiterpenes (5C)

isoprene (emitted during photosynthesis, function not clear)

isopentenyl side chains (such as for zeatin ?)

Monoterpenes (10C)

volatile steam distilled essential oils

(oil glands of mint family, pines, umbells- accumulation in specific trichome structures on epidermis; function in allelopathy. Some evidence for synthesis in trichomes) flower smells (eg geraniol) synthesized in petals glandular epidermis present in all species in lesser quantity. Functions include attractants, insecticidal, antiherbivory.

limonene

linalool

pinene

cineole

neptalacone (attractant in catnip, also aphid pheromone)

(evidence of terpenoid origin = feeding studies w MVA).

14C02, 14C MVA fed to plants- low incorporation, (except for petals). but: locations of radiolabel confirm isoprenoid origin. consistent finding that label appears at higher level in the IPP than the DMAPP-derived (except for petals). feed 14C MVA with 3H stereospecific label: 4proR 3H always retained, 4proS 3H always lost. (4R 1.99; 4S 1.25 = 14C/3H original MVA fed geraniol isolated was: 4R 1.97; 4S 0.05 Concludedt 4S hydrogen was lost during the original isomerization of IPP to DMAPP.

Most labeling studies show head-to-tail pattern...assume geranylpyrophosphate (GPP) is precursor. Geraniol=widespread. Linalool also widespread, also form non-enzymatically.

Sesquiterpenes

volatile (steam distilled) essential oils

found in high concentrations in oil glands.

universal, in fungi too, usually at lower concentrations than monoterpenes

200 carbon skeletal types; several thousand individual compounds identified so far.

allelopathic chemicals (furanoid phytoalexins, lactone antifeedants)

many structures with unknown function. also unknown biosynthetic origin. labeling

studies with stereospecific tritium show biosynthetic origin of most known

sesquiterpenes is via FPP.

capsidol

gossypol (dimer, found in cotton, used traditionally for birth control in China)
Abscisic acid, an exception, is derived from carotenoids

Diterpenes (20C)

GAs

casbene (antibiotic from castorbeans)

taxol (derivitized anti-cancer drug)

Triterpenes (30C)

brassinosteroids

squalene

sterols

oleanoic acid (cuticular wax component)

ecdysone (resembles insect molting hormone)

saponins (soap-like glycosides, very toxic, used for control of schistosomiasis)

digitalis (from Foxglove, used to treat congestive heart failure)

Tetraterpenes (40C)

phytoene

carotenoids

phytyl tail of chlorophyll

C45, C50 prenyl chains on

Ubiquinones, Plastoquinones

IPP condensations to form 10,15,20+...carbon chains

IPP \leftrightarrow DMAPP IPP isomerase

sulfhydryl enzyme, electrophilic attack by H⁺ from aqueous media to IPP, stereospecific loss of 2-proR hydrogen (this hydrogen is called the proS hydrogen of MVA; numbering of carbons changes from MVA to IPP)

DMAPP= starts chain (also head-to-middle condensing unit)

IPP provides repeating units

Isoprenoid synthesis requires ATP, NADPH

Three distinct prenyltransferases :

IPP/DMAPP \rightarrow GPP GGP synthase

IPP/DMAPP \rightarrow FPP FPP synthase

IPP/DMAPP \rightarrow GGPP GGPP synthase

Conversion of IPP into DMAPP into terpenoids occurs in 3 subcellular compartments:

cytoplasm, mitochondria and plastids.

Plastidic reactions

Isolated chromoplasts shown to be site of synthesis of:

- monoterpenes (e.g., daffodil fragrance).
- carotenoids, prenyl side chains of photosynthetic pigments (eg, chromoplasts of tomato, red pepper; castor bean proplastids).

GPP synthase-makes C10 isoprenoids

GGPP synthase-makes C20 isoprenoids

Phytoene synthetase complex: membrane peripheral protein, catalyzes two step reaction: 2

GGPP->prephytoene diphosphate->15-cis phytoene. MW 200,000 D

Chlorophyll synthase (a prenyl transferase that catalyzes the formation of chlorophyll).

GGPP reduced in plastid to form phytyl diphosphate, which prenylates tocopherol and phyloquinones

nonaprenyl diphosphate (C45) prenylates plastoquinone-9.

Fatty acid prenyl esters (fatty acids + isoprenoid carbon chains) formed in vitro, stored.

Cytoplasmic reactions:

FPP synthase (prenyl transferase that makes FPP a C15 isoprenoid) in cytoplasm/ER.

Prenyl transferase catalyzes head to middle condensation of 2 FPP->triterpenes eg sterols, squalene, brassinosteroids

Dolichols (C120), and rubber (GPP or FPP used as starter, then IPP added in *cis* configuration C 1000s) also made in cytoplasm.

Mitochondrial reactions

Prenyl units for ubiquinones mostly C45 units (ubiquinone -9), C50 units (ubiquinone 10) are derived from FPP with addition of subsequent C5 units.

Ubiquinones located in mitochondria

The regulation of the pathway is complex. It occurs by cellular and subcellular **compartmentation** of the various reactions. chloroplasts (spinach, pea, green algae acetabularia); etioplasts (etiochloroplasts, chloroplasts (mustard seedlings); leucoplasts (castor bean endosperm, calamondin fruits); chromoplasts (tomato, red pepper fruit, daffodil flowers)

formation of MVA

acetylCoA PHS via G3P-- --pyruvate---(pyruvate dehydrogenase-PDH)---acetylCoA

but-- a mutase activity not found in chloroplasts ?

pPDH is in plastids, acetyl-CoA made in plastids

Carbon to mitochondria, converted to pyruvate. PDH--AcetylCoA
acetylCoA hydrolase)--acetate(permeable through organelles, metabolically inactive till it reaches chloroplasts
(Acetyl CoA synthase makes acetylCoA in plastids

MVA--IPP, DMAPP =plastidic ?
proplastids permeable to MVA, acetate
chloroplasts not permeable to MVA, but permeable to acetate

Acetyl-CoA present in plastids
chlorophyll, carotenoids exclusively localized in plastids--led to idea that terpenoid biosynthesis might be plastidic
only

¹⁴C02 fed to isolated chloroplasts--5% incorporated, into fatty acids (1/2) and into terpenoids (1/2)
¹⁴C acetate fed to isolated chloroplasts--better yield, but all into fas (acetate --acetyl CoA by plastidic acetyl-CoA
synthetase).
therefore though acetate gets into plastids, acetyl-CoA may be compartmentalized with regards to its metabolism to
terpenoids, even within the plastid. In spinach plastids, unlabeled acetate does dilute specific activity incorporation of
¹⁴C02 into carotenoids, supporting above idea.

cytoplasm can synthesise IPP from MVA (old lit), although this data was not obtained with rigorous organelle
isolation techniques, and activities were low.
plastids are permeable to leu, but spinach plastids didnt incorporate ¹⁴Cleu into isoprenoids.

All three compartments convert IPP into isoprenoids. IPP isomerases present in each compartment.

Plastid IPP isomerases purified, characterized .MW 28-37 kD
some evidence for association with phytoene synthase, prephenyl transferase (phytoene synthase complex) (Lutow, M.
Beyer, P. 1988. The isopentenyl-diphosphate delta-isomerase and its relation to the phytoene synthase complex in
daffodil chromoplasts Biochim Biophys Acta 959:118-26.)

Isolated chromoplasts make monoterpenes from IPP (daffodil; these flowers are good to use for biochemical studies
since all cells are similar)
Presence of leucoplasts in 45 plant species correlated with and monoterpene secretion.
In contrast, sesquiterpene synthesis doesnt occur in plastid; sesquiterpenes arent accumulated in plastids,
cytoplasm/ER-containing fraction do synthesize sesquiterpene

G. fujikuroi-Using cultures, entire pathway elucidated. GAs formed from MVA, GGPP → *ent*-kaurene formed by single enzyme
provides means to obtain intermediates (radiolabeled) for biosynthetic studies

GA BIOSYNTHETIC PATHWAY CAN BE DIVIDED INTO FOUR PARTS IN HIGHER PLANTS:

- conversion of mevalonic acid to IPP: soluble enzymes in cytosol or conversion of 3-glycerophosphate plus pyruvate to IPP: in stroma
- conversion of IPP to *ent*-kaurene: stroma
- conversion of *ent*-kaurene to GA12 aldehyde : oxidative reactions in ER involving cytochrome P450.
- conversion and interconversions of C20 and C19 gibberellins. oxidative reactions in cytoplasm

Mutants with blocks in specific steps: maize, pea, Arabidopsis

Inhibitors:

- sulphhydryl reagents: MVA kinase
- compactin: HMGC_oA reductase
- AMO1618, CCC (less specific): CPP synthase and *ent*-kaurene synthase (GGPP to CPP)
- Phosphon D: *ent*-kaurene synthase
- 16 alpha,17 epoxy-*ent* kaurane- inhibits *ent*-kaurene conversion to GA3 in fungus; also inhibits *ent*-kaurene synthesis
- 7,7-Difluoro-*ent*-kaurenol - inhibits *ent*-kaurene to *ent*-kaurenoic acid in Cucurbit
- ancymidol (CytP450 monooxygenases): *ent*-kaurene → *ent*7 OH-kaurenoic acid
- GA-like fluoridated cpds inhibit GA12-aldehyde metabolism
- Prohexadione: interconversions of GAs (inhibits 2-oxoglutarate-dependent dioxygenases)

7 beta-hydroxy-ent-kaurenoic acid is converted to 6 beta, 7 beta-dihydroxyentkaurenoic acid and to GA 12 aldehyde, probably by the same enzyme in vitro. This may also occur in vivo, depending on conditions: as they're both formed from a common intermediate.

IPP to *ent*-kaurene

All enzymes have been at least partially purified. At least two prenyl transferases in pumpkin.

GGPP synthetase

- single enzyme in pumpkin endosperm, Arabidopsis
 - catalyses the successive addition of 3 IPPs to DMAPP to form GGPP.
- high ratio of Mn/Mg favors GGPP formation in cell free extract.

ent-K formed from GGPP in two step reaction

ent-copallyl-PP synthase (CPS, A activity)-

- proton-initiated formation of copallyl PP (CPP) from GGPP
- single-copy gene in Arabidopsis (*GA1 mutant in Arabidopsis*)

ent-kaurene synthase (KS, B activity)

- CPP cyclised to form *ent*-kaurene
- single-copy gene in Arabidopsis (*GA2 mutant in Arabidopsis*)

gal-3 (deletion mutant of KS in Arabidopsis) still accumulates low level of GA. Is this due to activity from a related still unidentified cyclase that normally catalyzes a different not identified reaction.

Reaction GGPP->ent-kaurene: pyrophosphate is lost, resulting in the formation of a carbonium ion, rearrangement of carbon bonds 12 and 16 and a shift from 12,16 to 12,13 bonds leads to formation of C and D rings. Radiolabeled CPP at carbon 15 is converted to 3H-14-ent-kaurene, supporting this mechanism. CPP found as low level product from feeds of GGPP in cell free systems of cuke. CPP is converted by cell free systems to ent-kaurene.

pH optima are different, inhibited by different growth retardants (A activity only : AMO 1618, AMO1618; both activities, phosphon D)

Cyclization of GGPP to *ent*-kaurene typically **first committed step** in pathway (and may play a role in regulating the pathway. *ent*-kaurene synthase activity and growth correlated in pea shoots, and maize seedling shoots)

Exceptions: other diterpenes derived from GGPP.

kaurene pine accumulates (+) kaurene. This was the original source of the name kaurene, and the reason *ent*-kaurene and its derived GA structures are termed ent is that they are enantiomers of the (+) kaurene found in pine.

castor bean

sandaracopimaradiene, beyerene, *ent*-trachylobane are synthesized by cell free extracts of castor bean from 14C GGPP or 14C CPP. These CPP-derived metabolites all follow from a similar reaction mechanism. Beyere and sandaracopimaradiene are synthesized by distinct enzymes. *ent*-trachylobane and *ent*-kaurene may be synthesized by the same enzyme.

Casbene is synthesized from 14C GGPP (but not 14C CPP) following infection of the castor beans, and is thought to be a phytoalexin.

All enzymes for casbene biosynthesis, and casbene itself are plastidic.

maize

ent-isokaurene in infected maize scutellum, coleoptiles after cessation of elongation

brown algae

brominated derivatives of CPP

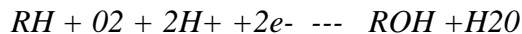
sunflower

ent-trachylobanic acid- major constituent of vascular fluid

ent-kaurene to GA 12 aldehyde

- successive oxidations at carbon 7, followed by ring contraction
- occurs on membrane (ER?), ent-kaurene must leave plastid
- ent-kaurene oxidase (EKO)
 - single copy gene in Arabidopsis (*GA3 mutant in Arabidopsis*):
 - membrane bound mono-oxygenase (=mixed-function oxidase)
 - Require NADPH, O₂ (18-O incorporated into ent-kaurenol)
 - CO inhibits oxidation, inhibition reversed by light with maximal effectiveness at 450 nm; cytochrome p450 flavin nucleotides act as cofactor.
 - sensitive to ancymidol (kaurene oxidation inhibited w $K_i=2 \times 10^{-9}$ M) *however*, all cytochrome p450 reactions are inhibited by ancymidol
 - Hard to purify and retain activity, since P450 membrane enzyme.

Regardless, GA3 mutant of Arabidopsis used to clone EKO (ent-kaurene oxidase), EKO expressed in heterologous system (yeast) and retained activity.



ultimate source of the electrons for P450 mixed function(= mono) oxidases is reduced pyridine nucleotides, which transfer their reducing equivalents via short chains to the final acceptor at the active site???????

First demonstrated biochemically in endosperm, *G. fujikuroi*. Cells free systems from shoots of maize seedlings can do similar conversion. In this case, the reactions can be shown to occur only one step at a time. i.e. substrate=ent-kaurene---converted to a predominant detectable cpd, ent-kaurenol. feed ent-kaurenol---converted to a single predominant cpd, ent-kaurenol. Identity of cpds demonstrated by GCMS. **Demonstrated genetically in Arabidopsis** (i.e., sequence of enzyme like a P450 oxidase, and

Feeds of ent-k etc to dwarfs
where is the block?

*why do GA12ald, GA53ald not give 100% responses?

Early intermediates may not be fully converted to GA because they may not get in, they may not get to right place to be converted, they may get metabolized to other (non-GA) cpds

Intermediates after a block might have activity because they have biological activity per se, or because they are converted to the cpd that is biologically active in vivo

Ring contraction -feeds of 7-beta,hydroxykaurenoic acid to *Cucurbita* endosperm and analysis of GA12, GA12-aldehyde, show stereospecific retention of 6 alpha H; loss of 6 beta.

Branch products from ent-kaurene:

sunflower

ent-12 beta-acetoxykaurenoic acid is major constituent of vascular tissues.

ent-kaurene esterified to phenol or **acetic acid**

Isodon shikokim

ketone derivatives

Stevia rebaudiana

steviol (a ent-kaurenoic acid glycoside)

Montanoa ptreropoda (fern).

17 derivatives of ent-kaurene or ent- kaurenoic acid

GA₁₂ aldehyde conversion to GAs

In cytoplasm

GA₁₂ aldehyde → GA₁₂ → GA₅₃

- **Mono-oxygenases**

(Pumpkin has additional di-oxygenase (see below) that catalyzes GA₁₂aldehyde → GA₁₂)

- single copy gene in Arabidopsis

Subsequent conversion of GA₅₃

- Catalyzed by **di-oxygenases**.
 - Soluble cytoplasmic enzymes
 - Require 2-oxoglutarate
 - Contain Fe
 - Use 2-oxoglutarate as substrate
 - *Remember ACC oxidase??*

GA 20 oxidase

- oxidizes at 20 position from CH₃ to COOH (C-20 is typically lost)
- 4+ member gene family in Arabidopsis (*GA5mutant in Arabidopsis*)

GA 3-beta hydroxylase

- oxidizes at 3 position- forms active GAs
- different GA 3-beta hydroxylases have different substrate kinetics, e.g., *GA4* of Arabidopsis and *Le* of pea, substrates have lower K_m if no C13-OH present (GA₉ is better substrate than GA₂₀).
- 4+ member gene family in Arabidopsis (*GA4 mutant in Arabidopsis*)
- can also have additional activities-e.g., bean embryos have an enzyme that also has 2 beta hydroxylase and 2,3 desaturase activity; one of pumpkins GA 3-beta hydroxylases (overexpressed in *E. coli*) also has low 2-beta hydroxylase activity

GA 2- hydroxylase

- oxidizes at 2 position-deactivates
- 6+ member gene family in Arabidopsis (mutants not yet identified in Arabidopsis)
- can make dwarf plants by overexpression of 2-hydroxylase transgene

other enzymes

- deactivation by formation of ketone at C3 (morning glory)

over 125 GAs identified

Grid of pathways determined by repeated feeds, re-feeds of radiolabeled GAs using wild-type and mutant plants

there is apparently a “grid” of GA biosynthetic pathways.

the major pathways through the grid can differ for different tissues, and species.

active GAs :GA₁, GA₄

Pathways (GRID) of synthesis worked out for several species/tissues.

Double label (¹⁴C /stable isotope) used because

- 1) recoveries can be calculated
- 2) the stable isotope can be detected and discerned from the endogenous compounds by GCMS
- 3) ¹⁴C useful to trace metabolites during fractionation

mutants and wild type feeding results compared

maize, pea (GA₁ is major active form)

arabidopsis, pumpkin endosperm and seedlings (GA₄ is major active form)

- Grids elucidated via a combination of identifying mutants in each particular gene, use of inhibitors for specific steps in pathway, and careful biochemical studies (eg feed radiolabeled, isotopically labeled GA putative precursors to mutants and analyze products by GC-MS.)
- Identify which GAs are ACTIVE in species, and or in the same species under different conditions. Apply putative GA precursors to mutants and determine which ones induce reversions to wild type phenotype. (Contrast to ethylene where pathway was worked out biochemically, genes were cloned by enzyme purification (ACC synthase) and by differential expression (ACC oxidase); and auxin and CK where we still don't know the pathway of synthesis, and there are no known mutants in biosynthetic genes)

Certain GA biosynthetic genes are feedback regulated by active GAs.

Negative feedback regulation at decrease in transcript accumulation of GA 20-oxidase

- Active GAs decrease accumulation of GA₄ transcripts (GA 20-oxidase) in Arabidopsis *regardless* of whether they have a 3OH group. Inactive GAs do not.
- *gai* (“GA-insensitive”) and *spy* (“spindly”), Arabidopsis GA response mutants, do not have GA-feedback repression of GA biosynthetic gene transcripts. Thus, repression of GA₄ transcripts may involve feedback regulation that requires signal transduction
- Levels of C19-GAs are very high in GA response mutants maize D8, wheat *Rht3*, Arabidopsis *gai*
- Treat peas with GA biosynthetic inhibitor (e.g., Paclobutrazol). Get increase in GA-20 oxidase mRNA. Add GA₃, get decrease in GA 20-oxidase mRNAs.

Negative feedback regulation at decrease in transcript accumulation of GA 3-hydroxylase (GA4 transcript= $GA3ox1$) but no decrease in GA4-H transcript = $GA3ox2$. $GA3ox2$ may be important for germination, which requires high concentration of GAs and doesn't need feedback regulation.

Positive feedback regulation

- GA2hydroxylase mRNA accumulation is up-regulated in GA-deficient *gal-2* mutants after treatment with GA

Certain GA biosynthetic genes are regulated by auxin

GA metabolism is regulated by environment: phytochrome bolting

- GAs induce bolting in LDP rosette plants (*Silene*, Arabidopsis, spinach). (bolt=rapid elongation of stem to form inflorescence stem preceding flowering)
- expose spinach to longer day length, increase C20 oxidation of GA_{19} , GA_{53} [fig 17.19](#) and ent-kaurene oxidase
- Transfer *Silene* to LDP: decrease GA_{53} and increase GA_1 in subapical region of stem where elongation will occur.

Flowering

May be role of GA in flowering of some species.

Bryophyllum (a LDP)

- GA_{20} is major GA of *Bryophyllum* leaves
- GA_3 applied to leaves of non-induced *Bryophyllum* → flowers in SD
- GA_3 applied to leaves of non-induced *Bryophyllum*; *Bryophyllum* leaves grafted onto non-induced *Echeveria*. *Echeveria* plant flowers in SD
- GA_3 applied to *Echeveria* in SD ...no flowers

growth

Many many studies of involvement of GA in growth-response to dark and FR light.

Results not clear, effect of phytochrome may be on GA signal transduction ??

seed germination

Lettuce seed germination induced by red light, effect reversed by a pulse of FR light.

Red light induces 3-beta hydroxylase activity, increased accumulation of transcript from one of two (known) C20 oxidases, and increased concentration of GA_1 , effect reversed by a pulse of FR light.

GA induces germination in absence of red light.

GA metabolism is regulated by environment: temperature vernalization

- *Thlaspi arvense* bolting and flowering induced by 4 week vernalization. Perceived in shoot tip
- GA eliminates vernalization requirement (most active= GA_9)

- Ent-kaurenoic acid level accumulates in shoot tip in cold, decreases in warmth.
- Ent-kaurenoic acid \rightarrow GA₉ increased in induced shoot tips
- Ent-kaurene and ent-kaurenoic acid \rightarrow GA₉ increased in microsomes

Overexpression of GAs:

GA 20-oxidase (35S promoter)

ARabidopsis-Reduced seed dormancy, longer hypocotyls, pale leaves, early flowering

Potato-reduced time required for induction of tuber,

Poplar-faster growth, more, longer, xylem fibers