Plant Hormone Signal Transduction Pathway: Cross talk between GA and ABA in plant

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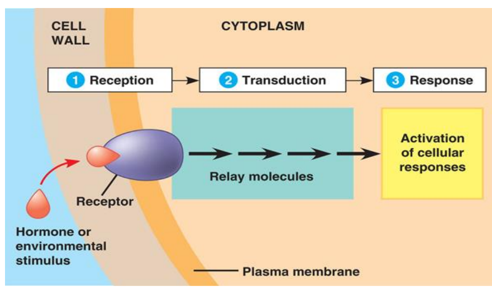


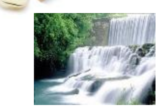
Talk Outline

I. General introduction about plant hormones

a. Types.
b. Physiological functions.
c. Differences compared with animal's hormones.
d. Ways or approaches of studies.

II. Molecular mechanism of various plant hormone signal transduction pathways

a. Ubiquitin-dependent protein degradation.
(negative regulator) e.g. GA



b. Double negative regulatory mechanism
(Ser/Thr phosphatase/kinase cascade) e.g. ABA

(繼電器)

c. Tow-component system (phospho-relay).
e.g. Ethylene, Cytokine (His/Asp-kinase)

Toward a Molecular Understanding of Plant Hormone Actions

---Molecular Plant 9, 1-3, January 2016

Plants rely on a diverse set of small-molecule hormones to regulate every aspect of their biological processes including development, growth, and adaptation. Since the discovery of the first plant hormone, auxin, hormones have always been at the frontier of plant biology. Although the physiological functions of most plant hormones have been studied for decades, the last 15–20 years have seen dramatic progress in our understanding of the molecular mechanisms of hormone actions. The publication of the whole-genome sequences of the model systems of *Arabidopsis* and rice, together with the advent of multidisciplinary approaches, has opened the door to successful experimentation on plant hormone actions.

Proteomics 2015, *15*, 1113–1126 DOI 10.1002/pmic.201400403 1113

REVIEW

Plant hormone signalling through the eye of the mass spectrometer

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Plant growth and development are regulated by hormones and the associated signalling pathways share several common steps, the first being the detection of the signal by receptor proteins. This typically leads to conformational changes in the receptor, thereby modifying its spectrum of interaction partners. Next, secondary signals are transmitted via rapid posttranslational cascades, such as targeted phosphorylation or ubiquitination, resulting in the activation/deactivation, relocalization or degradation of target proteins. These events finally give rise to the signal-dependent read-out, such as changes in gene expression and regulation of protein activity. So far, the majority of studies aimed at unravelling hormone signalling pathways in plants relied on genetic or transcriptomic approaches. During the last decade however, MS-driven proteomic methods became increasingly popular tools in plant research as they reveal the specific mechanisms controlled by phytohormones, which for a large part occur on the level of the proteome. Here, we provide an up-to-date review on the growing body of work in these areas using MS-based techniques, with a focus on nonpeptide plant hormones.

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Preface

Peptides take centre stage in plant signalling

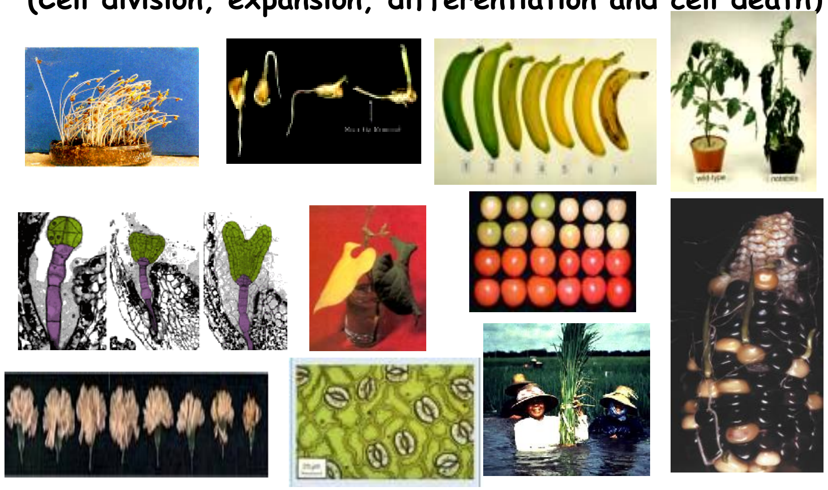
Due to the presence of cell-wall material separating the surface of neighbouring cells, plants were expected to require many mobile ligands to exchange information among cells, tissues, organs, and with their environment. In particular, the classical plant hormones auxin, cytokinins, gibberellins, abscisic acid, and ethylene have long been considered to regulate growth and developmental processes as extracellular signalling molecules (Kende and Zeevart 1997). Until the 18 amino acid Systemin was discovered by Pearce *et al.* (1991) peptides were not known as regulators of signalling events in plants. The availability of the first plant genome in 2000, the generation of huge RNAseq data sets, and improved biochemical isolation procedures and gene prediction tools have shown that plants possess thousands of genes encoding putative secreted extracellular peptide ligands. These have now been distinguished into two major classes as cysteine-rich peptides (CRPs) with a length of about 40 to more than 100 amino acid residues and post-translationally modified small-peptides of about 10 amino acid residues (nonCRPs) (Matsubayashi, 2014). Notably, while nonCRPs regulate many intercellular communication processes during vegetative development and stress responses, CRPs are particularly abundant during plant reproduction. This special issue of the *Journal of Experimental Botany* now tries to provide a state-of-the-art insight into the numerous roles of some major plant peptide classes including CRPs as well as various nonCRPs such as CEPs, CLEs, IDAs, PEPs, PSKs, and others.

I. General introduction about plant hormones (phytohormones)

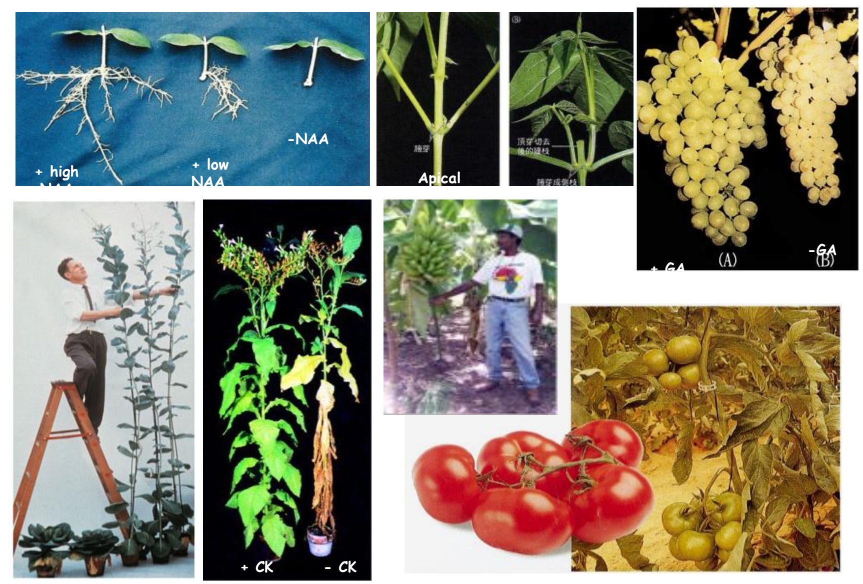
Phytohormones are important for plant growth and development

Embryogenesis → **Senescence**

(Cell division, expansion, differentiation and cell death)



Many plant growth regulators have been applied in agricultural production



Definition of plant hormone (phytohormone)

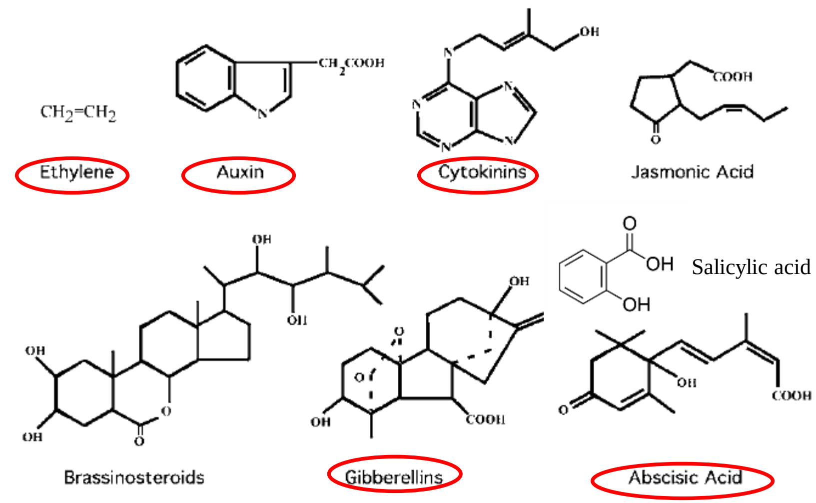
1. The word hormone is derived from the Greek verb meaning to excite.

2. Hormones are organic substances synthesized in one tissue and transported out where their presence results in physiological responses (not always true; may act at or close to synthesis site). They are required in minute amounts (10^{-6} to 10^{-8}M).

3. Each hormone may result in multiple effects -- the particular effect depending on a number of factors:
   (a) the presence of other hormones and the presence of activator molecules (calcium, sugars)
   (b) the amount of the hormone (dosage or concentration)
   (c) the sensitivity of that tissue to the hormone.
   (d) the condition of the plant itself is critical: what is the condition of the plant? its age?

Site of action x developmental stage x concentration of hormone? crosstalk and specificity?

Different types of the Plant Hormones



Salicylic acid, jasmonates, polyamines, nitric oxide, carbon monooxide, strigolactone, peptide hormones

Early stage

Update

Update progress

Observations of hormone-regulated plant physiological processes and responses

Understanding phytohormone biosynthesis, metaboloism and transportation

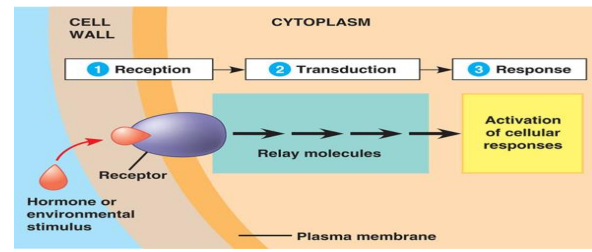
Elucidation of the molecular basis for hormone pertception and signal transduction

Analysis of mutants and transgenic plants: provided us with extensive "parts lists" of genes involved in hormone synthesis and catabolism or encoding receptors and signaling components for most classical hormones

Dissection of the cross talk between different hormones and biotic or abiotic stresses

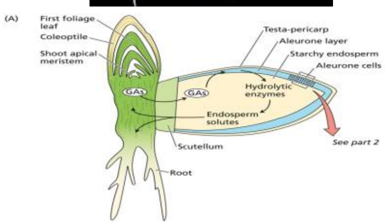
System biology approach: -OMICs

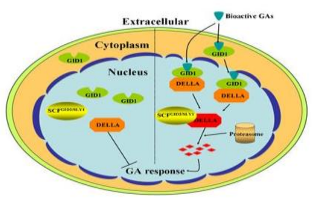
Many questions to ask regards plant hormone action

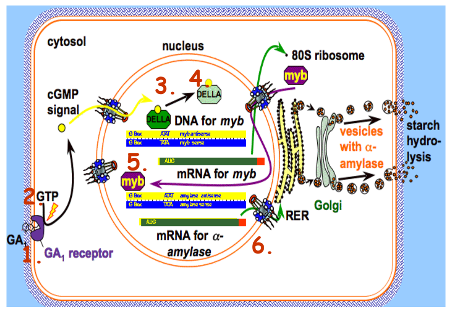


1. How the hormone is synthesized and degraded? (homeostasis state)
2. How the hormone is transported? (free form or conjugated form)
3. How the hormone exerts its specific or general function?
   hormonal regulation of organ patterning, development, and outgrowth
   hormonal regulation of plant responses to biotic and abiotic stresses
4. What are the hormone receptors ? (membrane or cytosol or ER or ?)
5. What are the signaling components (positive or negative regulators) in the transduction pathway?
6. What are the responsive or final targeted genes?
7. How the plant hormone effect is regulated?

GA induces alpha-amylase production via Ca2+ independent pathway.







1. GA binds to receptor
2. GA-receptor binds to G-protein
3. G-protein activates F-box protein
4. F-box protein binds to DELLA-domain repressor (which is degraded by SCF-ubiquitin ligase)
5. GAMYB gene expression is activated
6. GAMYB activates alpha-amylase expression

Main approaches for plant hormones studies

1. **Molecular genetics approach**
   hormone biosynthetic mutants vs. hormone insensitive mutants

2. **Biochemistry approach**
   hormone binding protein
   (photo-affinity probe, affinity chromatography, immuno-purification,protein-protein interation)

3. **Inhibitors approach**
   kinase/phosphatase, G-protein, PKC etc. inhibitors.

4. **Functional genomics approach**
   transgenic study by gene OE or KO, complementary rescue.

5. **Chemical genomics**

6. **System biology or synthetic biology (Omics- study)**

Chronological events and persons involved in identification of different hormone receptors

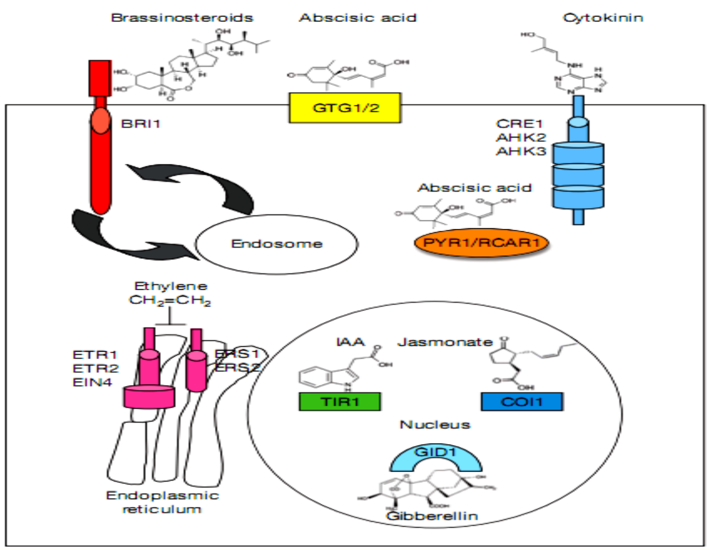
hormone	Substance found	Receptor identified	Scientists	Worked place
Ethylene	1900's	1993 (ETR1)	Elliot Meyerowitz	Cal Tech, USA
Auxin	1920's	2005 (TIR1)	Ottoline Leyser Mark Estelle	Univ of York, UK Indianna Univ, USA
Cytokinin	1950's	2001 (CRE1)	Tatsuo Kakimoto	Osaka Univ, Japan
Gibberellin	1930's	2005 (GID1)	Makoto Matsuoka	Nagoya Univ, Japan
Abscisic	1960's	2006 (FCA) 2007 (CHLH, GCR2)	Robert D. Hill Fawzi A. Razem	University of Manitoba, Canada
Brassinosteroid	1979	1997 (BRI1)	Joanne Chory	Salk Inst, USA

Currently identified different plant hormone receptors

Hormone	Receptor type	Receptors
Auxin	F-box protein	TIR1, AFBs
Abscisic acid	G-protein, Chelatase	GTG1, GTG2, GCR2*, CHLH*
Cytokinin	Two-component regulators	CRE1, AHK2, AHK3
Gibberellins	Hormone-sensitive lipase like	GID1
Ethylene	Two-component regulators	ETR1, ERS1, ETR2, EIN4, ERS2
Brassinosteroids	Leucine-rich repeat receptor-like kinases	BRI1
Jasmonic acid	F-box protein	COI1
Salicylic acid	Unknown	
Nitric oxide	Unknown	
Strigolactones	Unknown	

Nature 405:1071-1078 (2009)

Cellular locations of different plant hormone receptors

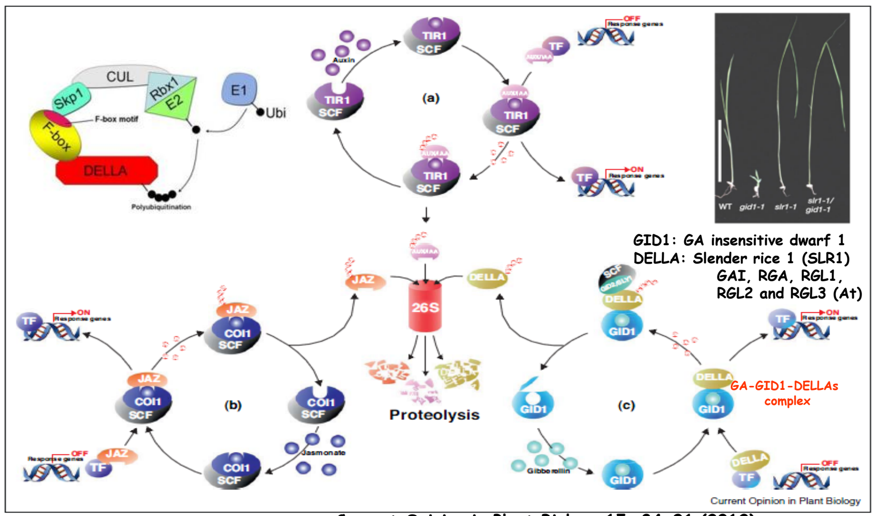


Nature 459:1071-1078 (2009)

II. Molecular mechanism of various plant hormone signal transduction pathways

(a) ubiquity-dependent protein degradation.
(negative regulator)

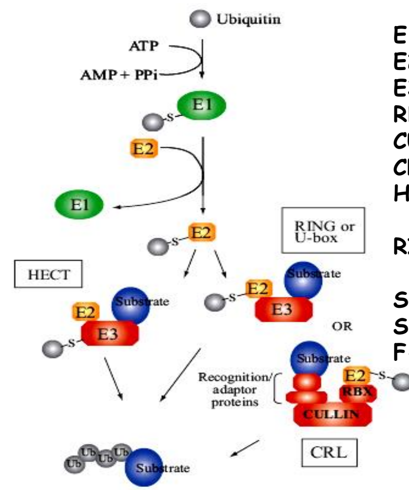
Gibberellin signaling pathways



Current Opinion in Plant Biology 15: 84-91 (2012)

Schematic diagram of auxin, jasmonate and gibberellin signaling pathways. Positive interactions are noted by arrows. 26S represents 26S proteasome, little red circles represent ubiquitin. **(A)** Auxin signaling pathway. TF: transcription factors including ARFs. **(B)** Jasmonate signaling pathway. TF: transcription factors including MYC2/3/4, MYB21/24/75, TT8, GL1, GL3, EGL3. **(C)** Gibberellin signaling pathway. GID1: GID1 in rice and GID1a/b/c in *Arabidopsis*; DELLA: the rice DELLA protein SLR1 and five *Arabidopsis* DELLA proteins (GAI, RGA, RGL1, RGL2 and RGL3); TF: transcription factors such as PIFs, ALC and SCL3.

An overview of the ubiquitination process in plants



E1; UBA, ubiquitin activating enzyme
E2: UBC, ubiquitin conjugating enzyme
E3: ubiquitin protein ligase
RBX: ring-box protein
CUL: cullin
CRL:cullin ring ligase
HECT: homologous to E6-AP COOH terminus
RING: really interesting new gene protein domain
SCF: SKP1-Cullin-F-box
SKP: S phase kinase associated protein
F-box: SKP/ASK interaction motif in cyclin F

Annals of Botany 99: 787-822 (2007)

Biological significance of ubiquitin/proteosome system (UPS) in plants

1. **UPS was involved in different processes of plant life, including organ initiation and embryogenesis patterning, light signaling , circadian clock regulation ,hormone production, perception and signal transduction, and in plant defense.**

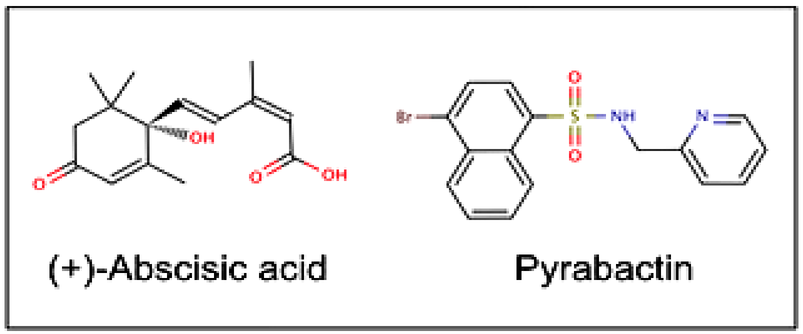
2. **The covalent attachment of ubiquitin to a substrate protein changes its fate. Notably, proteins typically tagged with a lysine 48-linked polyubiquitin chain become substrates for degradation by the 26S proteasome.**

3. **Over 6 % (1,500 genes) of the predicted *Arabidopsis* genome encodes proteins involved in the UPS, and analyses of other plant genomes demonstrate a similar abundance of UPS-related genes.**
   E1 (1); E2 (1); UEV (8); E3 (mast abundant): HECT (7); Ring (450); U-domain (61); cullin (11); F-box (700) BTB (80)

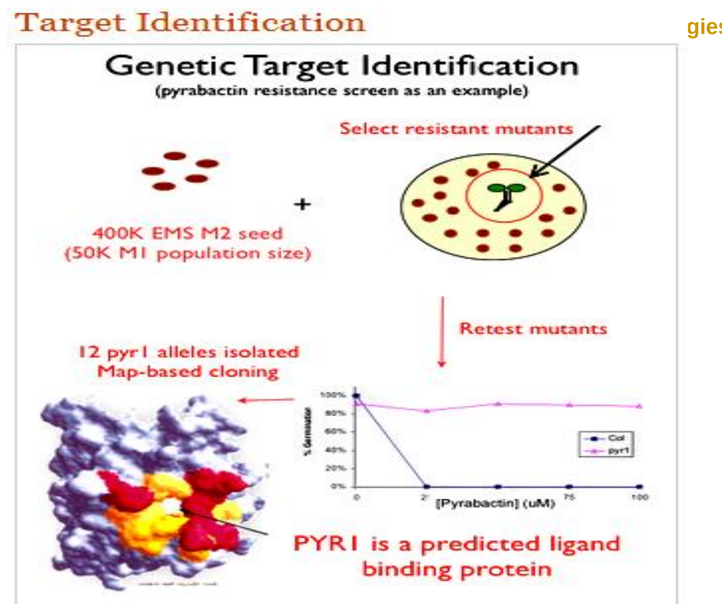
II. Molecular mechanism of various plant hormone signal transduction pathways

(b) double negative regulatory mechanism (protein phosphatase/kinase cascade)

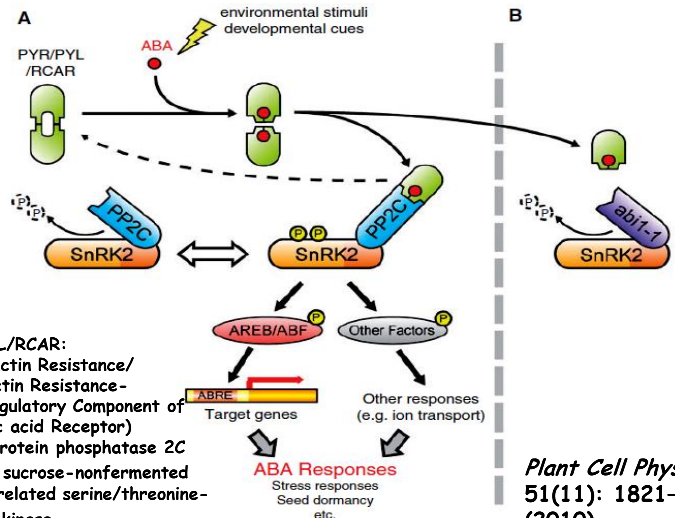
Chemical genomics approach to search for ABA receptor



a new naphthalene sulfonamide hypocotyl cell expansion inhibitor called pyrabactin (for pyridyl containing ABA activator)



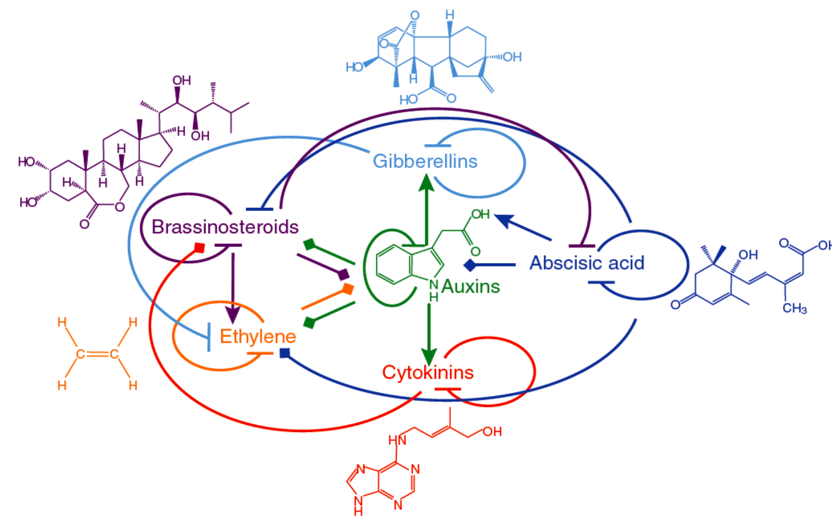
The PYR/PYL/RCAR, PP2C and SnRK2 form a signaling complex (ABA signalsome) in the major ABA signaling pathway



PYR/PYL/RCAR:
(PYrabactin Resistance/
Pyrabactin Resistance-
Like/Regulatory Component of
Abscisic acid Receptor)
PP2C: protein phosphatase 2C
SnRK2: sucrose-nonfermented
(SNF)-related serine/threonine-
protein kinase

Plant Cell Physiol.
51(11): 1821-1839 (2010)

How different plant hormones cross talk with each other?




Thanks for your attention!