Signalübertragung in der Phytohormonantwort II

Auxin, Gibberellinsäure, Jasmonsäure – Rolle des Proteasoms

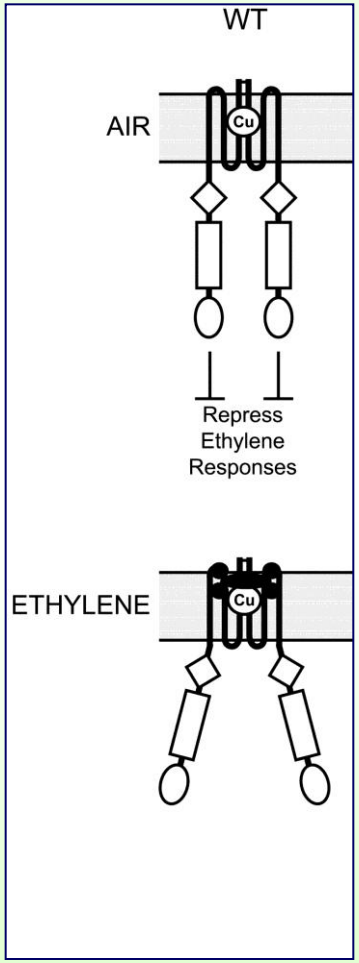
Crosstalk zwischen den Hormonsignalwegen?

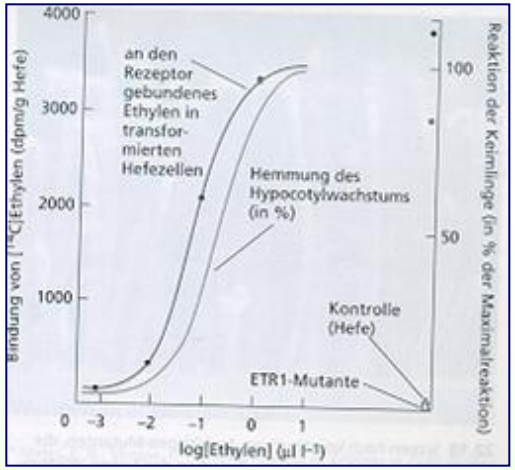
Evolution

Pflanzenphysiologie
PUR
Universität Rostock

ETR1 ist der erste identifizierte Ethylenrezeptor sowie der erste Rezeptor für ein Pflanzenhormon

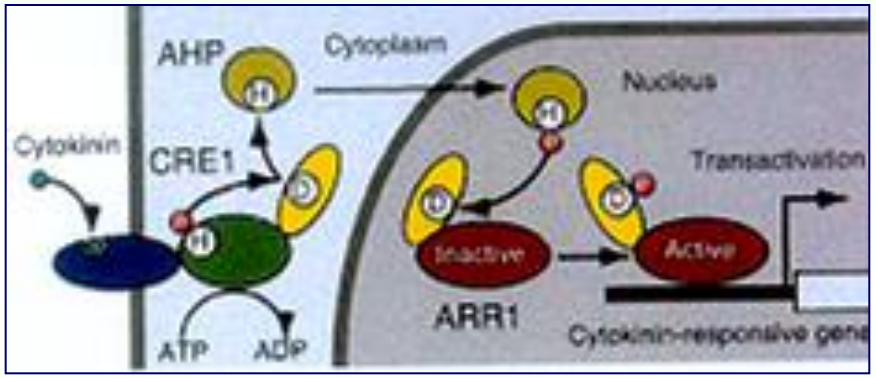
1. ETR1 hat 3 hydrophobe Domänen, die der Dimerisierung und Ethylenbindung dienen.
2. Expression von ETR1 in Hefe bewies Vermutung, da Ethylenbindung in Hefe messbar wurde.
3. Die Histidinkinase-Domäne befindet sich im Cytoplasma.
4. ETR1 hat auch eine Receiver-Domäne, d.h. einen fusionierten Response-Regulator.
5. Ethylenbindung inaktiviert einen negativen Regulator der Ethylen-Antwort, d.h. eine Mutation führt zu konstitutiven Ethylenmutanten.





Cytokinine – Signalerkennung ähnelt bakteriellem 2Komponentensystem, CRE1, AHP2, ARR1

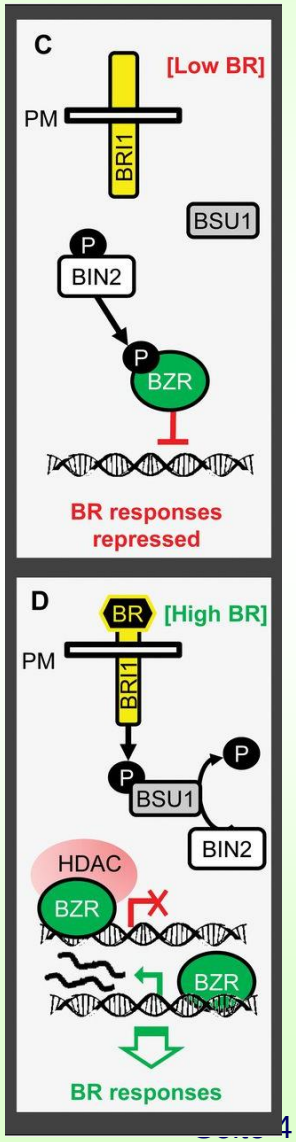
1. His-Kinase CRE1 bindet Cytokinin und autophosphoryliert sich
2. AHP2, Arabidopsis-Histidin-Phospho-Transferase interagiert mit CRE1 und übernimmt P-Gruppe.
3. AHP2 interagiert dann im Kern mit ARR1, das phosphoryliert Genexpression von Zielgenen aktiviert



Modell der Cytokinin-Regelkette

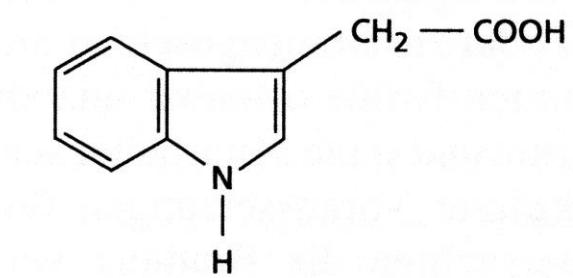
Der Brassinosteroid-Hormonrezeptor BRI1 ist eine Rezeptor-Kinase mit Ser/Thr-Kinase Spezifität

1. BRI1 wurde in einer Brassinolid-insensitiven Arabidopsis-Mutante gefunden.
2. Ohne Steroid inaktiv, dann ist Kinase BIN2 aktiv und inhibiert Brassinolid-abhängigen TF und Genexpression.
3. Brassinolidbindung stimuliert Autophosphorylierung, dann wird die Phosphatase BSU1 aktiv und inhibiert den negativen Regulator BIN2.



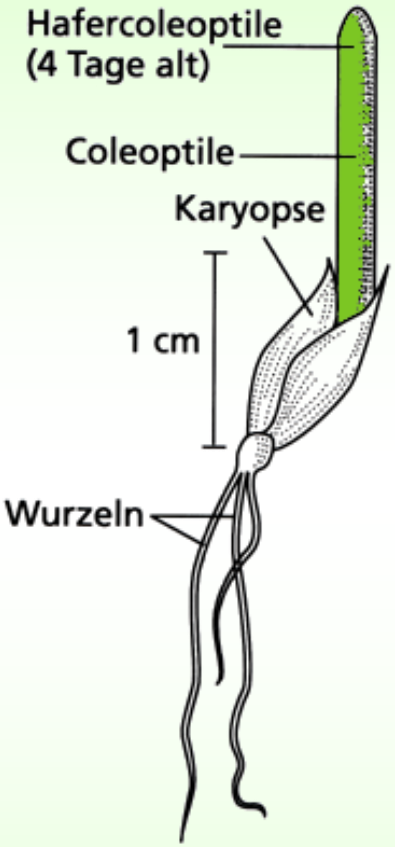
Auxin = Indol-3-Essigsäure

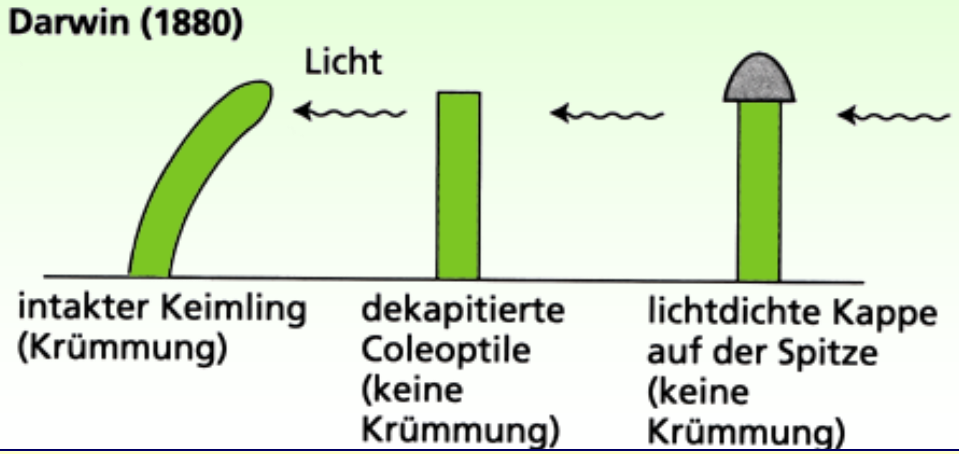
IAA ist das häufigste und physiologisch wichtigste Auxin.
Weitere natürlich vorkommende Auxine sind …

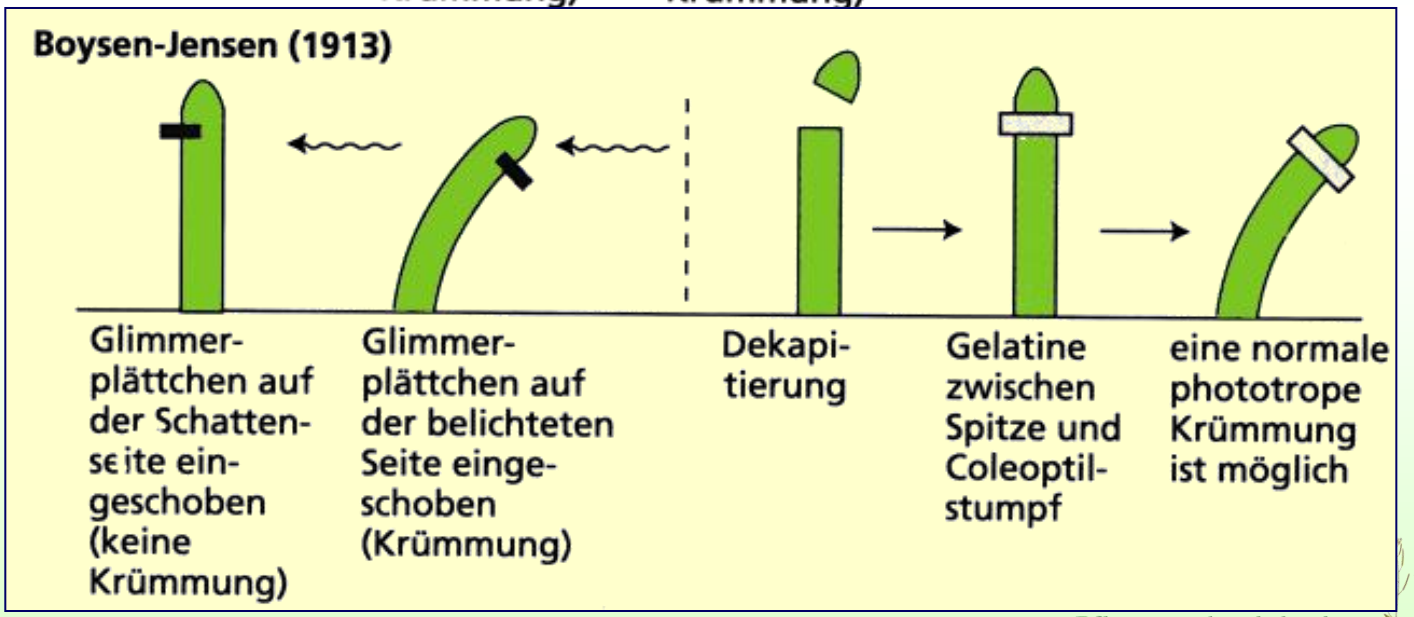


Indol-3-Essigsäure (IAA)

Entdeckung von Auxin: Phototropismus







Auxine

1. Biosynthese von Indol-3-Essigsäure (IAA) aus Tryptophan v.a. in der Sprossspitze.
2. Wird polar, basipethal transportiert.
3. Stimuliert Wachstum von Sprossen, hemmt Primärwurzelwachstum.
4. Säurewachstumshypothese für Zellvergrößerung
5. Ungleichverteilung ist Ursache für Photo- und Gravitropismus.
6. Auxin induziert Apikaldominanz und Seitenwurzeln.
7. WW mit hemmenden Hormonen.
8. Analoge (z.B. 2,4-D) dienen als Herbizide.
9. Auxine dienen dem Bewurzeln von Stecklingen.

Auxine und Cytokinine sind essentiell!
Zellteilung und Morphogenese

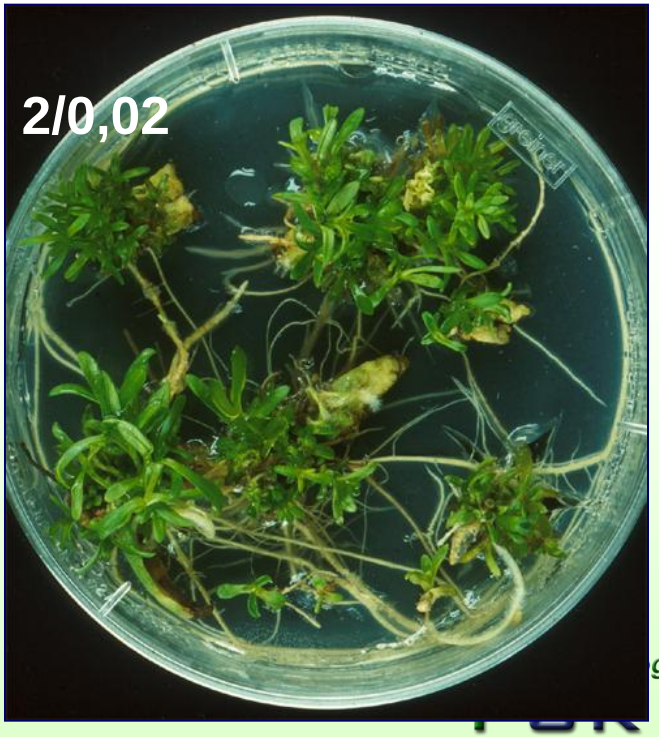
Das Verhältnis von Auxin zu Cytokinin steuert die Morphogenese in Gewebekulturen (Skoog & Miller 1965):

a. **Viel Cytokinin fördert Sprossbildung**

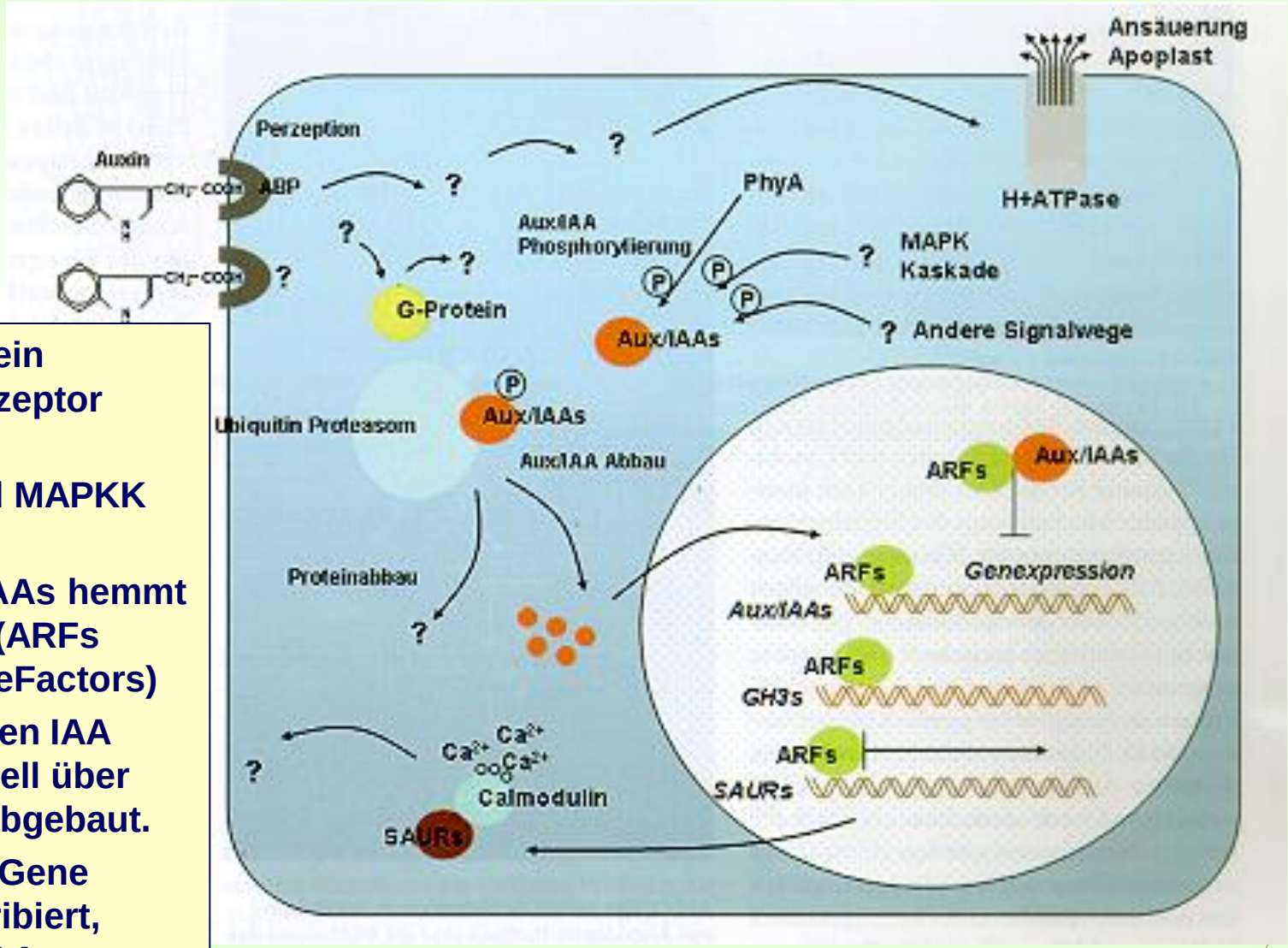
b. **Viel Auxin fördert Wurzelbildung**

Auxin/Cytokinin: 2/0,2

2/0,5



Bei der Auxin-Signalvermittlung spielt die IAA-abhängige Proteinstabilität eine Rolle



1. Bis 2005 war kein eindeutiger Rezeptor ermittelt
2. G-Proteine und MAPKK beteiligt?
3. Inhibitor Aux/IAAs hemmt Auxin-abh. TF (ARFs AuxinResponseFactors)
4. Aux/IAAs werden IAA stimuliert schnell über Ubiquitinweg abgebaut.
5. IAA-induzierte Gene werden transkribiert, darunter Aux/IAAs.
6. Rückkopplung

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ARTICLES

The *Arabidopsis* F-box protein TIR1 is an auxin receptor

Stefan Kepinski[1,2] & Ottoline Leyser[1]

Vol 435|26 May 2005|doi:10.1038/nature03543 nature

ARTICLES

The F-box protein TIR1 is an auxin receptor

Nihal Dharmasiri[1], Sunethra Dharmasiri[1] & Mark Estelle[1]

TIR – transport inhibitor response,
Genfamilie, in Arabidopsis 6 derartige Proteine, mindestens 4 sind IAA-Bindeproteine

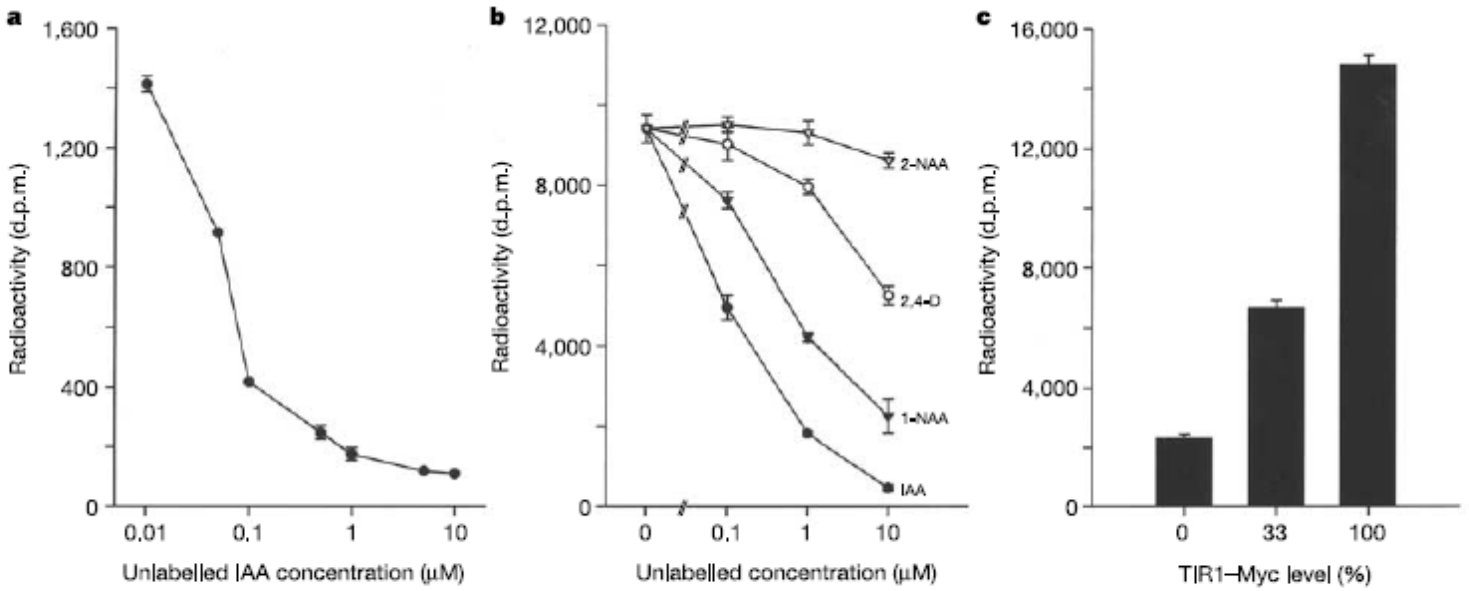
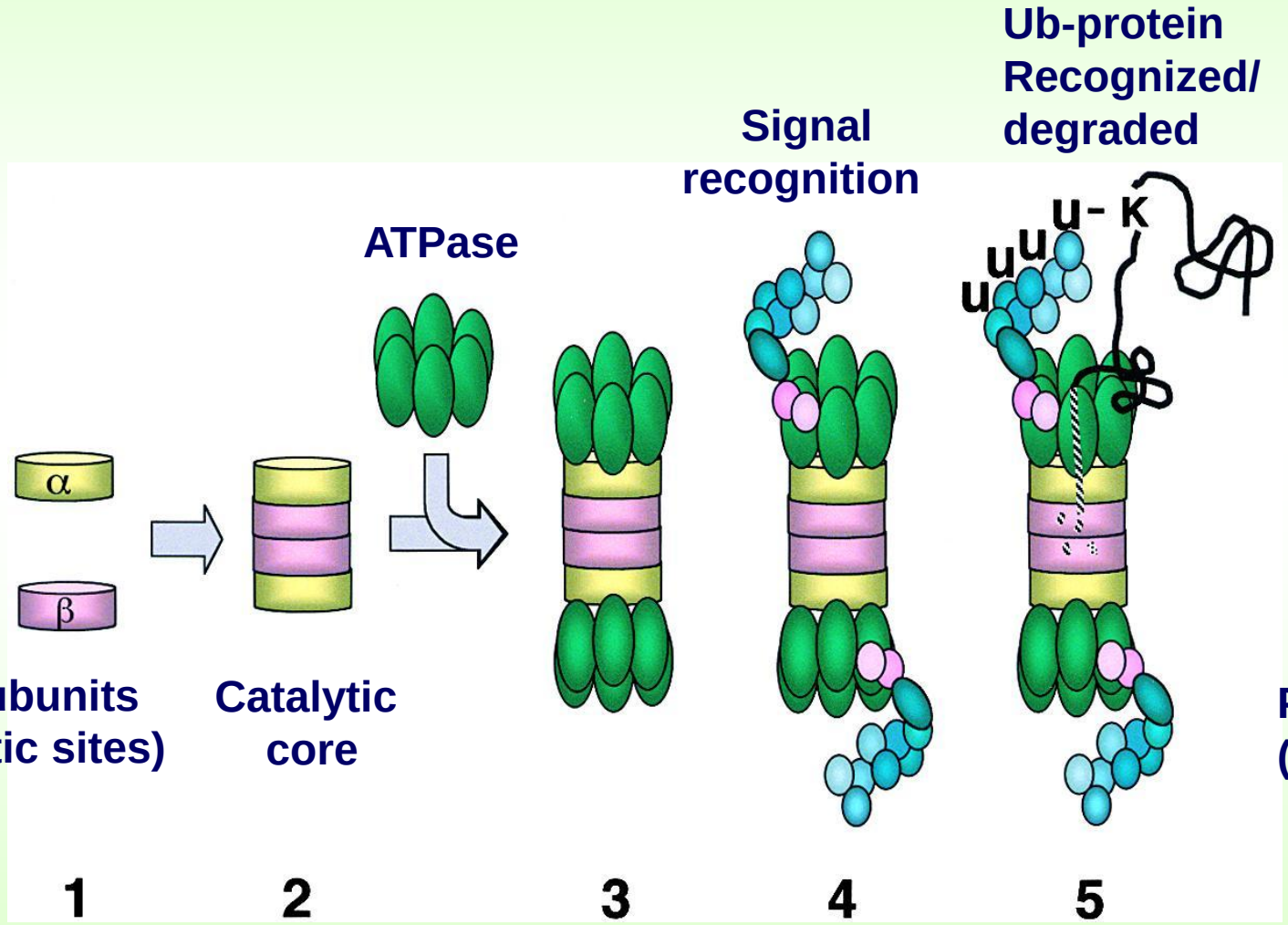


Figure 3 | The TIR1–Aux/IAA interaction involves direct auxin binding. **a**, GST–AXR2 pull-down assays from *tir1-1[TIR1–Myc]* seedling extract with 0.01 μM [^{3}H]IAA and increasing concentrations of unlabelled IAA. Binding of [^{3}H]IAA was assessed by scintillation counting and is expressed as disintegrations per minute (d.p.m.). **b**, As in **a**, except that the experiment was performed with the [^{3}H]IAA concentration held constant at 0.1 μM and with increasing concentrations of unlabelled IAA (filled circles), 2,4-D (open circles), 1-NAA (filled triangles) or 2-NAA (open triangles) as indicated. **c**, GST–AXR2 pull-down assays from otherwise identical *tir1-1* seedling extracts containing 0.1 μM [^{3}H]IAA and increasing levels of TIR1–Myc as indicated. Error bars indicate s.e.m. ($n = 3$).

Einschub – Proteinabbau

Proteine werden häufig am so-genannten Proteasom abgebaut



Verma and Deshaies (2000) Cell 101, 341-344

Pflanzenphysiologie
PUR
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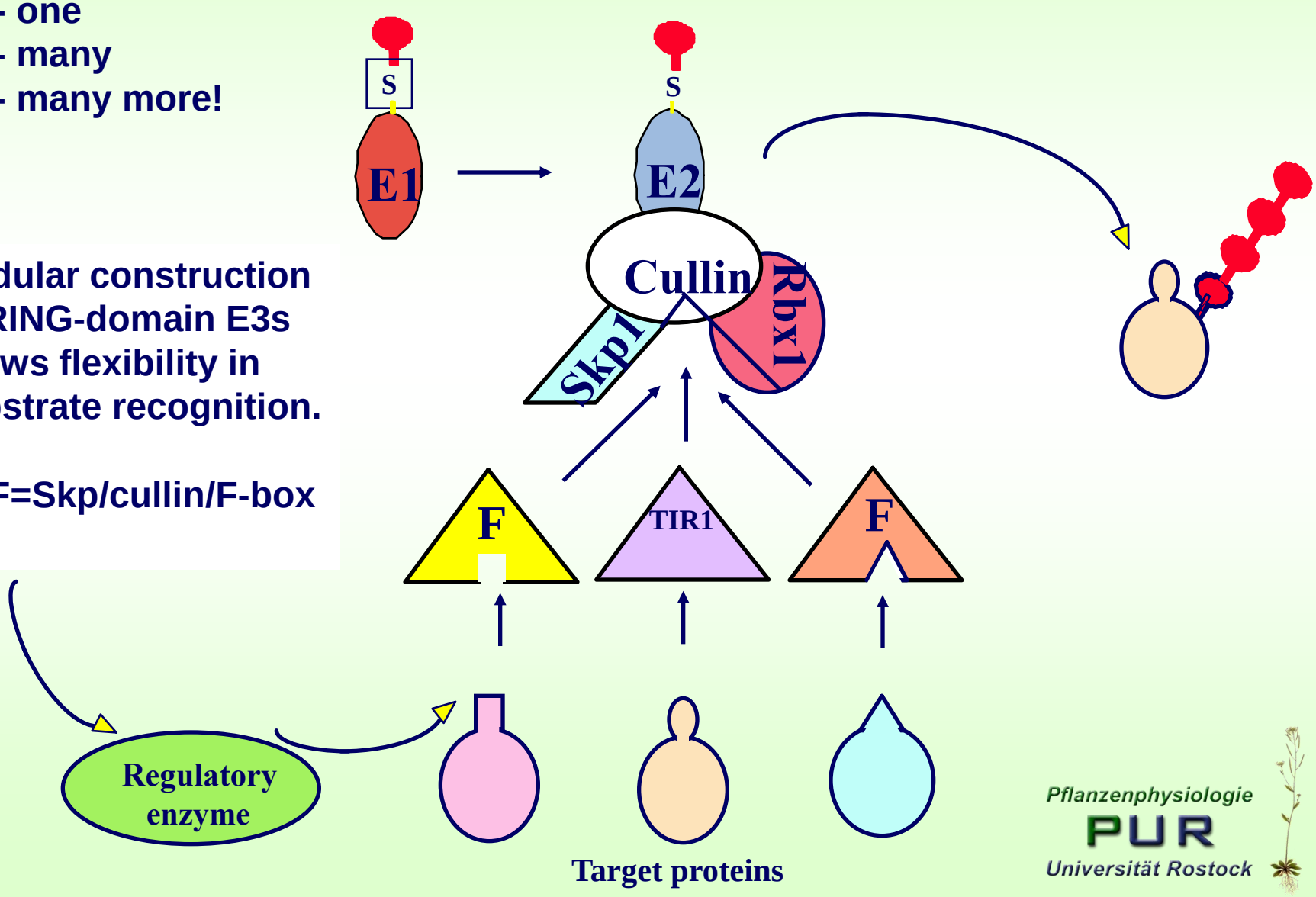
The SCF ubiquitin ligase model

E1- one
E2- many
E3- many more!

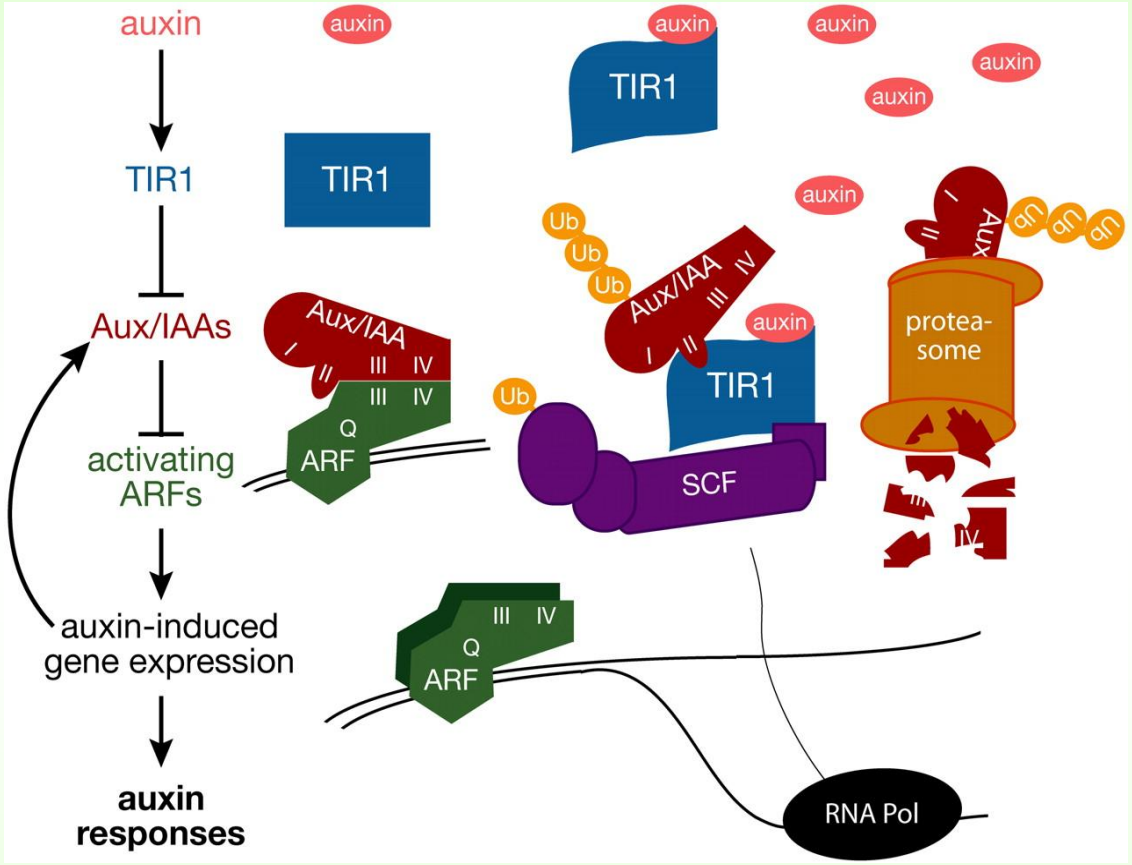
Modular construction of RING-domain E3s allows flexibility in substrate recognition.

SCF=Skp/cullin/F-box



Model for Auxin Response through the TIR1 Auxin Receptor Pathway

IAA-binding induce a TIR1/Aux/IAA complex which stimulate degradation of the inhibitor protein and result in auxin-regulated gene inductions



Woodward, A. W., et al. Plant Cell 2005;17:2425-2429

Skp, **C**ullin, **F**-box containing complex. A multi-protein complex catalyzing the ubiquitylation of proteins destined for proteasomal degradation. Protein substrates are recognized by an F-box protein and delivered to the E3 ligase through the bridging protein, Skp. Cullen is the major scaffold of the SCF, as dogma indicates its binding of Skp, the ring-finger catalytic protein (eg ROC1), and recruitment of E2.

A model of Auxin-regulated TIR-1 substrate interaction

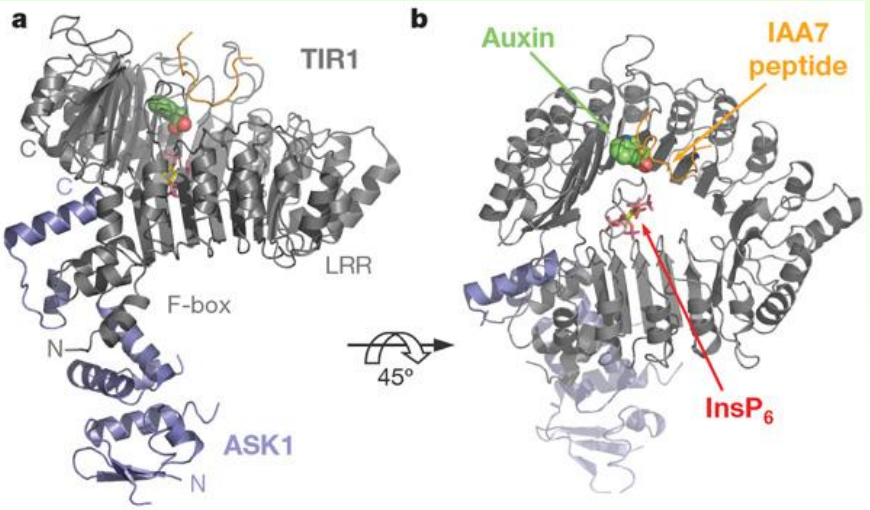
nature Vol 446|5 April 2007|doi:10.1038/nature05731

ARTICLES

Mechanism of auxin perception by the TIR1 ubiquitin ligase

Xu Tan[1], Luz Irina A. Calderon-Villalobos[2], Michal Sharon[3], Changxue Zheng[1], Carol V. Robinson[3], Mark Estelle[2] & Ning Zheng[1]

Auxin is a pivotal plant hormone that controls many aspects of plant growth and development. Perceived by a small family of F-box proteins including transport inhibitor response 1 (TIR1), auxin regulates gene expression by promoting SCF ubiquitin-ligase-catalysed degradation of the Aux/IAA transcription repressors, but how the TIR1 F-box protein senses and becomes activated by auxin remains unclear. Here we present the crystal structures of the *Arabidopsis* TIR1–ASK1 complex, free and in complexes with three different auxin compounds and an Aux/IAA substrate peptide. These structures show that the leucine-rich repeat domain of TIR1 contains an unexpected inositol hexakisphosphate co-factor and recognizes auxin and the Aux/IAA polypeptide substrate through a single surface pocket. Anchored to the base of the TIR1 pocket, auxin binds to a partially promiscuous site, which can also accommodate various auxin analogues. Docked on top of auxin, the Aux/IAA substrate peptide occupies the rest of the TIR1 pocket and completely encloses the hormone-binding site. By filling in a hydrophobic cavity at the protein interface, auxin enhances the TIR1–substrate interactions by acting as a 'molecular glue'. Our results establish the first structural model of a plant hormone receptor.



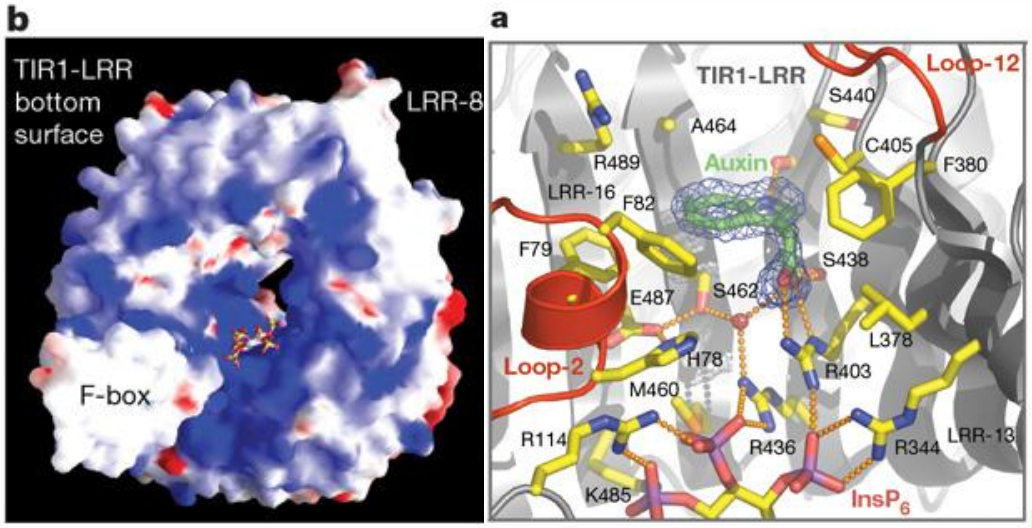


Figure 6 | A model of auxin-regulated TIR1–substrate interactions. A schematic diagram of auxin functioning as a 'molecular glue' to enhance TIR1–substrate interactions. In contrast to an allosteric mechanism, auxin binds to the same TIR1 pocket that docks the Aux/IAA substrate. Without inducing significant conformational changes in its receptor, auxin increases the affinity of two proteins by simultaneously interacting with both in a cavity at the protein interface.

Bei der Auxin-Signalvermittlung könnten noch weitere Rezeptoren eine Rolle spielen z.B. ABP1, Auxin-Binde-Protein

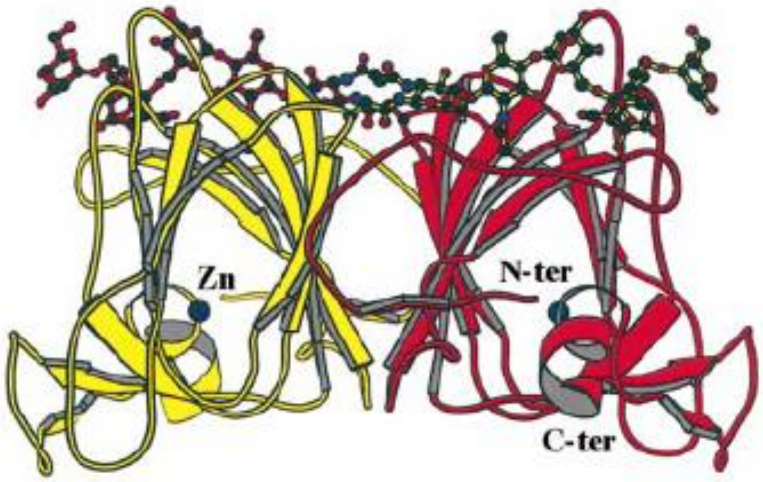
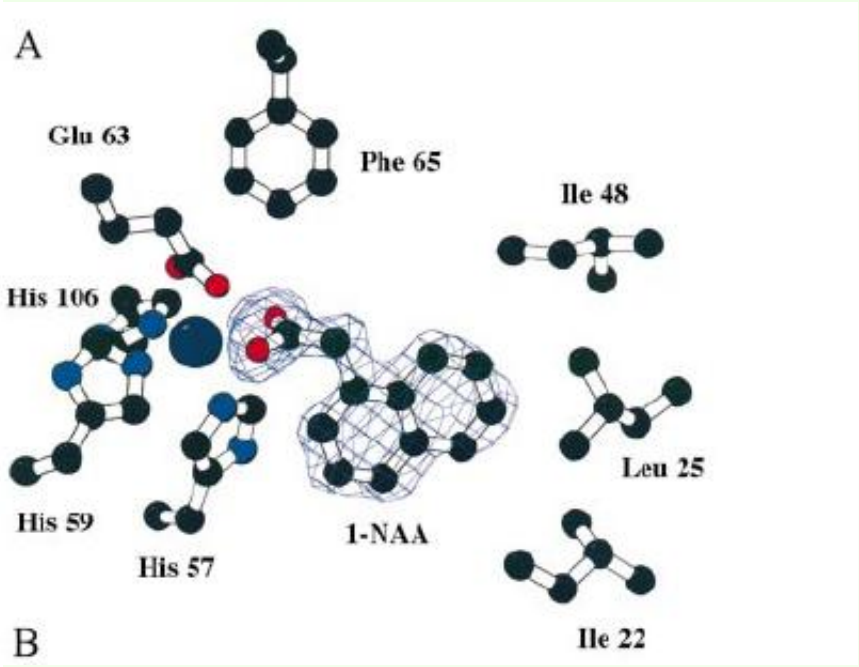
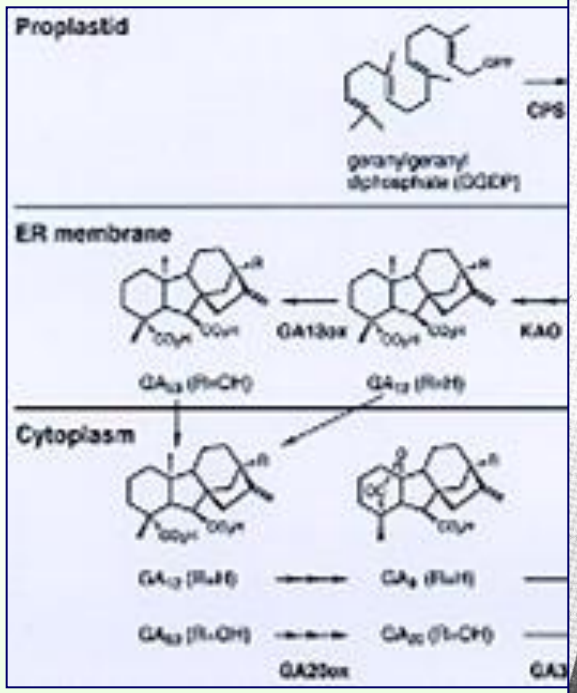


FIG. 4. Ribbon diagram showing the structure of an ABP1 dimer. The β-sheets are shown as broad arrows. ABP1 is *N*-glycosylated and some of the sugar residues are shown at the top of each monomer. Three C-terminal residues were not resolved and would extend the α-helices at the foot of the molecules. The zinc ion is shown in green. Reproduced from The EMBO Journal, Vol. 21 No. 12, pp. 2877–2885, 2002, with permission from Woo *et al.* (2002), Oxford University Press.



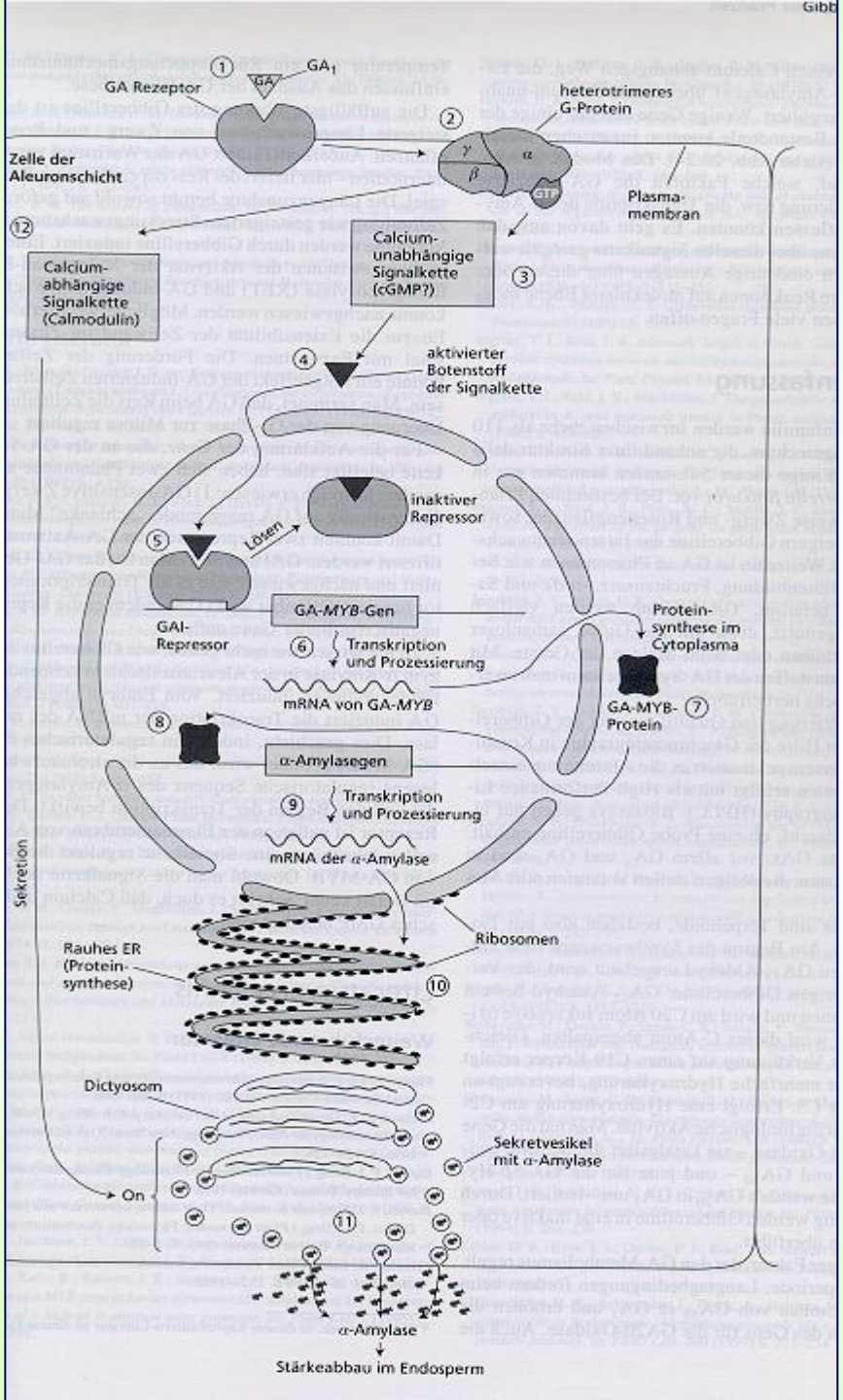
Gibberelline (GAs)

1. Mindestens 100 Gibberellíne bekannt, alle mit *ent*-Kauren-Ring
2. GAs stimulieren Sprosswachstum.
3. Induzieren Schossen und Blütenbildung
4. GAs bewirken die Mobilisierung von Reservestoffen im Endosperm - Bier.

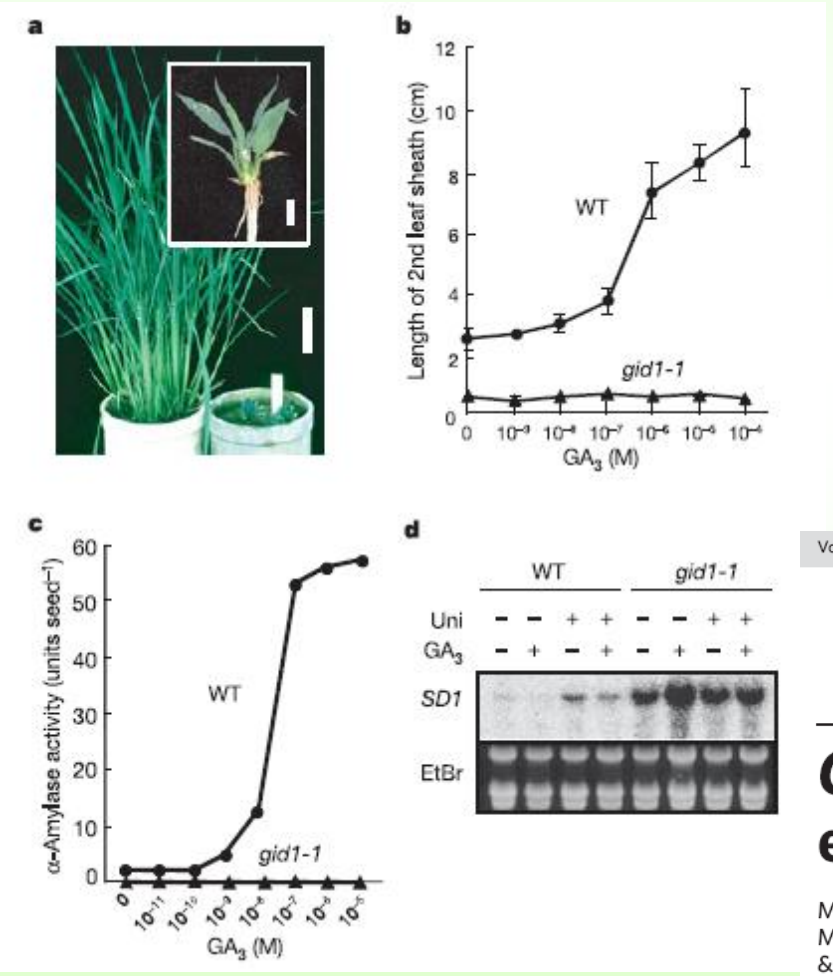


GA-Signalwandlung ist weitgehend unbekannt

1. Erst 2005 wurde der GA-Rezeptor identifiziert
2. Gibberellin-induzierte Promotoren enthalten GARE: TAACAAA.
3. Ein Repressor wird inaktiviert.
4. Ein myb-TF wird induziert, der an GARE bindet
5. Ca^{2+}, G-Proteine und cGMP sollten ursprünglich beteiligt sein. - aber



Der erste Gibberellin-Rezeptor wurde in Reis gefunden – GID1, (GA-insensitive dwarf)



Vol 437|29 September 2005|doi:10.1038/nature04028 nature

ARTICLES

GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin

Miyako Ueguchi-Tanaka[1]*, Motoyuki Ashikari[1]*, Masatoshi Nakajima[2]*, Hironori Itoh[1], Etsuko Katoh[3], Masatomo Kobayashi[4], Teh-yuan Chow[5]†, Yue-ie C. Hsing[5], Hidemi Kitano[1], Isomaro Yamaguchi[2,6] & Makoto Matsuoka[1]

GID1 ist ein kernlokalisiertes HSL-(hormone sensitive lipase)-Protein – interagiert mit anderen Proteinen

Lipase-Aktivität fehlt!

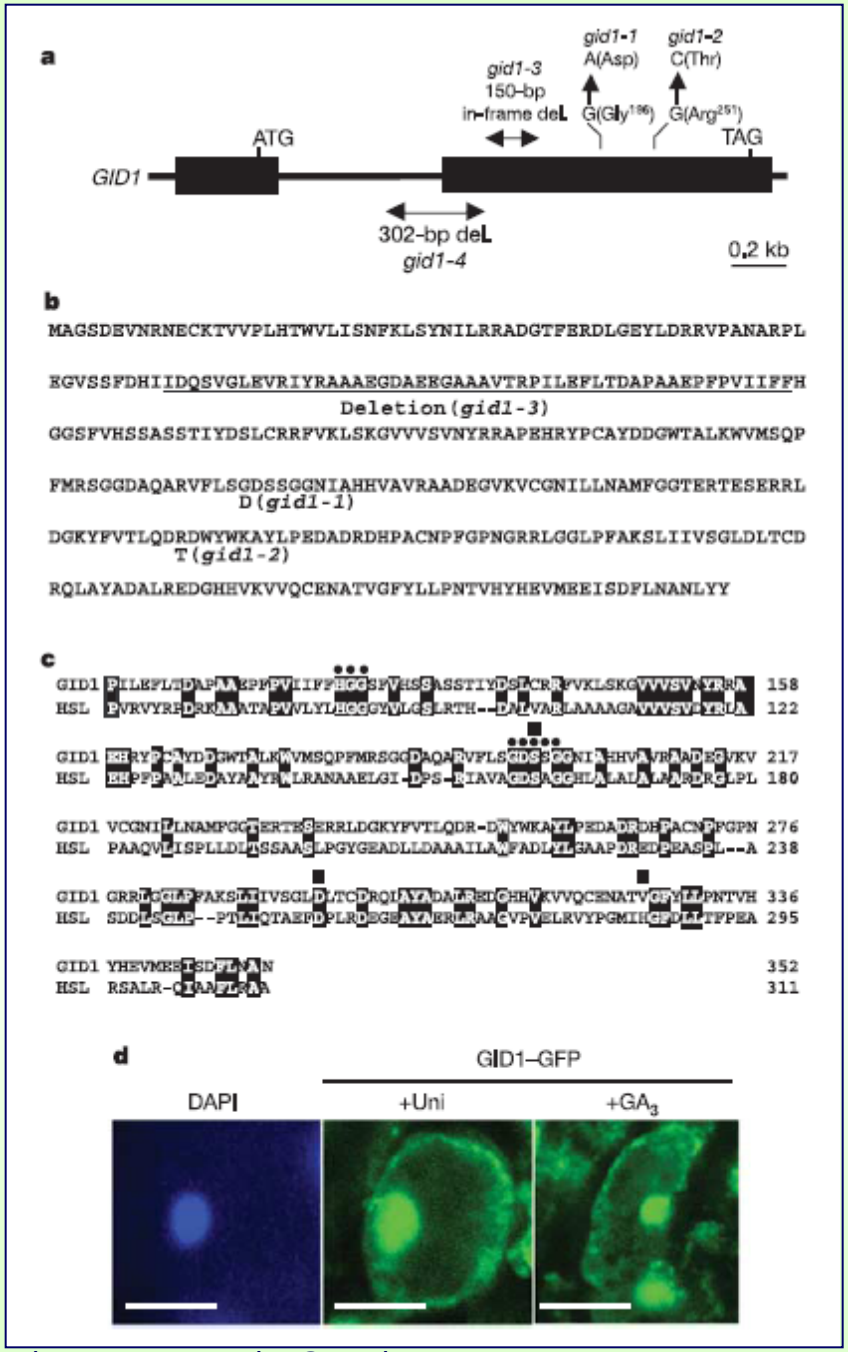


Figure 3 | Structure of GID1. **a**, Structure of the *GID1* gene and its mutation sites in the four *gid1* alleles. The *GID1* gene consists of two exons (thick lines) and one intron (thin line). Nucleotide deletions and substitutions in the four *gid1* alleles are indicated. **b**, The deduced amino acid sequence of *GID1*. The *gid1-1*, *gid1-2* and *gid1-3* mutations are also indicated. **c**, Comparison of amino acid sequences between GID1 and the HSL consensus sequence. Filled circles and squares represent conserved regions and the catalytic triad in the HSL family, respectively. Numbers indicate the position from the start codon. **d**, GFP fluorescence in leaf sections of transgenic rice carrying actin1 promoter−GID1−GFP. Plants were treated with 10^{-6} M uniconazol (+Uni) or 10^{-5} M GA_3 (+GA_3) for 1 week. The left panel represents DAPI staining of the central image. Scale bars, 5 μm.

GID1 bindet hochspezifisch biologisch aktive GA

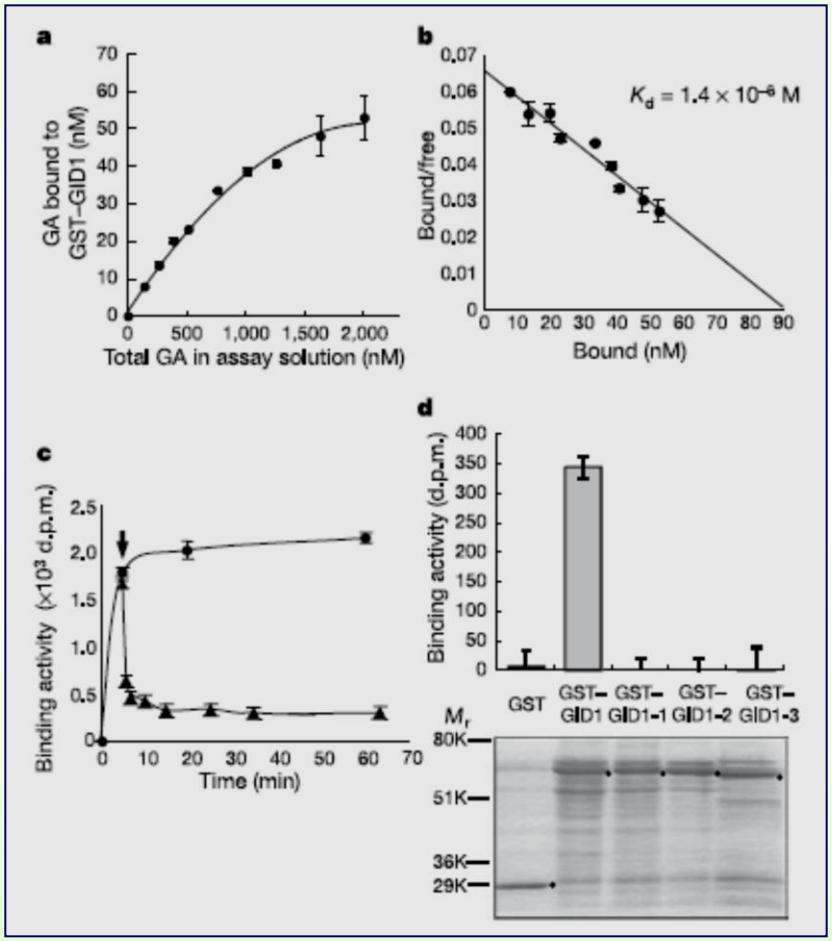
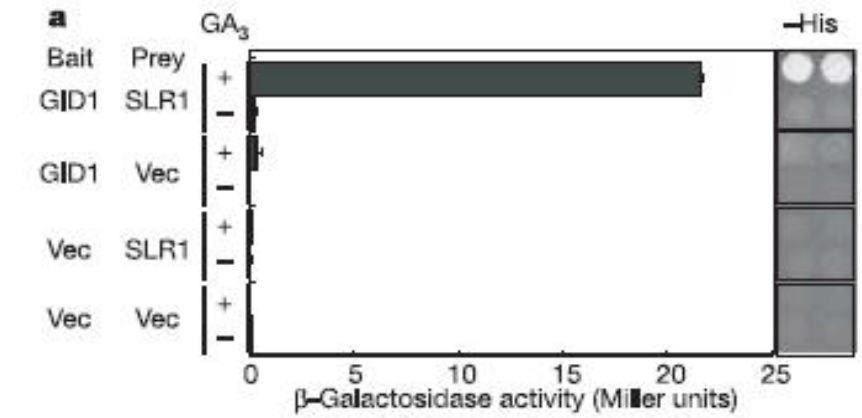
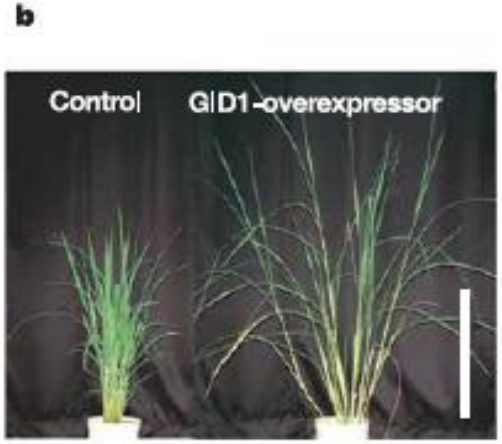


Figure 4 | GA-binding properties of GID1. **a**, GA-binding saturability of GST−GID1. GST−GID1 was incubated with 6 pmol $^3H_4$-16,17-dihydro-GA_4 and increasing concentrations of unlabelled 16,17-dihydro-GA_4 (mean ± s.d.; $n = 3$). Total binding of 16,17-dihydro-GA_4 (labelled plus unlabelled) was calculated from labelled ligand binding. **b**, Scatchard plot of binding data in **a**. K_d values were calculated from three independent experiments ($R^2 = 0.96$). Data are mean ± s.d., $n = 3$. **c**, Association/dissociation rates of $^3H_4$-16,17-dihydro-GA_4 and GST−GID1. Total binding of $^3H_4$-16,17-dihydro-GA_4 reached one-half of the maximum within 5 min (filled circles). Addition of the unlabelled GA_4 (0.125 mM, arrow) reduced $^3H_4$-16,17-dihydro-GA_4 binding to less than 10% within 5 min (filled triangles). d.p.m., disintegrations per minute. Data are mean ± s.d.; $n = 3$. **d**, Top panel: the three mutated GST−GID1 proteins (GST−GID1-1, GST−GID1-2 and GST−GID1-3) did not interact with GA_4. GST, GST tag alone. Data are mean ± s.d.; $n = 3$. Bottom: panel: CBB control. Dots indicate the GST−GID1 proteins or GST tag alone on SDS–PAGE. Approximately equal amounts of protein (about 3.2 μg) were used for the assay.

GID1 interagiert mit SLR1, DELLA-Protein, das TF für GA-abhängige Gene inhibiert





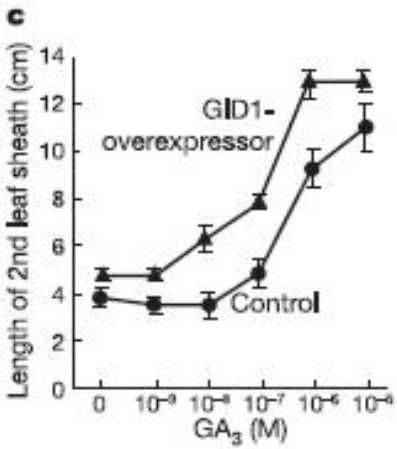
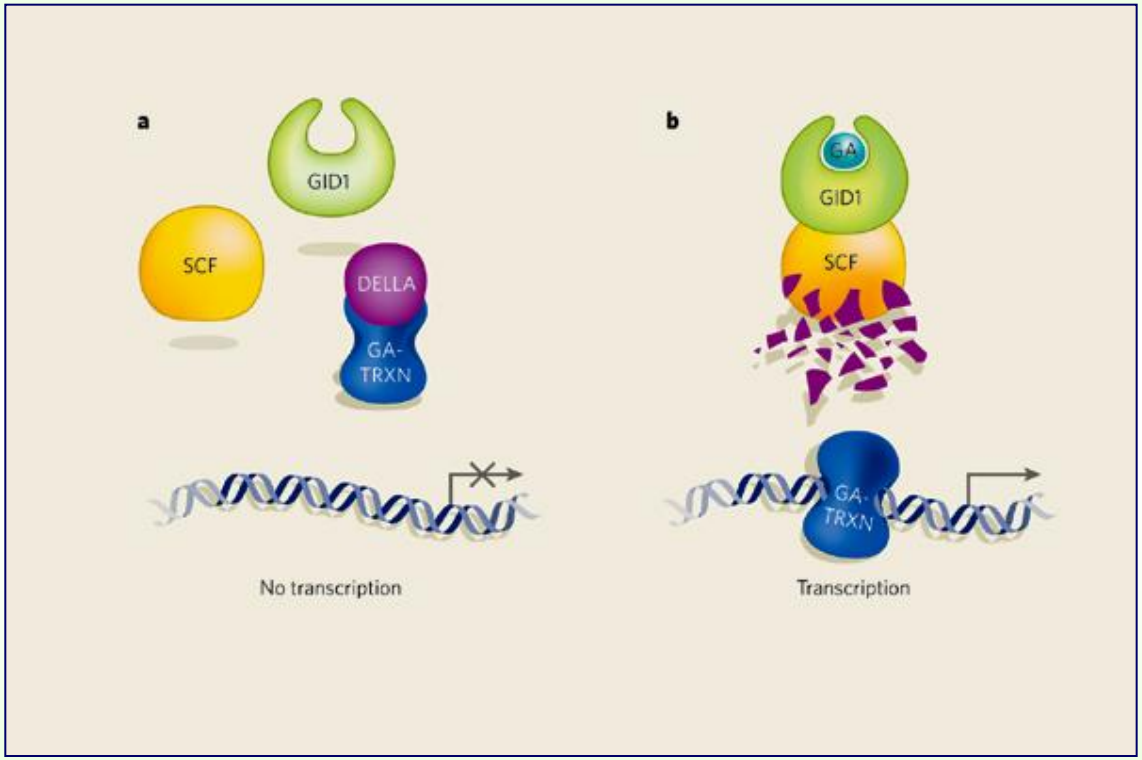
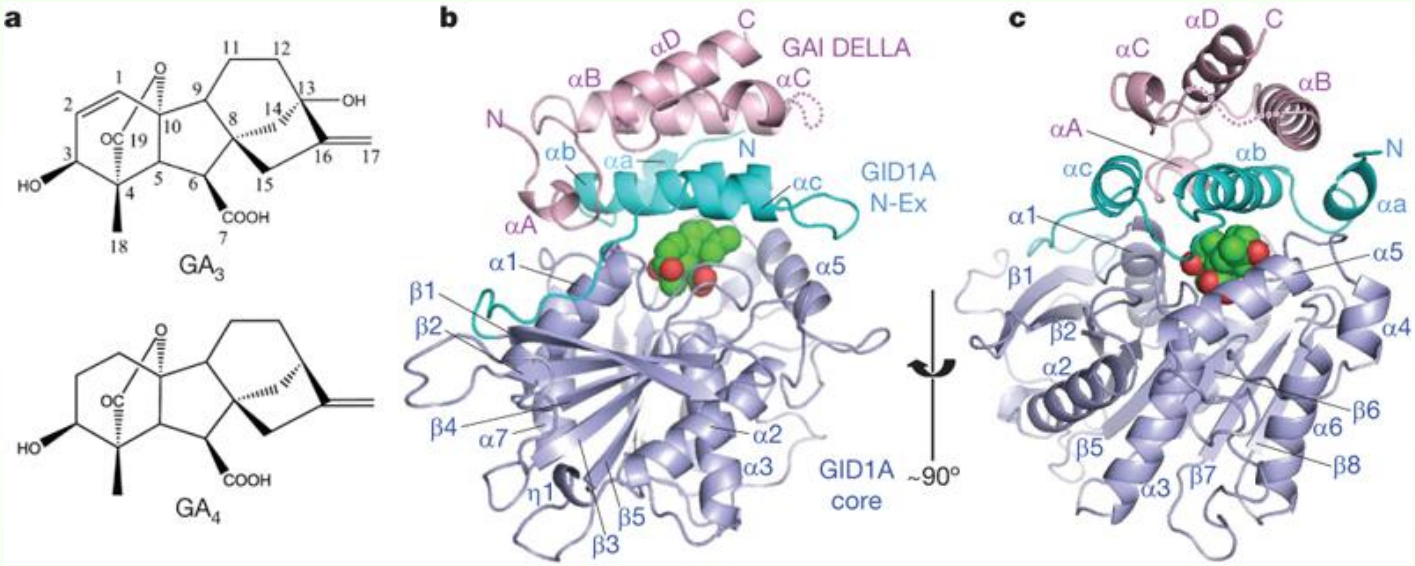


Figure 5 | Interaction between GID1 and SLR1, and the phenotype of a GID1 overexpressor. **a**, GID1 binds to SLR1 in a GA-dependent manner. Left: β-galactosidase activity detected in a liquid assay with Y187 transformants (mean ± s.d.; $n = 3$). Right: growth of HA109 transformants on a −His plate. **b**, Gross morphology of GID1-overexpressor and control plants. Scale bar, 50 cm. **c**, Elongation of the sheath of the second leaf with exogenous GA_3 (mean ± s.d.; $n = 5$).

Modell für die Wirkung von GID1
SLR1, ein DELLA-Protein wird von dem für GA-abhängige Gene zuständigen TF abgelöst und dem Abbau zugeführt

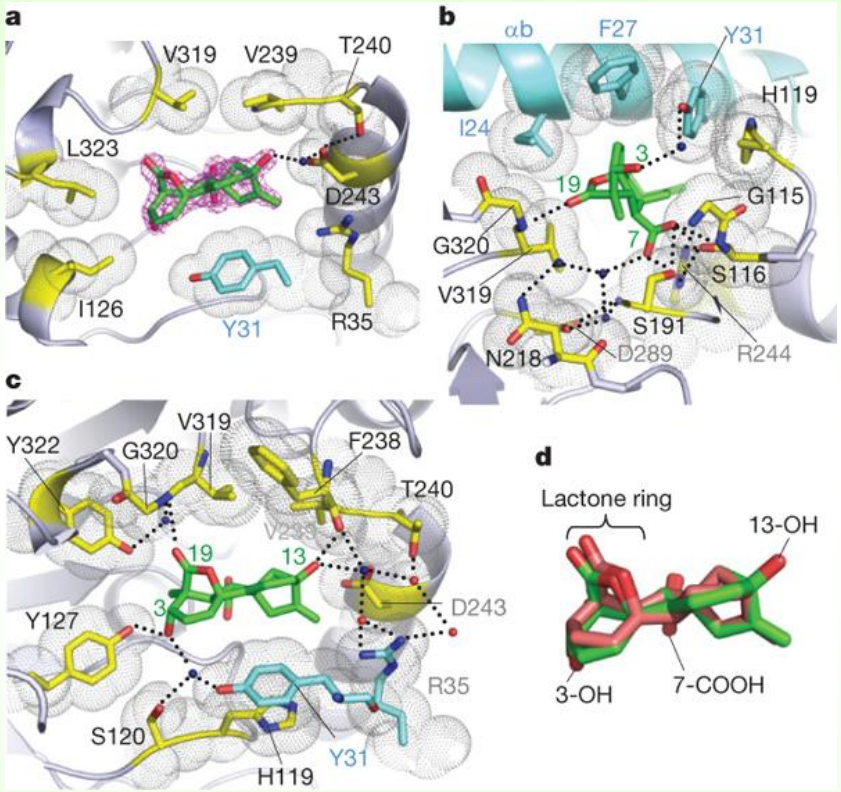


Struktur des GA_3–GID1A–DELLA Komplex.



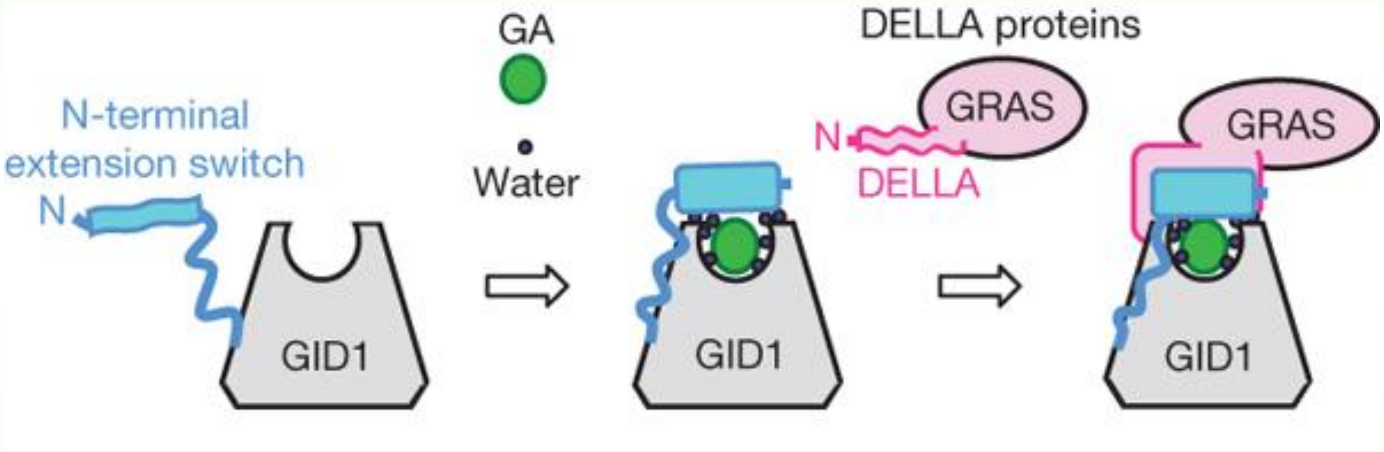
K Murase *et al. Nature* **456**, 459-463 (2008) doi:10.1038/nature07519

Erkennung von GA_3 durch GID1A.



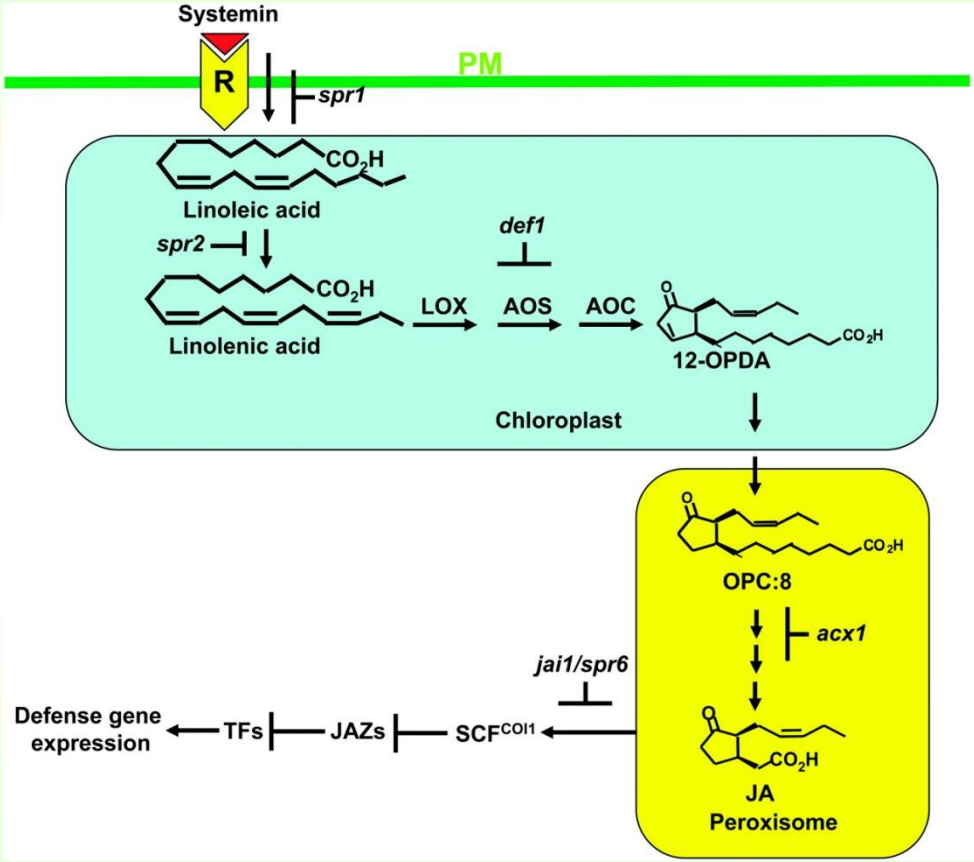
K Murase *et al. Nature* **456**, 459-463 (2008) doi:10.1038/nature07519

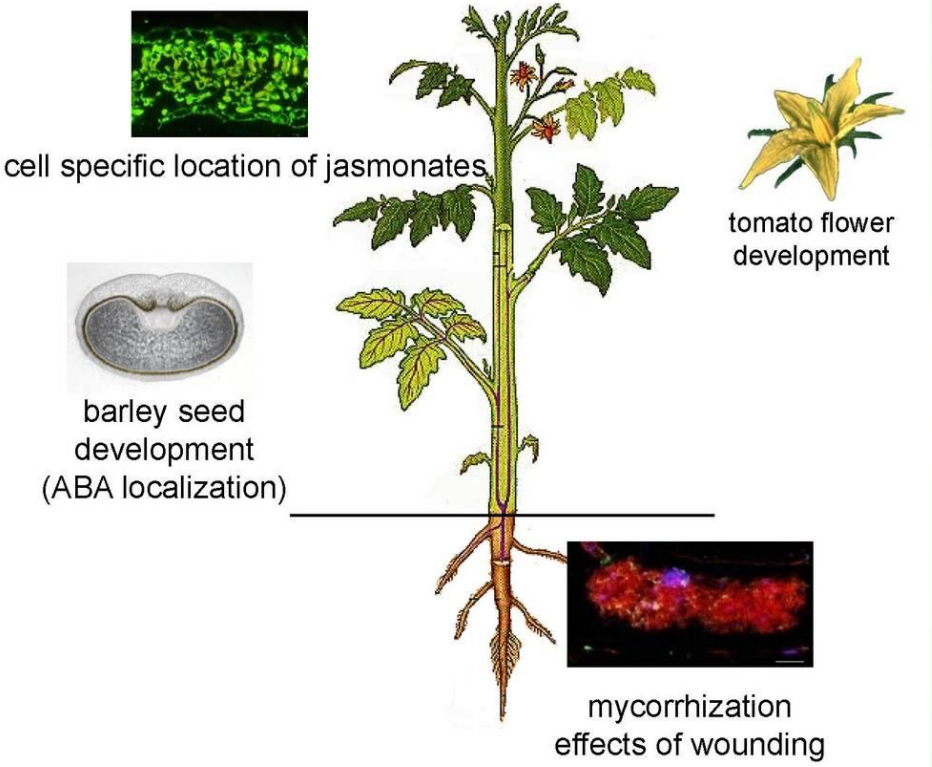
Modell der GA-regulierten GID1–DELLA Protein Interaktion



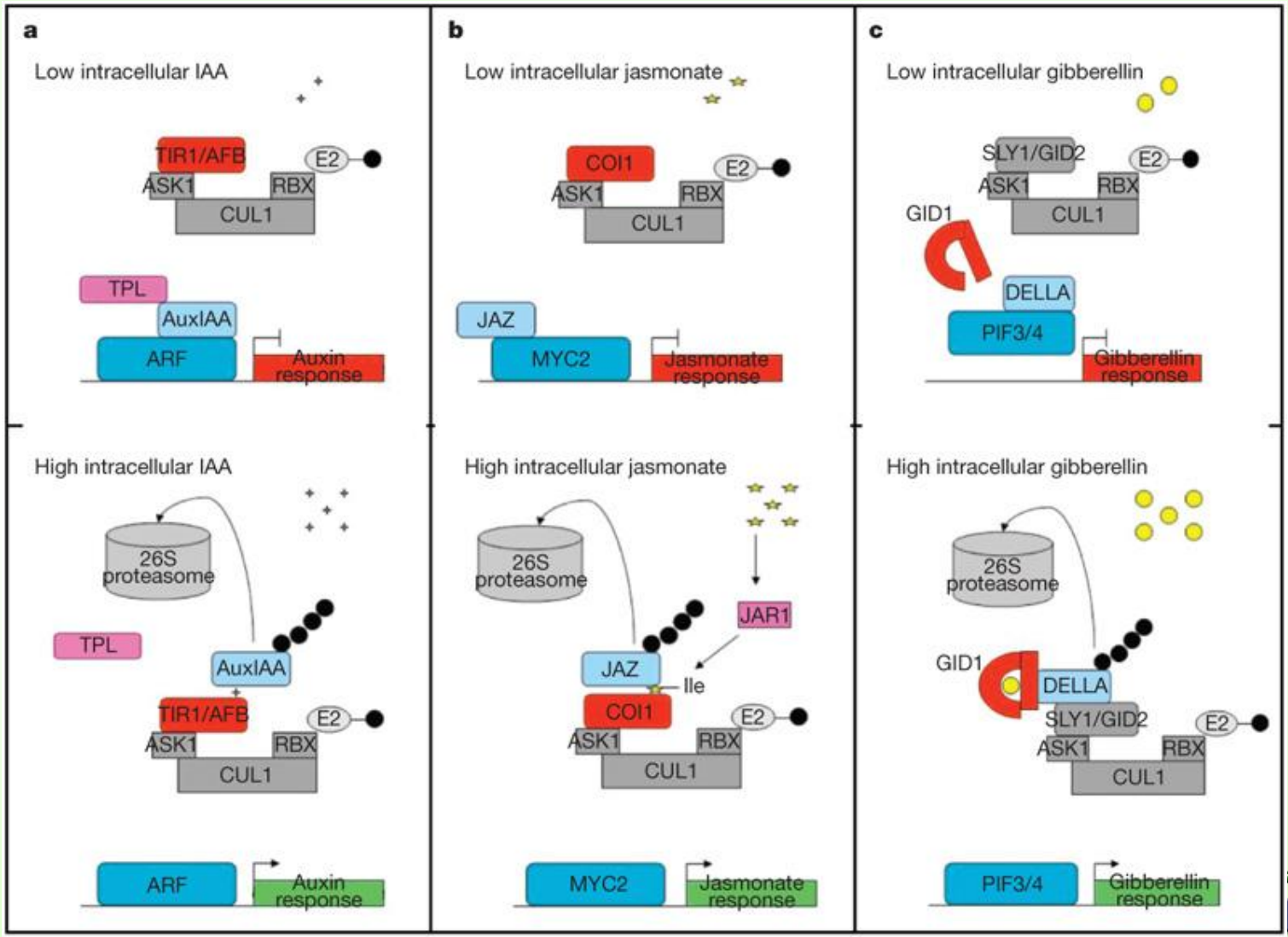
K Murase *et al. Nature* **456**, 459-463 (2008) doi:10.1038/nature07519

Jasmonsäure – Pflanzen-Pathogenabwehr etc.





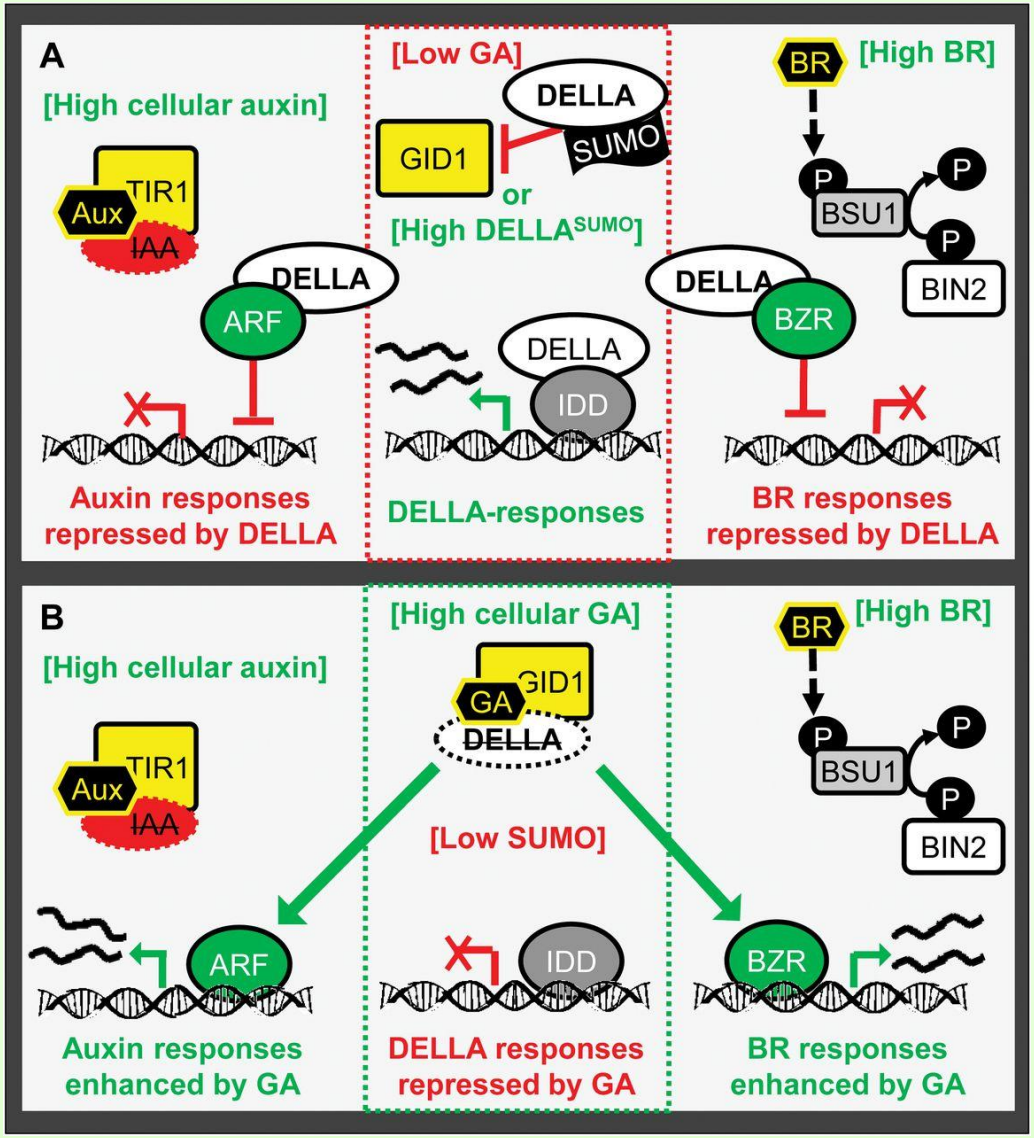
Mindestens 3 Phytohormonrezeptoren interagieren mit dem Ubiquitin-Komplex (in Pflanzen extrem viele F-Box-Proteine)



Crosstalk zwischen Hormonsignalwegen?
Crosstalk versus Wechselwirkung

1. Pflanzenhormone wirken fast immer in Kombinationen, Experimente mit Hormonzusätzen bzw. bestimmten Inhibitoren führen auch zu Effekten, die anderen Hormonen zugeschrieben wurden.
2. Biosynthesen beeinflussen sich gegenseitig (z.B. Auxin stimuliert ACC-Synthese, Ethylen stimuliert dann Aba-Synthese über verstärkte Spaltung von Epoxy-Carotenoiden)
3. Auch Signalwege interagieren, z.B. Mutanten im Ethylenrezeptor haben auch veränderte Reaktionen auf Aba, diese können Gewebe- und Entwicklungs-abhängig variieren.

Crosstalk zwischen GA und Auxin und BA Signalwegen



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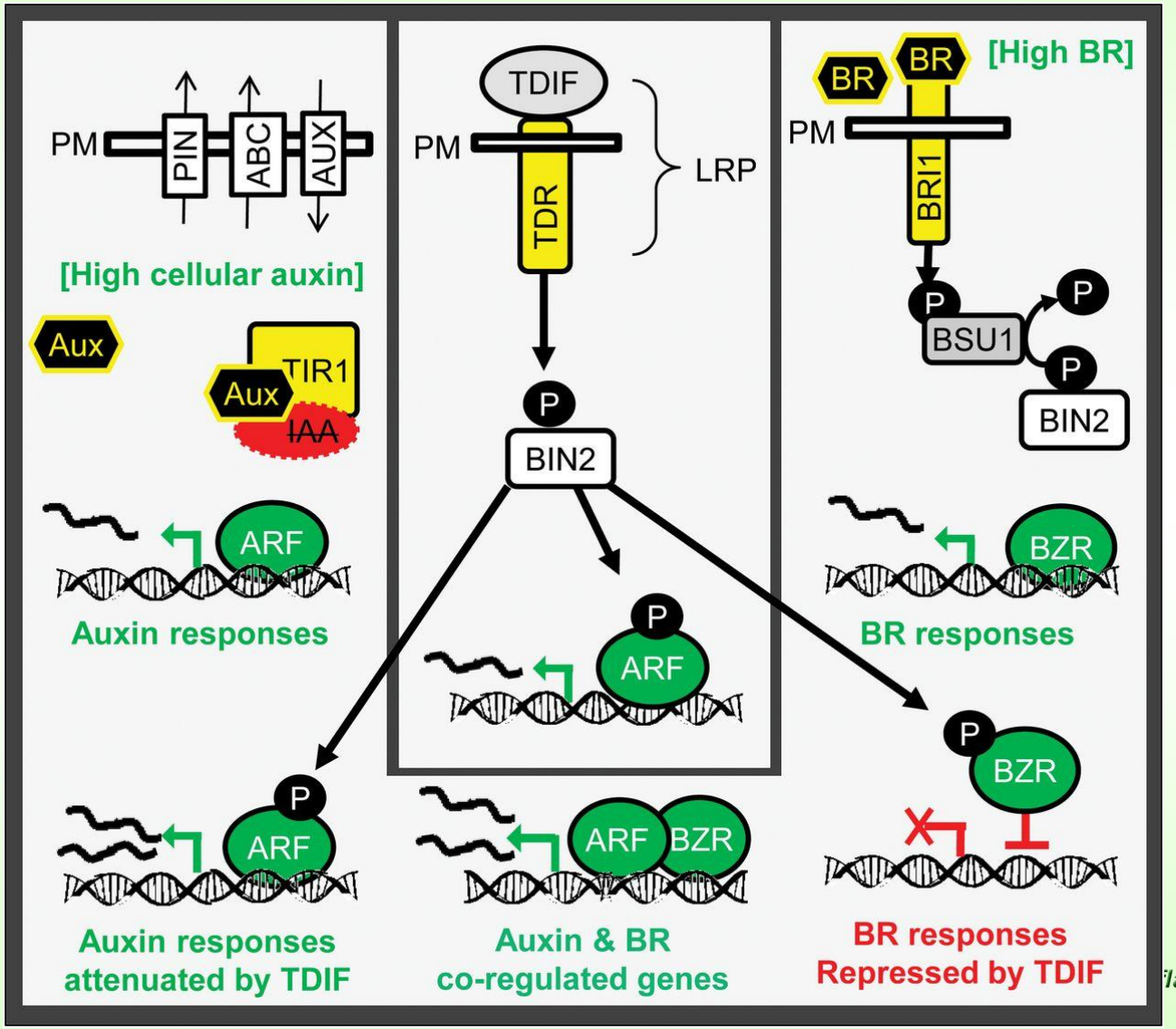
Crosstalk zwischen BA und Auxin Signalwegen



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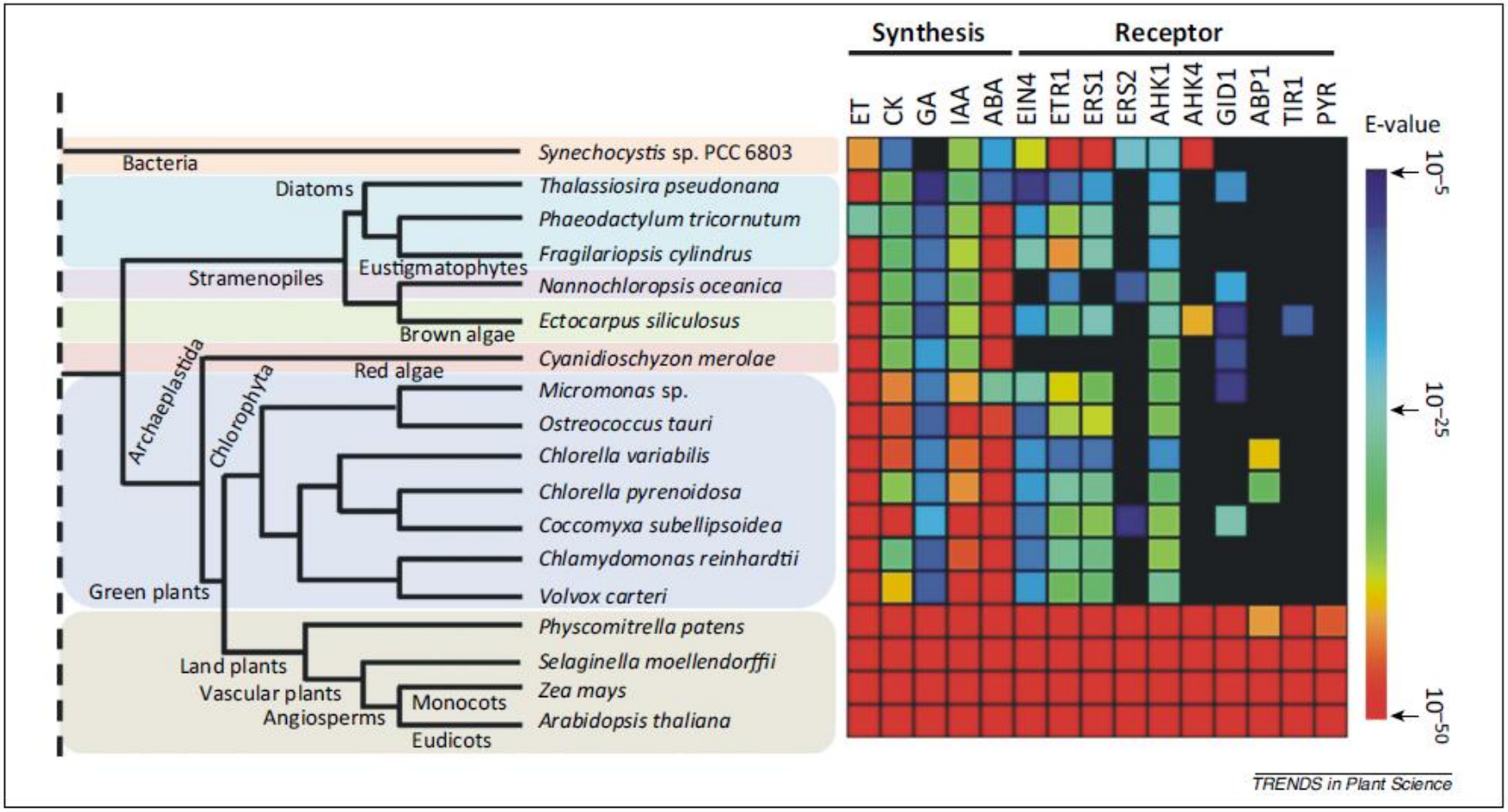

Figure 1. Distribution of phytohormone biosynthetic pathways and phytohormone receptors in microalgae. The phylogenetic tree was built based on ribosomal 18S RNA genes of microalgae using MEGA 6.0, followed by manual modification (according to Baldauf [86]). The color key indicates the similarity of a gene to its closest match, and ranges from low similarity (black) to high similarity (red). Black areas indicate that no BLASTp hits below the e-value threshold ($1e^{-5}$) were found. Red areas indicate orthologs with BLASTp e-values below $1e^{-50}$. For genes with multiple isoforms, homologs with the lowest BLASTp e-values were selected. The similarity of each of the phytohormone biosynthesis pathways between algae and *Arabidopsis* is indicated by the average e-value of all the selected genes. Owing to space limitations, this figure does not fully reflect the complexity of the biosynthetic pathways. For detailed diagrams of the biosynthetic pathways, please refer to the excellent reviews for auxin [38], abscisic acid (ABA) [10], cytokinins (CKs) [10], ethylene (ET) [87], and gibberellins (GAs) [88]. Green algae include *Micromonas* sp. RCC299, *Ostreococcus tauri*, *Chlorella variabilis* NC64A, *Chlorella pyrenoidosa*, *Coccomyxa subellipsoidea* C-169, and *Chlamydomonas reinhardtii*. The red algae are represented by the simple cellular architecture of *Cyanidioschyzon merolae*. Diatoms include *Fragilariopsis cylindrus*, *Phaeodactylum tricornutum*, and *Thalassiosira pseudonana*. Eustigmatophyte algae are represented by *Nannochloropsis oceanica*. The green alga *Volvox carteri* and the brown alga *Ectocarpus siliculosus* are included as representatives to probe the roles of phytohormones in the evolution of multicellularity and differentiation. The bryophyte *Physcomitrella patens* and the lycophyte *Selaginella moellendorffii* were selected to represent phylogenetically basal land plants, and the monocot maize (*Zea mays*) and the dicot *A. thaliana* were included as representatives of seed plants.

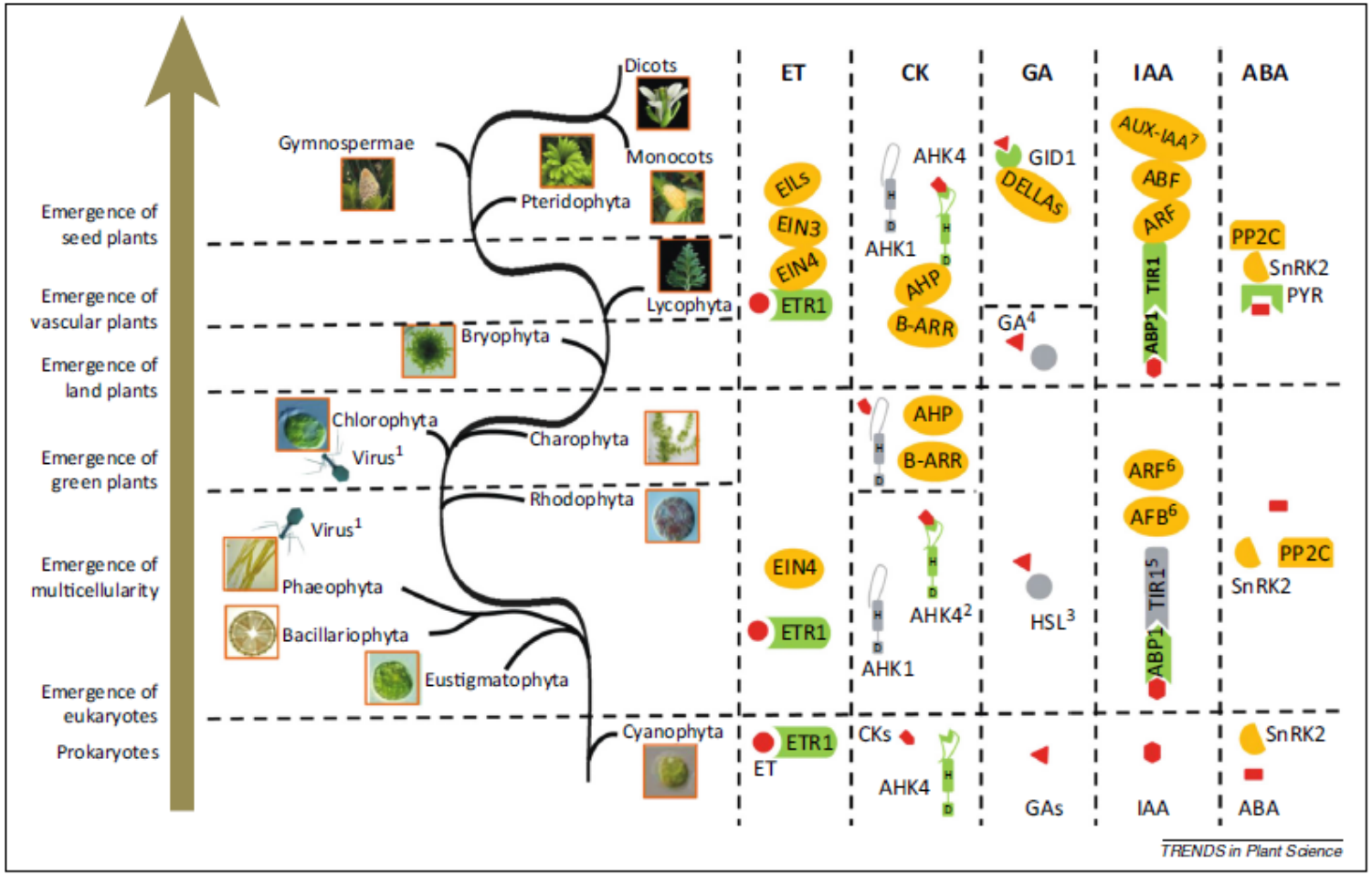

Figure 3. Proposed model for the evolution of phytohormone pathways: ethylene (ET), cytokinin (CK), gibberellin (GA), indole-3-acetic acid (IAA), and abscisic acid (ABA). Biosynthesis of these plant hormones may be largely inherited from cyanobacteria via endosymbiosis, whereas the signaling components may have acquired their current functions through stepwise evolution. Phytohormones, receptor precursors, receptors, and transcriptional factors are shown as red, grey, green, and yellow symbols, respectively. The superscript numbers denote: 1, lateral gene transfer of phytohormone-related elements may occur between viruses and microalgae (i.e., *Chlorella variabilis* NC64A and *Ectocarpus siliculosus*); 2, homologs of AHK4 are only found in cyanobacteria *Synechocystis* sp. PCC 6803 and the brown alga *Ectocarpus siliculosus*; 3, the ancient hormone-sensitive lipase (HSL) is the precursor of plant GID1, and the first functioning GID1 may have evolved in ancient lycophytes; 4, although GAs have not been found in moss, the moss (i.e., *P. patens*) produces and utilizes GA-type diterpenes as an endogenous regulator in development; 5, homologs of auxin receptor ABP1 are found in green algae *Chlorella variabilis* NC64A and *Chlorella pyrenoidosa*, whereas a homolog of auxin receptor TIR1 is found only in brown alga *Ectocarpus siliculosus* but lacks the auxin-binding motifs; 6, homologs of the auxin signaling components ARFs have been found in the green algae *Chlamydomonas reinhardtii*, *Coccomyxa subellipsoidea*, and *Volvox carteri*, and in the brown alga *Ectocarpus siliculosus*, while AFBs have been found in the green algae *Chlorella pyrenoidosa*, *Coccomyxa subellipsoidea*, and in the diatom *Thalassiosira pseudonana*; 7, Brophyta harbor an intermediate form of AUX-IAA that is unlikely to be an active factor in the early auxin response. Note that Bryophyta, Lycophyta and gymnosperms as represented here comprise several lineages and might not be monophyletic. The signaling components in microalgae indicated here have not been experimentally studied, thus whether they are *bona fide* signaling components remains to be validated. Moreover, the presence of homologs of the signaling pathways does not necessarily indicate the occurrence of classical phytohormone responses.