Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

Leveraging synthetic biology approaches in plant hormone research

Chengsong Zhao, Anna Yaschenko, Jose M Alonso and Anna N Stepanova

The study of plant hormones is critical to understanding development, physiology and interactions of plants with their environment. Synthetic biology holds promise to provide a new perspective and shed fresh light on the molecular mechanisms of plant hormone action and propel the design of novel biotechnologies. With the recent adoption of synthetic biology in plant sciences, exciting first examples of successful tool development and their applications in the area of plant hormone research are emerging, paving the way for new cadres to enter this promising field of science.

Address
Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC 27695, USA

Corresponding author: Stepanova, Anna N (atstepan@ncsu.edu)

Current Opinion in Plant Biology 2021, **60**:101998

This review comes from a themed issue on **Plant biotechnology**

Edited by **Yiping Qi** and **Jing-Ke Weng**

https://doi.org/10.1016/j.pbi.2020.101998

Introduction

Synthetic biology is a fairly new area of experimental science at the intersect of molecular biology and engineering. It holds promise to augment traditional approaches in basic research and propel scientific discovery, but plant scientists have been slow to adopt synthetic biology as a tool. There are isolated examples in recent literature of successful implementation of synthetic genetic devices in plant systems, such as Boolean logic gates [1] or an integrase-based memory switch [2], but these are still rare. The field of hormone biology has been one of the first in plant sciences to build synthetic transcriptional reporters [3], but efforts to rewrite or rewire hormone signaling or build *de novo* synthetic circuits have not yet become mainstream in plant biology research. In this review, we describe the current state of the art in plant synthetic biology as it relates to the study of plant hormones. We hope that this brief compilation of studies will serve as an urgent call for new investigators to enter the field of synthetic biology to address long-standing questions in the plant hormone arena.

Synthetic hormone reporters and biosensors

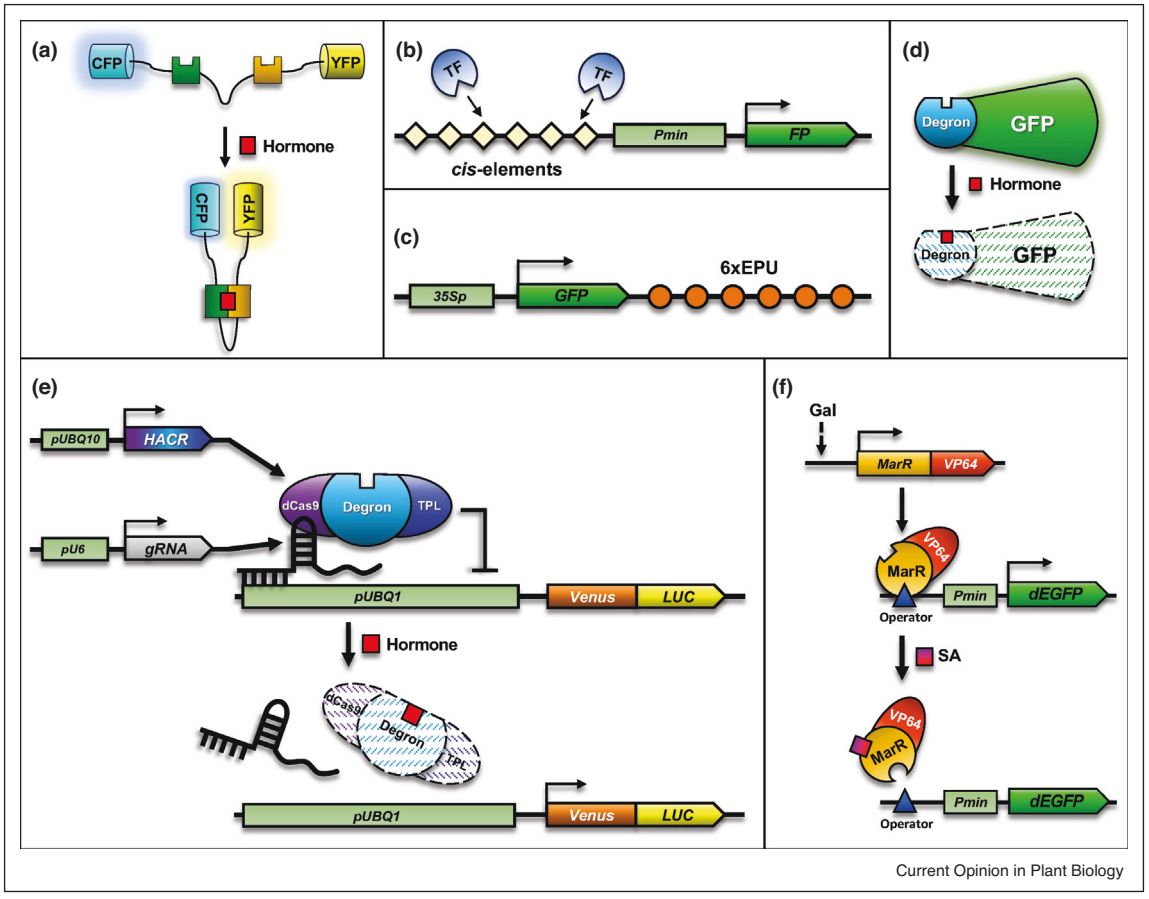
To study the roles of phytohormones in plant growth and development, genetically encoded sensors and reporters are commonly employed as an easy-to-use, widely accessible tool that enables tracking of plant hormone distribution, dynamics, and signaling. The generation of biosensors and reporters often relies on synthetic biology approaches to combine and/or multiplex native and synthetic DNA parts. Different types of genetically encoded hormone monitoring devices have been developed (reviewed in Refs. [4,5]), as described below, with each of them consisting of a sensory module (a part of the device that perceives either the growth regulator itself or a downstream signaling event) and a reporter (a part of the construct that converts that input signal into a visible output).

FRET-based sensors

Förster resonance energy transfer (FRET)-based hormone perception devices consist of two hormone-interacting protein domains linked to a pair of fluorescent protein (FP) reporters, such as CYAN FP (CFP) and YELLOW FP (YFP) (Figure 1a). Illuminating the sensor at the donor CFP's absorption wavelength results in the emission of light from CFP or partial energy transfer to the acceptor YFP and the emission of light in the YFP spectrum. How much energy is non-radiatively transferred to the acceptor depends on the conformation of the sensor, which in turn is affected by hormone binding that dictates the distance and orientation of the donor and acceptor modules with respect to one another. Hormone-binding triggers structural changes in the sensor, thus affecting FRET efficiency, with the CFP/YFP fluorescence emission ratio reflecting the hormone concentration. To date, FRET-based hormone biosensors have been developed for abscisic acid (ABA) [6•,7•,8], gibberellic acid (GA) [9], and auxin [10••] (Table 1).

What would it take to build FRET sensors for all plant hormones? The process of FRET sensor engineering is labor-intensive and time-consuming and often involves testing of several candidate ligand-binding sensory domains and multiple FRET pairs of fluorescent proteins as well as linkers. Extensive, stepwise optimization of the sensory module is typically required that

Figure 1



Phytohormone-responsive synthetic reporters and biosensors. **(a)** Intramolecular FRET sensors: the binding of a hormone to two hormone-interacting protein domains in the biosensor triggers a structural change, which affects the CFP/YFP emission ratio that reflects the hormone concentration. **(b)** A transcriptional reporter consists of a reporter gene (e.g., encoding an FP) driven by a synthetic promoter harboring a tandem of phytohormone-responsive *cis*-elements and a minimal promoter. **(c)** A synthetic translational reporter, 6xEPU, contains a *35S*-promoter-driven *GFP,* followed by a synthetic 3′ UTR harboring a tandem of U-rich *cis*-elements that enable translational inhibition of the reporter protein by hormone ethylene. **(d)** Protein degradation (degron)-based reporters: the binding of a hormone to the degron domain triggers the degradation of the fusion protein. **(e)** dCas9/degron-based reporters: a HACR is a synthetic transcriptional repressor that consists of dCas9, a phytohormone-inducible degron, and a repressor domain. HACR binds to a target constitutive promoter via a gRNA, thus blocking the expression of the downstream reporter gene. The binding of a phytohormone to the degron triggers HACR turnover and activates (de-represses) the transcription of the reporter gene. **(f)** Allosterically regulated synthetic biosensors: a phytohormone-sensing synthetic transcriptional activator consists of a bacterial MarR protein and a strong synthetic transcriptional activator VP64, which activates the reporter *dEGFP* expression by binding to the operator in the promoter. The activity of the synthetic transcriptional activator is antagonized by a phytohormone that binds, allosterically alters, and displaces MarR-VP64 from the promoter and stops reporter transcription.

entails site saturation mutagenesis and/or structure-guided design, with multiple amino acid substitutions usually needed to achieve high ligand affinity and binding specificity, to 'calibrate' the sensor to dynamically respond to physiologically relevant concentrations of the hormone and, ideally, make the binding of the ligand reversible to enable real-time FRET monitoring [11]. Some of the currently available FRET sensors are not optimal as they alter hormone sensitivity in transgenic plants harboring the reporter [6•,7•], bind the hormone irreversibly [9,12], and/or tend to get silenced in plants [6•,10••]. Building and optimizing FRET devices for all plant hormones would likely involve long-term efforts to continue to gradually improve these types of sensors.

Transcriptional reporters

In eukaryotic genes, proximal and distal promoters consist of an array of *cis*-elements recognized by gene-specific transcription factors (TFs) that control the recruitment of RNA polymerase II and general TFs to the core promoter. Synthetic promoters can be built by fusing a tandem of natural or synthetic *cis*-elements (or small

Table 1

Phytohormone biosensors, reporters, and circuits

Hormone	Construct, promoter, or microbial biosensor strain	References
FRET-based sensors		
Abscisic acid	ABACUS1-2μ	[6•]
	ABAleon2.1(5)	[7•]
	ABAleonSD1-3L21	[8]
Auxin	AuxSen	[10••]
Gibberellin	GPS1	[9]
Transcriptional reporter synthetic promoters		
Abscisic acid	*6xABRE_A* (*ABI1* version 6x (−1693 to −1664): *35S* (−90 to −1)	[16]
	6xABRE_R (*RD29A* version 6x (−173 to −144): *35S* (−90 to −1)	
	pD2-2 x DRE (*OsRAB16A* 2x *DRE* (−1080/−1034) plus *pD2* (−303 to −1 containing *pTATA-ABRE*))	[17]
Auxin	*DR5* (7x or 8x CCTttTGTCTC): *35S* (−46)	[3]
	DR5rev 8x (GAGACAaaAGG): *35S* (−46)	
	DR5v2 9x (CCGACAaaAGG): *35S* (−46)	[18]
	DR5 TC 9x (CCTTTTGTCTC): *35S* core	[19]
	DR5 CC 9x (CCTTTTGTCCC): *35S* core	
	DR5 GC 9x (CCTTTTGTCGC): *35S* core	
	DR5 AC 9x (CCTTTTGTCAC): *35S* core	
	pIAAmotif (three ∼50-bp TGTCCC-containing regions of *PtIAA1*, *AtIAA1*, and *AtIAA2*): *35S* (−49)	
Brassinosteroid	5x (GCAGAAACCCCCCGTGTGCCCACTCTCCCC): *35S* (−46)	[20]
Cytokinin	*TCS* (6 direct repeats of aaAATCTacaaAATCTttttGGATT-ttgtGGATTttctagc: *35S* (−46)	[21]
	TCSn (12 sites sharing a AA(A/G)GAT(C/T)TT): *35S* (−44)	[22]
Ethylene	*2(GCC)GUS* 2x (−1164 to 1118): *35S* (−46)	[23]
	EBS 5x (CCTCATGATCAAAGGGGGGATGCACTATTTAAGAT): *35S* (−46)	[24]
	ACE-35S $_{(-46/+8)}$ 2x (CCTAAACCCCAAAACAATC): *35S* (−46)	[25]
Gibberellin	*pGC*^{2} 2x (*OsRep1* −136 to −66): *35S* (−46)	[26]
Jasmonate	*Construct I* (*vspB* −585 to −535): *35S* (−88)	[27]
	pIB (*PDF1.2* −*250* to −*270*): *35S* (−46)	[28]
Salicylic acid	*4xWT* (SFR2 −501 to −473): *35S* (−46)	[29]
Translational reporters		
Ethylene	6x EPU	[31]
Protein degradation (degron)-based reporters		
Auxin	DII-Venus	[33]
	L2min17-Luc	[35]
	R2D2	[18]
Jasmonate	Jas9-Venus	[36]
Strigolactone	SMAX1$_{D2}$-LUC2; SMAX1$_{D2}$-gLUC	[34]
dCas9/degron-based reporter circuits		
Auxin, Gibberellin, Jasmonate	HACRs	[37••]
Bacterial allosterically regulated biosensor circuits		
Salicylic acid	*Acinetobacter baylyi* sp. ADPWH-*lux*: harbors a *Photorhabdus luminescens luxCDABE* gene cassette integrated in the native SA-inducible *salA* operon in the genome	[38,39]
	E. coli XL1-LUX: harbors a plasmid with *A. baylyi salR* gene and *P. luminescens luxCDABE* reporter driven by *A. baylyi salA* promoter	[43]
	E. coli DH5α harbors a plasmid with *Pseudomonas putida NahR* gene and firefly *LUC* reporter driven by *P. putida Psal* promoter	[40]
	Rhizobium leguminosarum Rlv3841: harbors a plasmid with *R. leguminosarum salR gene* and *P. luminescens luxCDABE* reporter driven by *R. leguminosarum salA* promoter	[42]
Yeast allosterically regulated synthetic biosensor circuits		
Auxin	*A. baumanii* lacR-VP64 activator, *dEGFP* driven by a minimal promoter containing an lacR binding site	[46••]
Salicylic acid	*E. coli* MarR-VP64 activator, *dEGFP* driven by a minimal promoter containing a MarR binding site	

promoter fragments containing *cis*-elements) with a minimal (aka core) promoter placed upstream of a reporter such as *GREEN FP (GFP),* ß-*GLUCURONIDASE (GUS)* or *LUCIFERASE (LUC)* (Figure 1b) (reviewed in Refs. [13,14]). The core promoter, exemplified by the commonly used cauliflower mosaic virus *35S* minimal promoter, −46 *CaMV35S*, harbors DNA sequences at which the basal transcription apparatus can assemble. The efficient recruitment of this machinery and, thus, transcription initiation rely on the help of gene-specific transcription factors that bind to proximal and distal promoter elements. By extracting and multiplexing stimulus-responsive *cis*-elements, transcriptional reporters can be built for any endogenous or environmental signal for which DNA response elements are known. Auxin-responsive *DR5* was the first published example of such a reporter [3]. To date, synthetic promoter-based hormone reporters have been described for all hormones but strigolactones (Table 1). Unlike FRET sensors, transcriptional reporters do not sense the growth regulator itself, but rather the level of hormone-induced signaling, since the input in that case is a hormone-activated transcription factor that binds to the synthetic promoter binding sites (reviewed in Ref. [15]). Thus, the ability to develop a synthetic hormone-specific transcriptional reporter for strigolactone and expansion of the toolbox for other plant growth regulators will rest on the identification of specific hormone-responsive *cis*-elements, their multiplexing and the optimization (or *vice versa*, diversification) of repeat sequences and their arrangement in synthetic promoter tandems.

Translational reporters

Regulation of gene expression in eukaryotes is not limited to the control of transcription, and post-transcriptional regulation also plays a key role. Translation of select transcripts in Arabidopsis has been shown to be inhibited by ethylene [30,31] and the first synthetic ethylene-repressible translational reporter, 6xEPU, has been described [31] (Figure 1c, Table 1). This *35S:GFP* reporter harbors a synthetic 3′ untranslated region (UTR) made of a tandem of six U-rich *cis*-elements derived from the 3′ UTR of the *EIN3-BINDING F BOX PROTEIN1 (EBF1)* transcript. That RNA *cis*-element has been shown to be required and sufficient for the ethylene-mediated translation inhibition of *EBF1* or *GFP* [31].

Currently, very little is known about gene-specific translational regulation in response to plant hormones beyond ethylene, but a major leap forward in that area is forthcoming with the adoption by plant scientists of genome-scale translatomic methods such as translating ribosome affinity purification (TRAP), ribosome profiling (Ribo-seq) and others (reviewed in Ref. [32•]). These state-of-the-art technologies, employed in conjunction with growth regulator treatments or hormonal mutants, should lead to the identification of sets of genes whose translational efficiency changes upon exogenous hormone application or in response to genetic perturbations of hormone biosynthesis or signaling [30,31]. Causal *cis*-elements could then be narrowed down using standard molecular biology approaches, and synthetic translational reporters then constructed by stacking candidate elements and introducing them into reporter constructs, as it has been done for 6xEPU [31].

Protein degradation (degron)-based reporters

Among the signaling pathways for plant hormones, those for auxins, GA, jasmonic acid (JA), and strigolactones have similar modes of hormone perception and signal transduction and rely on protein degradation of negative regulators of the pathways, INDOLE-3-ACETIC ACID INDUCIBLEs (Aux/IAAs), DELLAs, JASMONATE ZIM-DOMAIN PROTEINs (JAZs), and DWARF53-like SMAX1-LIKEs (SMXLs), upon hormone perception as a means of activating transcriptional responses (reviewed in Ref. [5]). Likewise, a hallmark of the ethylene pathway is the turnover of its positive signaling components, ETHYLENE INSENSITIVE2 (EIN2) and EIN3, in the absence of the hormone that allows for a rapid ground-state reset; and in brassinosteroid signaling, positive and negative regulators, BRASSINAZOLE RESISTANT1 (BZR1)/ BRI1-EMS SUPPRESSOR1 (BES1) and BRASSINOSTEROID INSENSITIVE2 (BIN2), are degraded in the absence and presence of brassinosteroids, respectively (reviewed in Ref. [5]). By leveraging hormone-regulated protein turnover, degradation-based reporters that harbor a full-length coding region of an unstable pathway component fused to a fluorescent or luminescent protein gene have been developed for all hormones (reviewed in Refs. [4,5,15]). Conversely, the minimal degron domains have been defined only for auxin and JA pathway repressor proteins, enabling construction of much more compact, synthetic, degron-based hormone sensors (Table 1). For example, to map auxin response and distribution, the DII-VENUS sensor was engineered by fusing the gene for the fast-maturing FP VENUS to the DII auxin-interaction domain of *IAA28* under the control of the constitutive *35S* promoter. In the presence of auxin, DII-VENUS is rapidly degraded through the SKP/CULLIN/F-BOX (SCF)-type E3 ubiquitin-ligase complexes $SCF^{TIR1/AFB1-5}$, resulting in the loss of fluorescence that reflects relative auxin abundance and distribution at cellular resolution [33] (Figure 1d). It remains to be tested if short, self-sufficient degrons can be defined for all other aforementioned hormones that rely on protein degradation. A recent attempt to narrow down the minimal domain of strigolactone-degradable SMAX1 lead to the discovery that for rapid protein degradation, a large C-terminal D2 domain is needed, but even D2 was not as effective as full-length SMAX1 at destabilizing LUC in

the context of two different ratiometric reporters [34] (Table 1). Engineering of synthetic, minimal-degron reporter constructs for GA, brassinosteroid and ethylene signaling proteins may face similar hurdles.

dCas9/degron-based reporter circuits

Another type of synthetic constructs that utilize a hormone-regulated degron domain but lead to reporter induction rather than turnover rely on a hormone-activated Cas9-based repressor (HACR) device [37••]. A HACR is an artificial transcriptional repressor whose degradation is triggered by a hormone (auxin, GA, or JA), much like the turnover of the DII-VENUS reporter protein described above (Figure 1d). In the HACR system, the reporter gene is driven by a constitutive promoter and the reporter protein is stable. However, in the absence of the hormone, the reporter is transcriptionally repressed via guide RNA (gRNA)-mediated recruitment of HACR to its promoter. In the presence of the hormone, degradation of HACR relieves the repression and induces the reporter (Figure 1e). For example, an auxin-responsive HACR is formed by fusing a deactivated Cas9 (dCas9) protein to a highly sensitive auxin-induced degron and a repressor domain (the first 300 amino acids of the TOPLESS (TPL) repressor) [37••] (Figure 1e, Table 1). A dual *VENUS-LUC* nuclear reporter is driven by the constitutive *UBIQUITIN1* (*UBQ1)* promoter, with the HACR targeted to it by a *U6* promoter-driven gRNA [37••]. Equivalent HACR devices have also been developed for GA and JA by replacing DII with full-length GA-responsive DELLA proteins GIBBERELLIC ACID INSENSITIVE (GAI) or REPRESSOR OF GA1 (RGA1) and a JA degron of JASMONATE-ZIM-DOMAIN PROTEIN9 (JAZ9), respectively [37••]. Expansion of the HACR toolbox to strigolactones, ethylene and brassinosteroids is easily foreseeable but would be much more elegant with the use of currently undefined minimal degron domains.

Outsourcing hormone detection to microbes

Since genetically encoded hormone sensors need to be either stably or transiently expressed in plants, they are not readily compatible with transformation-recalcitrant plant species. One way to circumvent this problem is to delegate hormone biosensing to a microbe, with the idea that a specialized bacterial or fungal strain can be engineered to detect a growth regulator of interest either directly in the plant or in a plant lysate. In the past 15 years, several salicylic acid (SA)-sensing strains of *Escherichia coli*, *Acinetobacter baylyi*, *Pseudomonas putida* and *Rhizobium leguminosarum* have been developed [38–43]. All of these engineered microbes leverage an allosterically controlled SA-binding LysR-type transcriptional regulator (LTTR) protein (salR or a related transcription factor, nahR) that interacts with the *salA* or *Psal* gene promoter and in the presence of SA turns on a downstream luminescent reporter gene (Table 1). Thus far, only one of these biosensor strains, *Acinetobacter* ADPWH-*lux*, was demonstrated as functional in plant tissues and enabled detection of free SA not only *in vitro* in Arabidopsis leaf lysates, but also *in planta* in the apoplastic space of infiltrated tobacco leaves [39,44,45].

The latest addition to the thus far limited collection of microbes harboring genetically encoded synthetic tracking devices for plant hormones are the man-made biosensor circuits developed by Garcia and co-authors for direct sensing of auxin and SA in yeast [46••] (Figure 1f). The hormone biosensors consist of a bacterial hormone-binding protein gene (*A. baumanii IacR* for auxin or *E. coli MarR* for SA) fused in frame to the herpes simplex virus-derived *VP64* transactivation domain, a nuclear localization signal, and a mouse ORNITHINE DECARBOXYLASE (ODC) degron for rapid protein turnover, together forming a hormone-sensing synthetic transcriptional activator gene that is driven by a yeast galactose-inducible promoter. A fluorescent reporter, *dEGFP*, in these devices is driven by a minimal promoter harboring IacR or MarR binding sites upstream of the TATA-box. dEGFP is intentionally made unstable via tagging with an uncleavable version of ubiquitin (G76V) in the reporter N-terminus. Hormone binding to the biosensors (auxin to IacR or SA to MarR) allosterically alters and dislodges the synthetic transcription factors from the promoter of *dEGFP*, thus stopping reporter transcription and leading to the loss of fluorescence upon dEGFP degradation [46••] (Figure 1f). Importantly, these new yeast devices have not yet been tested for responses to auxin- and SA-related compounds, so it remains to be seen whether the synthetic biosensor circuits can exclusively detect the active forms of the two hormones while being blind to inactive hormone conjugates, precursors or isomers. Likewise, the suitability of these yeast strains for *in planta* application is yet to be demonstrated.

Rebuilding hormone signaling pathways in yeast or protoplasts

Phytohormone signaling pathways are often complex, involve multiple functionally redundant family members, and interact with other signals. Hence, it is often challenging to analyze the functions of hormone-related genes in plants. One way to bypass this complexity is to move selected pathway components to a heterologous system such as yeast. Yeast cells lack auxin signaling but share the SCF-mediated degradation pathway with plants. Havens *et al.* [47] leveraged that property and successfully integrated Arabidopsis auxin co-receptors TRANSPORT INHIBITOR RESPONSE1 (TIR1)/AUXIN F-BOX PROTEIN2 (AFB2) and Aux/IAA into the yeast ubiquitin pathway making it feasible to evaluate plant Aux/IAA degradation dynamics in a yeast chassis. This semi-synthetic yeast auxin signaling system has then been employed to characterize the motifs flanking the auxin-inducible degron [47,48], study the effects of DII

domain mutations on Aux/IAA protein turnover [49], examine natural variation in auxin signaling F-box proteins from different Arabidopsis accessions [50], and monitor the dynamics of auxin-triggered maize Aux/IAA degradation [51].

Furthermore, Pierre-Jerome *et al.* [52••] generated a complete auxin response circuit in yeast (*Saccharomyces cerevisiae*) (ARC^{Sc}). The ARC consists of the full suite of auxin signaling components, from auxin perception to a transcriptional response: an F-box TIR1/AFB protein and an Aux/IAA (together functioning as co-receptors for auxin), TPL (functioning as a transcriptional co-repressor along with Aux/IAAs), a transcriptional activator AUXIN RESPONSE FACTOR (ARF), and a reporter comprised of a synthetic promoter harboring auxin-response *cis*-elements driving *GFP*. In this system, all other essential components for protein degradation, transcription, and translation are provided by the yeast chassis. This semi-synthetic ARC^{Sc} setup in yeast has been instrumental for the functional analysis of Arabidopsis auxin signaling components [52••,53] and has recently been replicated with maize proteins [54]. The beauty of these ARC^{Sc} systems is that they provide a convenient modular platform for the functional dissection of individual pathway components while keeping the rest of the machinery invariable [55]. Yeast chassis was also employed to study DNA binding preference of Arabidopsis and maize activator ARFs by constitutively expressing them alongside with multiple synthetic promoter variants driving a *VENUS* reporter gene to test the effect of copy number and orientation of auxin response elements on ARF binding [56].

The success of utilizing yeast to characterize the auxin signaling machinery suggests that similar strategies can be applied to dissecting other hormone signaling pathways. In fact, Ruschhaupt *et al.* [57•] have successfully reconstructed an Arabidopsis ABA signaling pathway in yeast to test the core signaling components, from the receptors to ABA-responsive transcription factors. While yeast is an excellent heterologous system, plant cells can also be used for ectopic expression of signaling components, although with the caveat that endogenous machinery may complicate data interpretation. Li *et al.* [58] have rebuilt the core JA signaling pathway in transiently transformed Arabidopsis protoplasts and leveraged this system to clarify the functions of specific sequence motifs in CORONATINE-INSENSITIVE1 (COI1) and JAZ1 co-receptors.

Rewriting plant hormone signaling

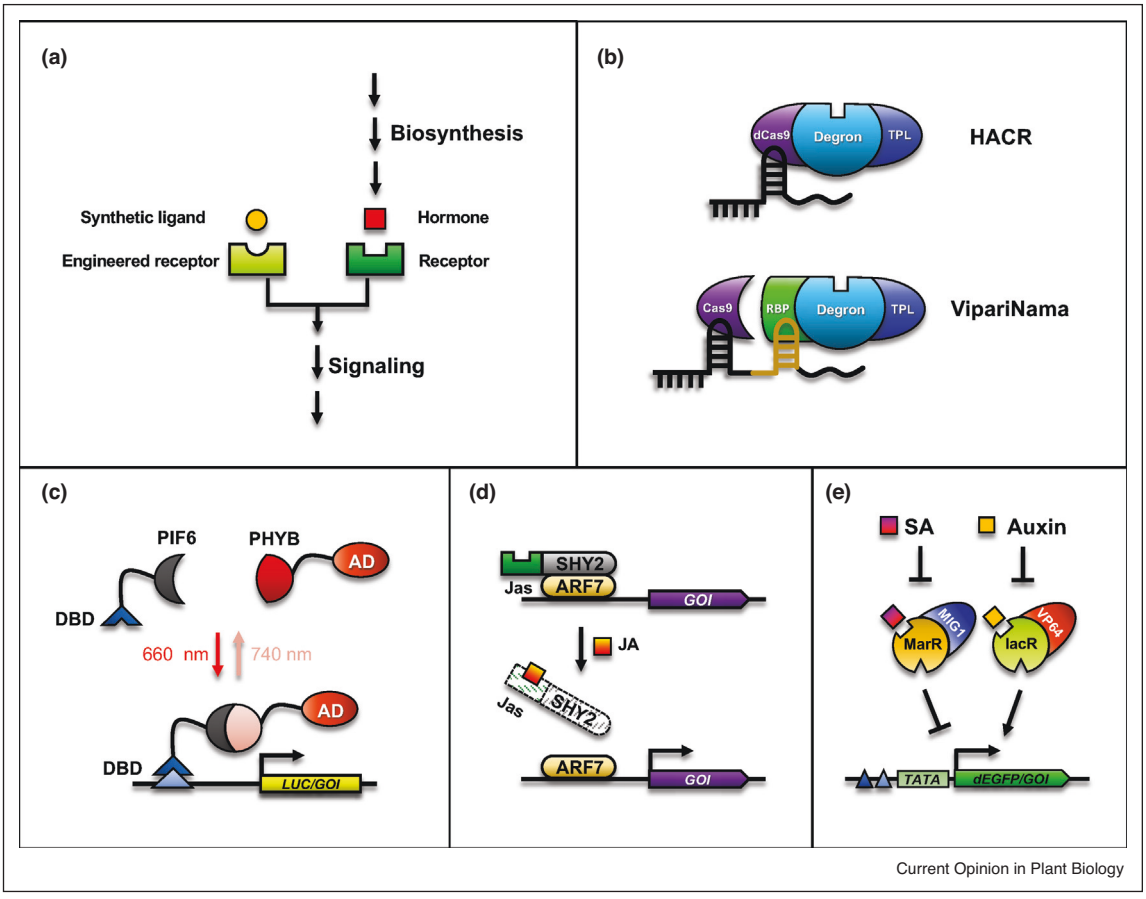
Engineering synthetic hormone receptors

While a long-term goal of plant synthetic biology is to engineer entire orthogonal hormone-signaling pathways that can be triggered in response to synthetic growth regulators, to date, good progress has been made only towards generating semi-synthetic hormone perception systems. Park *et al.* [59••] have succeeded at engineering an ABA receptor PYRABACTIN RESISTANCE1 (PYR1) to respond to a synthetic ligand instead of ABA. The experimental pipeline involved screening individual amino acid substitutions in the 25 residues that make up the PYR1 ligand-binding pocket for mutants that cannot bind to ABA (Figure 2a). Then directed mutagenesis of PYR1 was employed to optimize response sensitivities to mandipropamid, a synthetic ABA analog that is not perceived by natural PYR1. The resulting hextuple mutant, $PYR1^{MANDI}$, but not wild-type PYR1, was able to bind to its PROTEIN PHOSPHATASE 2C (PP2C) co-receptor HYPERSENSITIVE TO ABA1 (HAB1) in response to mandipropamid in *Nicotiana benthamiana*. In stably transformed *35S:PYR1*^{MANDI} Arabidopsis plants treated with mandipropamid, the synthetic receptor triggered ABA-like transcriptional responses, inhibited seed germination and root growth, and conferred drought tolerance, indicating that it worked through endogenous downstream ABA signaling machinery [59••].

Similarly, based on the crystal structure of the TIR1-IAA-AUX/IAA complex, Uchida *et al.* [60•] employed the bump-and-hole strategy to develop an orthogonal synthetic auxin-TIR1 pair, cvxIAA-ccvTIR1, that can trigger auxin-like responses in the presence of a synthetic auxin analog cvxIAA but not of native auxins. In ccvTIR1, a key residue of the auxin binding pocket was mutated (F79G) to make room (aka hole) for an aryl group (aka bump) added to IAA at the 5-position to make cvxIAA. Unlike cvxIAA, neither IAA nor its commonly used synthetic analogs, 1-naphthaleneacetic acid (1-NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D), were able to prompt the interaction between ccvTIR1 and its co-receptor IAA3. *In vivo* assays indicated that cvxIAA could activate auxin transcriptional outputs via ccvTIR1, but did not affect the endogenous auxin transport or response [60•].

Another remarkable example of plant hormone receptor engineering stems from an attempt to uncouple plant JA signaling from responses triggered by pathogenic bacteria. Interestingly, some strains of *Pseudomonas syringae* can secrete a JA-Isoleucine (JA-Ile)-mimicking toxin, coronatine (COR), to hijack JA signaling and promote disease susceptibility (reviewed in Ref. [61•]). One approach to stop these pathogens is to separate endogenous JA signaling from pathogen hijacking via COR. Zhang *et al.* [62] modified a JA-Ile coreceptor, COI1, by making a single amino acid substitution, A384V, in the Arabidopsis COI1 ligand-binding pocket, leading to a greatly reduced affinity for the bacterial COR but leaving endogenous JA-Ile binding and downstream signaling unaffected. Transgenic Arabidopsis plants expressing the engineered JA-Ile receptor, $COI1^{A384V}$, gain resistance against *P. syringae pv. tomato (Pst)* DC3000 and *P. syringae pv.*

Figure 2



Synthetic switches and regulatory circuits that rewrite phytohormone signaling.
(a) A hormone receptor is engineered to specifically perceive a synthetic ligand, but relies on native downstream signaling components. **(b)** A HACR and its split ViparNama derivative [in which the synthetic TF is reconstituted *in planta* with the help of an extended gRNA scaffold that recruits both Cas9 and an RNA-binding protein (RBP) fused to a degron and a transcriptional effector TPL] are designed to target hormone biosynthesis or signaling genes via gRNA. **(c)** Red light-regulated genetic toggle switch selectively induces or represses hormone signaling. Under red light (660 nm), the light photoreceptor PHYB interacts with PIF6, reconstituting the split TF that brings together a DNA binding domain (DBD) fused to PIF6 and the transcription activation domain (AD) fused to PHYB, to control a *LUC* reporter or hormone signaling gene. The system is reversible in the dark or in the presence of far-red light (740 nm). **(d)** By fusing a Jas-degron motif of JAZ1 to an Aux/IAA protein SHY2/IAA3, auxin-responsive gene expression can be regulated by JA. **(e)** Allosterically regulated synthetic biosensor circuits are designed to study the crosstalk between auxin and SA in yeast. An SA-sensing synthetic transcriptional repressor (MarR-MIG1) and an auxin-sensing transcriptional activator (lacR-VP64) block and induce the expression of the reporter gene (*dEGFP*), respectively, with the activity of these synthetic effectors antagonized by SA and auxin. Triangles upstream of the reporters or genes of interest (GOI) represent the DNA binding sites for the corresponding DNA-binding proteins.

maculicola (Psm) ES4326, while still maintaining a high level of defense against insects [62].

Rewiring plant hormone signaling events

The second direction that leverages synthetic biology tools to study plant hormone signaling and response has been to rewire specific signal transduction events or to tune hormone biosynthesis or transport using various types of synthetic genetic devices. For example, dCas9-based hormone-degradable HACRs that have been described above in the contest of reporters as effective tools to monitor endogenous hormone distribution can also be employed to target and repress endogenous genes of interest. Khakhar *et al.* [37••] manipulated auxin responses in Arabidopsis by recruiting with the help of a gRNA an auxin-degradable HACR to the promoter of an auxin-inducible auxin transporter gene *PIN-FORMED1* (*PIN1*). HACR expression reduced the strength of the transcriptional feedback of auxin on *PIN1* expression, leading to fewer branches in transgenic HACR-expressing Arabidopsis plants [37••]. Likewise, in a follow-up study, Khakhar *et al.* [63••] employed a GA-degradable HACR to downregulate a GA biosynthesis gene *GIBBERELLIN 20 OXIDASE1-3* (*GA20ox1-3*) in

N. benthamiana leading to dampened GA responses and reduced leaf size in the absence of exogenous GA (Figure 2b). The effect of GA treatment was not reported. One of the innovations of that study included developing viral RNA (ViN) vectors with sufficient cargo capacity to deliver gRNAs into plants stably transformed with a *GA-HACR* construct [63••]. Furthermore, a set of stable Arabidopsis transgenic lines (aka ViparíNama) was developed that express *Cas9* and a transcriptional effector construct that combines an *RNA-binding protein* (*RBP*) gene with either a repressor domain of *TPL*, a *VP64* activation domain, or *TPL* with an auxin degron (Figure 2b). In the presence of ViN-delivered gRNAs that harbor a 3′ sequence extension recognized by the specific RBP, these synthetic RNA-binding transcriptional effectors can be recruited to Cas9/gRNA, thus conferring a transcriptional activation or repression function to the tri-partite ribonucleoprotein complex. The nuclease activity of Cas9 is suppressed by reducing the length of the gRNA spacer sequence from 20 to 14 nt. By transiently expressing ViN gRNAs targeting endogenous GA receptor genes *GA INSENSITIVE DWARF1* (*GID*) in an Arabidopsis line stably co-expressing *Cas9* and the *RBP-TPL* construct, plants with reduced rosette size were obtained [63••]. A similar approach was also used to target and repress a tomato GA co-repressor *DELLA* gene *PROCERA* in a stably transformed tomato line expressing *Cas9*, except in that case the *RBD-TPL* construct and *PROCERA*-targeting gRNAs were both delivered transiently via ViN vectors. This led to an increase in both the internode length and leaf size in systemic leaves, consistent with the enhanced activation of the downstream GA responses in *DELLA*-depleted plants [63••]. This innovative study demonstrates the utility of synthetic Cas9-based split transcriptional effectors in rational manipulation of hormonal pathways.

Another illustrative example of how a synthetic device can control an endogenous hormone signaling pathway is a red light-regulated genetic toggle switch developed by Muller *et al.* [64•] to activate auxin signaling in tobacco. Initially utilized in mammalian cells [65], the device is based on the light photoreceptor PHYTOCHROME B (PHYB) and PHYTOCHROME-INTERACTING FACTOR6 (PIF6) that physically associate with one another in the presence of red light (660 nm) but fall apart under far-red light (740 nm). By fusing *PIF6* to a DNA-binding domain and *PHYB* to a transcriptional activation domain and providing a *LUC* reporter driven by a promoter recognized by the DNA binding domain, the switch was validated as inducible by red light and repressible by far-red light or darkness in tobacco [64•] (Figure 2c). The device was then employed to selectively induce the ectopic expression of *TIR1* auxin receptor or of *TIR1*-targeting $miRNA^{TIR1}$. As expected, red-light-stimulated production of extra *TIR1* resulted in the destabilization of a degron-based ratiometric auxin sensor L2min17-Luc (Table 1), whereas induction of $miRNA^{TIR1}$ led to the reporter stabilization, thus confirming the applicability of the synthetic light-regulated toggle switch to controlling hormone signaling in plants [64•].

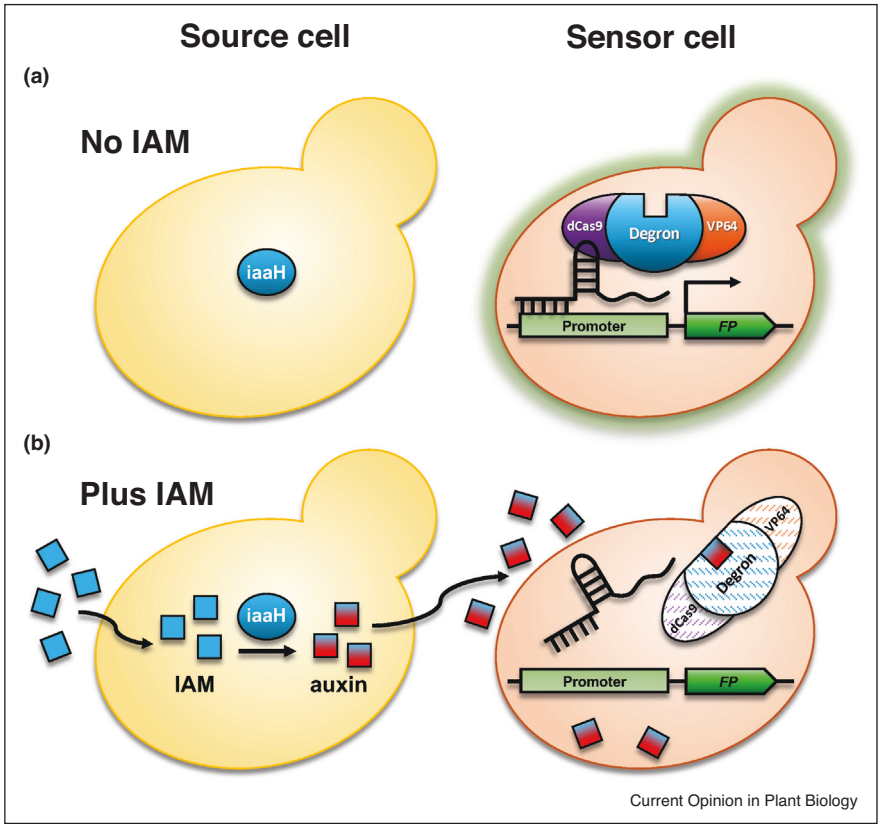
A conceptually different pathway rewiring strategy was recently reported by Li *et al.* [66]. Building on the similarity of the auxin and JA signaling pathways, where TIR1 or COI1 F-box receptor proteins form SCF complexes upon binding to their respective hormones and degrade co-receptor proteins Aux/IAA and JAZ, Li and co-authors merged the elements of the two pathways and conferred JA-mediated regulation to an auxin-response reporter in Arabidopsis protoplasts [66]. A 27-amino acid Jas-degron motif of JAZ1 was fused to an Aux/IAA protein SHORT HYPOCOTYL2 (SHY2/IAA3) and the resulting chimeric JaSHY protein became degradable by COI1 in a JA-dependent manner but also retained its transcription repression activity on a synthetic auxin reporter gene, *DR5:LUC* (Figure 2d). Thus, JaSHY is a first semi-synthetic device that combines upstream JA signaling events with the downstream auxin response and induces the auxin reporter in the presence of JA [66].

In addition to using whole-plant and protoplast models, much simpler microbial systems can be employed to study plant hormone interactions in a heterologous context. One interesting example of a synthetically triggered crosstalk between two phytohormone signal transduction pathways comes from merging the aforementioned allosterically regulated synthetic biosensor circuits for auxin and SA in yeast [46••]. The device consists of two galactose-inducible synthetic transcription factors, the IacR-VP64 activator described above (Figure 1f) and a MarR-MIG1 repressor [in which MarR is fused to the last 24 amino acids of the yeast transcriptional repressor protein MIG1, a nuclear localization signal, and a mouse ODC degron], co-regulating the expression of the aforementioned destabilized fluorescent *dEGFP* reporter driven by a synthetic promoter containing IacR and MarR binding sites (operators) upstream of the TATA-box (Figure 2e) (Table 1). The device displayed a robust response to dynamic hormone changes [46••]. The fact that this work was done in a yeast chassis that lacks endogenous auxin and SA perception machinery made for a largely interference-free experimental system.

Application of plant hormone signaling components in non-plant species

Auxin-induced degradation of Aux/IAAs has been leveraged in animal and fungal systems to confer IAA-triggered turnover to proteins of interest by tagging them with the IAA17 degron and ectopic expression of *TIR1* from Arabidopsis [67]. Initially developed over a decade ago and demonstrated to work in yeast, chicken and several mammalian systems, this is now a widely adopted synthetic biology tool successfully employed across a wide variety of non-plant species (reviewed in

Figure 3



An engineered cell-to-cell communication system in yeast. The source cell heterologously expresses the *iaaH* gene from *Agrobacterium tumefaciens* that converts IAM into auxin, while the receiver cell expresses an auxin-regulated dCas9-based synthetic transcription factor (made of dCas9, an auxin-inducible degron, and a transcriptional activation domain, VP64), an *FP* reporter gene, and a gRNA that targets the dCas9 effector to the promoter of the reporter. In the absence of IAM, no auxin is produced by the source cell, and the synthetic transcription factor in the sensor cell activates the reporter, leading to FP fluorescence. In the presence of IAM, auxin is produced/secreted by the source cell and uptaken by the sensor cell, leading to the degradation of the synthetic transcription factor, shutdown of the reporter gene, and the loss of fluorescence.

Ref. [68]). A recent improvement to this approach calls for the co-expression of a degron-binding PB1 domain of rice ARF16 to suppress the chronic depletion of degron-tagged proteins by the proteasome in the absence of auxin and to increase the rate of target protein turnover upon auxin treatment [69,70]. Furthermore, an auxin degron was utilized in yeast to control the stability and hence the function of a synthetic CRISPR-based transcription factor [71]. The latter synthetic protein consisted of dCas9 fused to a transcriptional activation domain of VP64 and an IAA17 degron and thus could be turned over in the presence of auxin upon co-expression of the F-box protein TIR1 (Figure 3). This yeast strain was used in combination with a second yeast strain that heterologously expresses a bacterial auxin biosynthesis enzyme iaaH that produces auxin out of indole-3-acetamide (IAM). The two yeast strains were co-cultured to trigger auxin synthesis and release by the source strain in the presence of IAM and the resulting synthetic transcriptional factor degradation and deactivation of its target *GFP* reporter in the sensor strain [71] (Figure 3). This was an exciting first attempt to imitate cell-to-cell communication between two strains of yeast using synthetic genetic devices based on the native plant auxin signaling machinery and a bacterial auxin biosynthetic gene.

Conclusions

As it can be inferred from this review, the field of plant hormone biology is still in the very early stages of adopting synthetic biology as an effective tool to dissect the function of individual hormone signaling components, shed light on specific signaling events or to study interactions between interconnected pathways. The cloning technologies to build simple genetic devices or construct synthetic circuits are now widely accessible and at least in theory readily implementable by trained molecular biologists, as evidenced by the recent surge in the number and diversity of hormone reporters and biosensors developed by plant scientists (Table 1). It is now time to

expand the reach of synthetic biology and develop more refined tools and multi-gene regulatory circuits to probe our understanding of the endogenous hormone regulation and engineer increasingly sophisticated synthetic hormone-triggered processes in plants. We hope the elegant examples described above will serve as an inspiration for young plant biologists to develop creative new ways of harnessing the power of synthetic biology for the benefit of basic and applied plant sciences alike.

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

Chengsong Zhao: Conceptualization, Writing - original draft, Visualization. **Anna Yaschenko:** Visualization. **Jose M Alonso:** Conceptualization, Writing - review & editing. **Anna N Stepanova:** Conceptualization, Writing - original draft, Writing - review & editing.

Acknowledgement

The work in the Alonso-Stepanova lab is supported by the NSF grants 1444561 and 1940829 to JMA and ANS, and 1750006 to ANS.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Cordero T, Rosado A, Majer E, Jaramillo A, Rodrigo G, Daròs J: **Boolean computation in plants using post-translational genetic control and a visual output signal**. *ACS Synth Biol* 2018, **7**:2322-2330 http://dx.doi.org/10.1021/acssynbio.8b00214.

2. Bernabé-Orts JM, Quijano-Rubio A, Vazquez-Vilar M, Mancheño-Bonillo J, Moles-Casas V, Selma S *et al.*: **A memory switch for plant synthetic biology based on the phage ϕC31 integration system**. *Nucleic Acids Res* 2020, **48**:3379-3394 http://dx.doi.org/10.1093/nar/gkaa104.

3. Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ: **Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements**. *Plant Cell* 1997, **9**:1963-1971 http://dx.doi.org/10.1105/tpc.9.11.1963.

4. Isoda R, Yoshinari A, Ishikawa Y, Sadoine M, Simon R, Frommer WB, Nakamura M: **Sensors for the quantification, localization and analysis of the dynamics of plant hormones**. *Plant J* 2020 http://dx.doi.org/10.1111/tpj.15096.

5. Waadt R: **Phytohormone signaling mechanisms and genetic methods for their modulation and detection**. *Curr Opin Plant Biol* 2020, **57**:31-40 http://dx.doi.org/10.1016/j.pbi.2020.05.011.

6. • Jones AM, Danielson JA, Manojkumar SN, Lanquar V, Grossmann G, Frommer WB: **Abscisic acid dynamics in roots detected with genetically encoded FRET sensors**. *eLife* 2014, **3**:e01741 http://dx.doi.org/10.7554/eLife.01741

A FRET-based ABA biosensor ABACUS suitable for the visualization of ABA is described.

7. • Waadt R, Hitomi K, Nishimura N, Hitomi C, Adams SR, Getzoff ED, Schroeder JI: **FRET-based reporters for the direct visualization of abscisic acid concentration changes and distribution in Arabidopsis**. *eLife* 2014, **3**:e01739 http://dx.doi.org/10.7554/eLife.01739

FRET-based ABAleon biosensors for the visualization of abscisic acid are described.

8. Waadt R, Köster P, Andrés Z, Waadt C, Bradamante G, Lampou K *et al.*: **Dual-reporting transcriptionally linked genetically encoded fluorescent indicators resolve the spatiotemporal coordination of cytosolic abscisic acid and second messenger dynamics in Arabidopsis**. *Plant Cell* 2020, **32**:2582-2601 http://dx.doi.org/10.1105/tpc.19.00892.

9. Rizza A, Walia A, Lanquar V, Frommer WB, Jones AM: **In vivo gibberellin gradients visualized in rapidly elongating tissues**. *Nat Plants* 2017, **3**:803-813 http://dx.doi.org/10.1038/s41477-017-0021-9.

10. •• Herud-Sikimic O, Stiel AC, Ortega-Perez M, Shanmugaratnam S, Hoecker B, Juergens GC: *Design of a Biosensor for Direct Visualisation of Auxin*. Cold Spring Harbor, United States Cold Spring Harbor, Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 2020 http://dx.doi.org/10.1101/2020.01.19.911735

The first auxin FRET sensor, AuxSen, is described. Unlike other FRET-based hormone biosensors, which rely on native plant hormone receptors/coreceptors for hormone binding, AuxSen is based on an engineered derivative of TrpR, an *E. coli* tryptophan repressor protein.

11. Okumoto S, Jones A, Frommer WB: **Quantitative imaging with fluorescent biosensors**. *Ann Rev Plant Biol* 2012, **63**:663-706 http://dx.doi.org/10.1146/annurev-arplant-042110-103745.

12. Rizza A, Walia A, Tang B, Jones AM: **Visualizing cellular gibberellin levels using the nlsGPS1 Förster resonance energy transfer (FRET) biosensor**. *J Vis Exp* 2019 http://dx.doi.org/10.3791/58739.

13. Aysha J, Noman M, Wang F, Liu W, Zhou Y, Li H, Li X: **Synthetic promoters: designing the cis regulatory modules for controlled gene expression**. *Mol Biotechnol* 2018, **60**:608-620 http://dx.doi.org/10.1007/s12033-018-0089-0.

14. Stewart CN Jr, Liu W: **Synthetic promoters for precise control of gene expression in plants**. *Chem Eng Progress* 2017, **113**:36-39.

15. Waadt R, Hsu P, Schroeder JI: **Abscisic acid and other plant hormones: methods to visualize distribution and signaling**. *BioEssays* 2015, **37**:1338-1349 http://dx.doi.org/10.1002/bies.201500115.

16. Wu R, Duan L, Pruneda-Paz JL, Oh D, Pound M, Kay S, Dinneny JR: **The 6xABRE synthetic promoter enables the spatiotemporal analysis of ABA-mediated transcriptional regulation**. *Plant Physiol* 2018, **177**:1650-1665 http://dx.doi.org/10.1104/pp.18.00401.

17. Min MK, Kim R, Moon S, Lee Y, Han S, Lee S, Kim B: **Selection and functional identification of a synthetic partial ABA agonist, S7**. *Sci Rep* 2020, **10**:1-10 http://dx.doi.org/10.1038/s41598-019-56343-9.

18. Liao C, Smet W, Brunoud G, Yoshida S, Vernoux T, Weijers D: **Reporters for sensitive and quantitative measurement of auxin response**. *Nat Methods* 2015, **12**:207-210 http://dx.doi.org/10.1038/nmeth.3279.

19. Lieberman-Lazarovich M, Yahav C, Israeli A, Efroni I: **Deep conservation of cis-element variants regulating plant hormonal responses**. *Plant Cell* 2019, **31**:2559-2572 http://dx.doi.org/10.1105/tpc.19.00129.

20. Gallego-Bartolomé J, Minguet EG, Grau-Enguix F, Abbas M, Locascio A, Thomas SG *et al.*: **Molecular mechanism for the interaction between gibberellin and brassinosteroid signaling pathways in Arabidopsis**. *Proc Natl Acad Sci U S A* 2012, **109**:13446-13451 http://dx.doi.org/10.1073/pnas.1119992109.

21. Müller B, Sheen J: **Cytokinin and auxin interaction in root stem-cell specification during early embryogenesis**. *Nature* 2008, **453**:1094-1097 http://dx.doi.org/10.1038/nature06943.

22. Zürcher E, Tavor-Deslex D, Lituiev D, Enkerli K, Tarr PT, Müller B: **A robust and sensitive synthetic sensor to monitor the transcriptional output of the cytokinin signaling network in planta**. *Plant Physiol* 2013, **161**:1066-1075 http://dx.doi.org/10.1104/pp.112.211763.

23. Ohme-Takagi M, Shinshi H: **Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element**. *Plant Cell* 1995, **7**:173-182 http://dx.doi.org/10.1105/tpc.7.2.173.

24. Stepanova AN, Yun J, Likhacheva AV, Alonso JM: **Multilevel interactions between ethylene and auxin in Arabidopsis roots**. *Plant Cell* 2007, **19**:2169-2185 http://dx.doi.org/10.1105/tpc.107.052068.

25. Wang C, Lin T, Wang M, Qi X: **An AC-rich bean element serves as an ethylene-responsive element in Arabidopsis**. *Plants (Basel, Switzerland)* 2020, **9** http://dx.doi.org/10.3390/plants9081033.

26. Sutoh K, Yamauchi D: **Two cis-acting elements necessary and sufficient for gibberellin-upregulated proteinase expression in rice seeds**. *Plant J* 2003, **34**:635-645 http://dx.doi.org/10.1046/j.1365-313x.2003.01753.x.

27. Mason HS, DeWald DB, Mullet JE: **Identification of a methyl jasmonate-responsive domain in the soybean *vspB* promoter**. *Plant Cell* 1993, **5**:241-251 http://dx.doi.org/10.1105/tpc.5.3.241.

28. Brown RL, Kazan K, McGrath KC, Maclean DJ, Manners JM: **A role for the GCC-box in jasmonate-mediated activation of the *PDF1.2* gene of Arabidopsis**. *Plant Physiol* 2003, **132**:1020-1032 http://dx.doi.org/10.1104/pp.102.017814.

29. Rocher A, Dumas C, Cock JM: **A W-box is required for full expression of the SA-responsive gene *SFR2***. *Gene* 2005, **344**:181-192 http://dx.doi.org/10.1016/j.gene.2004.09.016.

30. Merchante C, Brumos J, Yun J, Hu Q, Spencer KR, Enríquez P *et al.*: **Gene-specific translation regulation mediated by the hormone-signaling molecule EIN2**. *Cell* 2015, **163**:684-697 http://dx.doi.org/10.1016/j.cell.2015.09.036.

31. Li W, Ma M, Feng Y, Li H, Wang Y, Ma Y *et al.*: **EIN2-directed translational regulation of ethylene signaling in Arabidopsis**. *Cell* 2015, **163**:670-683 http://dx.doi.org/10.1016/j.cell.2015.09.037.

32. • Mazzoni-Putman SM, Stepanova AN: **A plant biologist's toolbox to study translation**. *Front Plant Sci* 2018, **9**:873 http://dx.doi.org/10.3389/fpls.2018.00873
The first synthetic ethylene-repressible translational reporter, 6xEPU, based on an ethylene-repressible *cis*-element from the 3' UTR of the Arabidopsis *EBF1* transcript is described.

33. Brunoud G, Wells DM, Oliva M, Larrieu A, Mirabet V, Burrow AH *et al.*: **A novel sensor to map auxin response and distribution at high spatio-temporal resolution**. *Nature* 2012, **482**:103-106 http://dx.doi.org/10.1038/nature10791.

34. Khosla A, Morffy N, Li Q, Faure L, Chang SH, Yao J *et al.*: **Structure-function analysis of SMAX1 reveals domains that mediate its karrikin-induced proteolysis and interaction with the receptor KAI2**. *Plant Cell* 2020, **32**:2639-2659 http://dx.doi.org/10.1105/tpc.19.00752.

35. Wend S, Dal Bosco C, Kämpf MM, Ren F, Palme K, Weber W *et al.*: **A quantitative ratiometric sensor for time-resolved analysis of auxin dynamics**. *Sci Rep* 2013, **3**:2052 http://dx.doi.org/10.1038/srep02052.

36. Larrieu A, Champion A, Legrand J, Lavenus J, Mast D, Brunoud G *et al.*: **A fluorescent hormone biosensor reveals the dynamics of jasmonate signalling in plants**. *Nat Commun* 2015, **6**:6043 http://dx.doi.org/10.1038/ncomms7043.

37. •• Khakhar A, Leydon AR, Lemmex AC, Klavins E, Nemhauser JL: **Synthetic hormone-responsive transcription factors can monitor and re-program plant development**. *eLife* 2018, **7** http://dx.doi.org/10.7554/eLife.34702
Taking advantage of dCas9-based synthetic transcription factors fused to a hormone-inducible degron, the authors developed several HACR devices responsive to auxin, GA or JA. HACRs were employed in conjunction with reporter genes to sense hormone levels, as well as a with an endogenous *PIN1* gene to regulate shoot branching.

38. Huang WE, Wang H, Zheng H, Huang L, Singer AC, Thompson I, Whiteley AS: **Chromosomally located gene fusions constructed in *Acinetobacter* sp. ADP1 for the detection of salicylate**. *Environ Microbiol* 2005, **7**:1339-1348 http://dx.doi.org/10.1111/j.1462-5822.2005.00821.x.

39. Huang WE, Huang L, Preston GM, Naylor M, Carr JP, Li Y *et al.*: **Quantitative *in situ* assay of salicylic acid in tobacco leaves using a genetically modified biosensor strain of *Acinetobacter* sp. ADP1**. *Plant J* 2006, **46**:1073-1083 http://dx.doi.org/10.1111/j.1365-313x.2006.02758.x.

40. Park HH: **NahR: effects of replacements at Asn 169 and Arg 248 on promoter binding and inducer recognition**. *Arch Biochem Biophys* 2005, **434**:67-74 http://dx.doi.org/10.1016/j.abb.2004.10.020.

41. Shin HJ: **Development of highly sensitive microbial biosensors by mutation of the *nahR* regulatory gene**. *J Biotechnol* 2010, **150**:246-250 http://dx.doi.org/10.1016/j.jbiotec.2010.09.936.

42. Pini F, East AK, Appia-Ayme C, Tomek J, Karunakaran R, Mendoza-Suárez M *et al.*: **Bacterial biosensors for *in vivo* spatiotemporal mapping of root secretion**. *Plant Physiol* 2017, **174**:1289-1306 http://dx.doi.org/10.1104/pp.16.01302.

43. Zhou Y, Memelink J, Linthorst HJM: **An *E. coli* biosensor for screening of cDNA libraries for isochorismate pyruvate lyase-encoding cDNAs**. *Mol Genet Genomics* 2018, **293**:1181-1190 http://dx.doi.org/10.1007/s00438-018-1450-5.

44. Ding Y, Shaholli D, Mou Z: **A large-scale genetic screen for mutants with altered salicylic acid accumulation in Arabidopsis**. *Front Plant Sci* 2015, **5**:763 http://dx.doi.org/10.3389/fpls.2014.00763.

45. Marek G, Carver R, Ding Y, Sathyanarayan D, Zhang X, Mou Z: **A high-throughput method for isolation of salicylic acid metabolic mutants**. *Plant Methods* 2010, **6**:21 http://dx.doi.org/10.1186/1746-4811-6-21.

46. •• Garcia SP, Navarrete MG, Sanchis DR, Navarro CP, Avdovic M, Pucciariello O, Wabnik K: *Synthetic Biosensor Circuits with a Synchronous Precision Response to Dynamic Phytohormone Inputs*. Cold Spring Harbor, United States Cold Spring Harbor, Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 2020 http://dx.doi.org/10.1101/2020.05.31.125997
By taking advantage of bacterial lacR and MarR repressors, the authors developed the first synthetic biosensor circuits for direct sensing of auxin and SA.

47. Havens KA, Guseman JM, Jang SS, Pierre-Jerome E, Bolten N, Klavins E, Nemhauser JL: **A synthetic approach reveals extensive tunability of auxin signaling**. *Plant Physiol* 2012, **160**:135-142 http://dx.doi.org/10.1104/pp.112.202184.

48. Moss BL, Mao H, Guseman JM, Hinds TR, Hellmuth A, Kovenock M *et al.*: **Rate motifs tune auxin/indole-3-acetic acid degradation dynamics**. *Plant Physiol* 2015, **169**:803-813 http://dx.doi.org/10.1104/pp.15.00587.

49. Guseman JM, Hellmuth A, Lanctot A, Feldman TP, Moss BL, Klavins E *et al.*: **Auxin-induced degradation dynamics set the pace for lateral root development**. *Development* 2015, **142**:905-909 http://dx.doi.org/10.1242/dev.117234.

50. Wright RC, Zahler ML, Gerben SR, Nemhauser JL: **Insights into the evolution and function of auxin signaling F-box proteins in *Arabidopsis thaliana* through synthetic analysis of natural variants**. *Genetics* 2017, **207**:583-591 http://dx.doi.org/10.1534/genetics.117.300092.

51. Galli M, Liu Q, Moss BL, Malcomber S, Li W, Gaines C *et al.*: **Auxin signaling modules regulate maize inflorescence architecture**. *Proc Natl Acad Sci U S A* 2015, **112**:13372-13377 http://dx.doi.org/10.1073/pnas.1516473112.

52. •• Pierre-Jerome E, Jang SS, Havens KA, Nemhauser JL, Klavins E: **Recapitulation of the forward nuclear auxin response pathway in yeast**. *Proc Natl Acad Sci U S A* 2014, **111**:9407-9412 http://dx.doi.org/10.1073/pnas.1324147111
The authors reconstituted the first phytohormone signaling pathway in yeast by heterologously expressing TIR/AFB and Aux/IAA co-receptors, a TPL repressor, an activator ARF and a reporter under the control of a plant auxin-inducible promoter harboring ARF binding sites.

53. Pierre-Jerome E, Moss BL, Lanctot A, Hageman A, Nemhauser JL: **Functional analysis of molecular interactions in synthetic auxin response circuits**. *Proc Natl Acad Sci U S A* 2016, **113**:11354-11359 http://dx.doi.org/10.1073/pnas.1604379113.

54. Ramos Báez R, Buckley Y, Yu H, Chen Z, Gallavotti A, Nemhauser JL, Moss BL: **A synthetic approach allows rapid characterization of the maize nuclear auxin response circuit**.

Plant Physiol 2020, **182**:1713-1722 http://dx.doi.org/10.1104/pp.19.01475.

55. Thiruppathi D: **Mix, match, and maize: a synthetic system for maize nuclear auxin response circuits**. *Plant Physiol* 2020, **183**:416-417 http://dx.doi.org/10.1104/pp.20.00489.

56. Lanctot A, Taylor-Teeples M, Oki EA, Nemhauser JL: **Specificity in auxin responses is not explained by the promoter preferences of activator ARFs(1[CC-BY])**. *Plant Physiol* 2020, **182**:1533-1536 http://dx.doi.org/10.1104/pp.19.01474.

57. • Ruschhaupt M, Mergner J, Mucha S, Papacek M, Doch I, Tischer SV *et al.*: **Rebuilding core abscisic acid signaling pathways of Arabidopsis in yeast**. *EMBO J* 2019, **38**:e101859 http://dx.doi.org/10.15252/embj.2019101859
The authors rebuilt the first ABA signaling pathway in yeast by expressing PYL/RCAR and PP2C co-receptors, a SnRK2 kinase, an ABF transcription factor and a synthetic ABRE-promoter driven reporter regulated by ABF.

58. Li N, Uhrig JF, Thurow C, Huang L, Gatz C: **Reconstitution of the jasmonate signaling pathway in plant protoplasts**. *Cells* 2019, **8** http://dx.doi.org/10.3390/cells8121532.

59. •• Park S, Peterson FC, Mosquna A, Yao J, Volkman BF, Cutler SR: **Agrochemical control of plant water use using engineered abscisic acid receptors**. *Nature* 2015, **520**:545-548 http://dx.doi.org/10.1038/nature14123
By mutating the ABA receptor's PYR1 ligand-binding pocket and screening chemical ligands for specific binding, a synthetic hormone perception system was built in plants.

60. • Uchida N, Takahashi K, Iwasaki R, Yamada R, Yoshimura M, Endo TA *et al.*: **Chemical hijacking of auxin signaling with an engineered auxin-TIR1 pair**. *Nat Chem Biol* 2018, **14**:299-305 http://dx.doi.org/10.1038/nchembio.2555
By employing the bump-and-hole strategy, the authors developed an orthogonal synthetic auxin-TIR1 pair, cvxIAA-ccvTIR1.

61. • Zhang L, Zhang F, Melotto M, Yao J, He SY: **Jasmonate signaling and manipulation by pathogens and insects**. *J Exp Bot* 2017, **68**:1371-1385 http://dx.doi.org/10.1093/jxb/erw478
By modifying a JA receptor so that the mutant form maintains sufficient endogenous JA perception and signaling but significantly disrupts the interaction with the bacterial JA-mimicking toxin coronatine, the authors demonstrate that host target modification can be employed to prevent plant disease.

62. Zhang L, Yao J, Withers J, Xin X, Banerjee R, Fariduddin Q *et al.*: **Host target modification as a strategy to counter pathogen hijacking of the jasmonate hormone receptor**. *Proc Natl Acad Sci U S A* 2015, **112**:14354-14359 http://dx.doi.org/10.1073/pnas.1510745112.

63. •• Khakhar A, Wang C, Swanson R, Stokke S, Rizvi F, Sarup S *et al.*: *ViparNama: RNA Vectors to Rapidly Reprogram Plant Morphology and Metabolism*. Cold Spring Harbor, United States Cold Spring Harbor, Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 2020 http://dx.doi.org/10.1101/2020.06.03.130179
The study describes a series of CRISPR-based transcriptional effectors and viral RNA vectors to control plant gene expression. The utility of these tools was validated by targeting a GA biosynthesis gene GA20ox1-3 in *Nicotiana benthamiana*, receptor *GIDs* in *Arabidopsis thaliana*, and a DELLA co-repressor *PROCERA* in tomato.

64. • Müller K, Siegel D, Rodriguez Jahnke F, Gerrer K, Wend S, Decker EL *et al.*: **A red light-controlled synthetic gene expression switch for plant systems**. *Mol Biosyst* 2014, **10**:1679-1688 http://dx.doi.org/10.1039/c3mb70579j
An optogenetic tool based on PHYB-PIF6 dimerization in red light was implemented in plants and employed to regulate expression of *TIR1* and *TIR1*-targeting miRNA.

65. Müller K, Engesser R, Schulz S, Steinberg T, Tomakidi P, Weber CC *et al.*: **Multi-chromatic control of mammalian gene expression and signaling**. *Nucleic Acids Res* 2013, **41**:e124 http://dx.doi.org/10.1093/nar/gkt340.

66. Li N, Cao L, Miu W, Cao R, Peng M, Wan W, Huang L-J: **Molecular rewiring of the jasmonate signaling pathway to control auxin-responsive gene expression**. *Cells* 2020, **9**:641 http://dx.doi.org/10.3390/cells9030641.

67. Nishimura K, Fukagawa T, Takisawa H, Kakimoto T, Kanemaki M: **An auxin-based degron system for the rapid depletion of proteins in nonplant cells**. *Nat Methods* 2009, **6**:917-922 http://dx.doi.org/10.1038/nmeth.1401.

68. Yesbolatova A, Natsume T, Hayashi K, Kanemaki MT: **Generation of conditional auxin-inducible degron (AID) cells and tight control of degron-fused proteins using the degradation inhibitor auxinole**. *Methods (San Diego, Calif.)* 2019, **164–165**:73-80 http://dx.doi.org/10.1016/j.ymeth.2019.04.010.

69. Sathyan KM, McKenna BD, Anderson WD, Duarte FM, Core L, Guertin MJ: **An improved auxin-inducible degron system preserves native protein levels and enables rapid and specific protein depletion**. *Genes Dev* 2019, **33**:1441-1455 http://dx.doi.org/10.1101/gad.328237.119.

70. Sathyan KM, Scott TG, Guertin MJ: **ARF-AID: a rapidly inducible protein degradation system that preserves basal endogenous protein levels**. *Curr Protoc Mol Biol* 2020, **132**:e124 http://dx.doi.org/10.1002/cpmb.124.

71. Khakhar A, Bolten NJ, Nemhauser J, Klavins E: **Cell-cell communication in yeast using auxin biosynthesis and auxin responsive CRISPR transcription factors**. *ACS Synth Biol* 2016, **5**:279-286 http://dx.doi.org/10.1021/acssynbio.5b00064.