achieved, controversies regarding the role of the LYP-Csk interaction need to be fully resolved. Though the data of Vang *et al.*[5] are a major step in this direction, some issues are still lingering. For instance, the notion that LYP is more efficient at warding off T-cell activation when it is freed from Csk has not been supported by studies with mouse LYP[3]. The latter studies showed that a mutant form of mouse LYP unable to bind Csk was less efficient at suppressing T-cell activation, thereby suggesting the opposite effect. This observation could simply be due to species differences. Moreover, it was recently reported that the R620W mutant and the homologous mutant protein in the mouse are not stable in cells[8]. As a result, the R620W mutant disappears faster from cells and is less active than wild-type LYP. If indeed the R620W mutant is less stable and less active than wild-type LYP, the use of LYP inhibitors to treat autoimmune diseases associated with R620W could be pointless. Contrary to this other recent report[8], however, Vang *et al.*[5] did not find that the R620W mutant is unstable. Hence, the matter regarding the stability of the R620W mutant is contentious, and additional work is needed to resolve the issue.

Autoimmune diseases are serious and potentially life-threatening conditions. The available treatments are not always efficacious and can cause severe side effects. Thus, development of new drugs that are more effective and less toxic is crucial. The findings reported by Vang *et al.*[5] provide evidence that LYP inhibitors could be new weapons against autoimmune diseases. Although more work is needed to confirm this idea, this notion will undoubtedly be of interest to many.

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Competing financial interests

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SYNTHETIC BIOLOGY

Automated design of RNA devices

RNA molecules have diverse functional roles, including silencing genes, catalyzing biochemical reactions and sensing chemicals that control gene expression. Biologists have drawn from nature's toolbox to construct engineered RNA molecules with versatile capabilities and can now begin to automate the design of libraries of regulatory RNAs.

Farren J Isaacs

Computer-aided design (CAD) had a disruptive impact on electronic chips by shifting the paradigm from a manual handcrafted art to an automated industry with design specification standards, large-scale fabrication pipelines and increasingly sophisticated microchips. Biological systems are intrinsically complex, and automating the design and synthesis of genetic and biomolecular components to drive programmable function remains a defining challenge. RNA, a versatile and programmable biomolecule[1], is an ideal substrate to engineer tailored biological function. Prior studies have primarily used manual approaches to rationally design RNA molecules that impart diverse functions[2], including regulators of gene expression[3,4], sensors that respond to target ligands[5,6] and molecular scaffolds that spatially organize enzymes[7]. However, these engineered RNA systems have been limited to small numbers of handcrafted functional RNA components. In this issue, Mutalik *et al.*[8] take an exciting step forward by creating a CAD-based tool that enables the design of a large library of regulatory RNAs with predictable function.

Biology has only recently entered an era in which the engineering of biological systems is attainable, and this pursuit is largely motivated by the prospect of harnessing engineered cells to solve global problems in medicine, energy supply and the environment. Achieving these lofty goals will not only lead to great societal transformations but also deepen our understanding of natural biological systems. These objectives define the emerging field of synthetic biology in which most studies attempt to synthesize new genetic and biomolecular components or edit existing systems in an attempt to mimic, or even co-opt, the exquisite regulatory capabilities of evolved cells toward new and desired functions. In practice, research efforts have focused on the development of enabling tools and technologies to design, construct and characterize biological systems. Though many of these efforts use protein-DNA interactions to modulate gene expression, the growing role of functional RNAs in natural systems inspires their use as a target for engineering. The predictability of base-pairing interactions, the capability to fold into complex shapes that bind metabolites and catalyze reactions and the ability to explore vast secondary structures that undergo conformational changes are the key chemical properties that render RNA an attractive biomolecule for custom design. These properties can be configured into modular domains that confer specific functions (for example, base-pair interactions and binding pockets for ligands) and have served as the basis for the design of versatile synthetic RNA control elements in genetic networks[2]. The synthetic biologist's toolbox and capabilities would be greatly enhanced by having quantitative models to automate the design and number of biomolecular elements that can interface and predictably alter cellular networks.

The work by Mutalik *et al.*[8] takes on just this goal, describing a beautifully integrated model-experiment strategy that enables the design of a library of engineered RNA regulators with predictable interactions and behaviors. Similar to prior work on engineered RNA regulators[2], the study uses a biologically inspired approach by rooting the design of engineered translational

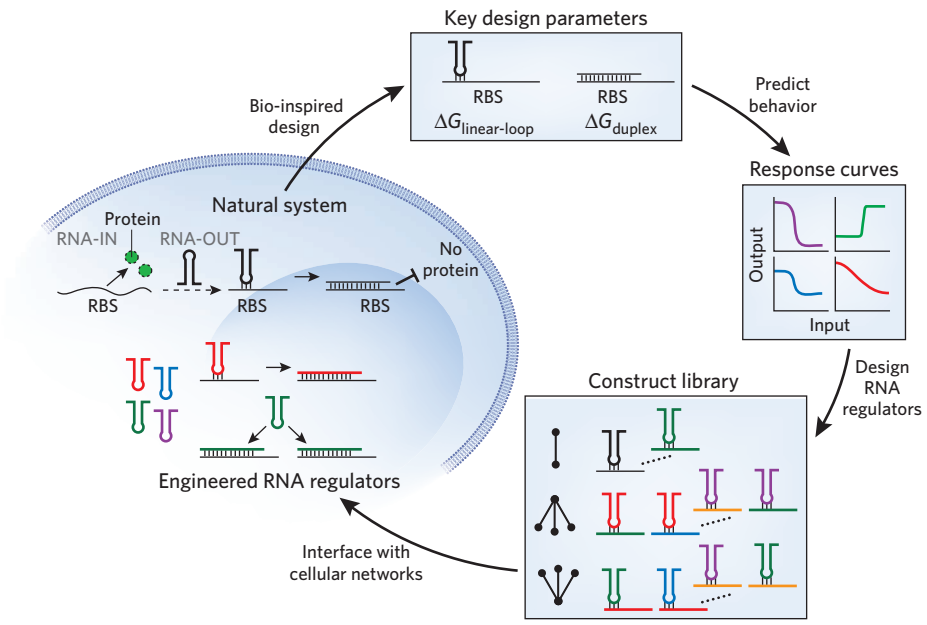

Figure 1 | Automating the design of RNA devices. The RNA-IN/OUT antisense RNA–mediated translation system from insertion sequence IS10 is used as the basis for automated design of engineered RNAs. The loop of the RNA-OUT antisense RNA binds the sense mRNA to interfere with initiation of translation by sequestering the ribosome-binding site (RBS). The hybridization energies (ΔG) of the initial linear-loop complex and entire duplex are the two key thermodynamic parameters that guide design of the library of RNA regulators. Large numbers of engineered RNA regulators were constructed to have different degrees of antisense-sense transcript orthogonality (for example, one-to-one, one-to-many or many-to-one) with predictable and customized response curves; these can then be integrated back into the cell to interact with existing biological pathways.

regulators in a natural system, the copy number control element of the IS10 insertion sequence. In this system, an antisense RNA (RNA-OUT) represses expression of a transposase mRNA (RNA-IN) by binding its ribosome-binding site and preventing translation initiation. The authors focused their engineering efforts on the linear-loop nucleotide interaction of the RNA-IN and RNA-OUT molecules (**Fig. 1**). They hypothesized that these nucleotides, which participate in the initial intermolecular binding event, could be targeted for specific and complementary mutations to generate many pairs of orthogonal, or non–cross-reacting, translational regulators.

Mutalik *et al.*[8] started by designing the sequences for 56 cognate pairs and used web-based computational software (http://mfold.rna.albany.edu/) to estimate the hybridization energies of all 3,136 antisense-sense combinations. As expected, the cognate pairs were found to be most stable, and 23 were selected for studies *in vivo*. The authors tested all pairwise combinations by measuring the ability of the antisense RNA-OUT transcript to repress the fluorescence of green fluorescent protein–expressing RNA-IN mRNAs. Though the result could be viewed as expected—the *in vivo* results match the thermodynamic predictions with most cognate sense-antisense pairs showing >80% repression—the authors extracted some additional insights from the experiments. First, they isolated antisense pairs with a wide dynamic range (5–90% repression), creating many regulator pairs that can be studied for identification of key design parameters. Second, they identified two classes of nonorthogonal, or cross-reacting, RNA pairs: (i) one antisense RNA repressing multiple sense mRNAs and (ii) one sense mRNA serving as a substrate for multiple antisense RNAs. These RNA regulators, along with the orthogonal RNA pairs, permit the design of customized network interactions. Third, the authors identified a key free energy threshold between interacting RNA species that serves as a quantitative predictor of the degree of repression and as an important metric in predicting the customized response of engineered RNA regulators (**Fig. 1**). To quantitatively formalize their findings, they developed two metrics—percentage of repression (%R) and percentage of cross-reactivity (%C)—that characterize dynamic range and degree of orthogonality, respectively. This quantitative framework allowed the authors to classify the antisense-sense pairs into groups with different behaviors (for example, high repression and low cross-reactivity), iterate back to the model to tease out key properties that determine behavior and validate their predictions by forward engineering two more sense-antisense pairs (**Fig. 1**).

The work by Mutalik *et al.*[8] represents an important advance toward a more automated synthetic biology. Excitingly, another recent study provides an additional indication that we are at a stage at which we can meaningfully extract information from data to develop design parameters for biomolecular interactions. In this work, Carothers *et al.*[9] combined biochemical models and biophysical kinetic RNA folding simulations to design engineered RNA devices that also control gene expression in *E. coli*. They built 28 engineered RNA regulators that showed strong quantitative agreement with model predictions and applied them to tune flux of p-AF, an important precursor of polymers.

Together, these studies present insights into the important biophysical parameters that quantitatively describe and predict the interactions of RNA molecules. Their quantitative strategies can be applied toward an increased understanding of endogenous RNA processes. From an engineering perspective, these studies set the stage for automating the design of large numbers of RNA devices with predictable behaviors, substantially expanding regulatory components in the synthetic biology toolbox. Now, the field faces the challenge of using directed evolution[10] and advances in genome engineering technologies[11] to evolve and integrate scores of independently operating RNA devices across entire networks to program more sophisticated biological function. Such efforts will bring synthetic biology a step closer toward the creation of artificial Darwinian systems that are capable of self-selecting new phenotypes and recapitulating emergent properties of natural biological systems.

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PLANT SIGNALING

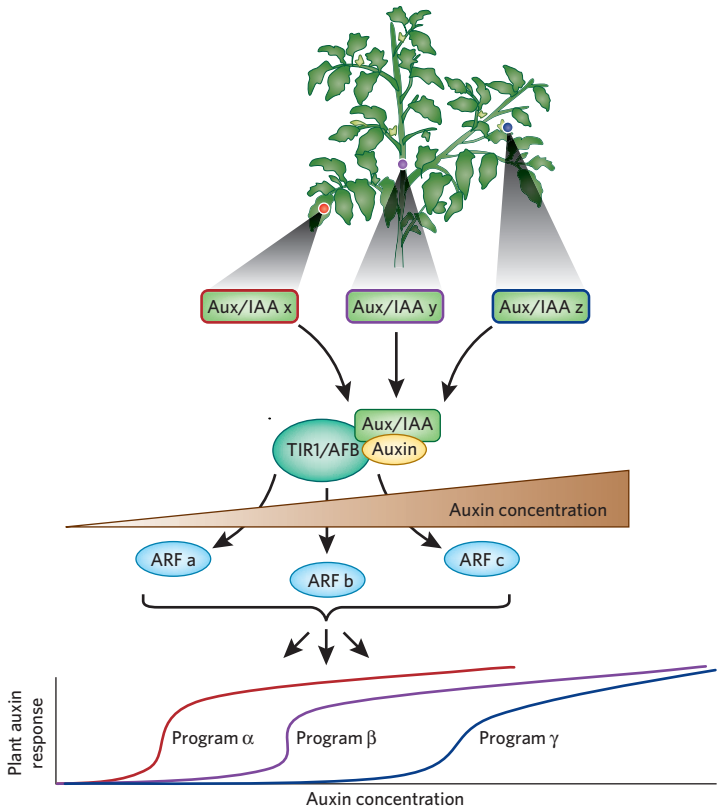
Deconstructing auxin sensing

Sensing of the plant hormone auxin involves formation of a co-receptor complex consisting of an F-box protein and an AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA) transcriptional repressor. Distinct co-receptor combinations might provide cells with an unexpectedly broad range of auxin-sensing capacities and contribute to diverse transcriptional programs activated by different auxin levels in various developmental contexts.

Steffen Vanneste & Jiří Friml

Auxin is one of the main plant growth-regulating hormones and is involved in nearly every aspect of plant growth and development. How such a simple tryptophan-like molecule can trigger such a wide array of responses is conceptually difficult to envision. Importantly, auxin's graded distribution within tissues is believed to be instructive in shaping plant growth and architecture. Moreover, cells can have different auxin sensitivities and show different responses depending on their context. As a consequence, cells within a gradient of auxin can be recruited to divergent developmental programs. However, how differential auxin sensitivity can be brought about is unclear. Here, Calderón Villalobos *et al.*[1] show that the combinatorial potential of co-receptor complexes brings about a broad range of auxin binding affinities and that this affinity mainly depends on the Aux/IAA component of the complex.

Recently, it was demonstrated that auxin perception depends on auxin's capacity to stabilize the interaction between a family of F-box components of E3 ligases (TRANSPORT INHIBITOR RESPONSE 1/AUXIN-SIGNALING F-BOX proteins 1–5 (TIR1/AFB1–AFB5)) and Aux/IAA transcriptional repressors and that such a heteromer constitutes an auxin co-receptor complex[2,3]. Instead of changing TIR1's conformation, auxin extends its interaction interface to increase binding affinity for Aux/IAAs[4], resulting in its ubiquitination and targeting for proteolytic degradation. This model of auxin-dependent degradation of Aux/IAAs explains how auxin can induce various transcriptional changes. Interestingly, both the F-box component (TIR1/AFB) and the Aux/IAA component of this co-receptor complex are encoded by relatively large gene families in plants[5,6]. For example, *Arabidopsis thaliana* has 6 TIR1/AFBs and 29 Aux/IAAs, allowing for an impressive combinatorial potential. So far, the physiological importance of this myriad of potential auxin-perceiving complexes has remained unclear.



Katie Vicari

Figure 1 | Single-cell auxin signaling for executing different plant developmental programs. Individual cells express different combinations of auxin signaling pathway components, in particular ARF transcription factors and Aux/IAA transcriptional repressors. Aux/IAA x, y and z represent different sets of Aux/IAAs expressed within individual cells in different developmental contexts (for example, leaf, stem or flower). These confer different auxin binding affinities to TIR1/AFB–Aux/IAA co-receptor complexes. Depending on the auxin concentration, different sets of ARF interaction partners (ARF a, b and c) are released from inhibition and activate distinct downstream transcriptional responses (program α, β and γ), as depicted in the graph.