

Designing cell function: assembly of synthetic gene circuits for cell biology applications

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Abstract | Synthetic biology is the discipline of engineering application-driven biological functionalities that were not evolved by nature. Early breakthroughs of cell engineering, which were based on ectopic (over)expression of single sets of transgenes, have already had a revolutionary impact on the biotechnology industry, regenerative medicine and blood transfusion therapies. Now, we require larger-scale, rationally assembled genetic circuits engineered to programme and control various human cell functions with high spatiotemporal precision in order to solve more complex problems in applied life sciences, biomedicine and environmental sciences. This will open new possibilities for employing synthetic biology to advance personalized medicine by converting cells into living therapeutics to combat hitherto intractable diseases.

Ectopic (over)expression
The forced expression of a particular gene in a cell type in which the gene is usually not expressed at a desired level.

Constitutive expression
The persistent production of a target protein by any cell that contains the encoding gene.

Gene switches
Any natural or synthetic system (for example, a promoter, an RNA molecule or a signalling pathway) that allows initiation, interruption or termination of target gene expression.

Since the earliest days of human civilization, microbial cells have been harnessed to produce fermented foods and beverages. Now, synthetic biology aims to engineer artificial cell functionalities that have never existed in nature. This aim has been underpinned by the advent in the late 20th century of recombinant DNA technologies, which have already revolutionized the pharmaceutical industry. For example, metabolic engineering has enabled the development of living cells as powerful production factories for high-value small molecules and proteins, for which chemical synthesis is too complex, too inefficient or too expensive¹. By ectopic (over)expression of a minimal set of transgenes coding for a protein or a specialized biosynthetic enzymatic cluster, host cells grown on inexpensive and renewable carbon sources can be programmed to produce pharmaceuticals^{2–5}, food additives⁶, feedstock and raw materials^{7,8} or biopolymers⁹, achieving high bio-process efficiency and environmental sustainability^{1,10}. To further optimize host cell productivity and increase profitability, we require gene circuits that can provide more precise control over spatiotemporal expression and activity of proteins than can be achieved with constitutive expression strategies. Importantly, very tight control of the activity of foreign genetic elements is also needed if these strategies are to be used in human therapy. For example, patient-specific T cells genetically modified to express cancer-targeting chimeric antigen receptors (CARs) are currently achieving unprecedented response rates in human clinical trials to treat blood cancers^{11,12}, but there is

still a need to increase treatment safety, efficacy and reliability by introducing more sophisticated control layers for precise regulation of CAR T cell activity and performance^{13,14}. Thus, the focus of synthetic biology is now on designing synthetic gene circuits consisting of interconnected gene switches to programme time-dependent and context-dependent target gene activities in living cells.

In this Review, we first discuss in detail the regulatory elements that are available for precise, remote control of gene expression in human cells and the ways in which they can be rationally, systematically and effectively assembled to form autonomous gene circuits. Then, we highlight the direct impacts of synthetic gene circuits in current stem cell research, molecular diagnostics, drug discovery and agriculture. Finally, in considering the prospects for synthetic biology, we conclude that the scenario of using human cells as living drugs for autonomous disease treatment in personalized medicine is close to realization.

Controlling gene expression

Gene expression in living cells is in most cases tightly regulated at the level of individual genes. To programme cell functions, it is desirable to gain precise control of gene expression using synthetic gene circuits, which comprise networks of multiple interconnected gene switches regulating target gene activities in a time-dependent and context-dependent manner and whose activity can be predicted and externally controlled.

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Natural and synthetic gene switches. From an engineering perspective, a gene switch can be regarded as any naturally evolved or rationally designed accession point that allows a scientist to 'dial' into a cell and decide whether gene expression at a particular level (DNA, RNA or protein) should be initiated, interrupted or terminated (FIG. 1A). In human cells, gene switches acting at the transcriptional level are operated by chimeric trans-regulators (Supplementary Box 1 (see the figure, part a)) consisting of a sequence-specific DNA-binding domain (DBD) that is fused to either a nonspecific epigenetic effector domain (also known as synthetic transcription factor; FIG. 1A, step 1) or a sequence-modifying nuclease domain (also known as designer nuclease; FIG. 1A, step 2; see also Supplementary Box 1 (see the figure, part b)). The DBD allows the trans-regulator to target a promoter region of either chromosomal genes (on the genome) or transgenes (on episomal vectors), where it triggers temporary activation, repression or silencing of target gene transcription (FIG. 1A, step 1) or permanent disruption or alteration of a nucleotide sequence (FIG. 1A, step 2). Genomic and transcriptional gene switches can also operate through CRISPR–Cas systems, where sequence targeting is mediated by guide RNAs (gRNAs) (see also Supplementary Box 1).

At the RNA level, protein-specific aptamer structures are central regulatory elements applicable for the design of site-specific gene switches. To regulate alternative splicing, for example, a single primary RNA transcript containing target-specific aptamers in key intronic regions can be used to produce different protein isoforms¹⁵. Depending on the presence of target proteins specifically binding to the aptamers (aptamer-binding proteins (ABPs); see also Supplementary Box 1 (see the figure, part c)) and masking the splice sites in the nucleus, different mRNA transcripts are generated (FIG. 1A, step 3). Similarly, translation-regulating gene switches use RNA aptamers as target-specific protein tethers. When incorporated upstream or downstream of a coding region in the mRNA, endogenous regulatory proteins^{16,17} or synthetic translation factors (see also Supplementary Box 1) are recruited by the aptamer to bind a target mRNA, which either facilitates or blocks ribosomal binding (FIG. 1A, step 4). Furthermore, RNAi is a naturally evolved translational gene switch operating in the cytoplasm of all eukaryotic cell types^{18,19}. Intronomically encoded small regulatory RNA (srRNA) molecules, such as short hairpin RNA (shRNA), siRNA or microRNA (miRNA), can be designed to bind complementary sequences of approximately 20 nucleotides in length on any target mRNA and to knock down gene expression by triggering RNA degradation²⁰ (FIG. 1A, step 5). Similarly, single-stranded antisense mRNA transcribed to produce an antiparallel configuration of a target gene can also inhibit translation through complementary base pairing²¹ (FIG. 1A, step 5). Lastly, protein-level switches are based on the control of localization and stability, post-translational modification and target binding. Manipulation of the chemical stability or cytosolic exposure of a nuclear localization signal²², nuclear export signal^{23,24}, prenylation motif²⁵, peroxisomal targeting sequence²⁶ or degron^{27,28} controls the trafficking of a protein to the nucleus, cytoplasm, plasma membrane, peroxisome or proteasome, respectively (FIG. 1A, step 6). Alternatively, the phosphorylation status can regulate nuclear permeability^{29,30} or the potency for transcriptional activation^{31,32} of a target protein.

Multiplexed control of gene expression. To simultaneously control multiple target genes in a single cell, which is a prerequisite for generating gene circuits of high complexity, a conventional strategy is to use mutually orthogonal trans-regulators that operate in parallel and on independent genetic events (FIG. 1B_a). Whereas chimeric transcription factors based on synthetic zinc-finger protein (ZFP) or transcriptional activator-like effector (TALE) scaffolds are inherently orthogonal to each other owing to their custom-designed and therefore predictable DBDs^{33,34}, naturally evolved transcription factors repurposed from the bacterial tetracycline-dependent repressor (TetR) protein family or the CRISPR–Cas system require context-dependent validation of functional orthologues^{35,36} (Supplementary Box 1 (see the figure, part b)). Specifically, the combined use of two catalytically dead Cas9 (dCas9) orthologues derived from *Staphylococcus aureus* (SaCas9) and *Streptococcus pyogenes* (SpCas9) fused to different fluorescent proteins (GFP and mCherry) allowed simultaneous targeting and imaging of different genomic loci³⁷ (FIG. 1B_b, left panel). Alternatively, by incorporating orthogonal sets of protein-binding aptamers into non-conserved regions of gRNA structures³⁸, a unique platform known as scaffold RNA (scRNA) was created for multiplexed gene regulation by CRISPR–Cas9. In scRNAs, effector domains need no longer be physically bound to a protein domain, as required in conventional trans-regulator designs (FIG. 1A, step 1; Supplementary Box 1 (see the figure, part a)), but can be flexibly attached to different gRNAs through specific ABP-aptamer interactions³⁹ (FIG. 1B_b, right panel; Supplementary Box 1). Multiple site-specific scRNAs programmed for different effector tasks (activation, repression or visualization) can then be read by the same Cas9 orthologue disseminated throughout the nucleus, thus enabling simultaneous Cas9-dependent control of different scRNA-selected targets^{39,40} (FIG. 1B_b, right panel).

Stimulus-dependent gene activity. In addition to multiplexed control of gene expression, gene switches responding to diverse molecular signals are instrumental in programming synchronized and interconnected gene or transgene activities^{41,42} (see also Supplementary Box 2). Whereas gene switches controlled by chemical ligands (for example, small molecules, ions or proteins) are advantageous for achieving feedback control by endogenous metabolites or other molecular cues, light-regulated gene switches offer the highest spatiotemporal resolution for experimental activation and termination^{43,44}. An optimal inducible gene switch should permit essentially no expression of the target gene in the absence of the trigger signal while enabling maximal expression in the activated state^{20,45}.

To engineer stimulus-responsive gene expression in human cells, conventional strategies include systematic

Trans-regulators
Chimeric regulatory proteins consisting of a trafficking domain (controls translocation to target DNA, RNA or protein destination) and a regulatory domain (specifies target-specific activity).

Episomal vectors
Carriers of coding genes that are not part of the endogenous chromosome, such as plasmid DNA, mini-circles or replicon RNA.

Guide RNAs
(gRNAs). Synthetic RNA molecules that bind and guide a specific Cas protein (CRISPR-associated protein) towards a gRNA-specific DNA or RNA target sequence through complementary base pairing; also known as single-guide RNAs (sgRNAs).

Aptamer
A single-stranded RNA or DNA sequence forming a secondary structure that undergoes a considerable conformational change upon binding to a specific chemical ligand (small molecules, ions or proteins) with high affinity.

Mutually orthogonal trans-regulators
A set of trans-regulators operating at parallel genetic targets that do not show cross-interaction in terms of direct binding or potential influence on each other's downstream targets.

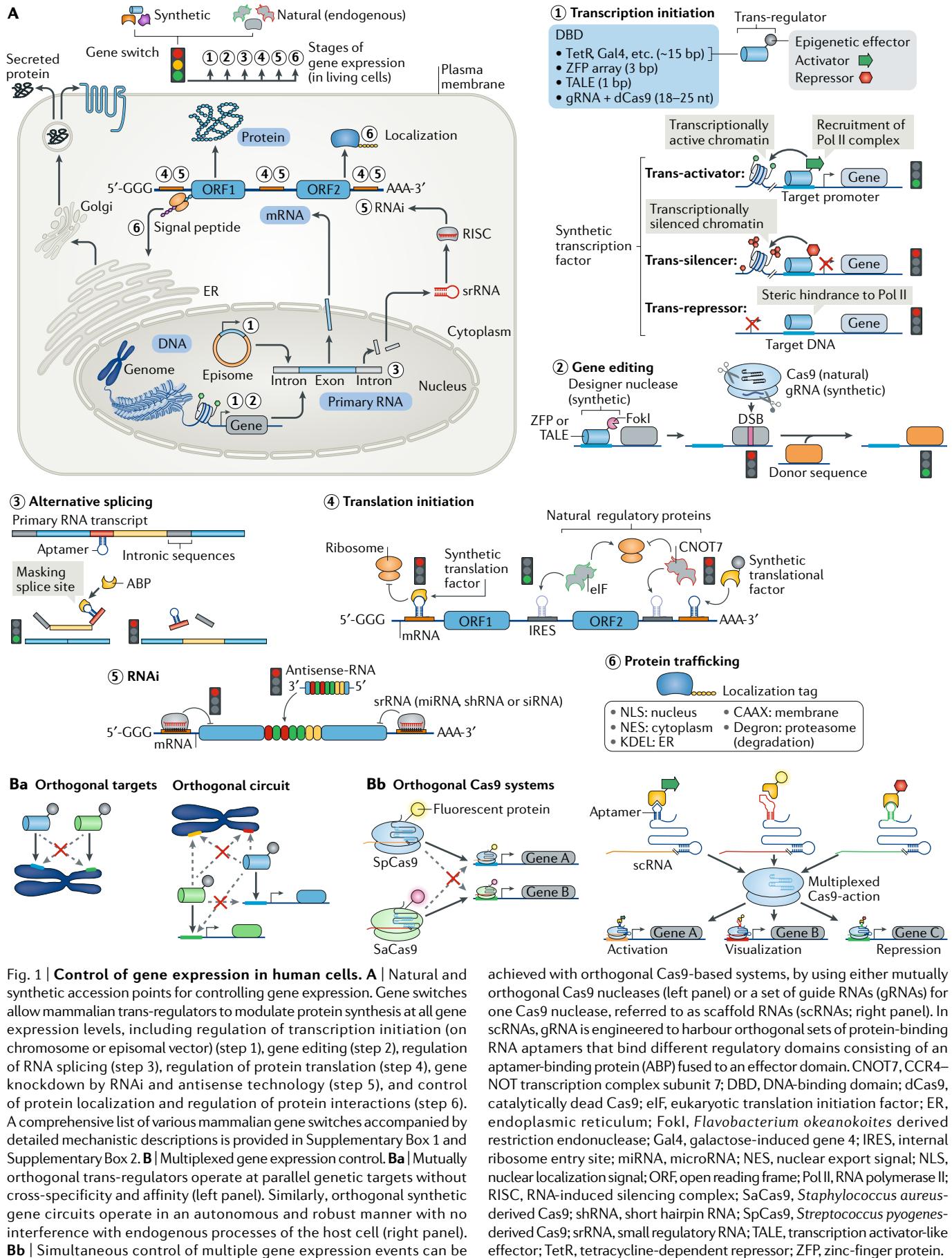


Fig. 1 | Control of gene expression in human cells. **A** | Natural and synthetic accession points for controlling gene expression. Gene switches allow mammalian trans-regulators to modulate protein synthesis at all gene expression levels, including regulation of transcription initiation (on chromosome or episomal vector) (step 1), gene editing (step 2), regulation of RNA splicing (step 3), regulation of protein translation (step 4), gene knockdown by RNAi and antisense technology (step 5), and control of protein localization and regulation of protein interactions (step 6). A comprehensive list of various mammalian gene switches accompanied by detailed mechanistic descriptions is provided in Supplementary Box 1 and Supplementary Box 2. **B** | Multiplexed gene expression control. **Ba** | Mutually orthogonal trans-regulators operate at parallel genetic targets without cross-specificity and affinity (left panel). Similarly, orthogonal synthetic gene circuits operate in an autonomous and robust manner with no interference with endogenous processes of the host cell (right panel). **Bb** | Simultaneous control of multiple gene expression events can be

use of ligand-responsive prokaryotic transcription factors as the DBD of eukaryotic trans-regulators^{46–48} (FIG. 2A,B), engineering of transcriptional gene switches responding to stimuli delivered to cell surface receptors via naturally occurring (FIG. 2Ca) or synthetic signalling pathways (FIG. 2Cb)^{49,50} and the design of RNA-level gene switches^{51,52} (FIG. 2D). The tightest transgene switch reported to date was created with the combination of two prokaryote-derived trans-repressors (TetR and lactose operon repressor (LacI)) together with RNAi (FIG. 2B). In this system, transcription of TetR and the target gene was repressed by LacI, allowing a TetR-repressible shRNA to be expressed to abolish basal expression of the target gene. Addition of the LacI-specific ligand isopropyl β-D-1-thiogalactopyranoside (IPTG) could relieve the expression of TetR and the gene of interest by inhibiting shRNA-mediated knockdown of the target gene. The extreme tightness of this LacI-TetR-RNAi (LTRi) switch was demonstrated by the survival of cells in which the LTRi-regulated transgene encoded diphtheria toxin-α — a protein so toxic that expression of a single molecule would have killed the host cell⁵³.

Whereas gene switches based on prokaryotic transcription factors are limited to cell-permeable trigger compounds for activation, cell surface receptors such as G protein-coupled receptors (GPCRs), receptor tyrosine kinases (RTKs), cytokine receptors and CARs respond to extracellular signals and require minimal exposure times to a ligand for activation. Various cell type-specific responses, such as endothelial cell motility^{54,55} or T cell activation^{13,56}, are transduced by these receptors. Thus, forced expression (for example, of CARs) or forced dimerization of the cell surface receptor (for example, of RTKs) in cell types that harbour the receptor-specific signalling cascade is reminiscent of using a ‘master key’ to kick off the complex but self-contained response^{12,54} (FIG. 2Ca; Pathway-specific transcription). Because many receptor-mediated signalling cascades activate specific endogenous promoters, engineering of synthetic promoters containing the same response elements as the endogenous promoter creates a ‘virtual clone’ on an episomal vector that captures pathway-specific endogenous signalling events with user-defined transgene readouts (FIG. 2Ca; Semi-synthetic approach). To create receptor-activated gene switches based on artificial signal transduction that do not interfere with endogenous signalling, various proteolysis-dependent design strategies inspired by the nuclear translocation of the Notch receptor intracellular domain (NICD) have been developed (FIG. 2Cb). In this paradigm, NICD is replaced by a synthetic transcription factor, which translocates to the nucleus upon receptor activation to regulate transgene expression^{57–60}. Replacement of the extracellular Notch domain by a single-chain antibody allows recognition and response to cell surface antigens while retaining the native *cis*-inhibition feature of Notch signalling. Incorporation of different synthetic transcription factors into the framework of such synthetic Notch receptors enables the sensing of antigen-specific cell contacts⁵⁷. To also allow soluble ligands to trigger proteolytic cleavage and activate transgene expression, GPCRs and RTKs were

fused to synthetic transcription factors through a transmembrane linker that contained synthetic cleavage sites for the tobacco etch virus (TEV) protease^{58–60}. Notably, the use of Cas9-based transcription factors is advantageous for simultaneous targeting of multiple genes when multiple gRNAs are co-expressed in the cell^{58–60}.

Inducible regulation of RNA-level gene activities commonly relies on the use of riboswitches, whereby a trigger (an RNA molecule or an aptamer-specific ligand) induces or stabilizes a conformational change in the target RNA^{51,52} (FIG. 2D). An elegant riboswitch principle was designed in *Escherichia coli*, where the ribosome-binding site (RBS) was masked with an engineered *cis*-acting hairpin structure that rendered the target mRNA inaccessible for ribosomal binding and hence translation⁶¹. This riboswitch enabled the detection of single-stranded trigger RNA, which bound to a complementary portion of the masking hairpin and induced a conformational change in the mRNA molecule, resulting in RBS unmasking and initiation of translation (FIG. 2Da). In human cells, a similar system based on aptamers was designed to engineer ligand-responsive gRNAs, also known as signal conductors⁴⁰. Because aptamers undergo a considerable conformational change upon ligand binding (FIG. 2Db, top panel), they can be used to control the conformation and hence activity of the RNA molecule they are attached to. Accordingly, gRNAs harbouring aptamers in their 3' hairpin regions were designed to adopt a secondary structure in which the DNA-targeting guide region was sealed by a synthetic complementary region engineered into the same gRNA molecule (FIG. 2Db, bottom panel). The presence of external ligands binding the aptamer triggered unmasking of the guide region and restored gRNA-dependent CRISPR-Cas activity, such as transcription control (FIG. 1A, step 1) or gene editing (FIG. 1A, step 2). Similarly, incorporation of aptamers into regions of srRNA precursors⁶² or self-cleaving aptazymes⁶³ enables regulation of target gene translation through ligand-dependent control over RNAi (Supplementary Box 2 (see the figure, part c)) or mRNA stability (Supplementary Box 2 (see the figure, part d)), respectively.

Trigger-mediated control of gene activity at the protein level is achieved by allosteric proteins^{64,65} and ligand-induced protein dimerization (Supplementary Box 1 (see the figure, part d), see also next subsection).

Spatiotemporal control elements. Whereas natural localization signals encoded in a protein structure are spontaneously recognized by the cellular trafficking machinery to guide the protein towards different target destinations following translation (FIG. 1A, step 6), protein dimerization — controlled by external signals, for example, chemical ligands or light (Supplementary Box 1 (see the figure, part d)) — can also be employed to create synthetic tethering motifs (FIG. 3A). For example, a dimerization partner could be fused to an anchor protein residing within the plasma membrane^{25,66} or at specific organelles (for example, endoplasmic reticulum²⁵ or mitochondria⁶⁷) to attract target proteins to these sites, thereby regulating intracellular cargo

Riboswitches

Regulatory segments within an mRNA that bind to specific metabolites and modify the expression of the protein product of the riboswitch-containing mRNAs.

Self-cleaving aptazymes

Products of a chimeric fusion between an aptamer and a (self-cleaving) ribozyme in which the ligand-dependent conformational change of the aptamer is also propagated to affect the activity of the ribozyme.

Allosteric proteins

Proteins in which an active or inactive conformation is reversibly triggered by ligand binding or other stimuli (such as light).

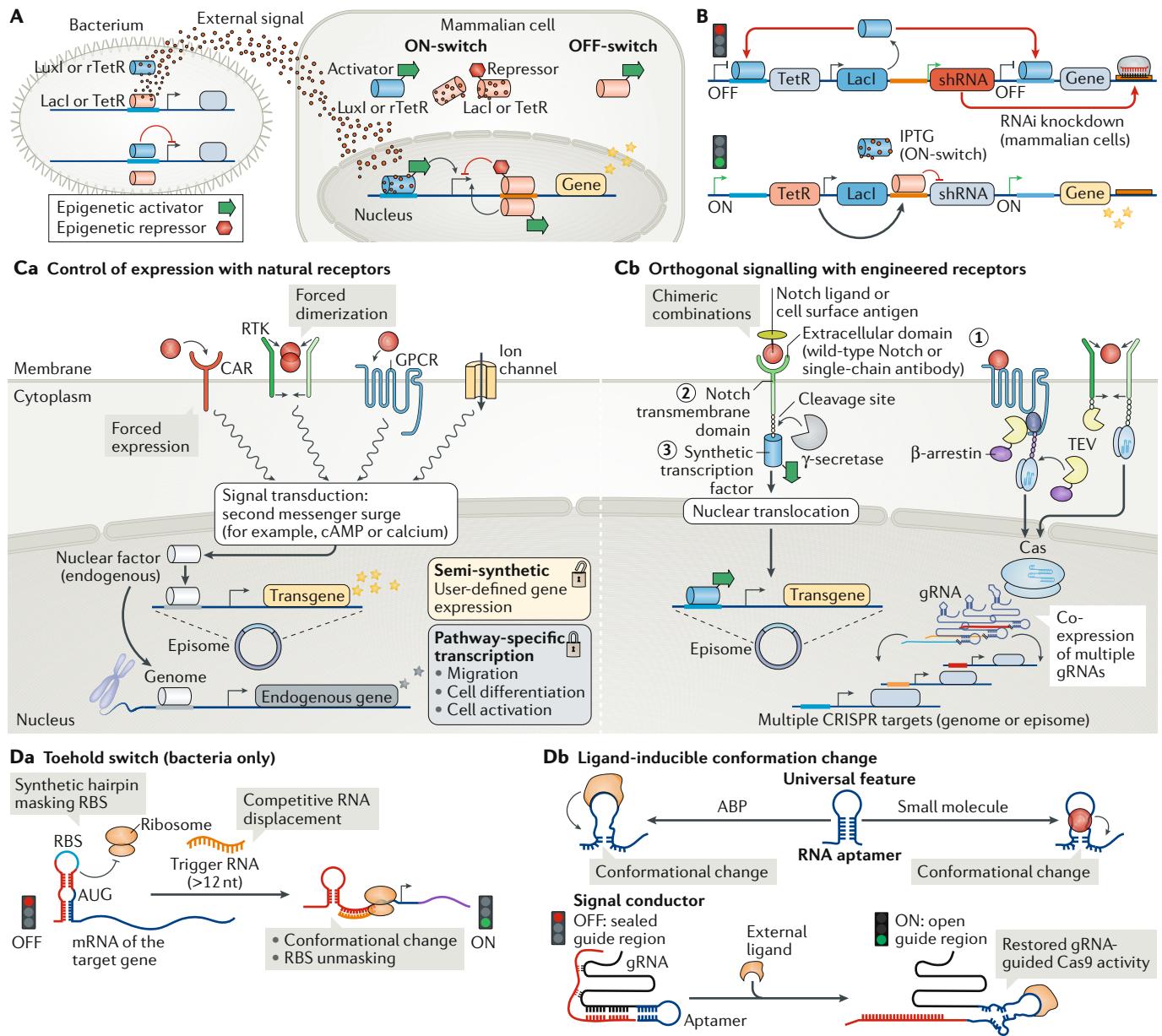


Fig. 2 | Engineering of stimulus-responsive human cell behaviour. **A** Gene switches based on prokaryotic transcription factors. The use of prokaryotic transcription factors (pTFs; for example, tetracycline-dependent repressor (TetR), lactose operon repressor (LacI) or transcriptional activator protein LuxR) in mammalian cells as the DNA-binding domain (DBD) of a synthetic transcription factor enables trigger-inducible regulation of synthetic target gene promoters that contain pTF-specific binding sites. The presence of a pTF-specific trigger compound renders the DBD either capable or incapable of binding DNA, and depending on the choice of the effector domain, this conformational change results in activation (ON-switch) or termination (OFF-switch) of transcription. **B** Principle of the LacI-TetR-RNAi (LTRi) switch. In the repressed state (OFF), transgene expression is abolished through LacI-dependent transcription repression and concomitant RNAi by a short hairpin RNA (shRNA) that targets the 3' untranslated region (UTR) of the transgene mRNA. Addition of isopropyl-β-D-thiogalactopyranoside (IPTG) derepresses LacI-specific promoters, resulting in transgene transcription and concomitant TetR-mediated repression of shRNA production. **C** Control of gene expression by cell surface receptors. Gene switches responsive to surface receptor-mediated signalling can be host cell-specific (receptor activation is sufficient to trigger pathway-specific transcription), semi-synthetic (transgene expression from

synthetic promoters engineered to contain endogenous response elements) (part **Ca**) or orthogonal (engineered receptors activate synthetic promoters through artificial signal transduction) (part **Cb**). For orthogonal approaches, engineered receptors consist of: (1) an extracellular ligand-binding domain (for example, single-chain antibodies, G protein-coupled receptors (GPCRs) or receptor tyrosine kinases (RTKs)), (2) a transmembrane domain containing target sites for proteolytic cleavage (for example, by Notch signalling-specific γ-secretase or by tobacco etch virus (TEV) protease) and (3) a synthetic transcription factor (based on pTFs or Cas9) that is released for nuclear translocation upon receptor-activated proteolysis. **D** RNA-based gene switches. **Da** In *Escherichia coli*, a 'toehold' switch was engineered on the basis of RNA displacement. A hairpin structure in the 5' UTR of mRNAs masks the ribosome-binding site (RBS) and prevents translation initiation, which is counteracted by binding of a single-stranded trigger RNA and conformational change of the mRNA. **Db** Because RNA aptamers undergo a considerable conformational change upon ligand binding (top panel), they can be integrated into different RNA regions to create ligand-responsive gene switches. For example, gRNAs can be designed to contain an aptamer so that the DNA-targeting guide region is unmasked upon ligand binding (bottom panel). ABP, aptamer-binding protein; CAR, chimeric antigen receptor; gRNA, guide RNA; nt, nucleotide; rTetR, reversed TetR.

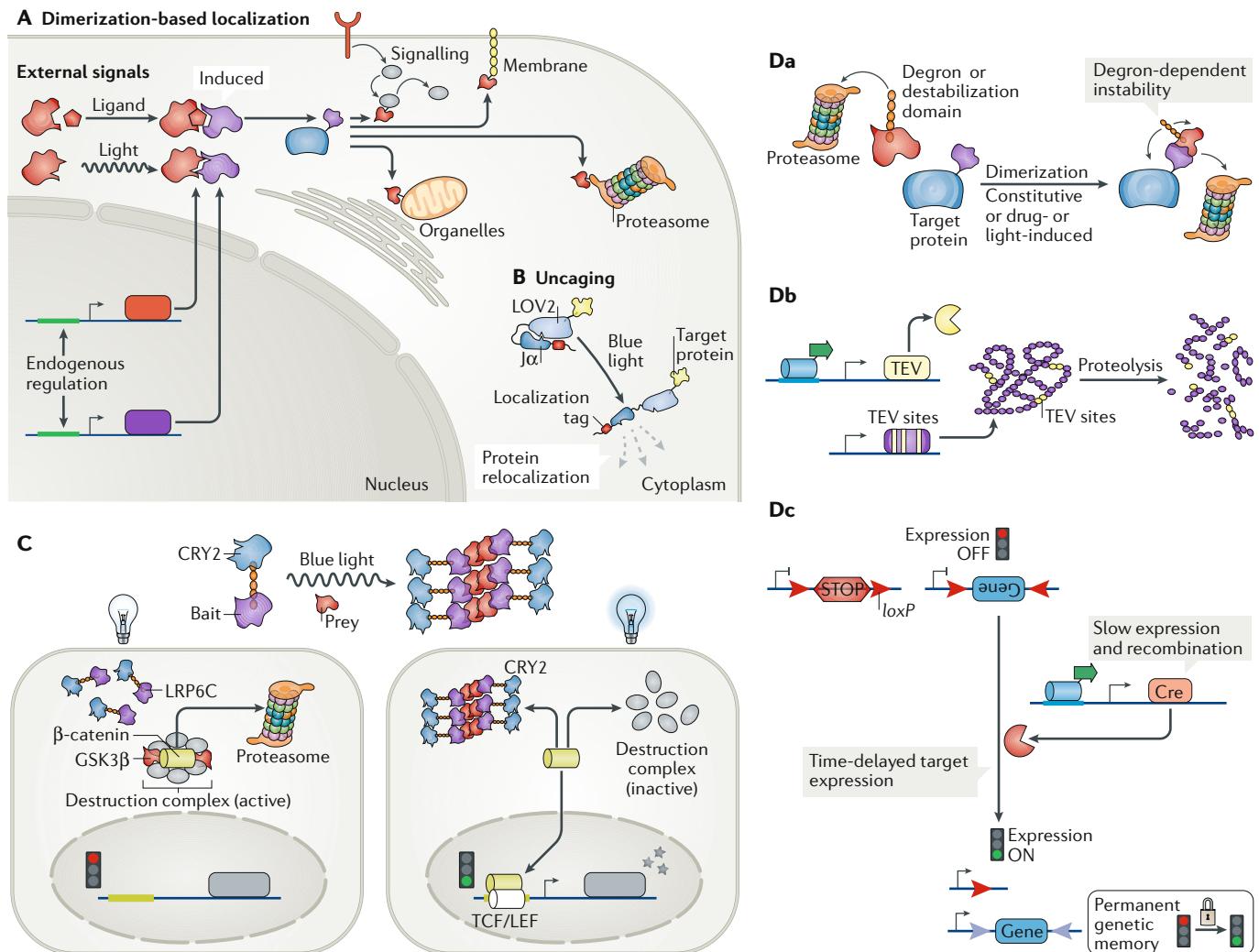


Fig. 3 | Spatiotemporal control. **A | Dimerization-based localization.** Chemical ligands or light can be used as triggers for driving protein–protein interactions (see also Supplementary Box 1 (see the figure, part d)). This enables a target protein harbouring a protein interaction domain to be targeted to a desired location that specifically expresses its interaction partner in a temporally controlled manner. Inducible dimerization can also be used for trigger-regulated reconstitution of transcription factors, nucleases, proteases or reporters (Supplementary Box 2). **B | Protein uncaging.** Blue light triggers ‘uncaging’ of the *Avena sativa* phototropin 1-derived LOV2 protein, resulting in dissociation and cytoplasmic exposure of a carboxy-terminal α-helix (Jα). By fusing localization signals to Jα, synthetic systems have been designed for conditional control of protein trafficking and degradation, whereby the target protein is fused to LOV2. **C | Oligomerization-based protein sequestration.** A target protein (prey; for example, glycogen synthase kinase-3β (GSK3β)) known to interact with a protein partner with high affinity (bait; for example, carboxy terminus of

low-density lipoprotein receptor-related protein 6 (LRP6C)) can be sequestered into a synthetic protein cluster formed by fusion of the bait protein to an oligomer-forming protein (for example, cryptochrome 2 (CRY2)). In this example, selective sequestration of GSK3β away from an endogenous destruction complex by blue light-triggered oligomerization of CRY2–LRP6C could inhibit the degradation complex of cytosolic β-catenin. **D | Fine-tuning temporal dynamics of gene expression.** **Da |** A conditionally stabilized degron or destabilization domain fused to a protein that specifically binds the target protein with high affinity can confer its conditional instability on the target protein, resulting in degradation. **Db |** Target proteins engineered to contain tobacco etch virus (TEV) protease cleavage sites can be degraded by proteolysis upon ectopic expression of the TEV protease. **Dc |** In a recombinase-mediated time-delay strategy, gene expression relying on Cre-mediated reconstitution of a functional transcription unit is temporally delayed by time-consuming Cre expression. TCF/LEF, T cell factor/lymphoid enhancer factor.

transport^{25,28,67,68} and/or modulation of cell signalling^{66,69} (FIG. 3A). Dimerization can also be used in split expression approaches to drive reconstitution of full, functional proteins from two individual fragments (Supplementary Box 2 (see the figure, part b)), or in two-hybrid systems to drive assembly of trans-regulators by combining a trafficking domain (such as a DBD) and an effector domain that are each attached to a dimer-forming protein (Supplementary Box 2 (see the figure, part a)). Spatiotemporal control of protein activity has

also been achieved by trigger-induced protein uncaging (FIG. 3B; Supplementary Box 2 (see the figure, part g)). Most commonly used in this context is the protein LOV2 derived from *Avena sativa* phototropin 1, which has been repurposed for various cell-engineering applications. LOV2 uncaging is driven by blue light. During this process, a carboxy-terminal α-helix (Jα) is dissociated from the protein core and exposed to the cytoplasm⁷⁰. By fusing localization signals to Jα, synthetic systems have been designed for conditional control of

protein trafficking^{22,71} and degradation^{43,64,72} (FIG. 3B). Similarly, fusion of a calcium-sequestering calmodulin-M13 domain to Jα afforded a synthetic optogenetic device controlling blue light-triggered calcium release⁷³.

Many signalling events in human cells are governed by spatial control elements that form physical barriers or scaffold structures to restrict localization and action of target proteins⁷⁴. For example, nuclear translocation of β-catenin during WNT signalling is negatively regulated by a destruction complex that sequesters cytoplasmic β-catenin for proteasomal degradation⁷⁵. To compete with this endogenous destruction complex, a synthetic clustering system was created on the basis of a synthetic fusion protein of cryptochrome 2 (CRY2) and carboxy terminus of low-density lipoprotein receptor-related protein 6 (LRP6C), which acts as a bait for destruction complex component glycogen synthase kinase-3β (GSK3β)⁷⁶. Blue light triggers oligomerization of CRY2-LRP6C, which by interacting with GSK3β sequesters it from its interaction partners and thereby disrupts the destruction complex⁷⁷ (FIG. 3C).

Protein-level switches generally offer rapid responsiveness to external stimuli⁷⁸. For example, an effective method for trigger-inducible depletion of a target protein is based on forced recruitment of a binding partner that contains a conditional degron, which drives proteasomal degradation of the entire protein complex⁴³ (FIG. 3D*a*). Proteasome-independent control of protein degradation can be achieved by incorporating cleavage sites for a conditionally activated TEV protease into a target protein⁷⁹ (FIG. 3D*b*). To enable temporal synchronization of input and output levels between different gene switches, synthetic control elements enabling accelerated or delayed response times are required. For example, a rapid-acting signal transduction module based on protein-level phosphorylation processes can be used to connect two slow-acting transcriptional systems to accelerate the dynamics of the entire circuit³². Site-specific recombinases such as Cre are often used to programme time-delayed transcription initiation (FIG. 3D*c*). By placing the coding region of a gene of interest in an antisense orientation flanked by antiparallel *loxP* sites^{80,81} or by placing a termination (STOP) signal between parallel *loxP* sites in a promoter region of the gene^{82,83}, reconstitution of a functional transcription unit and gene expression depend on time-consuming Cre expression and Cre-mediated sequence inversion or excision, which considerably slows down target gene expression.

Cryptochrome 2 (CRY2). A protein derived from *Arabidopsis thaliana* that undergoes reversible oligomerization or heterodimerization with CIBN (amino-terminal domain of cryptochrome-interacting basic helix-loop-helix) upon exposure to blue light.

Cre
A type I topoisomerase from bacteriophage P1 that catalyses site-specific recombination (inversion or deletion) of DNA between *loxP* sites

Memory buffers
Transient memory devices with a finite capacity for storing cellular information that ensure unperturbed functionality of a regulated subsystem when sufficiently charged.

Principles of prototype gene circuits

A long-term goal of synthetic biology is to develop sophisticated gene circuits that serve as a kind of ‘genetic software’ to programme cellular functions analogously to the case of electronic computers⁸⁴. To this end, various prototype circuits providing a standardized toolkit for the design and assembly of specific cell functions have been developed (FIG. 4).

Memory devices. Cells have evolved a variety of mechanisms to remember past experiences in the form of quantifiable memory^{10,85} (FIG. 4A*a*). Protein stability is an intrinsic memory element of any biological system.

For example, an induced transcription factor can keep activating a specific gene switch even after removal of the trigger signal, resulting in sustained and unperturbed target activity over a specific transient time window. Such memory buffers, which are reminiscent of the charge level of electronic capacitors, protect the robustness of a particular subsystem and have been characterized in both natural⁸⁶ and synthetic transcriptional contexts^{87–90}. To quantify the charge level of memory buffers in real-time, a synthetic gene network based on hybrid transcription factors was developed. In the default state (discharged memory; 0% charged), the hybrid transcription factor binds and activates a high-affinity promoter (P_A). Loading of the memory device with a trigger signal abolishes trans-activation of P_A but allows the transcription factor to activate a low-affinity promoter (P_B) (charged memory; 100% charged). Degradation of the load signal results in a gradual inactivation of P_B (buffer consumption) and concomitant re-initiation of P_A (full discharge)⁹¹. Quantification of reporter protein levels expressed from P_A and P_B enables precise monitoring of actual charge levels (FIG. 4A*b*). To create long-lasting memory devices, positive auto-regulatory feedback loops are effective because they generate reporter proteins at such high levels that they can be inherited through multiple rounds of cell division⁹².

Epigenetic bistable gene switches characterized by two stable expression states represent a naturally evolved regulatory pattern for dynamic, inheritable and resettable memory⁹³. The presence of a switching signal flips an arbitrarily set default state into the second state, which is maintained over multiple cell generations until a second stimulus toggles the gene switch back to the equally stable default state⁸⁹. In general, bistability in regulatory networks requires two mutually inhibitory gene switches, allowing a minimal derepressive stimulus to sufficiently trigger the prevalence of one expression programme over the other (FIG. 4A*c*). Synthetic toggle switches created in bacteria^{94,95} and human cells^{89,96} based on mutually repressible gene transcription were among the first engineered gene circuits inaugurating modern synthetic biology at the turn of the millennium. Gene expression triggered by derepression (gene induction by repressor inactivation; for example, B represses C, but A triggers C by inactivating B) is particularly effective for achieving tight regulation, pulse-like induction and ultra-high sensitivity to transient stimuli⁹⁵, which are essential for bistable gene expression. Bistable switch performance can be further optimized when gene expression in each state is also self-amplified with positive feedback loops^{93,97} (FIG. 4A*c*).

In contrast to epigenetic memory, genetic events triggering a permanent change in a DNA sequence result in irreversible genotypes. Because the biochemistry of DNA is inherently compatible with robust, scalable and stable storage of analogue data, permanent genetic memory devices based on DNA editing (FIG. 1A, step 2) can accumulate and convert transient molecular signals into long-lasting information, allowing data recovery and decoding even if the cells are disruptively harvested^{42,98,99}. By synchronizing the activity of site-specific recombinases with a user-defined stimulus,

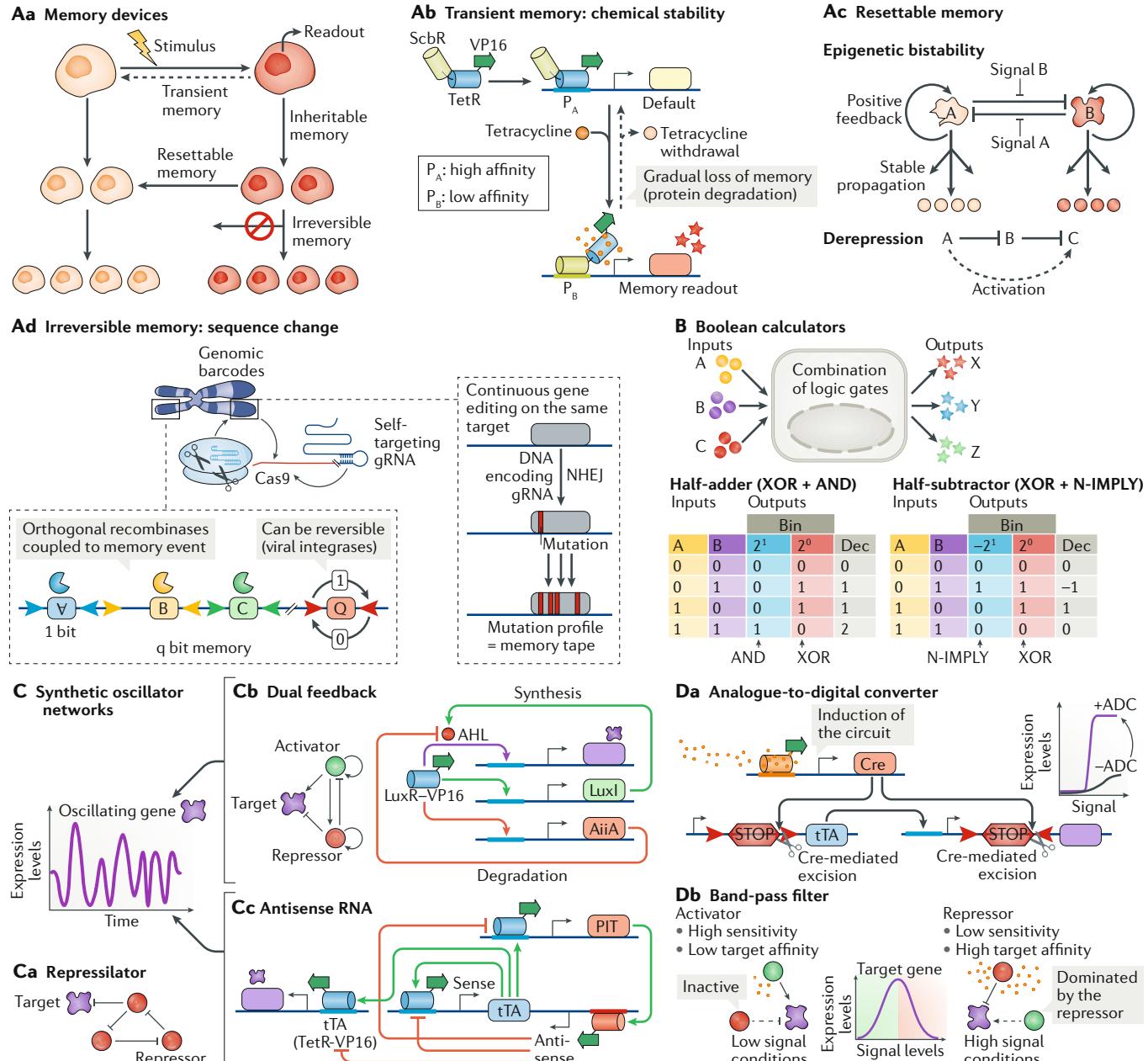


Fig. 4 | Prototype synthetic gene circuits. **A** | Synthetic memory devices. Memory devices allow a cell to remember a trigger-inducible cell state even after removal of the trigger signal (part **Aa**). Memory elements can be based on chemical stability, whereby memory is read out as protein expression driven by a trigger (part **Ab**), bistable gene switches (part **Ac**) and DNA sequence modification with recombinases and nucleases, which can be used to create synthetic barcodes in non-coding genomic regions (part **Ad**). **B** | Boolean calculators. Cells can be programmed to compute various inputs to define the output using combinations of logic gates (see also BOX 1). For example, a half-adder uses a combination of XOR and AND gates to calculate the binary addition of two Boolean numbers A and B, while a half-subtractor calculates the difference between A and B (A minus B) using XOR and A N-IMPLY B. Whereas the addition of 1 + 1 is shown as 2 in the conventional decimal system (Dec), the digit 2 is represented as '10' in the binary (Bin) system. **C** | Synthetic oscillator networks. **Ca** | Repressilator based on a triple-negative feedback ring architecture. **Cb** | Dual feedback consisting of an activator module (positive feedback) and a slower-acting repressor module (negative feedback). Implementations based on bacterial quorum-sensing components such as the acyl homoserine lactone (AHL)-inducible transcriptional activator protein LuxR-Herpes simplex-derived virion protein 16

(VP16) trans-activator (activator module) and AHL-degrading N-acyl homoserine lactonase AiiA (repressor module) encode pulse-like oscillations. **Cc** | Encoding oscillations with antisense technology. Two opposing synthetic promoters drive sense and antisense expression of a synthetic tetracycline-controlled trans-activator tTA (tetracycline-dependent repressor (TetR)-VP16). To generate oscillations, tTA drives its own sense expression (positive feedback), the expression of the oscillating gene and the expression of a second trans-activator (pristinamycin-induced protein (Pip)-dependent trans-activator (PIT)), which then drives the antisense tTA production (negative feedback). **D** | Synthetic cell-cell communication. To fine-tune signal processes in receiving cells, various strategies can be used. **Da** | Analogue-to-digital converters (ADCs) translate dose-dependent input signals into an all-or-nothing expression profile by amplifying time-delayed gene expression (FIG. 3Dc). **Db** | Synthetic band-pass filters permit only target gene expression at intermediate levels of a trigger signal by including regulation of the output by an activator that has a high sensitivity for the signal but low target affinity and a repressor that has low sensitivity for the signal but high target affinity. gRNA, guide RNA; LuxI, *Vibrio fischeri*-derived acyl homoserine synthase; NHEJ, non-homologous end joining; ScbR, *Streptomyces coelicolor* butyrolactone-dependent repressor.

irreversible DNA rearrangement can be programmed at defined transcription units to create permanently altered promoter architectures and therefore new gene expression profiles⁸¹ (FIG. 3Dc). Non-coding sequence readouts can be inscribed on a user-defined DNA stretch integrated into a target genomic locus, generating an internal barcode region that continuously captures cellular history (FIG. 4Ad). Barcode regions created by multiple sets of orthogonal recombinases controlled by synthetic trigger-inducible gene switches store cellular memory as a combination of single ‘information bits’ formed by binary DNA orientations of individual sequence segments that are flanked by specific recombinase target sites. Incorporation of n pairs of orthogonal recombinase target sites on one DNA stretch creates a data register with a memory capacity of n bits, which is capable of distinguishing 2^n cellular events synchronized with the activity of each trigger-inducible recombinase^{98,99} (FIG. 4Ad). Barcode regions can also be generated by CRISPR–Cas9 systems programmed for iterative self-targeting and random induction of point mutations^{42,100,101}. For example, self-targeting gRNAs guiding the Cas9 nuclease to its own genomic locus and inducing site-specific non-homologous end joining (NHEJ)-dependent DNA repair allow Cas9-induced mutations to continuously rewrite a gRNA-specific barcode region with unlimited memory capacity. Barcode regions created by recombinases or CRISPR–Cas9 systems are not designed to code for cellular functions and must therefore be decoded with conventional omics technologies such as whole-genome sequencing or transcriptome profiling^{42,100–102}. Barcode-based memory devices could become an attractive strategy for basic research applications to map genomic changes originating from diseases or associated with cell differentiation.

Non-homologous end joining

(NHEJ). An error-prone endogenous DNA repair mechanism for double-stranded breaks that is usually initiated when a correct DNA template is not provided.

Boolean logic gates

Synthetic (bio)computing devices that convert multiple input signals into a smaller number of outputs according to a defined logic algorithm.

Quorum sensing

A cell–cell communication mechanism evolved in many bacterial species that allows specific (sub)populations to measure their local density (by production, release, accumulation and detection of a signalling molecule) and subsequently coordinate gene expression.

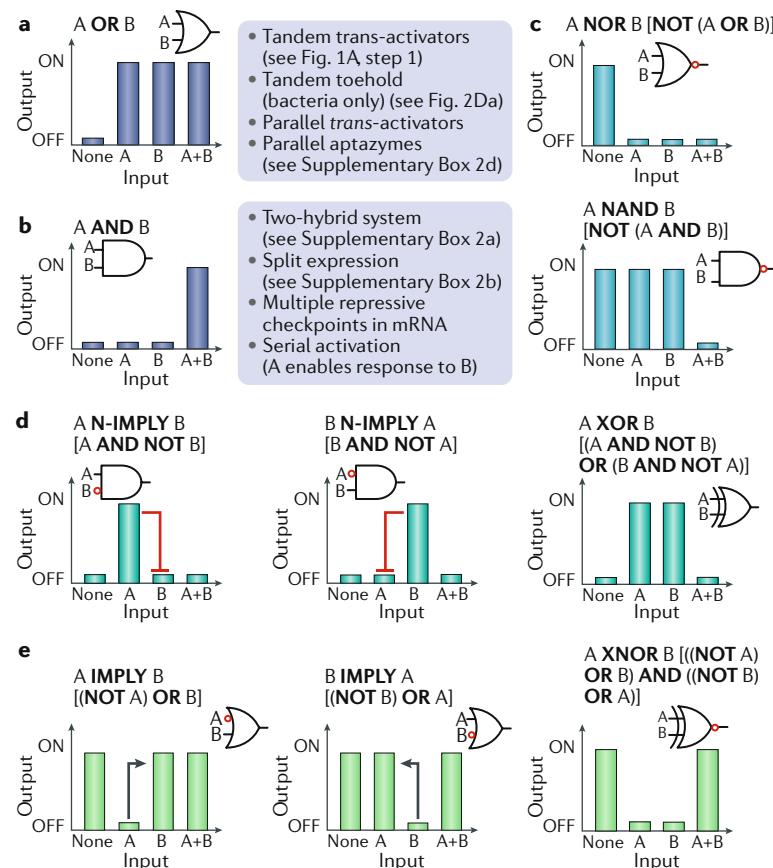
programming all logic operations into a single gene expression layer^{83,84,106,107}.

Synthetic oscillators. Oscillating gene expression controls and programmes rhythmic cellular activities such as the circadian clock^{108,109}, glycolysis¹¹⁰ or the cell cycle¹¹¹. The repressilator, which is based on a triple-negative feedback ring architecture consisting of three mutually repressive control elements (FIG. 4Ca), was the first attempt to engineer a synthetic oscillator that programmes rhythmic production of target proteins¹¹². However, subsequent computer-aided studies revealed that a dual-feedback topology consisting of a positive auto-feedback module activating the expression of all interaction partners in a network, combined with a slower-acting negative feedback module repressing the same targets, was most effective to programme autonomous, sustained and tunable gene oscillations^{90,113–115}. One of the well-established systems for generating oscillations is based on bacterial quorum-sensing systems¹¹⁶ (FIG. 4Cb). In *Vibrio fischeri*, acyl homoserine lactone synthase (LuxI) produces acyl homoserine lactone (AHL) as a quorum signal that diffuses across the cell population, providing a marker for population density. Upon passing a critical threshold of population density, accumulated AHL activates the TetR family transcription factor LuxR to induce transcriptional responses at cognate LuxR-specific promoters. Because many quorum-induced cell responses are released in an all-or-nothing manner^{117–119}, synthetic oscillators engineered with LuxR-dependent positive feedback modules typically exhibit pulse-like gene expression dynamics¹²⁰. In this system, a synthetic trans-activator based on LuxR regulates the expression of the oscillating gene and LuxI (positive feedback) as well as the AHL-degrading enzyme *N*-acyl homoserine lactonase AiiA derived from *Bacillus thuringiensis* (negative feedback)^{121,122} (FIG. 4Cb). In contrast to pulse-like oscillators, harmonic alternation of positive and negative feedback modules can be programmed with antisense technology¹²³ or RNAi¹²⁴ in mammalian cells. Using two opposing synthetic promoters driving sense or antisense expression of a synthetic tetracycline-controlled trans-activator (tTA; TetR–*Herpes simplex*-derived virion protein 16 (VP16)), pendulum-like oscillations were generated when basal tTA expression could simultaneously activate its own expression (positive feedback), the expression of the oscillating gene (target) and the expression of a second trans-activator (pristinamycin-induced protein (Pip)-dependent transactivator (PIT)) that triggered tTA knockdown through antisense mRNA production (negative feedback) until tTA was depleted enough to not allow efficient expression of PIT, but basal tTA expression remained sufficient to re-initiate another oscillation cycle¹²³ (FIG. 4Cc).

Intercellular communication. Precise control over cell–cell communication is important to achieve higher-order behaviours in multicellular organisms such as humans. To engineer synthetic intercellular communication systems, gating mechanisms in receiver cells that not only

Box 1 | Logic gates and their application to gene regulation

Logic gates are synthetic gene circuits programmed to permit the expression of an output protein only when a strictly defined signature of input signals is matched. OR gates (see the figure, part a), which permit expression of the output protein whenever either of two input signals (A or B) is present, can be encoded by promoter^{103,193} or mRNA⁸⁴ architectures containing tandem binding sites for different mutually orthogonal activators or by multiple activator-specific promoters¹⁹⁴ or mRNAs¹⁹⁵ driving the expression of the same target gene. AND gates (see the figure, part b) permit output gene expression only when all input signals are present. All two-hybrid and split expression technologies (see also Supplementary Box 2) inherently encode an AND gate, in which input-defining events are synchronized through constitutive protein dimerization^{104,192}, intein splicing¹⁹⁶ or chaperone-assisted protein folding¹⁹⁷. Alternatively, mRNAs that could be translated into mature proteins are modified to contain different input-dependent checkpoints, such as aptazymes^{63,198}, inhibitory hairpins⁸⁴ or target sites for translational repressors⁶³ and self-encoded small regulatory RNA (srRNA)⁵⁶. Output protein is expressed when all inhibitory checkpoints are cleared. AND gates can also be programmed by serial activation of input-specific targets, in which the presence of one input drives the expression of a receptor for the next input^{170,179} or induces an active conformation of a target protein specific for the other input¹⁹⁹. NOT inverters (see the figure, part c) specifically invert the computing logic of any AND or OR gate component. Conventional strategies are integration of a repressor-based subsystem into an activated gene expression motif^{103,194}, the use of synthetic promoters containing tandem binding sites for different trans-repressors¹⁹³ and mRNAs containing multiple target sites for srRNAs^{195,200}. AND NOT (N-IMPLY; also known as NOT IF) gates (see the figure, part d) can also be created with tandem trans-regulator binding sites, in which one trans-regulator is an activator and the other trans-regulator is a repressor that overrides the regulatory function of the activator by binding to the same target¹⁹⁴. N-IMPLY gates can be further assembled to form exclusive OR gates (XOR) to permit only the presence of one input signal for output gene expression. Similarly, exclusive NOR gates (XNOR), which process only the simultaneous presence or absence of both input signals can be assembled by connecting two IMPLY gates ($A \text{ IMPLY } B = (\text{NOT } A) \text{ OR } B$; $B \text{ IMPLY } A = (\text{NOT } B) \text{ OR } A$) (see the figure, part e).



sense but also process and adjust signals produced from sender cells are essential. To programme digital signal processing devices that initiate all-or-nothing responses in receiver cells when the input signal has passed a critical threshold level, a synthetic network consisting of a time-delayed gene expression module and a synthetic trans-activator (such as tTA) was designed. Specifically, insertion of a terminator signal (STOP) flanked by parallel target sites of Cre recombinase between a synthetic tTA-specific promoter and the coding region of a target gene allows tTA to accumulate to saturating levels at its promoter during Cre-mediated terminator excision, permitting maximal trans-activation of target gene expression only upon reconstitution of the transcription unit⁸² (FIG. 4Da). Unlike such synthetic analogue-to-digital converters (ADCs), band-pass filters allow only a specific intermediate range of input signals to trigger gene expression and repress gene expression outside of this defined concentration window¹¹³ (FIG. 4Db). In both bacterial¹²⁵ and mammalian^{126,127} systems, the key design principle for synthetic band-pass filters is the use of two antagonistic gene switches regulating the same target gene but with different target affinity and signal sensitivity. Low and intermediate levels of the trigger signal allow a gene switch of higher sensitivity — but lower affinity — to regulate target gene expression in a conventional dose-dependent manner until the low-sensitivity gene switch, which is activated at high signal levels, overrides the regulatory activity of the high-sensitivity gene switch owing to higher affinity for the target gene (FIG. 4Db).

Because the intensity of trigger signals produced from a sender cell population negatively correlates with the distance to receiver cell populations in synthetic cell-cell communication systems, synthetic band-pass filters allow receiver cells to fine-tune distance-dependent gene expression¹²⁵. In most cases, soluble communication signals such as quorum-derived lactones^{103,116–120,122}, cytokines¹²⁸, amino acids^{128,129} or vitamins¹³⁰ are used to programme cell-cell communication. In contrast to soluble molecules, gaseous signals are typically far-reaching but are relatively weak and short-lived because of the low physical stability of the gaseous species *in vitro*^{122,130}. Nevertheless, upon implantation of individual populations of sender cells and receiver cells into mice, synthetic intercellular communication *in vivo* mediated by volatile aldehydes achieved comparable signalling dynamics to those of the native endocrine system¹³⁰. To sense physical cell contacts, two strategies were recently developed on the basis of synthetic Notch signalling⁵⁷ (FIG. 2Cb) and semi-synthetic transcriptional readout of synthetic CD45-dependent Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signalling¹³¹ (FIG. 2Ca).

Present applications

Prototype synthetic gene circuits represent an ideally simplified replicate of complex regulatory motifs used by native cells, serving as a high-level starting point for in-depth analysis of related medical conditions and/or the development of more advanced solutions. In other words, prototype gene circuits render complex cellular processes

accessible to engineering (BOX 1; FIG. 4) and allow the design of application-specific cell functions (FIGS 5,6).

Molecular diagnostics. Transcriptional gene switches based on prokaryotic transcription factors (FIG. 2A,B) are widely used in metabolic engineering for real-time monitoring of difficult-to-trace metabolites¹³² or to increase bioprocess efficiency in the production of protein therapeutics¹⁰. Similarly, biosensors based on ligand-regulated nucleic acids¹³³ (FIG. 2D) and ligand-responsive reporter proteins¹³⁴ (FIG. 3B; see also Supplementary Box 2) transferred from living cell systems into cell-free contexts^{135,136} are being used to develop point-of-care diagnostics for personalized medicine¹³⁷. Although human cells carrying natural, receptor-mediated gene switches (FIG. 2Ca) responding to histamine¹³⁸ or tumour necrosis factor (TNF)¹³⁹ also showed impressive diagnostic capacities for detecting allergic responses and bacterial infections in human blood in vitro, synthetic gene circuits can improve the biosensing precision of living cells by avoiding the risks of false negatives potentially caused by signal fluctuations. For example, encapsulated bacteria harbouring genetic software consisting of gene switches (FIG. 2A), ADCs (FIG. 4Da), two-input logic gates (BOX 1) and a recombinase-based permanent memory register (FIG. 4Ad) were engineered to iteratively detect and compute pathological levels of short-lived metabolites such as nitrogen oxides and glucose from clinical urine samples, offering an attractive diagnostic approach for metabolic diseases such as diabetes¹⁴⁰.

Intein

A protein motif that is irreversibly excised from a larger protein structure when specific peptide domains are brought into close proximity.

Epigenetic memory

A type of resettable memory of living cells that is based on inheritable post-translational protein modifications and DNA conformations rather than a change in nucleotide sequences.

Transient transfection

Delivery of foreign gene elements into host cells through episomal vectors that do not permanently integrate into the genome and therefore reside in the cells for only a few rounds of cell division.

Drug target mimetics

Simplified replicates of biological targets created outside of their natural environment that retain all basic molecular functionalities important for (high-throughput) drug screening and development.

Anti-targets

Biological targets that should not be activated by a potential drug candidate.

synchronized to cell stage-specific PDX1 expression and β-cell-specific MAFA transcription was shown to differentiate pancreatic progenitor cells into β-cells with unprecedented efficiency¹²⁷. In short, this system — known as a lineage control network — is reminiscent of a genetic software that uses vanillic acid as the sole trigger compound to control differentiation-stage-dependent NGN3 expression. When this system is incorporated into pancreatic progenitor cells, addition of intermediate concentrations of vanillic acid triggers a surge in the second messenger cAMP, which drives expression of a vanillic acid-dependent trans-activator (vanillic acid repressor VanR fused to VP16 (VanR-VP16)) from a high-sensitivity, low-affinity cAMP-responsive element-binding protein 1 (CREB1)-specific promoter. VanR-VP16 then triggers expression of both ectopic NGN3 and an shRNA that knocks down endogenous PDX1 expression, which is essential for the transition from pancreatic progenitor cell state to endocrine progenitor fate. To control the subsequent differentiation stage of β-cell maturation characterized by high-level expression of PDX1 and MAFA and low-level expression of NGN3, high concentrations of vanillic acid were added to simultaneously terminate VanR-VP16-dependent trans-activation of NGN3 and activate a low-sensitivity, high-affinity CREB1-specific promoter driving expression of ectopic PDX1 and MAFA. Notably, this gene circuit was operational on episomal vectors in pancreatic progenitor cells following transient transfection, which triggered permanent differentiation of pancreatic β-cells by interacting with endogenous master transcription factors without the need for gene editing (FIG. 5a). Therefore, regulation of cell fate using rationally designed gene circuits might become a new gold standard in stem cell research, but the moderately high costs associated with biopharmaceutical manufacturing of stem cell-derived therapeutic cell products might limit the applicability for clinical and commercial purposes¹⁴⁶.

Drug discovery and development. Using synthetic gene circuits, functional drug target mimetics and anti-targets can be engineered and assembled on user-defined drug screening platforms and with customizable phenotypic outputs. For example, the ethionamide-dependent repressor EthR of *Mycobacterium tuberculosis* is an attractive drug target for anti-tuberculosis pharmacotherapy. EthR represses the expression of flavin-containing monooxygenase EthA, which is necessary for converting the prodrug ethionamide — the last line of treatment in tuberculosis — into an active compound. Thus, drugs that suppress EthR activity could greatly improve the treatment of tuberculosis, counteracting resistance to ethionamide. The development of a screening assay in a human cell line operating a synthetic EthR-based gene switch, including EthR-VP16 (EthR coupled to the trans-activating domain of VP16) and an EthR-responsive promoter driving expression of a reporter protein (FIG. 5b), resulted in the discovery of the US Food and Drug Administration (FDA)-approved food additive 2-phenylethyl butyrate as a new lead compound that interferes with EthR-DNA binding and thus can be used to assist

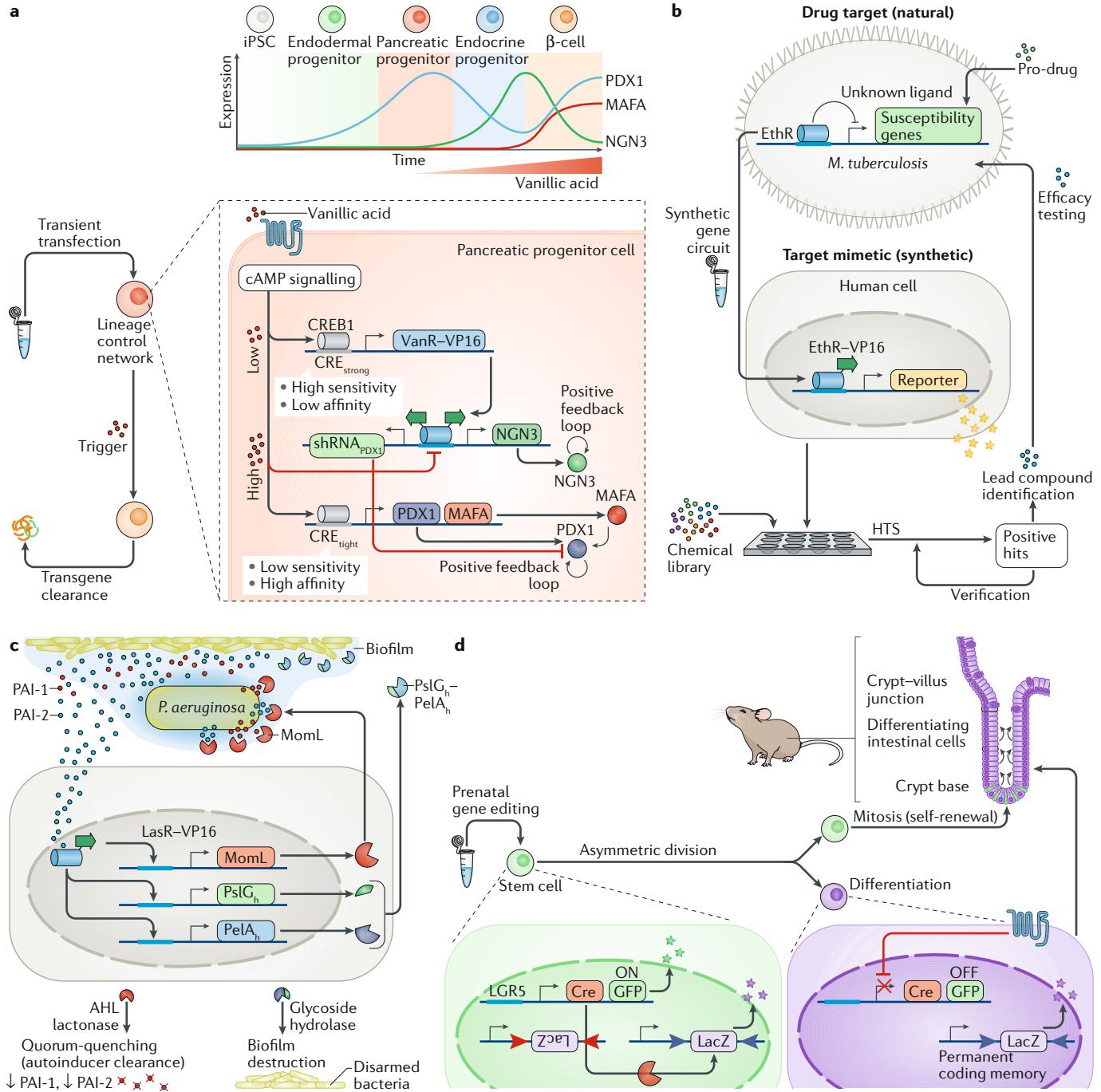


Fig. 5 | Present applications of synthetic gene circuits. **a** | Stem cell differentiation. A synthetic lineage control network transiently integrated into the endogenous transcription network of stem cells can control differentiation into target cell types, for example, differentiation of induced pluripotent stem cells (iPSCs) into pancreatic β -cells with high robustness, reliability and efficiency. See the main text for a detailed mechanistic description. **b** | Drug screening. Functional mimetics of native drug targets can be created by custom-designing synthetic gene circuits. In this example, a synthetic ethionamide-dependent repressor (EthR)-based gene switch operating in mammalian cells sufficiently mimicked a drug-resistance operon in *Mycobacterium tuberculosis* and resulted in the identification of 2-phenylethyl butyrate as a new lead compound for anti-tuberculosis pharmacotherapy. **c** | Drug development. Using a synthetic cell-cell communication system, a new quorum-quenching strategy for treating *Pseudomonas aeruginosa* was developed. Human cells were engineered to sense the quorum signal PAI-1 and in

response to produce antibacterial enzymes that trigger quorum signal destruction (through expression of the *N*-acyl homoserine lactonase MomL) as well as destruction of bacterial biofilms (through expression of the bipartite glycoside hydrolase PslG_h–PelA_h). **d** | Lineage tracing and retrograde labelling. Cre recombinase-based memory and logic are important tools for lineage tracing in transgenic animal models. For example, this method was used to identify leucine-rich repeat-containing G protein-coupled receptor 5 (LGR5)-positive cells as bona fide stem cells of the intestinal crypts. See the main text for a detailed mechanistic description. AHL, acyl homoserine lactone; CRE, cAMP-responsive element; CREB1, cAMP-responsive element-binding protein 1; HTS, high-throughput sequencing; LacZ, β -galactosidase; LasR, transcriptional activator protein from *P. aeruginosa*; MAFA, v-maf musculoaponeurotic fibrosarcoma homologue A; NGN3, neurogenin 3; PDX1, pancreas/duodenum homeobox protein 1; VanR, vanillic acid repressor; VP16, Herpes simplex-derived virion protein 16.

in the treatment of tuberculosis¹⁴⁷. Synthetic gene circuits can also be applied on abiotic screening platforms^{135,148}, which results in higher throughput and sensitivity to target compounds but does not take into account potential issues such as cytotoxicity or tissue permeability, as a cell-based system would.

Additionally, drug target mimetics created with synthetic gene circuits not only enable the discovery of new drugs but also allow in-depth validation of novel treatment strategies (FIG. 5c). For example, chronic *Pseudomonas aeruginosa* infections are a considerable clinical concern because the bacteria generate antibiotic-resistant biofilms in vivo. During infections, the pathogens communicate through quorum signals such as PAI-1 (*Pseudomonas* autoinducer 1; also known as N-3-oxododecanoyl-L-homoserine lactone (3O-C₁₂-HSL)) and PAI-2 (also known as C₄-HSL) to establish survival mechanisms or to produce virulence factors. Capitalizing on quorum sensing, a synthetic cell–cell communication system has been engineered in which human cells respond to PAI-1 by expressing the synthetic transcription factor LasR–VP16, which triggers an anti-infective response upon binding to PAI-1. Specifically, PAI-1-inducible expression of a bipartite glycoside hydrolase PslG_h–PelA_h and the AHL lactonase MomL (both mediated by LasR–VP16) results in the destruction of biofilms (by PslG_h–PelA_h) and degradation of PAI-1 and PAI-2 (by MomL)¹⁴⁹ (FIG. 5c). This destruction of quorum signals compromised key resistance mechanisms of a clinical isolate (PA01 strain), restoring antibiotic susceptibility and reducing the cytotoxicity in infected human tissues. In contrast to previous attempts using PAI-1-responsive *E. coli* to kill *P. aeruginosa*¹⁵⁰, a key advantage of this quorum-quenching strategy is a reduced risk of triggering drug-resistance mechanisms. This could be an effective basis for developing new strategies for fighting infections as soon as technologies for safe delivery of therapeutic gene circuits into host-specific tissues in vivo become available.

Gene editing. Integration of the principles of single-layer logic gates and permanent recombinase-based memory (BOX 1; FIG. 3Dc) has enabled the generation of transgenic mice stably expressing different variants of Cre recombinase as powerful research tools for mechanistic studies and disease profiling¹⁵¹. For example, a synthetic gene circuit was created in which the expression of Cre and GFP was regulated from a stem cell-specific leucine-rich repeat-containing GPCR5 (LGR5)-responsive promoter, while the expression of a blue pigment generated by β-galactosidase (LacZ) was dependent on Cre-mediated sequence inversion (FIG. 5d). Once the transcription unit was reconstituted in LGR5-positive stem cells, pigment production was permanently locked in a constitutive state even when the cells differentiated into LGR5-negative progeny. Because tissues marked with the dark pigment must have differentiated from a progenitor population producing both GFP and LacZ, this approach enabled the discovery of multipotent stem cells in the crypt base of the small intestine, establishing LGR5-positive cells as multipotent intestinal stem cells¹⁵². Similar lineage tracing and retrograde labelling

studies have elucidated basic mechanisms of cardiac development¹⁵³ and identified sleep neurons¹⁵⁴ in mammals. Owing to ethical concerns, however, approaches based on gene editing might remain restricted to the permanent modification of individual cells *in vitro* (for example, the creation of stable cell lines for metabolic engineering or correction of patients' own blood cells for autologous transfusion therapies), genetic modification of agricultural plants and the generation of transgenic animal models for research purposes.

Agriculture and the environment. To support the production of isobutanol, a next-generation biofuel, synthetic gene circuits conferring ionic liquid resistance can increase the robustness and survival of bacterial production strains¹⁵⁵. Improved productivity for feedstock and raw materials has also been achieved by using a synthetic cell–cell communication strategy consisting of a fungal specialist population converting lignocellulosic biomass into soluble saccharides and a bacterial fermentation specialist that metabolizes soluble saccharides into desired products, such as isobutanol¹⁵⁶. Such synthetic consortia, in which a complex task is divided among multiple subpopulations that perform simple tasks with high robustness, are extremely effective for achieving high workforce productivity. For proof of concept, blueprints of synthetic consortia performing population-level oscillations¹⁵⁷ (FIG. 4C) or Boolean calculations^{158,159} (BOX 1) were created using individual activator and repressor strains¹⁵⁷ or cell populations equipped with different Boolean logic gates^{158,159}. To achieve control over reproduction of agricultural animals, a synthetic communication system was applied to achieve 'synthetic artificial' insemination¹⁶⁰. Cows were implanted with cellulose capsules loaded with bull sperm and mammalian cells transgenic for luteinizing hormone (LH)-specific cellulase production. Using this device, systemic surges of LH during the cow's natural ovulation cycle could trigger capsule breakdown and sperm release. Lastly, synthetic gene circuits were created to control mosquito spread and virulence^{161,162}. However, the potential impact of synthetic gene drive systems on entire biotopes raises similar ethical concerns to those involved in generating transgenic mammals.

Towards next-generation therapeutics

Early diagnosis is critical for successful disease treatment. However, patients usually seek medical advice in response to nonspecific symptoms such as pain, nausea or diarrhoea, and many diseases do not show early symptoms. Thus, implantation of living cells engineered for disease-specific biomarker sensing systems coupled to the expression of a quantifiable readout or a therapeutic protein would greatly facilitate the diagnosis and/or treatment of many asymptomatic disease states.

Therapeutic bacteria. Bacteria engineered to contain synthetic memory devices (FIG. 4A) can reside in the mammalian gut for months, collecting information about their environment (including changes associated with disease) and translating it into permanent memory^{87,163–165}. Following isolation of these diagnostic

Biofilms

Sessile communities of virulent bacteria encased in an extracellular matrix adhering to a solid surface and showing increased survival compared with free-floating bacteria.

Gene drive systems

Systems that enable biased inheritance of a genetic element so that offspring within a population have a >50% chance of inheritance of a given trait.

REVIEWS

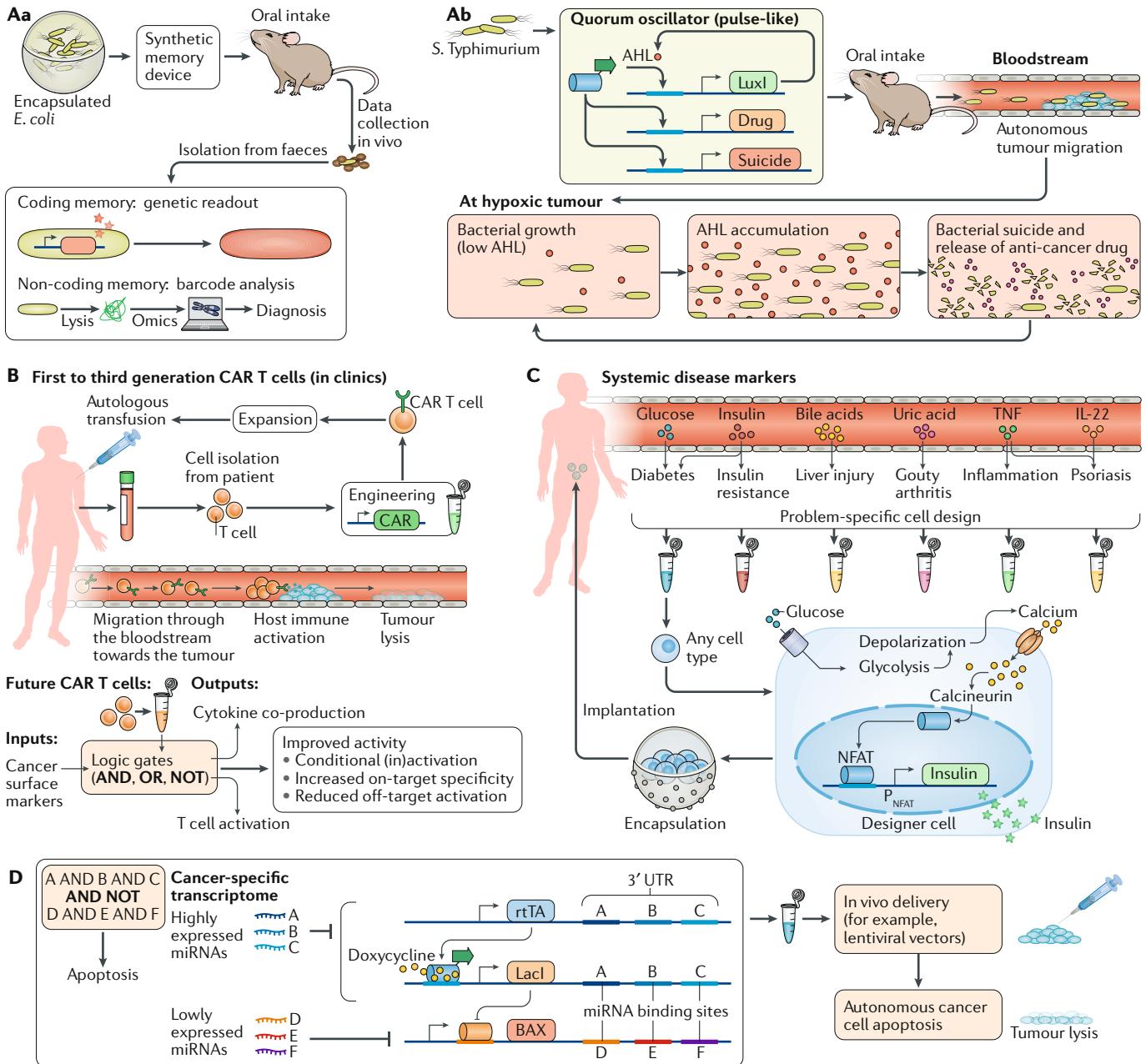


Fig. 6 | Cell and gene therapy as next-generation precision therapeutics.

A | Therapeutic bacteria. **Aa** Use in diagnostics. Bacteria engineered with synthetic memory devices can collect and remember environmental signals while residing in the gut. Following isolation of the diagnostic bacteria from faeces, profiles of the gastrointestinal tract can be read with a reporter protein or computed by decrypting pre-programmed memory barcodes, providing information about potential pathological changes. **Ab** Use as therapeutics. Orally administered bacteria can migrate to certain tumours in vivo and release drugs in a user-defined manner, which is here based on a pulse-like oscillator controlling periodic cycles of bacterial proliferation, bacterial suicide and concomitant drug release (see also FIG. 4Cb).

B | Chimeric antigen receptor (CAR) T cell-based therapy. Clinically approved CAR T cell systems are based on CAR-mediated activation of T cell signalling that allows tumour-specific and autonomous cell killing. The current focus of CAR T cell development is on improving their killing activity and on the reduction of on-target, off-tumour cell killing by trigger-inducible CAR expression (conditionally activated CARs). These functionalities can be designed by following the principles of Boolean logic gates (see also BOX 1).

C | Designer cell implants. Any cell type can be engineered to carry a

custom-designed synthetic circuit for the treatment of a specific metabolic disease, such as diabetes or immune disorders. Following encapsulation into a clinically licensed implant device, the designer implant residing at a vascularized site can coordinate diagnosis (by detecting specific chemical markers) with treatment and/or prevention of diseases (by releasing therapeutic agents) in an autonomous and/or remote-controlled manner.

D | Cancer biocomputers. Synthetic gene circuits controlling apoptosis in cancer cells are based on multi-input logic gates integrating a cancer-specific expression profile. One strategy is to use a cancer-specific profile of microRNAs (miRNAs) to positively (through highly expressed miRNAs) and negatively (through lowly expressed miRNAs) control the expression of a pro-apoptotic protein such as apoptosis regulator BAX. Application of such cancer biocomputers in vivo requires transfer of circuits through gene therapy, for example, involving lentiviral vectors. AHL, acyl homoserine lactone; *E. coli*, *Escherichia coli*; IL-22, interleukin 22; LacI, lactose operon repressor; LuxL, *Vibrio fischeri*-derived acyl homoserine synthase; NFAT, nuclear factor of activated T cells; P_{NFAT}, NFAT-responsive promoter; rtTA, reversed tetracycline-dependent trans-activator; *S. Typhimurium*, *Salmonella enterica* subsp. *enterica* serovar *Typhimurium*; UTR, untranslated region.

bacteria from faeces, an environmental profile of the gastrointestinal tract can then be reconstituted by user-defined analysis of genetic readouts or memory barcodes (FIGS 4Ad, 6Aa). By using synthetic intercellular communication networks, therapeutic bacteria can also be programmed to sense pathogenic strains and directly trigger antimicrobial responses¹⁶⁶. Certain bacterial species are also excellent vehicles for targeting hypoxic tumour microenvironments in vivo^{167,168}. By synchronizing a synthetic quorum-coordinated cell death programme with the dynamics of a pulse-like oscillator (FIG. 4Cb), *Salmonella enterica* subsp. *enterica* serovar Typhimurium carrying an antitumour toxin was programmed to invade colorectal tumour tissues in mice, resulting in periodical drug release and a significant reduction in tumour mass¹¹⁸ (FIG. 6Ab).

Chimeric antigen receptor T cell-based anticancer therapy. Human T lymphocytes stably expressing CARs are a clinically validated cell therapy for treating chronic and acute lymphoid leukaemia^{11,12}. Capitalizing on the design strategy of receptor-mediated activation of T cell signalling (FIG. 2Ca), patient-specific T cells are modified in vitro to recognize cancer-specific antigens through CARs. Upon transfusion into a patient, autologous CAR T cells autonomously migrate to target tissues and recruit the endogenous immune system for local destruction of tumour cells (FIG. 6B). The first three generations of CAR designs have focused on the development of an optimal receptor structure that can effectively trigger T cell signalling upon binding to single surface antigens and on the prevention of graft-versus-host reactions by deleting endogenous T cell receptors through gene editing¹⁶⁹ (FIG. 1A, step 2). Current development of CAR T cells focuses on the improvement of tumour specificity by adopting synthetic logic gate principles¹⁷⁰ (BOX 1) allowing synchronization of T cell activation with other, user-defined gene expression programmes¹⁷¹, including co-production of cytokines¹⁷¹ and ion channels¹⁷² to improve tumour cell-killing potency, co-expression of a chemokine receptor to facilitate migration towards solid tumours¹⁷³ and trigger-inducible CAR expression¹⁷⁰ to improve the on-target activity ratio. Implementations of CAR T cells with AND logic gates are based on reconstitution of CAR-dependent signal transduction through drug-induced protein dimerization¹⁷⁴, incorporation of bi-specific antibodies into the CAR framework¹⁷⁵ or triggering of CAR transcription through activation of another cell surface receptor¹⁷⁰. By using a T cell-suppressing CAR variant as a NOT inverter (BOX 1), CAR T cells following an A AND NOT B logic (A: surface marker of tumour; B: similar surface marker on healthy tissue) have also been developed to increase tumour specificity¹⁷⁶.

Designer cell implants. CAR T cell technology has already validated the use of engineered human cells to detect and treat cancer. However, cell-based therapies can in principle be generalized and metabolic diseases are particularly well-suited for these applications. To treat endocrine and immune disorders that do not require physical contact between the therapeutic cells and the drug target, an effective approach would be to use designer

implants consisting of encapsulated human cells engineered for automated sensing of systemic disease markers and production of a disease-specific therapeutic protein. Placement at a vascularized site would enable the implant to communicate constantly with the host via the bloodstream, allowing it to autonomously coordinate automated diagnosis (through measurement of different systemic disease markers as trigger signals) with treatment and/or prevention of latent diseases (through trigger-inducible gene switches controlling drug release) (FIG. 6C). For example, human cells engineered with a synthetic gene switch based on glucose-dependent calcium entry coupled to a calcium-responsive promoter driving insulin expression autonomously restored glucose and insulin homeostasis in diabetic mice through closed-loop control of glucose-sensing and insulin secretion¹⁷⁷. Similarly, closed-loop control of insulin detection and adiponectin secretion effectively targeted the latent and asymptomatic disease stage of insulin resistance and attenuated the development of obesity-induced diabetes¹⁷⁸. Additionally, a serially assembled AND gate (BOX 1) allowing simultaneous profiling of TNF and interleukin-22 (IL-22) and controlling the secretion of the anti-inflammatory cytokines IL-4 and IL-10 achieved remarkable therapeutic efficacy in treating psoriasis-related skin rashes¹⁷⁹. Various closed-loop control systems acting as therapeutic biocomputers have been developed for controlling liver injuries¹⁸⁰, gouty arthritis¹⁸¹, hypertension¹⁸², diabetic ketoacidosis¹⁸³, obesity¹⁸⁴ and Graves disease¹⁸⁵ (FIG. 6C).

Although closed-loop systems of CAR T cells and designer implants can operate in an autonomous manner in vivo, it would be desirable for a human (the patient or doctor) to be able to interrupt or fine-tune the autonomous therapeutic programme with user-defined control signals in the clinical context. Classical implementations of such safety switches are based on controllable induction of cell death^{186,187} or interruption of target gene transcription^{178,184} using clinically licensed drugs. Future systems for remote control of therapeutic cells in vivo could involve safety-approved trigger signals such as cosmetics and food additives or smartphones¹⁸⁸.

Cancer biocomputers. Although future advances in CAR T cell technology could allow successful targeting of any cell surface tumour antigen, the intracellular transcriptome of cancer cells represents an even more important target for early diagnosis and intervention in cancer¹⁸⁹. Oncogenic states at the transcriptional level often precede the expression of cell surface markers, and not all cancer cells effectively express tumour-specific antigens¹⁹⁰. To address this issue, a multi-input biocomputer was engineered by integrating cancer-specific miRNA markers into an LTRi-like gene switch¹⁹¹ (FIG. 2B). In this circuit, expression of pro-apoptotic regulator BAX was placed under control of the reversed tetracycline-controlled trans-activator (rtTA; reversed TetR (rTetR)-VP16) and LacI. Importantly, for all elements of the circuit, target sites for miRNAs distinctive for cancer cells were incorporated into 3' untranslated mRNA regions: target sites for miRNAs that are chronically upregulated in cancer (A, B and C) were included in rtTA and LacI transcripts, whereas target sites for miRNAs

Graft-versus-host reactions
Medical complications related to the immunological adverse effects in a patient (host) caused by implanted or infused therapeutics (graft).

Adiponectin
An adipocyte-derived protein hormone that increases insulin sensitivity in target tissues, such as fat, muscle or liver.

Graves disease
An autoimmune disorder characterized by hyperthyroidism in which autoantibodies constitutively trigger thyroid hormone production from the thyroid gland.

that are silenced and remain low in cancer (D, E and F) were added to the BAX transcript. This establishes an (A AND B AND C) AND NOT (D AND E AND F) logic gate that produces output specifically in cancer cells, resulting in autonomous induction of apoptosis. (FIG. 6D). Similarly, various other AND logic gates based on simultaneous cancer-specific promoter activities^{56,192} or protein levels⁴⁰ have been engineered and validated in different cancer cell lines in vitro. Although the concept of programming cancer cells for autonomous apoptosis might seem an ideal cancer treatment strategy, there are major technical limitations regarding safety and efficacy of delivery of such circuits into patients. Nevertheless, in pioneering work, local lentiviral delivery of an ovarian cancer-specific AND logic gate has shown remarkable treatment success in mice⁵⁶. In this circuit, simultaneous activation of two cancer-specific promoters was coupled to T cell recruitment, whereby one cancer-specific promoter regulated the immunomodulatory gene cassette with simultaneous expression of an autoinhibitory miRNA for this gene cassette, while the other promoter controlled the expression of an miRNA ‘sponge’ that was necessary to relieve the autoinhibition (clearance of

inhibitory checkpoint; see also BOX 1, figure part b) and completed the AND logic.

Conclusions and perspectives

Almost two decades after the first synthetic toggle switch and oscillator circuits were created in bacteria, synthetic biology now encompasses an extensive toolkit of knowledge, devices and design strategies that in principle allow the engineering and programming of essentially any cell functionality with user-defined complexity (FIG. 4) and purpose (FIGS 5,6). For example, current breakthroughs in cell-based therapies taking advantage of synthetic genetic circuits already indicate that it will be possible to successfully treat hitherto intractable diseases, including cancers and diabetes, using such synthetic biology approaches. Synthetic biology clearly has the potential to make an enormous impact on the world’s health-care, agriculture and environmental systems. It will be important to promote public understanding of the technology and to discuss its ethical implications in order to ensure its general acceptance.

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- Nielsen, J. & Keasling, J. D. Engineering cellular metabolism. *Cell* **164**, 1185–1197 (2016).
- Ajikumar, P. K. et al. Isoprenoid pathway optimization for Taxol precursor overproduction in *Escherichia coli*. *Science* **330**, 70–74 (2010).
- Galanie, S., Thodey, K., Trenchard, I. J., Filisinger Interrante, M. & Smolke, C. D. Complete biosynthesis of opioids in yeast. *Science* **349**, 1095–1100 (2015).
- Paddon, C. J. et al. High-level semi-synthetic production of the potent antimalarial artemisinin. *Nature* **496**, 528–532 (2013).
- Wurm, F. M. Production of recombinant protein therapeutics in cultivated mammalian cells. *Nat. Biotechnol.* **22**, 1393–1398 (2004).
- Eichenberger, M. et al. Metabolic engineering of *Saccharomyces cerevisiae* for de novo production of dihydrochalcones with known antioxidant, antidiabetic, and sweet tasting properties. *Metab. Eng.* **39**, 80–89 (2017).
- Atsumi, S., Hanai, T. & Liao, J. C. Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. *Nature* **451**, 86–89 (2008).
- Yang, X., Xu, M. & Yang, S. T. Metabolic and process engineering of *Clostridium cellulovorans* for biofuel production from cellulose. *Metab. Eng.* **32**, 39–48 (2015).
- Widmaier, D. M. et al. Engineering the *Salmonella* type III secretion system to export spider silk monomers. *Mol. Syst. Biol.* **5**, 309 (2009).
- Auslander, S., Auslander, D. & Fussenegger, M. Synthetic biology — the synthesis of biology. *Angew. Chem. Int. Ed Engl.* **56**, 6396–6419 (2017).
- Maude, S. L. et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N. Engl. J. Med.* **371**, 1507–1517 (2014).
- Porter, D. L., Levine, B. L., Kalos, M., Bagg, A. & June, C. H. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N. Engl. J. Med.* **365**, 725–733 (2011).
- Fesnak, A. D., June, C. H. & Levine, B. L. Engineered T cells: the promise and challenges of cancer immunotherapy. *Nat. Rev. Cancer* **16**, 566–581 (2016).
- Chakravarti, D. & Wong, W. W. Synthetic biology in cell-based cancer immunotherapy. *Trends Biotechnol.* **33**, 449–461 (2015).
- Culler, S. J., Hoff, K. G. & Smolke, C. D. Reprogramming cellular behavior with RNA controllers responsive to endogenous proteins. *Science* **330**, 1251–1255 (2010).
- Paek, K. Y. et al. Translation initiation mediated by RNA looping. *Proc. Natl Acad. Sci. USA* **112**, 1041–1046 (2015).
- Van Etten, J. et al. Human Pumilio proteins recruit multiple deadenylases to efficiently repress messenger RNAs. *J. Biol. Chem.* **287**, 36370–36383 (2012).
- Fire, A. et al. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806–811 (1998).
- Ipsaro, J. J. & Joshua-Tor, L. From guide to target: molecular insights into eukaryotic RNA-interference machinery. *Nat. Struct. Mol. Biol.* **22**, 20–28 (2015).
- Greber, D., El-Baba, M. D. & Fussenegger, M. Intronic encoded siRNAs improve dynamic range of mammalian gene regulation systems and toggle switch. *Nucleic Acids Res.* **36**, e101 (2008).
- Fux, C. et al. Streptogramin- and tetracycline-responsive dual regulated expression of p27(Kip1) sense and antisense enables positive and negative growth control of Chinese hamster ovary cells. *Nucleic Acids Res.* **29**, E19 (2001).
- Niopek, D. et al. Engineering light-inducible nuclear localization signals for precise spatiotemporal control of protein dynamics in living cells. *Nat. Commun.* **5**, 4404 (2014).
- Beyer, H. M. et al. Red light-regulated reversible nuclear localization of proteins in mammalian cells and zebrafish. *ACS Synth. Biol.* **4**, 951–958 (2015).
- Niopek, D., Wehler, P., Roensch, J., Eils, R. & Di Ventura, B. Optogenetic control of nuclear protein export. *Nat. Commun.* **7**, 10624 (2016).
- Chen, D., Gibson, E. S. & Kennedy, M. J. A light-triggered protein secretion system. *J. Cell Biol.* **201**, 631–640 (2013).
- Spiltoir, J. I., Strickland, D., Glotzer, M. & Tucker, C. L. Optical control of peroxisomal trafficking. *ACS Synth. Biol.* **5**, 554–560 (2016).
- Finley, D. Recognition and processing of ubiquitin-protein conjugates by the proteasome. *Annu. Rev. Biochem.* **78**, 477–513 (2009).
- Janse, D. M., Crosas, B., Finley, D. & Church, G. M. Localization to the proteasome is sufficient for degradation. *J. Biol. Chem.* **279**, 21415–21420 (2004).
- Sandri, M. et al. Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* **117**, 399–412 (2004).
- Macian, F. NFAT proteins: key regulators of T cell development and function. *Nat. Rev. Immunol.* **5**, 472–484 (2005).
- Keeley, M. B., Busch, J., Singh, R. & Abel, T. TetR hybrid transcription factors report cell signaling and are inhibited by doxycycline. *Biotechniques* **39**, 529–536 (2005).
- Mishra, D., Rivera, P. M., Lin, A., Del Vecchio, D. & Weiss, R. A load driver device for engineering modularity in biological networks. *Nat. Biotechnol.* **32**, 1268–1275 (2014).
- Garg, A., Lohmueller, J. J., Silver, P. A. & Armel, T. Z. Engineering synthetic TAL effectors with orthogonal target sites. *Nucleic Acids Res.* **40**, 7584–7595 (2012).
- Thakore, P. I., Black, J. B., Hilton, I. B. & Gersbach, C. A. Editing the epigenome: technologies for programmable transcription and epigenetic modulation. *Nat. Methods* **13**, 127–137 (2016).
- Stanton, B. C. et al. Systematic transfer of prokaryotic sensors and circuits to mammalian cells. *ACS Synth. Biol.* **3**, 880–891 (2014).
- Esveld, K. M. et al. Orthogonal Cas9 proteins for RNA-guided gene regulation and editing. *Nat. Methods* **10**, 1116–1121 (2013).
- Chen, B. et al. Expanding the CRISPR imaging toolset with *Staphylococcus aureus* Cas9 for simultaneous imaging of multiple genomic loci. *Nucleic Acids Res.* **44**, e75 (2016).
- Briner, A. E. et al. Guide RNA functional modules direct Cas9 activity and orthogonality. *Mol. Cell* **56**, 333–339 (2014).
- This paper analyses different structural motifs of gRNAs in great detail and provides an excellent resource for the design of novel CRISPR–Cas-dependent functions, such as the engineering of scRNA for multiplexed transcriptional regulation (see also reference 39) or the construction of signal conductors (see also reference 40).
- Zalatan, J. G. et al. Engineering complex synthetic transcriptional programs with CRISPR RNA scaffolds. *Cell* **160**, 339–350 (2015).
- Liu, Y. et al. Directing cellular information flow via CRISPR signal conductors. *Nat. Methods* **13**, 938–944 (2016).
- Kashida, S., Inoue, T. & Saito, H. Three-dimensionally designed protein-responsive RNA devices for cell signaling regulation. *Nucleic Acids Res.* **40**, 9369–9378 (2012).
- Perli, S. D., Cui, C. H. & Lu, T. K. Continuous genetic recording with self-targeting CRISPR-Cas in human cells. *Science* **353**, aag0511 (2016).
- Muller, K., Zurbriggen, M. D. & Weber, W. An optogenetic upgrade for the Tet-OFF system. *Biotechnol. Bioeng.* **112**, 1483–1487 (2015).
- Nihongaki, Y., Kawano, F., Nakajima, T. & Sato, M. Photoactivatable CRISPR-Cas9 for optogenetic genome editing. *Nat. Biotechnol.* **33**, 755–760 (2015).
- Nedermann, P. et al. A novel, inducible, eukaryotic gene expression system based on the quorum-sensing transcription factor TraR. *EMBO Rep.* **4**, 159–165 (2003).
- Auslander, S. & Fussenegger, M. From gene switches to mammalian designer cells: present and future prospects. *Trends Biotechnol.* **31**, 155–168 (2013).
- Dominguez, A. A., Lim, W. A. & Qi, L. S. Beyond editing: repurposing CRISPR–Cas9 for precision

- genome regulation and interrogation. *Nat. Rev. Mol. Cell Biol.* **17**, 5–15 (2016).
48. Khalil, A. S. et al. A synthetic biology framework for programming eukaryotic transcription functions. *Cell* **150**, 647–658 (2012).
 49. Schukur, L. & Fussenegger, M. Engineering of synthetic gene circuits for (re-)balancing physiological processes in chronic diseases. *Wiley Interdiscip. Rev. Syst. Biol. Med.* **8**, 402–422 (2016).
 50. Heng, B. C., Aubel, D. & Fussenegger, M. G protein-coupled receptors revisited: therapeutic applications inspired by synthetic biology. *Annu. Rev. Pharmacol. Toxicol.* **54**, 227–249 (2014).
 51. Auslander, S. & Fussenegger, M. Synthetic RNA-based switches for mammalian gene expression control. *Curr. Opin. Biotechnol.* **48**, 54–60 (2017).
 52. Chappell, J., Watters, K. E., Takahashi, M. K. & Lucks, J. B. A renaissance in RNA synthetic biology: new mechanisms, applications and tools for the future. *Curr. Opin. Chem. Biol.* **28**, 47–56 (2015).
 53. Deans, T. L., Cantor, C. R. & Collins, J. J. A tunable genetic switch based on RNAi and repressor proteins for regulating gene expression in mammalian cells. *Cell* **130**, 363–372 (2007).
 54. Karlsson, M. et al. Pharmacologically controlled protein switch for ON-OFF regulation of growth factor activity. *Sci. Rep.* **3**, 2716 (2013).
 55. Park, J. S. et al. Synthetic control of mammalian-cell motility by engineering chemotaxis to an orthogonal bioinert chemical signal. *Proc. Natl Acad. Sci. USA* **111**, 5896–5901 (2014).
 56. Nissim, L. et al. Synthetic RNA-based immunomodulatory gene circuits for cancer immunotherapy. *Cell* **171**, 1138–1150.e15 (2017). **This work uses lentiviral delivery of synthetic gene circuits in mice to illustrate a therapeutic strategy building on the concept of cancer biocomputers (see also references 191 and 192) and represents an important step towards clinical application.**
 57. Morsut, L. et al. Engineering customized cell sensing and response behaviors using synthetic Notch receptors. *Cell* **164**, 780–791 (2016). **By incorporating an antibody domain and a synthetic transcription factor into the modular framework of the Notch receptor, the authors of this study introduce a novel gene switch design for sensing direct cell contacts with programmable transgene readouts in neurons and T lymphocytes.**
 58. Baeumler, T. A., Ahmed, A. A. & Fulga, T. A. Engineering synthetic signaling pathways with programmable dCas9-based chimeric receptors. *Cell Rep.* **20**, 2639–2653 (2017).
 59. Barnea, G. et al. The genetic design of signaling cascades to record receptor activation. *Proc. Natl Acad. Sci. USA* **105**, 64–69 (2008).
 60. Schwarz, K. A., Daringer, N. M., Dolberg, T. B. & Leonard, J. N. Rewiring human cellular input-output using modular extracellular sensors. *Nat. Chem. Biol.* **13**, 202–209 (2017).
 61. Green, A. A., Silver, P. A., Collins, J. J. & Yin, P. Toehold switches: de-novo-designed regulators of gene expression. *Cell* **159**, 925–939 (2014).
 62. Beisel, C. L., Chen, Y. Y., Culler, S. J., Hoff, K. G. & Smolke, C. D. Design of small molecule-responsive microRNAs based on structural requirements for Drosha processing. *Nucleic Acids Res.* **39**, 2981–2994 (2011).
 63. Auslander, S. et al. A general design strategy for protein-responsive riboswitches in mammalian cells. *Nat. Methods* **11**, 1154–1160 (2014).
 64. Bonger, K. M., Rakshit, R., Payumo, A. Y., Chen, J. K. & Wandless, T. J. General method for regulating protein stability with light. *ACS Chem. Biol.* **9**, 111–115 (2014).
 65. Strickland, D. et al. Rationally improving LOV domain-based photoswitches. *Nat. Methods* **7**, 623–626 (2010).
 66. Levskaya, A., Weiner, O. D., Lim, W. A. & Voigt, C. A. Spatiotemporal control of cell signalling using a light-switchable protein interaction. *Nature* **461**, 997–1001 (2009).
 67. Hughes, R. M. et al. Optogenetic apoptosis: light-triggered cell death. *Angew. Chem. Int. Ed.* **54**, 12064–12068 (2015).
 68. 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* **6**, 917–922 (2009).
 69. Kawano, F., Suzuki, H., Furuya, A. & Sato, M. Engineered pairs of distinct photoswitches for optogenetic control of cellular proteins. *Nat. Commun.* **6**, 6256 (2015).
 70. Wu, Y. I. et al. A genetically encoded photoactivatable Rac controls the motility of living cells. *Nature* **461**, 104–108 (2009).
 71. Splittoor, J. I., Strickland, D., Glotzer, M. & Tucker, C. L. Optical control of peroxisomal trafficking. *ACS Synth. Biol.* **5**, 554–560 (2016).
 72. Renicke, C., Schuster, D., Usherenko, S., Essen, L. O. & Taxis, C. A. LOV2 domain-based optogenetic tool to control protein degradation and cellular function. *Chem. Biol.* **20**, 619–626 (2013).
 73. Fukuda, N., Matsuda, T. & Nagai, T. Optical control of the Ca²⁺ concentration in a live specimen with a genetically encoded Ca²⁺-releasing molecular tool. *ACS Chem. Biol.* **9**, 1197–1203 (2014).
 74. Wang, Y. H., Wei, K. Y. & Smolke, C. D. Synthetic biology: advancing the design of diverse genetic systems. *Annu. Rev. Chem. Biomol. Eng.* **4**, 69–102 (2013).
 75. Kahn, M. Can we safely target the WNT pathway? *Nat. Rev. Drug Discov.* **13**, 513–532 (2014).
 76. Metcalfe, C., Mendoza-Topaz, C., Miesczanek, J. & Biezen, M. Stability elements in the LRP6 cytoplasmic tail confer efficient signalling upon DIX-dependent polymerization. *J. Cell Sci.* **123**, 1588–1599 (2010).
 77. Bugaj, L. J., Choksi, A. T., Mesuda, C. K., Kane, R. S. & Schaffer, D. V. Optogenetic protein clustering and signaling activation in mammalian cells. *Nat. Methods* **10**, 249–252 (2013).
 78. Wehr, M. C. et al. Monitoring regulated protein-protein interactions using split TEV. *Nat. Methods* **3**, 985–993 (2006).
 79. Copeland, M. F., Politz, M. C., Johnson, C. B., Markley, A. L. & Pfleider, B. F. A transcription activator-like effector (TALE) induction system mediated by proteolysis. *Nat. Chem. Biol.* **12**, 254–260 (2016).
 80. Lapique, N. & Benenson, Y. Digital switching in a biosensor circuit via programmable timing of gene availability. *Nat. Chem. Biol.* **10**, 1020–1027 (2014).
 81. Prochazka, L., Angelici, B., Haefliger, B. & Benenson, Y. Highly modular bow-tie gene circuits with programmable dynamic behaviour. *Nat. Commun.* **5**, 4729 (2014).
 82. Muller, M. et al. Designed cell consortia as fragrance-programmable analog-to-digital converters. *Nat. Chem. Biol.* **13**, 309–316 (2017).
 83. Weinberg, B. H. et al. Large-scale design of robust genetic circuits with multiple inputs and outputs for mammalian cells. *Nat. Biotechnol.* **35**, 453–462 (2017). **Capitalizing on a selection of ten orthogonal site-specific recombinases, the authors programme single promoter-driven transcription units into complex Boolean calculators that process different recombinase-specific input signals according to half-adder or half-subtractor (two inputs) and full-adder or full-subtractor (three inputs) logics.**
 84. Green, A. A. et al. Complex cellular logic computation using ribocomputing devices. *Nature* **548**, 117–121 (2017). **By re-engineering the toehold switch (see reference 61) to become conditionally activated by multiple trigger RNAs, the authors demonstrate that any complex (bio)computational task can be programmed on the basis of two-input AND, OR and NOT logic gates.**
 85. Burrill, D. R. & Silver, P. A. Making cellular memories. *Cell* **140**, 13–18 (2010).
 86. Covert, M. W., Leung, T. H., Gaston, J. E. & Baltimore, D. Achieving stability of lipopolysaccharide-induced NF-κB activation. *Science* **309**, 1854–1857 (2005).
 87. Myhrvold, C., Kotula, J. W., Hicks, W. M., Conway, N. J. & Silver, P. A. A distributed cell division counter reveals growth dynamics in the gut microbiota. *Nat. Commun.* **6**, 10039 (2015).
 88. Weber, W. et al. A synthetic time-delay circuit in mammalian cells and mice. *Proc. Natl Acad. Sci. USA* **104**, 2643–2648 (2007).
 89. Kramer, B. P. & Fussenegger, M. Hysteresis in a synthetic mammalian gene network. *Proc. Natl Acad. Sci. USA* **102**, 9517–9522 (2005).
 90. Hussain, F. et al. Engineered temperature compensation in a synthetic genetic clock. *Proc. Natl Acad. Sci. USA* **111**, 972–977 (2014).
 91. Folcher, M., Xie, M., Spinnler, A. & Fussenegger, M. Synthetic mammalian trigger-controlled bipartite transcription factors. *Nucleic Acids Res.* **41**, e134 (2013). **Using different hybrid transcription factors composed of multiple TetR family repressors, this work provides an in-depth characterization of the most widely used trans-regulators in synthetic biology (for example, TetR, VanR, ScbR or TtgR)**
 92. Burrill, D. R., Inniss, M. C., Boyle, P. M. & Silver, P. A. Synthetic memory circuits for tracking human cell fate. *Genes Dev.* **26**, 1486–1497 (2012).
 93. Yao, G., Tan, C., West, M., Nevins, J. R. & You, L. Origin of bistability underlying mammalian cell cycle entry. *Mol. Syst. Biol.* **7**, 485 (2011).
 94. Gardner, T. S., Cantor, C. R. & Collins, J. J. Construction of a genetic toggle switch in *Escherichia coli*. *Nature* **403**, 339–342 (2000). **This paper describes a design of a genetic toggle switch, which inaugurated the modern era of synthetic biology featuring the development of a standardized and reusable ‘engineering language’ to programme complex cell functions.**
 95. Kobayashi, H. et al. Programmable cells: interfacing natural and engineered gene networks. *Proc. Natl Acad. Sci. USA* **101**, 8414–8419 (2004).
 96. Kramer, B. P. et al. An engineered epigenetic transgene switch in mammalian cells. *Nat. Biotechnol.* **22**, 867–870 (2004).
 97. Graf, T. & Enver, T. Forcing cells to change lineages. *Nature* **462**, 587–594 (2009).
 98. Bonnet, J., Subsoontorn, P. & Endy, D. Rewritable digital data storage in live cells via engineered control of recombination directionality. *Proc. Natl Acad. Sci. USA* **109**, 8884–8889 (2012). **In contrast to most other permanent memory devices, where each data register can be written by a site-specific recombinase only once, this work shows that additional expression of an excisionase in bacteria to restore the recombinase-specific recognition sites can generate resettable memory registers.**
 99. Yang, L. et al. Permanent genetic memory with >1-byte capacity. *Nat. Methods* **11**, 1261–1266 (2014).
 100. Kalhor, R., Mali, P. & Church, G. M. Rapidly evolving homing CRISPR barcodes. *Nat. Methods* **14**, 195–200 (2017).
 101. Frieda, K. L. et al. Synthetic recording and in situ readout of lineage information in single cells. *Nature* **541**, 107–111 (2017).
 102. Farzadfar, F. & Lu, T. K. Synthetic biology. Genomically encoded analog memory with precise *in vivo* DNA writing in living cell populations. *Science* **346**, 1256272 (2014).
 103. Tamsir, A., Tabor, J. J. & Voigt, C. A. Robust multicellular computing using genetically encoded NOR gates and chemical ‘wires’. *Nature* **469**, 212–215 (2011).
 104. Wong, A., Wang, H., Poh, C. L. & Kitney, R. I. Layering genetic circuits to build a single cell, bacterial half adder. *BMC Biol.* **13**, 40 (2015).
 105. Auslander, S., Auslander, D., Muller, M., Wieland, M. & Fussenegger, M. Programmable single-cell mammalian biocomputers. *Nature* **487**, 123–127 (2012).
 106. Bonnet, J., Yin, P., Ortiz, M. E., Subsoontorn, P. & Endy, D. Amplifying genetic logic gates. *Science* **340**, 599–603 (2013).
 107. Siuti, P., Yazbek, J. & Lu, T. K. Synthetic circuits integrating logic and memory in living cells. *Nat. Biotechnol.* **31**, 448–452 (2013).
 108. Imanishi, M. et al. Construction of a rhythm transfer system that mimics the cellular clock. *ACS Chem. Biol.* **7**, 1817–1821 (2012).
 109. Chivil, D. & Fussenegger, M. Toward construction of a self-sustained clock-like expression system based on the mammalian circadian clock. *Biotechnol. Bioeng.* **87**, 234–242 (2004).
 110. Fung, E. et al. A synthetic gene-metabolic oscillator. *Nature* **435**, 118–122 (2005).
 111. Toettcher, J. E., Mock, C., Batchelor, E., Loewer, A. & Lahav, G. A synthetic-natural hybrid oscillator in human cells. *Proc. Natl Acad. Sci. USA* **107**, 17047–17052 (2010).
 112. Elowitz, M. B. & Leibler, S. A synthetic oscillatory network of transcriptional regulators. *Nature* **403**, 335–338 (2000).
 113. Atkinson, M. R., Savageau, M. A., Myers, J. T. & Ninfa, A. J. Development of genetic circuitry exhibiting toggle switch or oscillatory behavior in *Escherichia coli*. *Cell* **113**, 597–607 (2003).
 114. Stricker, J. et al. A fast, robust and tunable synthetic gene oscillator. *Nature* **456**, 516–519 (2008). **This work reports the key principles for the design of an essentially ideal synthetic oscillator; robust, persistent and tunable gene oscillations are enabled by a positive feedback module that activates all modules in a gene circuit and a slower-acting negative feedback module that represses the very same targets.**

115. Mondragon-Palomino, O., Danino, T., Selimkhanov, J., Tsirring, L. & Hasty, J. Entrainment of a population of synthetic genetic oscillators. *Science* **333**, 1315–1319 (2011).
116. Weber, W. et al. *Streptomyces*-derived quorum-sensing systems engineered for adjustable transgene expression in mammalian cells and mice. *Nucleic Acids Res.* **31**, e71 (2003).
117. You, L., Cox, R. S. 3rd, Weiss, R. & Arnold, F. H. Programmed population control by cell–cell communication and regulated killing. *Nature* **428**, 868–871 (2004).
118. Din, M. O. et al. Synchronized cycles of bacterial lysis for in vivo delivery. *Nature* **536**, 81–85 (2016). **In this study, the pulse-like gene expression dynamics of the synthetic quorum-based oscillator are repurposed to programme *S. Typhimurium* for self-autonomous cancer targeting, lysis and drug release, resulting in a 50% increase in survival in a mouse model of colorectal cancer when combined with common clinical chemotherapy.**
119. Liu, C. et al. Sequential establishment of stripe patterns in an expanding cell population. *Science* **334**, 238–241 (2011).
120. Danino, T., Mondragon-Palomino, O., Tsirring, L. & Hasty, J. A synchronized quorum of genetic clocks. *Nature* **463**, 326–330 (2010).
121. Ryback, B. M. et al. Design and analysis of a tunable synchronized oscillator. *J. Biol. Eng.* **7**, 26 (2013).
122. Prindle, A. et al. A sensing array of radically coupled genetic ‘biopixels’. *Nature* **481**, 39–44 (2011).
123. Tigges, M., Marquez-Lago, T. T., Stelling, J. & Fussenegger, M. A tunable synthetic mammalian oscillator. *Nature* **457**, 309–312 (2009).
124. Tigges, M., Denervaud, N., Greber, D., Stelling, J. & Fussenegger, M. A synthetic low-frequency mammalian oscillator. *Nucleic Acids Res.* **38**, 2702–2711 (2010).
125. Basu, S., Gerchman, Y., Collins, C. H., Arnold, F. H. & Weiss, R. A synthetic multicellular system for programmed pattern formation. *Nature* **434**, 1130–1134 (2005).
126. Greber, D. & Fussenegger, M. An engineered mammalian band-pass network. *Nucleic Acids Res.* **38**, e174 (2010).
127. Saxena, P. et al. A programmable synthetic lineage-control network that differentiates human iPSCs into glucose-sensitive insulin-secreting beta-like cells. *Nat. Commun.* **7**, 11247 (2016). **In this study, a synthetic gene circuit, termed a lineage control network, based on a vanillic-acid-regulated band-pass filter controlling cell stage-specific NGN3 expression coupled to PDX1 repression and MAFA activation differentiates pancreatic progenitor cells into mature β-like cells with a higher efficiency than could be achieved with conventional methods such as ectopic overexpression of PDX1, NGN3 and MAFA (see references 142 and 143) or chemical cultivation methods (see references 144 and 145).**
128. Kolar, K. et al. A synthetic mammalian network to compute population borders based on engineered reciprocal cell–cell communication. *BMC Syst. Biol.* **9**, 97 (2015).
129. Bacchus, W. et al. Synthetic two-way communication between mammalian cells. *Nat. Biotechnol.* **30**, 991–996 (2012).
130. Weber, W., Daoud-El Baba, M. & Fussenegger, M. Synthetic ecosystems based on airborne inter- and intrakingdom communication. *Proc. Natl Acad. Sci. USA* **104**, 10435–10440 (2007).
131. Kojima, R., Scheller, L. & Fussenegger, M. Nonimmune cells equipped with T cell-receptor-like signaling for cancer cell ablation. *Nat. Chem. Biol.* **14**, 42–49 (2018).
132. Skoedt, M. L. et al. Engineering prokaryotic transcriptional activators as metabolite biosensors in yeast. *Nat. Chem. Biol.* **12**, 951–958 (2016).
133. Slomovic, S. & Collins, J. J. DNA sense-and-respond protein modules for mammalian cells. *Nat. Methods* **12**, 1085–1090 (2015).
134. Schena, A., Griss, R. & Johnsson, K. Modulating protein activity using tethered ligands with mutually exclusive binding sites. *Nat. Commun.* **6**, 7830 (2015).
135. Pardee, K. et al. Paper-based synthetic gene networks. *Cell* **159**, 940–954 (2014). **This work shows that synthetic gene circuits not only operate in living cells but also retain most of their functionality when the relevant coding genes and cell lysates are incorporated into abiotic material, such as paper.**
136. Gootenberg, J. S. et al. Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science* **356**, 438–442 (2017).
137. Griss, R. et al. Bioluminescent sensor proteins for point-of-care therapeutic drug monitoring. *Nat. Chem. Biol.* **10**, 598–603 (2014).
138. Auslander, D. et al. A designer cell-based histamine-specific human allergy profiler. *Nat. Commun.* **5**, 4408 (2014).
139. Schukur, L., Geering, B. & Fussenegger, M. Human whole-blood culture system for ex vivo characterization of designer-cell function. *Biotechnol. Bioeng.* **113**, 588–597 (2016).
140. Courbet, A., Endy, D., Renard, E., Molina, F. & Bonnet, J. Detection of pathological biomarkers in human clinical samples via amplifying genetic switches and logic gates. *Sci. Transl. Med.* **7**, 289ra83 (2015).
141. Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663–676 (2006). **This milestone paper, showing that ectopic overexpression of the master transcription factors OCT4, SOX2, KLF4 and MYC is sufficient to confer a stem cell-like identity upon any somatic cell type, features the Nobel Prize-winning discovery of induced pluripotent stem cells.**
142. Ariyachet, C. et al. Reprogrammed stomach tissue as a renewable source of functional beta cells for blood glucose regulation. *Cell Stem Cell* **18**, 410–421 (2016).
143. Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J. & Melton, D. A. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature* **455**, 627–632 (2008).
144. Zhu, S. et al. Human pancreatic beta-like cells converted from fibroblasts. *Nat. Commun.* **7**, 10080 (2016).
145. Pagliuca, F. W. et al. Generation of functional human pancreatic beta cells in vitro. *Cell* **159**, 428–439 (2014).
146. Teague, B. P., Guye, P. & Weiss, R. Synthetic morphogenesis. *Cold Spring Harb. Perspect. Biol.* **8**, a023929 (2016).
147. Weber, W. et al. A synthetic mammalian gene circuit reveals antituberculosis compounds. *Proc. Natl Acad. Sci. USA* **105**, 9994–9998 (2008).
148. Menzel, A., Gubel, R. J., Guder, F., Weber, W. & Zacharias, M. Detection of real-time dynamics of drug target interactions by ultralong nanowalls. *Lab Chip* **13**, 4173–4179 (2013).
149. Sedlmayer, F., Jaeger, T., Jenal, U. & Fussenegger, M. Quorum-quenching human designer cells for closed-loop control of *Pseudomonas aeruginosa* biofilms. *Nano Lett.* **17**, 5043–5050 (2017).
150. Saedi, N. et al. Engineering microbes to sense and eradicate *Pseudomonas aeruginosa*, a human pathogen. *Mol. Syst. Biol.* **7**, 521 (2011).
151. Livet, J. et al. Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system. *Nature* **450**, 56–62 (2007).
152. Barker, N. et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* **449**, 1003–1007 (2007). **In this paper, a synthetic recombinase-based memory device based on stem cell-specific Cre expression and Cre-dependent expression of β-galactosidase is repurposed for lineage tracing and enables the discovery and characterization of adult intestinal stem cells in mice.**
153. Lescroart, F. et al. Early lineage restriction in temporally distinct populations of Mesp1 progenitors during mammalian heart development. *Nat. Cell Biol.* **16**, 829–840 (2014).
154. Chung, S. et al. Identification of preoptic sleep neurons using retrograde labelling and gene profiling. *Nature* **545**, 477–481 (2017).
155. Ruegg, T. L. et al. An auto-inducible mechanism for ionic liquid resistance in microbial biofuel production. *Nat. Commun.* **5**, 3490 (2014).
156. Minty, J. J. et al. Design and characterization of synthetic fungal-bacterial consortia for direct production of isobutanol from cellulose biomass. *Proc. Natl Acad. Sci. USA* **110**, 14592–14597 (2013).
157. Chen, Y., Kim, J. K., Hirning, A. J., Josic, K. & Bennett, M. R. Emergent genetic oscillations in a synthetic microbial consortium. *Science* **349**, 986–989 (2015). **In this study, the dual-feedback architecture proposed in reference 114 is validated at the intercellular level to create oscillating bacterial populations (termed synthetic consortia), which consist of specialized activator and repressor strains.**
158. Regot, S. et al. Distributed biological computation with multicellular engineered networks. *Nature* **469**, 207–211 (2011).
159. Auslander, D. et al. Programmable full-adder computations in communicating three-dimensional cell cultures. *Nat. Methods* **15**, 57–60 (2018). **This work marks the pinnacle of complexity in the design of prototype gene circuits; synthetic consortia consisting of individual human cell populations transgenic for specific Boolean logic functions are programmed to operate robust full-adder computations of environmental signals.**
160. Kemmer, C. et al. A designer network coordinating bovine artificial insemination by ovulation-triggered release of implanted sperms. *J. Control. Release* **150**, 23–29 (2011).
161. Windbichler, N. et al. A synthetic homing endonuclease-based gene drive system in the human malaria mosquito. *Nature* **473**, 212–215 (2011).
162. Hammond, A. et al. A CRISPR–Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. *Nat. Biotechnol.* **34**, 78–83 (2016).
163. Kotula, J. W. et al. Programmable bacteria detect and record an environmental signal in the mammalian gut. *Proc. Natl Acad. Sci. USA* **111**, 4838–4843 (2014).
164. Riglar, D. T. et al. Engineered bacteria can function in the mammalian gut long-term as live diagnostics of inflammation. *Nat. Biotechnol.* **35**, 653–658 (2017).
165. Whitaker, W. R., Shepherd, E. S. & Sonnenburg, J. L. Tunable expression tools enable single-cell strain distinction in the gut microbiome. *Cell* **169**, 538–546.e12 (2017).
166. Borrero, J., Chen, Y., Dunny, G. M. & Kaznessis, Y. N. Modified lactic acid bacteria detect and inhibit multiresistant enterococci. *ACS Synth. Biol.* **4**, 299–306 (2015).
167. Wright, C. M., Wright, R. C., Eshleman, J. R. & Ostermeier, M. A protein therapeutic modality founded on molecular regulation. *Proc. Natl Acad. Sci. USA* **108**, 16206–16211 (2011).
168. Swofford, C. A., Van Dessel, N. & Forbes, N. S. Quorum-sensing *Salmonella* selectively trigger protein expression within tumors. *Proc. Natl Acad. Sci. USA* **112**, 3457–3462 (2015).
169. Torikai, H. et al. A foundation for universal T cell based immunotherapy: T cells engineered to express a CD19-specific chimeric antigen-receptor and eliminate expression of endogenous TCR. *Blood* **119**, 5697–5705 (2012).
170. Roybal, K. T. et al. Precision tumor recognition by T cells with combinatorial antigen-sensing circuits. *Cell* **164**, 770–779 (2016).
171. Roybal, K. T. et al. Engineering T cells with customized therapeutic response programs using synthetic Notch receptors. *Cell* **167**, 419–432.e6 (2016).
172. Eil, R. et al. Ionic immune suppression within the tumour microenvironment limits T cell effector function. *Nature* **537**, 539–543 (2016).
173. Moon, E. K. et al. Expression of a functional CCR2 receptor enhances tumor localization and tumor eradication by retargeted human T cells expressing a mesothelin-specific chimeric antibody receptor. *Clin. Cancer Res.* **17**, 4719–4730 (2011).
174. Wu, C. Y., Roybal, K. T., Puchner, E. M., Onuffer, J. & Lim, W. A. Remote control of therapeutic T cells through a small molecule-gated chimeric receptor. *Science* **350**, aab4077 (2015). **In this work, the autonomous tumour recognition and destruction programme of CAR T cells is rendered conditionally activatable by small molecule drugs; initiation of CD3ζ-dependent T cell signalling relies on chemically induced protein dimerization.**
175. Grada, Z. et al. TanCAR: a novel bispecific chimeric antigen receptor for cancer immunotherapy. *Mol. Ther. Nucleic Acids* **2**, e105 (2013).
176. Fedorov, V. D., Themeli, M. & Sadelain, M. PD-1- and CTLA-4-based inhibitory chimeric antigen receptors (iCARs) divert off-target immunotherapy responses. *Sci. Transl. Med.* **5**, 215ra172 (2013).
177. Xie, M. et al. Beta-cell-mimetic designer cells provide closed-loop glycemic control. *Science* **354**, 1296–1301 (2016). **This work shows that expression of voltage-gated calcium channels is decisive for glucose sensing in non-endocrine human cell types and indicates that synthetic gene circuits programming human cells for closed-loop control of glucose homeostasis could provide an important alternative to β-cell differentiation (see references 127 and 145) in future cell-based diabetes treatments.**

178. Ye, H. et al. Self-adjusting synthetic gene circuit for correcting insulin resistance. *Nat. Biomed. Eng.* **1**, 0005 (2017).
179. Schukur, L., Geering, B., Charpin-El Hamri, G. & Fussenegger, M. Implantable synthetic cytokine converter cells with AND-gate logic treat experimental psoriasis. *Sci. Transl. Med.* **7**, 318ra201 (2015).
180. Bai, P. et al. A synthetic biology-based device prevents liver injury in mice. *J. Hepatol.* **65**, 84–94 (2016).
181. Kemmer, C. et al. Self-sufficient control of urate homeostasis in mice by a synthetic circuit. *Nat. Biotechnol.* **28**, 355–360 (2010).
182. Rossger, K., Charpin-El Hamri, G. & Fussenegger, M. Reward-based hypertension control by a synthetic brain-dopamine interface. *Proc. Natl. Acad. Sci. USA* **110**, 18150–18155 (2013).
183. Auslander, D. et al. A synthetic multifunctional mammalian pH sensor and CO₂ transgene-control device. *Mol. Cell* **55**, 397–408 (2014).
184. Rossger, K., Charpin-El Hamri, G. & Fussenegger, M. A closed-loop synthetic gene circuit for the treatment of diet-induced obesity in mice. *Nat. Commun.* **4**, 2825 (2013).
185. Saxena, P., Charpin-El Hamri, G., Folcher, M., Zulewski, H. & Fussenegger, M. Synthetic gene network restoring endogenous pituitary-thyroid feedback control in experimental Graves' disease. *Proc. Natl. Acad. Sci. USA* **113**, 1244–1249 (2016).
186. Di Stasi, A. et al. Inducible apoptosis as a safety switch for adoptive cell therapy. *N. Engl. J. Med.* **365**, 1673–1683 (2011).
187. Chan, C. T., Lee, J. W., Cameron, D. E., Bashor, C. J. & Collins, J. J. 'Deadman' and 'Passcode' microbial kill switches for bacterial containment. *Nat. Chem. Biol.* **12**, 82–86 (2016).
188. Shao, J. et al. Smartphone-controlled optogenetically engineered cells enable semiautomatic glucose homeostasis in diabetic mice. *Sci. Transl. Med.* **9**, eaal2298 (2017).
- By integrating software engineering and synthetic biology**, the authors of this study create a telemedicine concept for future personalized cell-based diabetes therapy; in their design, smartphone-controlled light-emitting diode (LED) implants regulate the release of insulinogenic hormones by human cells transgenic for red light-inducible gene expression.
189. Lim, W. A. & June, C. H. The principles of engineering immune cells to treat cancer. *Cell* **168**, 724–740 (2017).
190. Klebanoff, C. A., Rosenberg, S. A. & Restifo, N. P. Prospects for gene-engineered T cell immunotherapy for solid cancers. *Nat. Med.* **22**, 26–36 (2016).
191. Xie, Z., Wroblewska, L., Prochazka, L., Weiss, R. & Benenson, Y. Multi-input RNAi-based logic circuit for identification of specific cancer cells. *Science* **333**, 1307–1311 (2011).
192. Nissim, L. & Bar-Ziv, R. H. A tunable dual-promoter integrator for targeting of cancer cells. *Mol. Syst. Biol.* **6**, 444 (2010).
193. Gaber, R. et al. Designable DNA-binding domains enable construction of logic circuits in mammalian cells. *Nat. Chem. Biol.* **10**, 203–208 (2014).
194. Kramer, B. P., Fischer, C. & Fussenegger, M. BioLogic gates enable logical transcription control in mammalian cells. *Biotechnol. Bioeng.* **87**, 478–484 (2004).
195. Rinaudo, K. et al. A universal RNAi-based logic evaluator that operates in mammalian cells. *Nat. Biotechnol.* **25**, 795–801 (2007).
196. Liernert, F. et al. Two- and three-input TALE-based AND logic computation in embryonic stem cells. *Nucleic Acids Res.* **41**, 9967–9975 (2013).
197. Moon, T. S., Lou, C., Tamsir, A., Stanton, B. C. & Voigt, C. A. Genetic programs constructed from layered logic gates in single cells. *Nature* **491**, 249–253 (2012).
198. Win, M. N. & Smolke, C. D. Higher-order cellular information processing with synthetic RNA devices. *Science* **322**, 456–460 (2008).
199. Wang, W. et al. A light- and calcium-gated transcription factor for imaging and manipulating activated neurons. *Nat. Biotechnol.* **35**, 864–871 (2017).
200. Leisner, M., Bleris, L., Lohmueller, J., Xie, Z. & Benenson, Y. Rationally designed logic integration of regulatory signals in mammalian cells. *Nat. Nanotechnol.* **5**, 666–670 (2010).

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