Metabolic Engineering 16 (2013) 33–41

Contents lists available at SciVerse ScienceDirect

Metabolic Engineering

journal homepage: www.elsevier.com/locate/ymben

Minireview

Engineering of synthetic intercellular communication systems

William Bacchus [a], Martin Fussenegger [a,b,*]

[a] ETH Zurich, Department of Biosystems Science and Engineering (D-BSSE), Mattenstrasse 26, CH-4058 Basel, Switzerland
[b] Faculty of Science, University of Basel, Basel, Switzerland

ARTICLE INFO

Article history:
Received 8 October 2012
Received in revised form
3 December 2012
Accepted 5 December 2012
Available online 14 December 2012

Keywords:
Synthetic biology
Intercellular communication
Engineered gene control circuits
Multicellular consortia

ABSTRACT

The introduction of synthetic devices that provide precise fine-tuning of transgene expression has revolutionized the field of biology. The design and construction of sophisticated and reliable genetic control circuits have increased dramatically in complexity in recent years. The norm when creating such circuits is to program the whole network in a single cell. Although this has been greatly successful, the time will soon come when the capacity of a single cell is no longer adequate. Therefore, synthetic biology-inspired research has started to shift towards a multicellular approach in which specialized cells are constructed and then interconnected, enabling the creation of higher-order networks that do not face the same limitations as single cells. This approach is conceptually appealing in many respects. The fact that overall workload can be easily divided between cells eliminates the problem of limited program capacity of a single cell. Furthermore, engineering of specialized cells will enable a plug-and-play approach in which cells are combined into multicellular consortia depending on the requested task. Recent advances in synthetic biology to implement intercellular communication and multicellular consortia have demonstrated an impressive arsenal of new devices with novel functions that are unprecedented even in engineered single cells. Engineering of such devices have been achieved in bacteria, yeast and mammalian cells, all of which is covered in this review. The introduction of synthetic intercellular communication into the cell engineering toolbox will open up new frontiers and will greatly contribute to the future success of synthetic biology and its clinical applications.

1. Introduction

The accumulation of genetic data obtained from biological studies, together with reliable recombinant DNA technologies, helped pave the way for the biotechnological breakthroughs of the 1980s and 1990s. In its aftermath, the establishment of new interdisciplinary fields such as bioinformatics, systems biology, and synthetic biology helped to make sense of the immense amount of biological data that had been collected. The establishment of such new interdisciplinary fields, overlapping all natural sciences, has significantly promoted the understanding of biological systems. In recent years, the ability to rationally design and construct synthetic regulatory circuits using this obtained information has led to great advances in areas such as functional genomics (Kawaguchi et al., 2002; Malleret et al., 2001; Mansuy and Bujard, 2000), tissue engineering (Sanchez-Bustamante et al., 2006; Weber and Fussenegger, 2004), biopharmaceutical manufacturing (Ulmer et al., 2006; Weber and Fussenegger, 2007), drug discovery (Aubel et al., 2001; Sharpless and Depinho, 2006; Weber et al., 2008), and prototype gene therapy studies (Gitzinger et al., 2009; Kemmer et al., 2010). The use of genetic design principles and validated genetic parts enables the design of functionally interoperable synthetic control devices (Purnick and Weiss, 2009). The central building blocks in these circuits are composed of engineered ligand-responsive transgene systems, which are able to respond to a specific stimulus in a reliable and predictable manner (Weber and Fussenegger, 2011b). The implementation of gene networks in the topology of highly complex biological systems has led to the creation of an immense number of synthetic devices. In as little as 10 years, what started out as groundbreaking proof-of-concept examples for synthetic biology, with the construction of relatively simple genetic circuits, resulting in the first bacterial toggle switch (Gardner et al., 2000) and repressilator (Elowitz and Leibler, 2000), has developed into a haven of complex and robust genetically encoded circuits that are able to perform highly complex regulatory tasks. These include functions such as counters (Friedland et al., 2009), logic formulas (Rinaudo et al., 2007), switches (Atkinson et al., 2003; Kramer et al., 2004), timers (Danino et al., 2010; Ellis et al., 2009; Fussenegger, 2010), time-delay circuits (Weber et al., 2007b), oscillators (Atkinson et al., 2003; Danino et al., 2010; Tigges et al., 2009), biocomputers (Auslander et al., 2012), and has even resulted in potential biomedical applications (Kemmer et al., 2010, 2011; Ruder et al., 2011; Weber and Fussenegger, 2012; Ye et al., 2011). Although these devices have been a great overall success, they have

* Corresponding author at: Department of Biosystems Science and Engineering (D-BSSE), ETH Hoenggerberg, HPT D74, Mattenstrasse 26, CH-4058 Basel, Switzerland. Fax: +41 61 387 39 88.
E-mail address: fussenegger@bsse.ethz.ch (M. Fussenegger).

1096-7176/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ymben.2012.12.001

been limited to function only in individual cells. Due to their increased complexity, these devices suggest that the limit of what a single cell can accomplish might soon be reached. To that end, engineering of specialized cells in distinct sub-populations and interconnecting them represents a robust and reliable method for building complex higher-order control networks, which would be harder, if not impossible, to achieve using a conventional single-cell approach. The idea is conceptually appealing in many aspects. The ability to administer different tasks to different cells would enable the division of the overall metabolic workload, and would also reduce the number of genetic components needed in constituent sub-populations (Brenner et al., 2008; Li and You, 2011; Shong et al., 2012). As the populations communicate via intercellular signals, the genetic response to a certain concentration of that signal would be on a population level instead of a single-cell level, which would enable a coordinated response as well as noise reduction (Koseska et al., 2009). Reduction of noise allows for the construction of more robust genetic switches by decreasing the negative effect of random fluctuations between individual cells. Furthermore, as more and more genetic switches are combined into larger circuits, noise reduction of each component will inevitably result in more robust and predictable behaviors of the overall circuits. Intercellular communication also allows for spatial separation of the cells, which may foster advances in biomedical applications (Macia et al., 2012). Furthermore, precise intercellular crosstalk represents a naturally exciting phenomenon, overlapping the biological kingdoms, to control important physiological activities (Bassler and Losick, 2006; Blair, 2007; Freeman, 2000; Gromada et al., 1997; Johnson and Tabin, 1997; Papetti and Herman, 2002; Yarilina et al., 2008). Constructing such synthetic variants could help increase our understanding of their natural counterparts.

Engineering reliable intercellular communication devices enables entire cell populations to receive, process, and transfer metabolic information and, in so doing, communicate with each other (Hooshangi and Bentley, 2008; Weber and Fussenegger, 2011a). The building blocks for such a communication device should be designed in a rational fashion in order to facilitate the interconnection of additional signaling elements, thereby dramatically increasing the size and complexity of the intercellular communication network. This review addresses the advances made to sustain the initial success era of synthetic biology by rationally shifting the focus from synthetic single cell devices towards intercellular counterparts. Recent achievements made in bacteria, yeast and mammalian cells will be covered.

2. Synthetic intercellular communication in bacteria

Bacterial communication utilizes intercellular chemical signaling molecules. These quorum-sensing (QS) molecules allow the bacteria to act with a multicellular response in order to regulate gene expression, depending on the cell density (Bassler and Losick, 2006; Hooshangi and Bentley, 2008). Many gram-negative bacteria communicate through various LuxR/LuxI homologous systems in which acyl-homoserine lactone (AHL) autoinducers are the freely diffusible intercellular signaling molecules. AHLs are species-specific metabolites synthesized by LuxI homologues, which at sufficiently high concentrations are further utilized by LuxR homologues to regulate the gene expression of target genes in an AHL-dependent manner (Waters and Bassler, 2005). Synthetic biologists have utilized this feature in bacteria to construct synthetic intercellular genetic systems. The most widely used QS mechanism is based on the LuxR/LuxI system from *Vibrio fischeri*. This gram-negative bacterium regulates bioluminescence in a cell-density-dependent manner. LuxI mediates production of the AHL 3-oxohexanoyl-HSL (3OC6HSL), which upon accumulation, activates LuxR to bind to its target genes for bioluminescence only when a larger number of the bacteria are present (Bassler, 1999; Egland and Greenberg, 1999). Another QS system utilized to build synthetic intercellular communication is that of the opportunistic human pathogen *Pseudomonas aeruginosa*. In this system, the QS is composed of the LuxR/LuxI homologues LasR/LasI and RhlR/RhlI, which act in a sequential manner to activate target virulence genes in response to increased bacterial cell density. LasI and RhlI produce AHLs 3-oxododecanoyl-HSL (3OC12HSL) and butanoyl-HSL (C4HSL), which are utilized by the LasR and RhlR, respectively, enabling the activation of multiple target genes. Furthermore, LasR acts positively on the gene expression of RhlI (Camara et al., 2002; Pesci et al., 1997; Viretta and Fussenegger, 2004).

The construction of synthetic intercellular communication in bacteria has made its entrance into synthetic biology over the past decade, introducing an impressive arsenal of novel features. These synthetic intercellular communication devices are either autonomous in nature (programming of a single-cell population) or a combination of two or more programmed cell populations that are interconnected using QS.

2.1. Synthetic autonomous intercellular systems

Synthetic autonomous intercellular systems are composed of a single-cell population in which individuals of that population can communicate using engineered QS. The systems constructed so far have demonstrated the potential of coupling intercellular signaling to a specific and coordinated gene response across a bacterial population. The preferred QS mechanism to implement has been the one of the LuxR/LuxI from *V. fischeri*.

You et al. constructed a synthetic gene network for programmed population control. *Escherichia coli* were engineered with the LuxR/LuxI components from *V. fischeri* controlling the cell density-dependent gene expression of a killer gene, ccdB. At sufficiently high cell densities, AHL was produced acting on LuxR to activate ccdB expression, thereby killing the bacteria. This population-controlled circuit enabled the autonomous regulation of the cell density (Fig. 1A) (You et al., 2004).

Anderson et al. demonstrated another example of engineered bacterial population control utilizing the LuxR/LuxI QS system from *V. fischeri*. *E.coli* was engineered with a genetic circuit, enabling invasion of cultured tumor cells in a population density-dependent manner. After the bacterial population reached a threshold size, LuxR initiates a positive feedback loop, resulting in increased LuxI and LuxR expression, which amplifies the production of AHL and activates the expression of the *Yersinia pseudotuberculosis* invasin gene (*inv*) (Fig. 1B). The expression of invasin by the bacteria enables invasion of the tumor cells by tumor-specific integrin receptors (Anderson et al., 2006).

The coupling of intercellular communication with a genetic circuit responsive to red light made it possible to construct a bacterial edge-detection device (Tabor et al., 2009). The circuit was constructed so that a red light would inhibit the expression of LuxI as well as the transcriptional repressor Cl. Furthermore, LacZ expression was under negative regulation by Cl and positive regulation by LuxR. The result of this was that only bacteria exposed to light and located close enough to bacteria grown in dark was able to express LacZ. These bacteria did not express Cl and were able to receive the AHL inducer from the neighboring bacteria grown in the dark (Fig. 1C).

Using their synchronized oscillator, Danino et al. demonstrated the intriguing aspect of connecting intercellular communication, which is broadcast over an entire cell population, with a determined genetic response that enables united and synchronized actions. The LuxR/LuxI system of *V.fischeri* was designed to drive the expression of a green fluorescent protein as well as the *Bacillus Thurigiensis*-derived

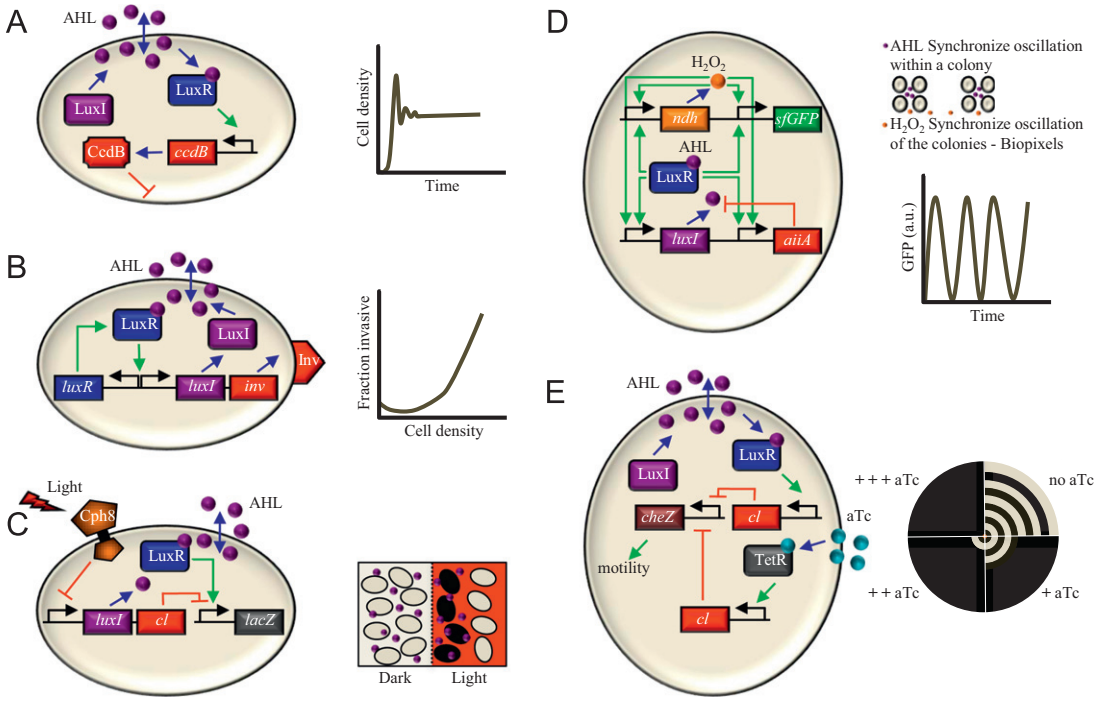


Fig. 1. Synthetic intercellular communication in bacteria based on the LuxR/LuxI components derived from *Vibrio fischeri*. At sufficiently high cell densities, LuxI produces enough AHL for the LuxR/AHL complex to activate the gene expression of (A) a killer gene, ccdB, which results in programmed population control of the cell density (You et al., 2004) and (B) an invasin gene, *inv*, that enables invasion of tumor cells by tumor-specific integrin receptors (Anderson et al., 2006). (C) Edge-detection device. Red light sensed by the Cph8 receptor inhibits the expression of LuxI and of the transcriptional repressor Cl. Bacteria grown in the dark expresses LuxI, thereby producing AHL and Cl that repress LacZ expression. Bacteria grown in light are not able to produce AHL. When close enough to bacteria grown in the dark, the bacteria grown in the light receive AHL,which enables the LuxR/AHL-complex to activate LacZ expression (Tabor et al., 2009). (D) Synchronized oscillation. The protease AiiA derived from *Bacillus Thurigensis*, whose expression is dependent on AHL, catalyzes the cleavage of AHL produced by LuxI, resulting in a synchronized and oscillating genetic response of sfGFP (superfolder variant of GFP) as its expression is AHL-dependent. NADH dehydrogenase II (ndh), the expression of which is also dependent on AHL, generates H_2O_2 vapor, which is broadcast between colonies enabling synchronized oscillations of the colonies (Prindle et al., 2012). (E) Striped pattern formation. At high cell density the production of the repressor Cl is activated due to increased AHL levels. Cl in turn repress *cheZ* expression thereby resulting in nonmotile cell phenotype at high cell density. A suspension of exponentially growing engineered *E. coli* was inoculated at the center of a dish. This enables the cells to form periodic stripes of high (light stripes) and low (dark stripes) cell densities over time. Additional transcriptional repression of *cheZ* by anhydrotetracycline (aTc) enabled the control of the number of formed stripes as increasing aTc concentrations (indicated with increasing numbers of +) resulted in less number of strips (Liu et al., 2011). Green arrows indicate activation and blunt red arrows indicate repression or killing. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

AiiA, a protease that catalyzes the cleavage of AHL. This resulted in a synchronized and oscillating genetic response (Danino et al., 2010). Adding a coupling circuit by introducing genetic components to regulate H_2O_2 production enabled synchronized oscillations, not only within but also between bacterial populations. H_2O_2vapor diffuses between the cell populations, thereby synchronizing them, as H_2O_2 is an activator of the LuxI promoter (Fig. 1D) (Prindle et al., 2012).

Liu et al. implemented the LuxR/LuxI system of *V. fischeri* to control cell motility of *E. coli* by regulating the transcription of *cheZ*. Deletion of *cheZ* results in a nonmotile phenotype. The LuxR/LuxI components were designed to activate the production of the repressor Cl in an AHL-dependent manner at high cell densities. Cl in turn represses *cheZ* expression thereby resulting in non-motile cells as a consequence of the high cell density. This enabled the cells to form periodic stripes of high and low cell densities. Additional transcriptional repression of *cheZ*, under anhydrotetracycline (aTc) regulation, enabled the control of the amount of formed stripes as increasing aTc concentrations resulted in reduced number of strips (Fig. 1E).

2.2. Multiple population-based synthetic intercellular systems

The engineering of synthetic intercellular communication is not limited to single-cell populations. In fact, the use of such intercellular signaling allows to functionally interconnect an unlimited number of cell populations. Basu et al. implemented such an approach by separating the LuxR/LuxI components into two different cell populations. A sender cell population was engineered to express LuxI, which produced AHL; meanwhile, a receiver cell population contained the LuxR component that, upon receiving sufficient levels of AHL from the sender cell population, activated the gene expression of GFP and the repressor Cl. Continued expression of Cl resulted in the threshold required to repress GFP being eventually reached. Separating the genetic components of the LuxR/LuxI QS system into two sub-populations made it possible to construct a pulse-generator with spatiotemporal behavior (Fig. 2A) (Basu et al., 2004).

Basu et al. went even further, constructing a band-pass circuit in which a sender cell population is engineered to produce AHL and the receiver cell population is equipped with genetic circuits that enable gene expression only at intermediate AHL levels. Upon high levels of AHL, LuxR activates the expression of Cl and $LacI_{M1}$ (modified version of the LacI repressor). The expression of $LacI_{M1}$ inhibits GFP expression. At low levels of AHL, the LuxR is unable to sufficiently activate the Cl repressor, which acts by repressing the LacI. This results in LacI being expressed and GFP expression being inhibited. At intermediate AHL levels, a sufficient amount of Cl is expressed to inhibit LacI expression, while $LacI_{M1}$ is unable to inhibit GFP expression. This results in GFP expression only at intermediate AHL concentrations. The AHL concentration dependency on GFP expression corresponds to the distance dependency between the two populations, as AHL is diffusible. This made it possible to construct various patterns depending on the positioning of the AHL-producing and -responding cells (Fig. 2B) (Basu et al., 2005).

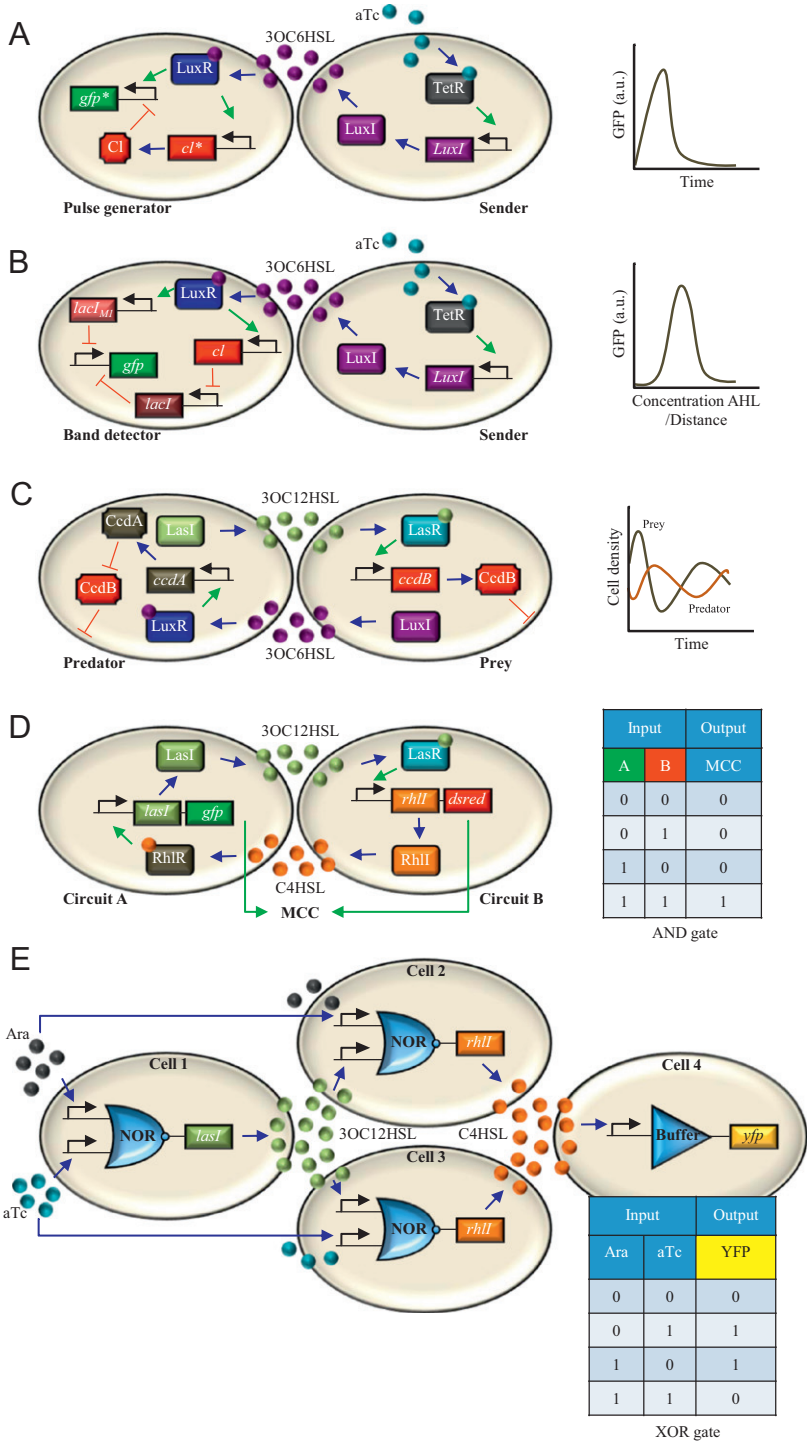


Fig. 2. Multiple-population-based synthetic intercellular communication in bacteria based on the LuxR/LuxI components derived from *Vibrio fischeri*and on the LasR/LasI and RhlR/RhlI components derived from *Pseudomonas aeruginosa*. (A, B) Sender cells are engineered to produce AHL. The addition of anhydrotetracycline (aTc) liberates the repression of TetR on LuxI expression, which enables AHL production (3OC6HSL). The produced AHL is sensed by (A) pulse generating cells or (B) band detector cells. For the pulse generating cells, AHL enable the activation of both GFP and Cl, where * indicates a destabilized version. Continued AHL transmission results in concentrations of Cl required to repress GFP expression (Basu et al., 2004). The band detector cells enable GFP expression only at intermediate AHL concentrations. At high AHL concentrations, the LuxR/AHL complex activates the $LacI_{M1}$ (modified version of the LacI repressor), which in turn represses GFP expression. At low AHL levels, the Cl repressor is not expressed, which leads to the derepression of LacI. This, in turn, represses GFP expression. At intermediate AHL levels, the LuxR/AHL-complex activates Cl, which represses the LacI. The levels of $LacI_{M1}$produced are not sufficient to repress GFP, which leads to expression of GFP (Basu et al., 2005). (C) Predator–prey system. The predator cells require AHL (3OC6HSL) produced by the prey cells for their survival as that enables the LuxR/AHL complex to activate the expression of CcdA, which inhibits the killer effect of CcdB. The predator cells also produce AHL (3OC12HSL), which enable the LasR/AHL complex to activate and induce the expression of the killer protein CcdB, thereby killing the cells required for predator survival (Balagadde et al., 2008). (D) Microbial consensus consortium (MCC). In the presence of AHL (C4HSL) produced by the cells containing circuit B, the RhlR/AHL complex activates the expression of LasI and GFP in the cells containing circuit A. In a similar fashion, AHL (3OC12HSL) produced by the cells containing circuit A enables the LasR/AHL-complex to activate the expression of RhlI and DsRed in the cells containing circuit B. Each cell population containing either circuit can be considered one input in a logic AND gate operation (Brenner et al., 2007). (E) Multicellular computing. Cell population 1 is engineered with a simple NOR gate responding to arabinose (ara) and aTc. The output of the NOR gate is the expression of LasI and, consequently, the production of AHL (3OC12HSL). This AHL functions as one of the two inputs for the NOR gate in cell population 2 and 3 in combination with ara (cell population 2) or aTc (cell population 3). The output of the NOR gates in both these cell populations is RhlI, which allows for the production of AHL (C4HSL). Cell population 4 functions as a buffer and converts the input signal into YFP expression. This multicellular assembly allows for logic XOR gate operations with ara and aTc as input (Tamsir et al., 2011). Green arrows indicate activation; blunt red arrows indicate repression or killing. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Although the LuxR/LuxI QS system from *V. fischeri* is predominantly used when constructing intercellular communication in bacteria, one signaling molecule is not sufficient when constructing two-way communication or building higher-order of intercellular circuits that consist of more than two cell populations. The LasR/LasI and RhlR/RhlI QS system from *P. aeruginosa* has also been utilized to assist in that task. The synthetic predator–prey system developed by Balagadde et al. (2008). was designed so that the predator population required metabolic information from the prey population in order to survive. At the same time the predator population sent metabolic information to the prey population, which killed those cells. LuxR/LuxI QS from *V. fischeri,* in combination with the LasR/LasI QS from *P. aeuginosa,* was used to construct such a bidirectional system in *E. coli*. The predator cells were engineered to express a killer protein (CcdB), the effect of which could be antagonized by the expression of an anti-toxin (CcdA). LuxR positively regulated the expression of the anti-toxin in the presence of the AHL 3OC6HSL. The prey cells were engineered to produce 3OC6HSL by expressing LuxI, thereby rescuing the predator. On the contrary, the predator produced the AHL 3OC12HSL that allowed LasR in the prey to activate the expression of CcdB, thereby killing the prey (Fig. 2C) (Balagadde et al., 2008). The constructed predator–prey ecosystem made it possible to study various ecological aspects and represents a clear example of the implementation of synthetic intercellular communication in microorganisms that makes it possible to understand complex natural biological systems (Song et al., 2009). A similar synthetic microbial ecosystem was constructed to investigate how environmental factors influence the overall ecosystem by implementing antibiotic selection pressure on the populations (Hu et al., 2010).

Bidirectional intercellular communication was also engineered between two populations of *E. coli,* which enabled consensus gene expression responses in a microbial biofilm consortium. One population was engineered to express LasI, which produced 3OC12HSL and target A (GFP) in a RhlR-dependent manner. The second population was engineered to express RhlI, which produced C4HSL and target B (dsRed) in a LasR-dependent manner. Together, these circuits compose a microbial consensus consortium (MCC) that responded as a logical AND gate when implementing the distinct cell populations as the input parameters (Fig. 2D) (Brenner et al., 2007).

Tamsir et al. demonstrated the ease of implementing distinct cellular consortiums to construct programmable cellular units capable of performing complex computational tasks. Simple logic NOR gates were engineered in distinct cellular populations. Interconnecting individual cells, capable of performing these simple computations using QS allowed bacterial consortiums to build up and perform complex computational operations such as XOR functions. The individual NOR gates were constructed by placing the expression of the Cl repressor under the regulation of two tandem inducible promoters. The Cl repressor further inactivates promoters driving LasI or RhlI expression, thereby indirectly regulating the production of the corresponding AHL. These AHLs were designed to act as one of the two input signals for the following NOR gate (Fig. 2E). This plug-and-play approach allowed all 16 possible two-input Boolean logic gates to be constructed by arranging and combining individual cell populations, programmed with a simple genetic circuit, in different spatial configurations interconnected by QS molecules (Tamsir et al., 2011).

3. Synthetic intercellular communication in eukaryotes

As with engineered intracellular circuits, most of the constructed intercellular systems have been engineered in bacteria. However, the rationale behind building and implementing intercellular systems instead of the traditional intracellular ones is not limited to such simple organisms. Reports have illustrated excellent achievements in yeast that utilizes engineered intercellular signaling; this mindset has even found its way into the more complex mammalian cells. The ease of use and the extraordinary accomplishments in bacteria doubtlessly remains true for higher orders of organisms.

3.1. Synthetic intercellular communication in yeast

The first constructed intercellular communication system implemented in yeast was composed of genetic material from *Arabidopsis thaliana*. The sender cell population consisted of yeast cells engineered with the isopentenyltransferase AtIPT4, derived from *A. thaliana*. These cells were able to synthesize the plant hormone cytokine isopentenyladenine (IP) by converting ATP to IP. The receiver cells were composed of yeast cells engineered with a hybrid exogenous/endogenous phosphorylation signaling pathway and the corresponding *A. thaliana*-derived AtCRE1 cytokinin receptor. Upon receiving IP produced by the sender cells, the AtCRE1 receptor of the receiver cells initiates a signaling cascade, which results in GFP expression (Fig. 3A) (Chen and Weiss, 2005).

A recent report by Regot et al. presented the construction of a library of 16 yeast strains, each of which is genetically engineered to perform a simple logic-gate computation. Depending on the composition of the cells chosen from the engineered library, the yeast consortium could perform a broad range of complex Boolean logics. Utilizing five of these engineered strains resulted in a yeast consortium that was able to perform a one-bit adder with carrier computations (Fig. 3B) (Regot et al., 2011).

Designer two-way communication has also been achieved in yeast by engineering a synthetic cooperative ecosystem in which two non-mating strains rely on each other for their survival. This was accomplished by engineering each strain to produce and supply a metabolite that was essential to the other. While the first strain was equipped to produce the amino acid lysine but lacked the ability to produce the amino acid adenine, the second strain was able to produce adenine but lacked the ability to produce lysine; this resulted in increased viability of the individual strains in the presence of the other (Fig. 3C) (Shou et al., 2007).

3.2. Synthetic intercellular communication in mammalian cells

Despite mammalian cells having a more complex nature than bacteria and yeast, synthetic mammalian intercellular communication has also been established. Weber et al. constructed a mammalian intercellular communication system based on the volatile metabolite acetaldehyde. Sender cells, engineered to express mouse-derived alcohol dehydrogenase (ADH), converted supplemented ethanol into acetaldehyde. Receiver cells, which were engineered with transcriptional control of the human placental-secreted alkaline phosphatase (SEAP) by the *Aspergillus nidulans*-derived synthetic acetaldehyde-inducible promoter, enabled SEAP expression of the receiver cells to be proportional to the cell density of the sender cells. Inter-kingdom communication was established by exchanging the mammalian sender cells to those of *E. coli* and *S. cerevisiae*. Both of these organisms produced acetaldehyde in a population size-dependent manner and were able to broadcast the signal across species and kingdom barriers to the mammalian receiver cells. The mammalian intercellular communication system was also shown to function in mice in a manner similar to that of hormones. Mice were implanted with the sender and receiver cells. When ethanol was supplemented in the drinking water of the mice, the sender cells converted the ethanol into acetaldehyde, which was broadcast to the receiver cells, triggering SEAP expression (Fig. 4A). The constructed inter-kingdom interactions were utilized to construct

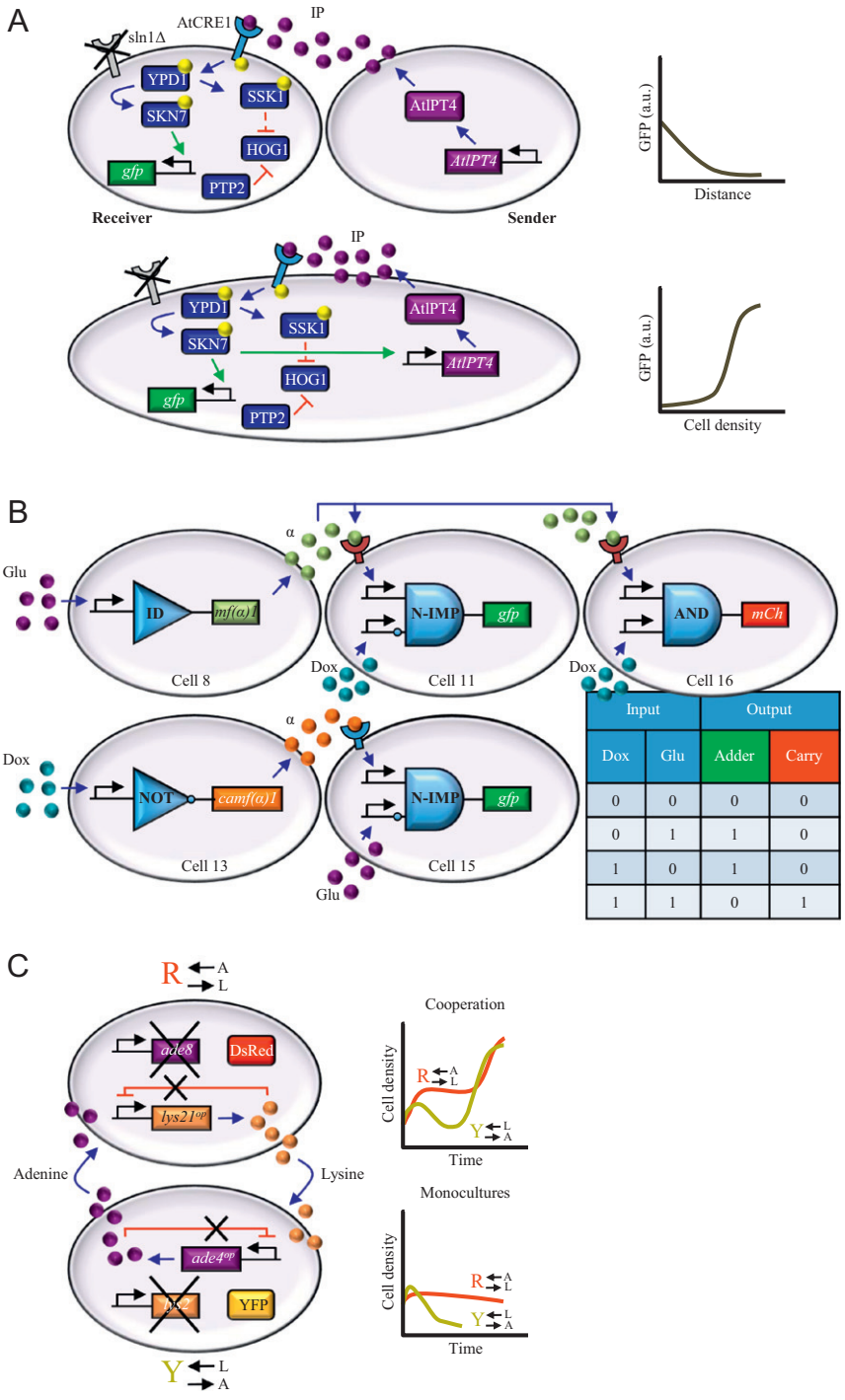
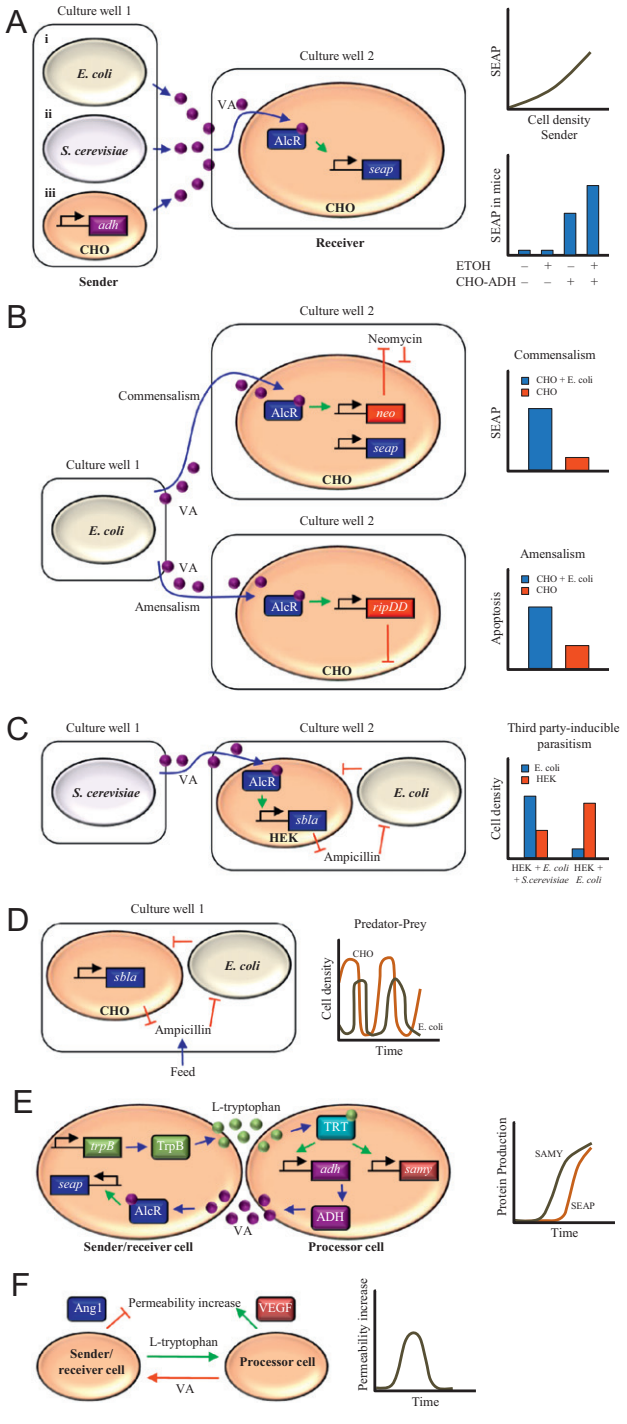


Fig. 3. Synthetic intercellular communication in yeast. (A) Sender cells engineered to express the isopentenyltransferase AtIPT4 from *A. thaliana* enable the cells to synthesize and secrete IP from ATP. Receiver cells engineered to express the cytokine receptor AtCRE1 from *A. thaliana* enable signal transduction over the yeast YPD1 and SKN7 signaling proteins,which activates GFP expression. To abolish any interference of the AtCRE1-dependent signaling, a sln1Δ yeast strain was used in combination with over-expression of PTP2, an endogenous HOG1 protein phosphatase, thereby inactivating the HOG1 pathway and rescuing the sln1Δ lethal phenotype. The expression of GFP was dependent on the distance between the sender and receiver cells. Implementing all genetic components into a single cell configuration allowed for the GFP expression to be cell density-dependent (Chen and Weiss, 2005). Green arrows indicate activation; blunt red arrows indicate repression. (B) Multicellular computing. A library of 16 engineered yeast strains was constructed in which each strain was able to perform different simple logic gate computations. Implementing five of these 16 engineered yeast strains, connected by intercellular communication, enabled the consortium to perform complex one-bit adder with carrier computations with glucose (Glu) and doxycycline (Dox) as the input parameters. To enable intercellular interactions, the alpha factor of *Saccharomyces cerevisiae* (mf(α)1) and of *Candida albicans* (camf(α)1) was implemented with their corresponding receptors. GFP and mCherry were used as output signals (Regot et al., 2011). (C) Synthetic cooperation. $R^{\rightarrow A}_{\rightarrow L}$; engineered with lys21^{op} enabled production of lysine without end-product feedback inhibition and lacking ade8, which removed the possibility of synthesizing adenine. $Y^{\rightarrow L}_{\rightarrow A}$; engineered with ade4^{op} enable production of adenine without end-product feedback inhibition and lacking lys2, thereby removing the possibility of synthesizing lysine. The two yeast strains rely on each other to receive the crucial metabolite as they are incapable of synthesizing it themselves. ($\rightarrow$) indicates that the strain can synthetize and release the corresponding amino acid. ($\leftarrow$) indicates that the strain cannot synthesize the corresponding amino acid. The two yeast strains were either cultured together (cooperation) or grown in monocultures. R indicate that the strain express DsRed and Y indicate that the strain express YFP (Shou et al., 2007). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

a number of synthetic ecosystems that were not limited to a single organism. Commensalism, which was constructed by engineering mammalian receiver cells to be resistant to neomycin in an acetaldehyde-inducible manner, was established, as was amensalism, which was constructed by engineering mammalian receiver cells to express RipDD in an acetaldehyde-inducible manner, thereby inducing apoptosis. *E. coli* was used in both of these settings as sender cells to produce the necessary acetaldehyde signal (Fig. 4B). Bidirectional synthetic ecosystems were constructed by engineering mammalian cells to express β-lactamase, which promote the survival of co-cultured *E.coli* in media containing ampicillin. The permitted growth of the *E. coli* by the mammalian cells broadcast back on the mammalian cells, which inhibited their own growth. The implementation of these characteristics enabled the construction of a range of ecosystem features, such as third-party-inducible parasitism and predator–prey behavior (Fig. 4C–D) (Weber et al., 2007a).

Bacchus et al. further utilized the acetaldehyde-based signaling mechanism and merged it with a L-tryptophan-based signaling mechanism. This enabled the construction of highly complex intercellular networks, including the first two-way communication device in mammalian cells. Sender/receiver cells were engineered to convert indole into L-tryptophan by expressing tryptophan synthase (TrpB) as well as expressing the reporter SEAP under acetaldehyde regulation. The processor cells were engineered to have a genetic response to L-tryptophan, expressing secreted α-amylase (SAMY) as a reporter, as well as ADH, thereby indirectly controlling the production of acetaldehyde in a L-tryptophan-dependent manner. The acetaldehyde produced by the processor cells is broadcast back to the sender/receiver cells, enabling the expression of SEAP as reporter. When following the communication events by monitoring the protein expression profiles, a SAMY increase is observed in response to the produced L-tryptophan, followed by a SEAP increase in response to the produced acetaldehyde (Fig. 4E). By replacing SEAP with angiopoietin-1 (Ang1) and replacing SAMY with vascular endothelial growth factor (VEGF) – two factors that act in sequential order to produce mature blood vessels – the two-way communication device was used to create an incoherent feed-forward loop programmed to regulate temporal permeability in vascular endothelial cell layers (Fig. 4F) (Bacchus et al., 2012).



4. Conclusion

In recent years, synthetic circuits implementing intercellular communication have expanded the scoop of synthetic biology.

Fig. 4. Synthetic intercellular communication in mammalian cells. (A) Interkingdom communication. Sender cells composed of either (i) bacteria (*E. coli*), (ii) yeast (*S. cerevisiae*), or (iii) mammalian cells (Chinese hamster ovary cells, CHO) engineered to express mouse-derived alcohol dehydrogenase (adh) produce volatile acetaldehyde (VA). The receiver cells engineered with AlcR, derived from *Aspergillus nidulans*, activate gene expression of its cognate promoter upon VA, thereby expressing secreted alkaline phosphatase (SEAP). The size of the sender cell populations determines the genetic response of the receiver cells. In mice implanted with the mammalian sender and receiver cells, administration of ethanol in the drinking water enables hormone-like characteristics as the sender cells signal via acetaldehyde to the receiver cells, which respond with corresponding SEAP production. (B) Commensalism and amensalism. Sender cells composed of *E. coli* produce VA, which is broadcast over the gas phase to the receiver cells. This allows the receiver cell population to survive by allowing for resistance to neomycin (commensalism) or by killing the receiver cells by expression of RipDD (amensalism). (C) Third-party-inducible parasitism. *S. cerevisiae* produce VA, which is broadcast to the mammalian cells (human embryonic kidney cells, HEK) engineered to produce β-lactamase (*sbla*) upon VA sensing, thereby promoting the survival of co-cultured *E. coli*. This, in turn, impairs the survival of the mammalian cells. (D) Predator–prey. CHO cells are engineered to produce β-lactamase, which promotes the survival of co-cultured *E. coli*. The increased concentration of *E. coli* impairs the survival of the mammalian cells, resulting in predator–prey-like characteristics (Weber et al., 2007a, 2007b). (E, F) Two-way communication. Mammalian sender/receiver cells convert indole supplemented in the media into L-tryptophan by expressing tryptophan synthase, TrpB, derived from *E. coli*. The produced L-tryptophan signal is broadcast to the processor cells, which are engineered with a L-tryptophan responsive circuit where the L-tryptophan-dependent transactivator (TRT) activates the expression of its cognate promoter, enabling expression of secreted α-amylase (SAMY) as recognition of the L-tryptophan signal as well as the ADH that enable VA production. VA is broadcast back to the sender/receiver cells that are engineered with the acetaldehyde-responsive elements described in **(A)**, and consequently produce SEAP. Green arrows indicate activation (E). Expressing vascular endothelial growth factor (VEGF) instead of SAMY and angiopoietin-1 (Ang1) instead of SEAP enables timely control of vascular permeability of endothelial cell monolayers. Green arrows, positive signal path increasing permeability; red arrows, negative signal path decreasing permeability **(F)** (Bacchus et al., 2012). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The entry of systems implementing such intercellular communications represents a novel trend and the advantages are clear. Intercellular communication systems may provide a solution that helps prepare for the construction of future synthetic circuits, which are likely to be more complex than present circuits (Moon et al., 2012). The genetic material can be divided into the different sub-populations, which allows for a plug-and-play approach that chooses specialized cells whose sum will result in any predefined overall function. This approach will ensure that advances in synthetic circuits are maintained even when one cell is no longer adequate. As more and more devices are built, the implementation of intercellular communication and microbial consortia of such will broaden (Mitchell et al., 2011) with potential future roles in various industrial biotechnological scenarios (Kobayashi et al., 2004; Shong et al., 2012; Tsai et al., 2010). Intercellular communication provides the possibility for spatial separation of the cell populations, which could lead to new possibilities in various biomedical applications (Duan and March, 2010). One intriguing scenario would be multiple cell implants that are connected through intercellular signaling, thereby enabling remote control of each other's functions. More and more intercellular signals will be available as increasing number of natural signals are being identified and characterized (Schaefer et al., 2008). Introducing synthetic intercellular communication, which is not based on metabolic information, has also been developed (Ortiz and Endy, 2012). Although the progress of engineered intercellular communication in mammalian cells has not been as explicit as it has for bacteria and yeast, there is no reason to doubt its future success. Two different intercellular communication systems, one utilizing arginine as metabolic information (Hartenbach et al., 2007; Weber et al., 2009) and one utilizing nitric oxide (Wang et al., 2008), have been constructed in mammalian cells and more are likely to follow. Circuits that are built and interconnected to construct systems of higher order will dramatically increase their complexity and utility.

The move from intracellular to intercellular communication systems is a major tool that will enable future advances in synthetic biology. These intercellular systems are likely to outperform any intracellular counterpart. The future will require greater processing capacity, as more complex networks and circuits are being established, which will dramatically increase performance characteristics. Therefore, the division of metabolic workload of the overall system between cells is crucial, as one cell is unlikely to be sufficient. Just as computers are assembled using different standardized electrical hardware coupled in a rational way to increase a system's overall performance, the assembly of distinct biological cellular machineries will be achieved in a similar manner.

References

Anderson, J.C., Clarke, E.J., Arkin, A.P., Voigt, C.A., 2006. Environmentally controlled invasion of cancer cells by engineered bacteria. J. Mol. Biol. 355, 619–627.

Atkinson, M.R., Savageau, M.A., Myers, J.T., Ninfa, A.J., 2003. Development of genetic circuitry exhibiting toggle switch or oscillatory behavior in *Escherichia coli*. Cell 113, 597–607.

Aubel, D., Morris, R., Lennon, B., Rimann, M., Kaufmann, H., Folcher, M., Bailey, J.E., Thompson, C.J., Fussenegger, M., 2001. Design of a novel mammalian screening system for the detection of bioavailable, non-cytotoxic streptogramin antibiotics. J Antibiot (Tokyo) 54, 44–55.

Auslander, S., Auslander, D., Muller, M., Wieland, M., Fussenegger, M., 2012. Programmable single-cell mammalian biocomputers. Nature 487, 123–127.

Bacchus, W., Lang, M., Daoud-El Baba, M., Weber, W., Stelling, J., Fussenegger, M., 2012. Synthetic two-way communication between mammalian cells. Nat. Biotechnol. 30, 991–996.

Balagadde, F.K., Song, H., Ozaki, J., Collins, C.H., Barnet, M., Arnold, F.H., Quake, S.R., You, L., 2008. A synthetic *Escherichia coli* predator–prey ecosystem. Mol. Syst. Biol. 4, 187.

Bassler, B.L., 1999. How bacteria talk to each other: regulation of gene expression by quorum sensing. Curr. Opin. Microbiol. 2, 582–587.

Bassler, B.L., Losick, R., 2006. Bacterially speaking. Cell 125, 237–246.

Basu, S., Gerchman, Y., Collins, C.H., Arnold, F.H., Weiss, R., 2005. A synthetic multicellular system for programmed pattern formation. Nature 434, 1130–1134.

Basu, S., Mehreja, R., Thiberge, S., Chen, M.T., Weiss, R., 2004. Spatiotemporal control of gene expression with pulse-generating networks. Proc. Nat. Acad. Sci. U.S.A. 101, 6355–6360.

Blair, S.S., 2007. Wing vein patterning in drosophila and the analysis of intercellular signaling. Annu. Rev. Cell Dev. Biol. 23, 293–319.

Brenner, K., Karig, D.K., Weiss, R., Arnold, F.H., 2007. Engineered bidirectional communication mediates a consensus in a microbial biofilm consortium. Proc. Nat. Acad. Sci. U.S.A. 104, 17300–17304.

Brenner, K., You, L., Arnold, F.H., 2008. Engineering microbial consortia: a new frontier in synthetic biology. Trends. Biotechnol. 26, 483–489.

Camara, M., Williams, P., Hardman, A., 2002. Controlling infection by tuning in and turning down the volume of bacterial small-talk. Lancet Infect. Dis 2, 667–676.

Chen, M.T., Weiss, R., 2005. Artificial cell–cell communication in yeast *Saccharomyces cerevisiae* using signaling elements from *Arabidopsis thaliana*. Nat. Biotechnol. 23, 1551–1555.

Danino, T., Mondragon-Palomino, O., Tsimring, L., Hasty, J., 2010. A synchronized quorum of genetic clocks. Nature 463, 326–330.

Duan, F., March, J.C., 2010. Engineered bacterial communication prevents *Vibrio cholerae* virulence in an infant mouse model. Proc. Nat. Acad. Sci. U.S.A. 107, 11260–11264.

Egland, K.A., Greenberg, E.P., 1999. Quorum sensing in *Vibrio fischeri*: elements of the luxl promoter. Mol. Microbiol. 31, 1197–1204.

Ellis, T., Wang, X., Collins, J.J., 2009. Diversity-based, model-guided construction of synthetic gene networks with predicted functions. Nat. Biotechnol. 27, 465–471.

Elowitz, M.B., Leibler, S., 2000. A synthetic oscillatory network of transcriptional regulators. Nature 403, 335–338.

Freeman, M., 2000. Feedback control of intercellular signalling in development. Nature 408, 313–319.

Friedland, A.E., Lu, T.K., Wang, X., Shi, D., Church, G., Collins, J.J., 2009. Synthetic gene networks that count. Science 324, 1199–1202.

Fussenegger, M., 2010. Synthetic biology: synchronized bacterial clocks. Nature 463, 301–302.

Gardner, T.S., Cantor, C.R., Collins, J.J., 2000. Construction of a genetic toggle switch in *Escherichia coli*. Nature 403, 339–342.

Gitzinger, M., Kemmer, C., El-Baba, M.D., Weber, W., Fussenegger, M., 2009. Controlling transgene expression in subcutaneous implants using a skin lotion containing the apple metabolite phloretin. Proc. Nat. Acad. Sci. U.S.A. 106, 10638–10643.

Gromada, J., Bokvist, K., Ding, W.G., Barg, S., Buschard, K., Renstrom, E., Rorsman, P., 1997. Adrenaline stimulates glucagon secretion in pancreatic A-cells by increasing the Ca^{2+} current and the number of granules close to the L-type Ca^{2+} channels. J. Gen. Physiol. 110, 217–228.

Hartenbach, S., Daoud-El Baba, M., Weber, W., Fussenegger, M., 2007. An engineered L-arginine sensor of *Chlamydia pneumoniae* enables arginine-adjustable transcription control in mammalian cells and mice. Nucleic Acid Res. 35, e136.

Hooshangi, S., Bentley, W.E., 2008. From unicellular properties to multicellular behavior: bacteria quorum sensing circuitry and applications. Curr. Opin. Biotechnol. 19, 550–555.

Hu, B., Du, J., Zou, R.Y., Yuan, Y.J., 2010. An environment-sensitive synthetic microbial ecosystem. PLoS One 5, e10619.

Johnson, R.L., Tabin, C.J., 1997. Molecular models for vertebrate limb development. Cell 90, 979–990.

Kawaguchi, N., Xu, X., Tajima, R., Kronqvist, P., Sundberg, C., Loechel, F., Albrechtsen, R., Wewer, U.M., 2002. ADAM 12 protease induces adipogenesis in transgenic mice. Am. J. Pathol. 160, 1895–1903.

Kemmer, C., Fluri, D.A., Witschi, U., Passeraub, A., Gutzwiller, A., Fussenegger, M., 2011. A designer network coordinating bovine artificial insemination by ovulation-triggered release of implanted sperms. J. Control Release. 150, 23–29.

Kemmer, C., Gitzinger, M., Daoud-El Baba, M., Djonov, V., Stelling, J., Fussenegger, M., 2010. Self-sufficient control of urate homeostasis in mice by a synthetic circuit. Nat. Biotechnol. 28, 355–360.

Kobayashi, H., Kaern, M., Araki, M., Chung, K., Gardner, T.S., Cantor, C.R., Collins, J.J., 2004. Progammable cells: interfacing natural and engineered gene networks. Proc. Nat. Acad. Sci. U.S.A. 101, 8414–8419.

Koseska, A., Zaikin, A., Kurths, J., Garcia-Ojalvo, J., 2009. Timing cellular decision making under noise via cell–cell communication. PLoS One 4, e4872.

Kramer, B.P., Viretta, A.U., Daoud-El-Baba, M., Aubel, D., Weber, W., Fussenegger, M., 2004. An engineered epigenetic transgene switch in mammalian cells. Nat. Biotechnol. 22, 867–870.

Li, B., You, L., 2011. Synthetic biology: division of logic labour. Nature 469, 171–172.

Liu, C., Fu, X., Liu, L., Ren, X., Chau, C.K., Li, S., Xiang, L., Zeng, H., Chen, G., Tang, L.H., Lenz, P., Cui, X., Huang, W., Hwa, T., Huang, J.D., 2011. Sequential establishment of stripe patterns in an expanding cell population. Science 334, 238–241.

Macia, J., Posas, F., Sole, R.V., 2012. Distributed computation: the new wave of synthetic biology devices. Trends Biotechnol. 30, 342–349.

Malleret, G., Haditsch, U., Genoux, D., Jones, M.W., Bliss, T.V., Vanhoose, A.M., Weitlauf, C., Kandel, E.R., Winder, D.G., Mansuy, I.M., 2001. Inducible and reversible enhancement of learning, memory, and long-term potentiation by genetic inhibition of calcineurin. Cell 104, 675–686.

Mansuy, I.M., Bujard, H., 2000. Tetracycline-regulated gene expression in the brain. Curr. Opin. Neurobiol. 10, 593–596.
Mitchell, R.J., Lee, S.K., Kim, T., Ghim, C.M., 2011. Microbial linguistics: perspectives and applications of microbial cell-to-cell communication. BMB Rep 44, 1–10.
Moon, T.S., Lou, C., Tamsir, A., Stanton, B.C., Voigt, C.A., 2012. Genetic programs constructed from layered logic gates in single cells. Nature 491, 249–253.
Ortiz, M.E., Endy, D., 2012. Engineered cell–cell communication via DNA messaging. J. Biol. Eng. 6, 16.
Papetti, M., Herman, I.M., 2002. Mechanisms of normal and tumor-derived angiogenesis. Am. J. Physiol. Cell Physiol 282, C947–C970.
Pesci, E.C., Pearson, J.P., Seed, P.C., Iglewski, B.H., 1997. Regulation of las and rhl quorum sensing in *Pseudomonas aeruginosa*. J. Bacteriol. 179, 3127–3132.
Prindle, A., Samayoa, P., Razinkov, I., Danino, T., Tsimring, L.S., Hasty, J., 2012. A sensing array of radically coupled genetic 'biopixels'. Nature 481, 39–44.
Purnick, P.E., Weiss, R., 2009. The second wave of synthetic biology: from modules to systems. Nat. Rev. Mol. Cell Biol. 10, 410–422.
Regot, S., Macia, J., Conde, N., Furukawa, K., Kjellen, J., Peeters, T., Hohmann, S., de Nadal, E., Posas, F., Sole, R., 2011. Distributed biological computation with multicellular engineered networks. Nature 469, 207–211.
Rinaudo, K., Bleris, L., Maddamsetti, R., Subramanian, S., Weiss, R., Benenson, Y., 2007. A universal RNAi-based logic evaluator that operates in mammalian cells. Nat. Biotechnol. 25, 795–801.
Ruder, W.C., Lu, T., Collins, J.J., 2011. Synthetic biology moving into the clinic. Science 333, 1248–1252.
Sanchez-Bustamante, C.D., Kelm, J.M., Mitta, B., Fussenegger, M., 2006. Heterologous protein production capacity of mammalian cells cultivated as monolayers and microtissues. Biotechnol. Bioeng. 93, 169–180.
Schaefer, A.L., Greenberg, E.P., Oliver, C.M., Oda, Y., Huang, J.J., Bittan-Banin, G., Peres, C.M., Schmidt, S., Juhaszova, K., Sufrin, J.R., Harwood, C.S., 2008. A new class of homoserine lactone quorum-sensing signals. Nature 454, 595–599.
Sharpless, N.E., Depinho, R.A., 2006. The mighty mouse: genetically engineered mouse models in cancer drug development. Nat. Rev. Drug Dis. 5, 741–754.
Shong, J., Jimenez Diaz, M.R., Collins, C.H., 2012. Towards synthetic microbial consortia for bioprocessing. Curr. Opin. Biotechnol..
Shou, W., Ram, S., Vilar, J.M., 2007. Synthetic cooperation in engineered yeast populations. Proc. Nat. Acad. Sci. U.S.A. 104, 1877–1882.
Song, H., Payne, S., Gray, M., You, L., 2009. Spatiotemporal modulation of biodiversity in a synthetic chemical-mediated ecosystem. Nat. Chem. Biol. 5, 929–935.
Tabor, J.J., Salis, H.M., Simpson, Z.B., Chevalier, A.A., Levskaya, A., Marcotte, E.M., Voigt, C.A., Ellington, A.D., 2009. A synthetic genetic edge detection program. Cell 137, 1272–1281.
Tamsir, A., Tabor, J.J., Voigt, C.A., 2011. Robust multicellular computing using genetically encoded NOR gates and chemical 'wires'. Nature 469, 212–215.
Tigges, M., Marquez-Lago, T.T., Stelling, J., Fussenegger, M., 2009. A tunable synthetic mammalian oscillator. Nature 457, 309–312.
Tsai, S.L., Goyal, G., Chen, W., 2010. Surface display of a functional minicellulosome by intracellular complementation using a synthetic yeast consortium and its application to cellulose hydrolysis and ethanol production. Appl. Environ. Microbiol. 76, 7514–7520.
Ulmer, J.B., Valley, U., Rappuoli, R., 2006. Vaccine manufacturing: challenges and solutions. Nat. Biotechnol. 24, 1377–1383.
Viretta, A.U., Fussenegger, M., 2004. Modeling the quorum sensing regulatory network of human-pathogenic *Pseudomonas aeruginosa*. Biotechnol. Progr. 20, 670–678.
Wang, W.D., Chen, Z.T., Kang, B.G., Li, R., 2008. Construction of an artificial intercellular communication network using the nitric oxide signaling elements in mammalian cells. Exp. Cell Res. 314, 699–706.
Waters, C.M., Bassler, B.L., 2005. Quorum sensing: cell-to-cell communication in bacteria. Annu. Rev. Cell Dev. Biol.. 21, 319–346.
Weber, W., Daoud-El Baba, M., Fussenegger, M., 2007a. Synthetic ecosystems based on airborne inter- and intrakingdom communication. Proc. Nat. Acad. Sci. U.S.A. 104, 10435–10440.
Weber, W., Fussenegger, M., 2004. Approaches for trigger-inducible viral transgene regulation in gene-based tissue engineering. Curr. Opin. Biotechnol. 15, 383–391.
Weber, W., Fussenegger, M., 2007. Inducible product gene expression technology tailored to bioprocess engineering. Curr. Opin. Biotechnol. 18, 399–410.
Weber, W., Fussenegger, M., 2011a. Design of synthetic mammalian quorum-sensing systems. Methods Mol. Biol. 692, 235–249.
Weber, W., Fussenegger, M., 2011b. Molecular diversity–the toolbox for synthetic gene switches and networks. Curr. Opin. Chem. Biol. 15, 414–420.
Weber, W., Fussenegger, M., 2012. Emerging biomedical applications of synthetic biology. Nat. Rev. Genet. 13, 21–35.
Weber, W., Schoenmakers, R., Keller, B., Gitzinger, M., Grau, T., Daoud-El Baba, M., Sander, P., Fussenegger, M., 2008. A synthetic mammalian gene circuit reveals antituberculosis compounds. Proc. Nat. Acad. Sci. U.S.A. 105, 9994–9998.
Weber, W., Schuetz, M., Denervaud, N., Fussenegger, M., 2009. A synthetic metabolite-based mammalian inter-cell signaling system. Mol. Biosyst. 5, 757–763.
Weber, W., Stelling, J., Rimann, M., Keller, B., Daoud-El Baba, M., Weber, C.C., Aubel, D., Fussenegger, M., 2007b. A synthetic time-delay circuit in mammalian cells and mice. Proc. Nat. Acad. Sci. U.S.A. 104, 2643–2648.
Yarilina, A., Park-Min, K.H., Antoniv, T., Hu, X., Ivashkiv, L.B., 2008. TNF activates an IRF1-dependent autocrine loop leading to sustained expression of chemokines and STAT1-dependent type I interferon-response genes. Nat. Immunol. 9, 378–387.
Ye, H., Daoud-El Baba, M., Peng, R.W., Fussenegger, M., 2011. A synthetic optogenetic transcription device enhances blood-glucose homeostasis in mice. Science 332, 1565–1568.
You, L., Cox 3rd, R.S., Weiss, R., Arnold, F.H., 2004. Programmed population control by cell–cell communication and regulated killing. Nature 428, 868–871.