Synthetic ecosystems based on airborne inter- and intrakingdom communication

Wilfried Weber*, Marie Daoud-El Baba†, and Martin Fussenegger*‡

*Institute for Chemical and Bioengineering, Eidgenössiche Technische Hochschule, HCI F 115, Wolfgang-Pauli-Strasse 10, CH-8093 Zurich, Switzerland; and †Institut Universitaire de Technologie, Département Génie Biologique, F-69622 Villeurbanne Cedex, France

Edited by Charles R. Cantor, Sequenom, Inc., San Diego, CA, and approved April 25, 2007 (received for review February 14, 2007)

Intercellular communication within an organism, between populations, or across species and kingdoms forms the basis of many ecosystems in which organisms coexist through symbiotic, parasitic, or predator–prey relationships. Using multistep airborne communication and signal transduction, we present synthetic ecosystems within a mammalian cell population, in mice, or across species and kingdoms. Inter- and intrakingdom communication was enabled by using sender cells that produce volatile aldehydes, small vitamin-derived molecules, or antibiotics that diffuse, by gas or liquid phase, to receiver cells and induce the expression of specific target genes. Intercellular and cross-kingdom communication was shown to enable quorum sensing between and among mammalian cells, bacteria, yeast, and plants, resulting in precise spatiotemporal control of IFN-β production. Interconnection of bacterial, yeast, and mammalian cell signaling enabled the construction of multistep signal transduction and processing networks as well as the design of synthetic ecosystems that mimic fundamental coexistence patterns in nature, including symbiosis, parasitism, and oscillating predator–prey interactions.

biological circuit | gene switch | synthetic biology

Intercellular cross-talk either within or between organisms of the same or different species represents a fundamental communication process that is responsible not only for orchestrating vital functions within multicellular life forms but also for determining the manner in which different organisms coexist. Whereas the most sophisticated intercellular communication networks manage processes such as cellular differentiation and pattern formation during development or use hormonal and neural circuitries to adapt responses by individual organisms to endogenous and exogenous stimuli, higher-order ecosystems are organized by basic interaction patterns known as symbiosis, parasitism, or predator–prey interactions. Pioneering advances in the design and study of synthetic multicellular systems have focused entirely on cross-talk within the same species. Quorum-sensing prokaryotic variants, engineered to produce lactones and broadcast this cell-density signal across a population, have been used to establish automated population control (1), programmed pattern formation (2, 3), or metabolic information processing in bacteria (4). Similar intrapopulation communication has also been successfully engineered in yeast using *Arabidopsis thaliana*-derived signaling pathways (5). Although these monospecies networks probe the design principles of their more complex natural counterparts, their extrapolation to mammalian cell communities or interspecies and interkingdom cross-talk remains limited. Furthermore, the use of nonvolatile small-molecule inducers as the broadcast signal in existing synthetic intercellular cross-talk systems necessitates that both sender and receiver cells reside in the same liquid environment. Sender cells transgenic for expression of alcohol dehydrogenase (ADH) produced acetaldehyde, which was diffused by the gas or liquid phase to receiver cells engineered for acetaldehyde-inducible transgene expression (6). Acetaldehyde-based intercellular transcription cross-talk was used to design time-, distance-, and population density-dependent communication across species and kingdoms as well as hormone-like information processing in mice. The combination of acetaldehyde-triggered transfer of transcription protocols with vitamin- and antibiotic-based communication systems enabled multistep information processing networks comprising input, processor, and output cells, as well as the design of synthetic ecosystems emulating naturally occurring symbiotic multispecies interactions known as commensalisms, mutualism, and oscillating predator–prey relationships.

Results and Discussion

Inter- and Intrakingdom Quorum Sensing, Longimetry, and Chronometry. We have designed an airborne communication system [airborne transmission of transcription (AT&T)] by engineering mammalian sender cells (CHO-K1 and CHO-ADH) for constitutive expression of the mouse ADH (7), which enables them to convert ethanol into volatile acetaldehyde and broadcast this airborne signal (boiling point: 21°C) to receiver cells ($_{AIR}$CHO-SEAP), in which it triggers transcription of the human placental secreted alkaline phosphatase (SEAP) driven by the *Aspergillus nidulans*-derived synthetic acetaldehyde-inducible promoter [P_{AIR} (6)] (Fig. 1*A*). Because AT&T broadcasting intensity is proportional to the sender cell concentration, a precise density sensing of the remote mammalian cell population was triggered (Fig. 1*A*). To validate AT&T's interpopulation, cross-species and interkingdom compatibility, CHO-K1 cells transgenic for AT&T reception ($_{AIR}$-CHO-SEAP) were placed proximate to (*i*) human embryonic kidney cells (HEK293-T) engineered for mouse ADH expression (HEK-ADH) and cultivated in medium supplemented with 1‰ ethanol, (*ii*) *Escherichia coli* growing on ethanol-containing (2.5%) LB agar, (*iii*) *Saccharomyces cerevisiae* growing on yeast extract/peptone/dextrose (YPD) agar, and (*iv*) whole *Lepidium sativum* plants (garden cress) maintained in water containing 1% ethanol (Fig. 1*A*). In all of these configurations, the sender cells/organisms produced acetaldehyde in a population density-dependent manner and broadcast the airborne transcription signal across species and kingdom barriers to trigger quorum-sensing transgene expression in the remote mammalian cell population. The communication potential of AT&T is not restricted to cell culture but was also shown to function in mice in a manner similar to that of hormones. Thus, CHO-ADH sender and $_{AIR}$CHO-SEAP receiver cells were independently microencapsulated in alginate-poly-L-lysine-alginate capsules and separately injected i.p. into mice. Metabolic ethanol, converted into acetaldehyde by sender CHO-ADH, triggered SEAP production in receiver cells resulting in increased SEAP

Author contributions: W.W., M.D.-E.B., and M.F. designed research; W.W. and M.D.-E.B. performed research; W.W. and M.F. analyzed data; and W.W. and M.F. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

Abbreviations: SEAP, secreted alkaline phosphatase; AT&T, airborne transmission of transcription; YPD, yeast extract/peptone/dextrose; ES, E-streptavidin; BLA, β-lactamase; sBLA, secreted mammalian BLA; ADH, alcohol dehydrogenase; BTD, biotinidase.

‡To whom correspondence should be addressed. E-mail: fussenegger@chem.ethz.ch.

This article contains supporting information online at www.pnas.org/cgi/content/full/0701382104/DC1.

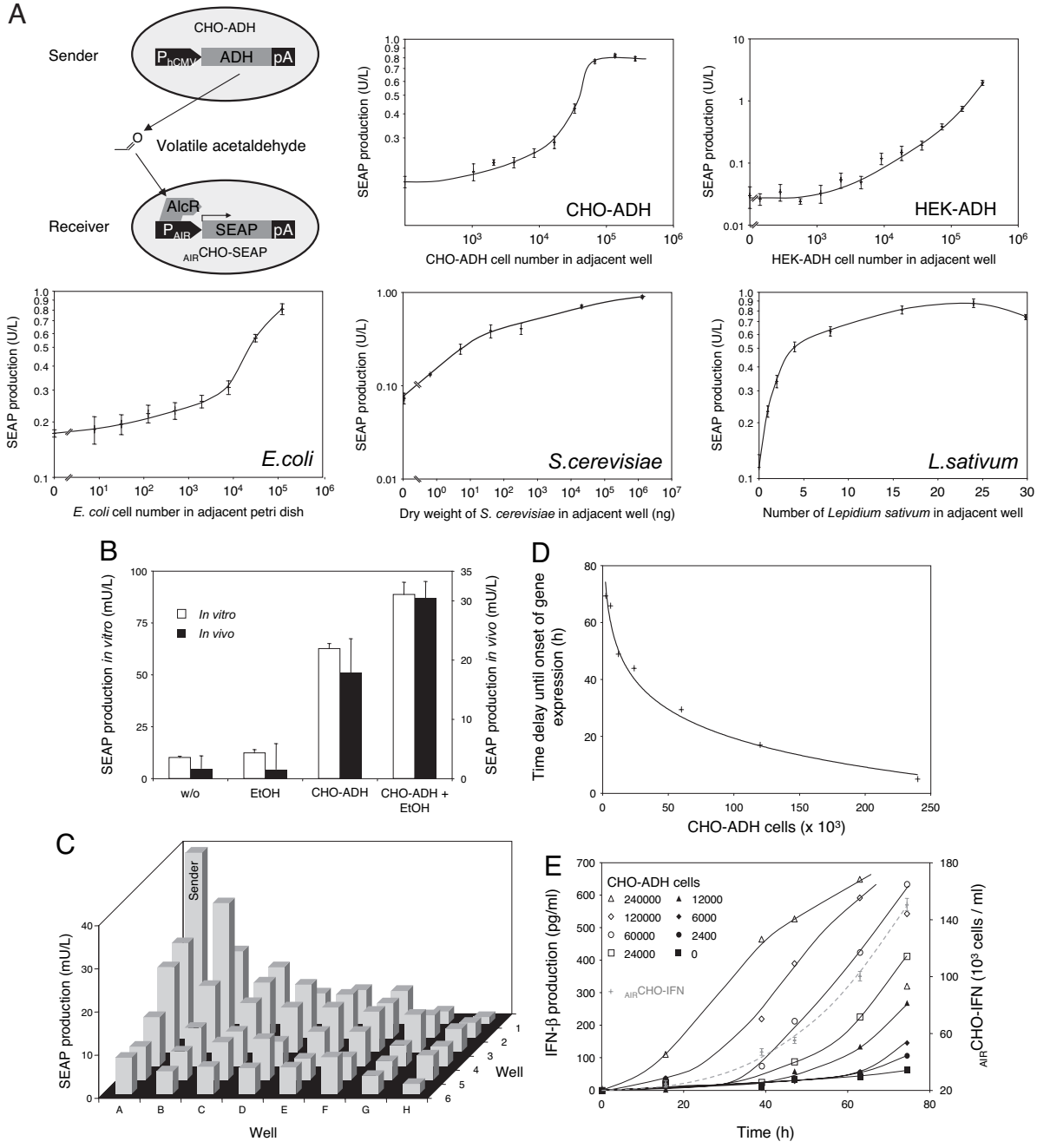

Fig. 1. AT&T. (*A*) Principle of airborne intra- and interkingdom signaling. Sender cells, which naturally express ADH or are transgenic for constitutive ADH expression (e.g., CHO-ADH), metabolize ethanol to volatile acetaldehyde, which diffuses by the gas phase to receiver cells (e.g., $_{AIR}$CHO-SEAP cells), which have been engineered to express a target gene such as the human placental SEAP under the control of the acetaldehyde-inducible regulation system (AIR; AlcR, acetaldehyde-dependent transactivator; P_{AIR}, AlcR-specific acetaldehyde-responsive promoter). Inside receiver cells, acetaldehyde triggers AlcR-dependent P_{AIR}-driven SEAP expression in a dose-dependent manner. $_{AIR}$CHO-SEAP receiver cells (30,000) were cultivated next to increasing numbers of sender cell populations derived from different organisms for 48 h before SEAP quantification: CHO-ADH (Chinese hamster ovary cells transgenic for mouse ADH cultivated in 1‰ ethanol-containing medium), HEK-ADH [human embryonic kidney cells (HEK293-T) transgenic for mouse ADH cultivated in 1‰ ethanol-containing medium], *E. coli* cells (cultivated on an LB-agar plate supplemented with 2.5% ethanol), *S. cerevisiae* (cultivated on a YPD-agar plate), and 5-day-old *L. sativum* (garden cress) plantlets cultivated in 1% ethanol. (*B*) Synthetic cell-to-cell communication in mice. Mice were i.p.-injected with microencapsulated $_{AIR}$CHO-SEAP cells (200 cells per capsule, 2×10^4 capsules per mouse) and, after 1 h, half of the mice were further injected with CHO-ADH (2×10^6 cells per mouse). Mice were kept with or without ethanol in their drinking water (uptake: 1.5 g/kg per 24 h) for 72 h before profiling of SEAP levels in the serum of both groups. Parallel assays were performed *in vitro* where microencapsulated $_{AIR}$CHO-SEAP (2×10^4 capsules; 200 cells per capsule) populations were cultivated in 20 ml of medium in the presence and absence of CHO-ADH (2×10^6 cells) and/or ethanol (1‰, vol/vol). (*C*) AT&T-based longimetry between mammalian sender and receiver cells. CHO-ADH (5,000) were seeded in the top-left well of a 96-well plate containing medium supplemented with 1‰ (vol/vol) ethanol. All wells were seeded with 10,000 $_{AIR}$CHO-SEAP receiver cells, and the 96-well plate was incubated for 48 h before quantification of SEAP production. (*D*) AT&T-based chronometry between mammalian sender and receiver cells. Increasing CHO-ADH populations were cocultivated with 20,000 cells/ml $_{AIR}$CHO-IFN in medium containing 1‰ ethanol (vol/vol), and the onset of IFN-β expression by the receiver cells was determined. (*E*) Timing of biopharmaceutical production by AT&T-based quorum sensing in the production culture. Serum-free suspension cultures of CHO-K1 cells transgenic for AIR-controlled expression of the multiple sclerosis therapeutic IFN-β ($_{AIR}$CHO-IFN) were cocultured with differently sized CHO-ADH populations in 10-ml cultures containing 1‰ ethanol. IFN-β production and $_{AIR}$CHO-IFN cell density were monitored for 72 h. Data are represented as mean ± SD.

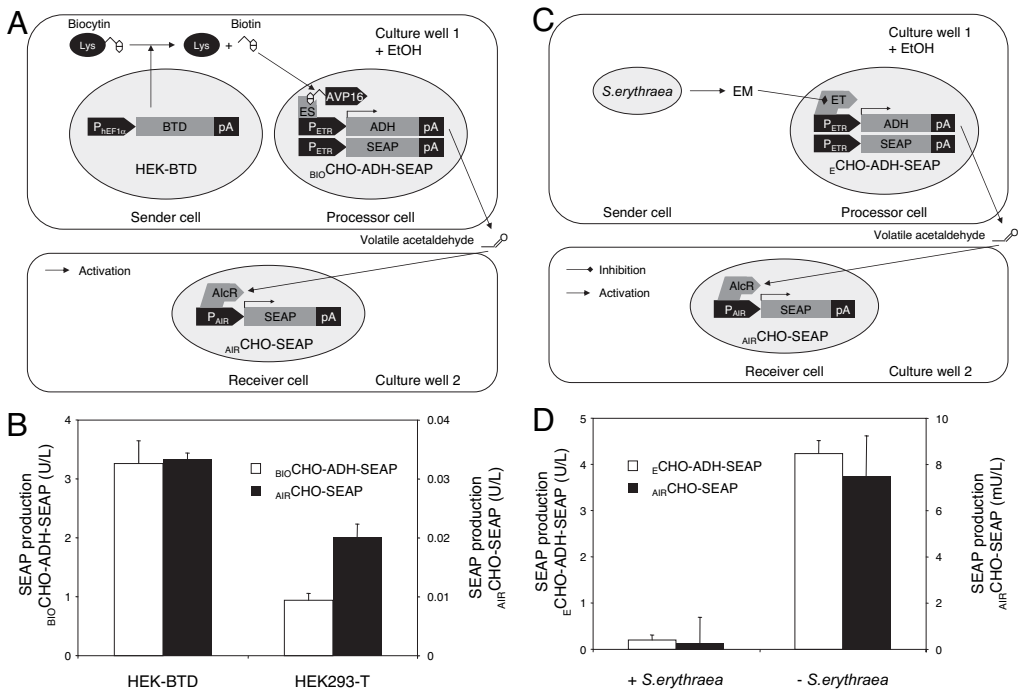

Fig. 2. AT&T-based multistep intra- and interkingdom signaling. (*A*) Three-step mammalian cell-based signaling and information processing. Sender cells (HEK-BTD) engineered for constitutive secretion of BTD hydrolyze biocytin [N(e)-(+)-biotinyl-L-lysine; 100 nM] and resulting biotin diffuses into processor cells ($_{BIO}$CHO-ADH-SEAP), where it heterodimerizes a synthetic transactivator (ES-Biotin-AVP16) triggering P_{ETR}-driven ADH and SEAP expression. ADH expression in $_{BIO}$CHO-ADH-SEAP cells results in the production of volatile acetaldehyde, which is broadcast to neighboring well 2 and induces AIR-controlled SEAP expression in the receiver cells ($_{AIR}$CHO-SEAP). (*B*) SEAP production profiles of the three-step mammalian cell-based signaling and information processing cascade shown in *A*. HEK-BTD, or native non-BTD-producing HEK293-T as control (30,000) sender and 30,000 processor cells ($_{BIO}$CHO-ADH-SEAP) were cocultivated in the presence of 100 nM biocytin and 1‰ ethanol in well 1, whereas 30,000 receiver cells ($_{AIR}$CHO-SEAP) were cultivated in well 2 for 48 h before profiling SEAP production in both wells. (*C*) Three-step bacteria-mammalian cell-based signaling and information processing. *S. erythraea* naturally produces erythromycin, which triggers erythromycin-responsive ADH and SEAP expression in the processor cells ($_{E}$CHO-ADH-SEAP) cultivated in well 1. ADH expression in the processor cells ($_{E}$CHO-ADH-SEAP) results in the production of volatile acetaldehyde, which is broadcast to the neighboring well 2 and induces AIR-controlled SEAP expression in the receiver cells ($_{AIR}$CHO-SEAP). (*D*) SEAP production profiles of the three-step bacteria-mammalian cell-based signaling and information processing cascade shown in *C*. An agar plug of an *S. erythraea* culture (1 mm in diameter) and 30,000 processor cells ($_{E}$CHO-ADH-SEAP) were cocultivated in the presence of 1‰ ethanol in well 1, whereas 30,000 receiver cells ($_{AIR}$CHO-SEAP) were cultivated in well 2 for 48 h before profiling SEAP production in both wells. Data are represented as mean ± SD.

levels circulating in the mouse bloodstream. SEAP production was significantly increased when mice were given ethanol in their drinking water (1.5 g/kg for 24 h), confirming that an exogenous signal (ethanol) triggered quorum-sensing cross-talk (acetaldehyde) between different cell populations within the mouse body ultimately resulting in fine-tuning of heterologous protein levels (SEAP) in the bloodstream of these animals (Fig. 1*B*). Synthetic hormone-like information-processing devices might be used in the future as clinical implants that capture and process pathologic signals or infection and coordinate therapeutic responses or pathogen defense throughout the body (8).

Because a well defined sender cell population produces a specific concentric gradient of gaseous acetaldehyde, which triggers dose-dependent transgene expression of receiver cells, the distance between sender and receiver cells is expected to proportionally impact transgene expression levels. Such molecular longimetry was tested by seeding receiver cells into each well of a multiwell plate with the top left well also inoculated with AT&T sender cells (Fig. 1*C*). SEAP expression profiling after 48 h showed a precise inverse correlation between sender–receiver cell distance and SEAP production (Fig. 1*H*) that is reminiscent of naturally occurring intercell longimetry, which is involved in polarity and pattern formation during development (9). In this case, however, intercellular communication occurred by air and did not involve liquid-phase contact between the communicating cell populations.

It was predicted that the speed of signal transmission between sender and receiver cells kept at a constant distance should depend upon the size of the sender population. By plotting the onset of transgene expression against sender population size in mixed sender/receiver cell populations, a graded response was observed with longer transmission times correlating with smaller sender populations (Fig. 1*D*). Linking this chronometric circuitry to production of the multiple sclerosis therapeutic, IFN-β ($_{AIR}$CHO-IFN), created a prototype bioprocess in which protein production kinetics could be precisely and autonomously forecast and controlled by a quorum-sensing cross-talk involving the production cell lines (Fig. 1*E*). Cell density-controlled gene expression is of immediate interest for the manufacturing of difficult-to-produce protein therapeutics (10) and could be foreseen to control the size or differentiation programs of synthetic organs in future tissue engineering scenarios (18).

Synthetic Signal Transduction and Processing Networks. Endocrine systems are essential for orchestration of complex physiological events in entire organisms (11). Their generic design consists of small sensor cell populations which process exogenous or physiologic stimuli and convert them into a systemic release of hormones which trigger defined transcription responses in specific target cells (11). Similar signal processing cascades have recently been implemented in *E. coli* to control sustained biofilm formation in response to the population density and to DNA damage (4). We have designed an endocrine signaling replica consisting of (i) a signal-generating sender cell (HEK-BTD, HEK293-T transgenic for constitutive production of human biotinidase (BTD) (12), which releases the signal molecule biotin (vitamin H) from biocytin [(N(ε)-(+)-biotinyl-L-lysine)], (*ii*) a central biotin-processing cell [$_{BIO}$CHO-ADH-SEAP; CHO-K1 engineered for constitutive BirA-mediated ligation of biotin to the Avitag-VP16 fusion protein (AVP16) resulting in heterodimerization with coexpressed E-streptavidin (ES) components to a chimeric ES-biotin-avitag-VP16 transactivator which induces P_{ETR}-driven ADH and SEAP expression and triggers transmission of the AT&T broadcasting signal acethaldehyde (13)], and (*iii*) a receiver cell line ($_{AIR}$CHO-SEAP; CHO-K1 harboring a P_{AIR}-driven SEAP expression unit converting the acetaldehyde gradient into a SEAP production level) (Fig. 2 *A* and *B*). Bacteria can also communicate their presence by antibiotic-mediated signaling, as demonstrated by creation of a three-step

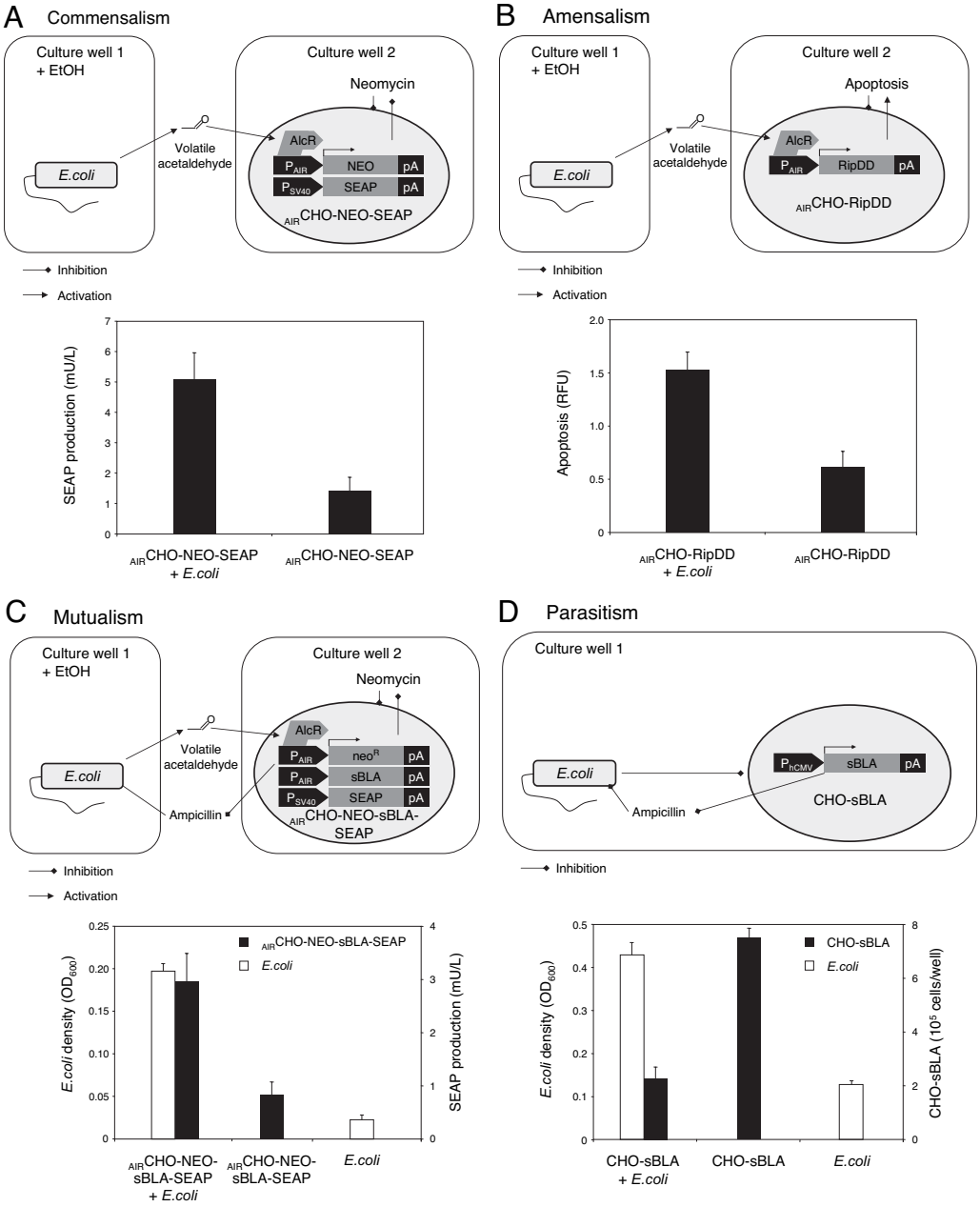

Fig. 3. (*Figure continues on the opposite page.*)

synthetic input–processing–output cascade consisting of (*i*) the erythromycin-producing *Streptomyces Saccharopolyspora erythraea*, (*ii*) a central erythromycin processing cell [$_{E}$CHO-ADH-SEAP; CHO-K1 engineered for macrolide-responsive P_{ETR}-driven ADH and SEAP expression (13)], and (*iii*) a receiver cell line ($_{AIR}$CHO-SEAP; CHO-K1 harboring a P_{AIR}-driven SEAP expression unit converting the acetaldehyde gradient into a SEAP production output) (Fig. 2 *C* and *D*). These examples illustrate how molecular modules with standardized cellular communication interfaces can rapidly be assembled to information-processing circuits in a "plug-and-play" manner reminiscent of the fabrication of electronic devices (4, 14).

Synthetic Ecosystems. Bidirectional interspecies communication systems are essential for designing synthetic ecosystems that emulate fundamental patterns of symbiotic coexistence such as commensalism (one partner profits, whereas the other is unaffected), amensalism (association is disadvantageous for one partner, whereas the other is unaffected), mutualism (both partner profit from association), parasitism (association beneficial to one partner, disadvantageous for the other), or predator–prey interactions (resulting in antagonistically oscillating populations of both partners) (15, 16). We have used AT&T's communication potential to construct fundamental synthetic ecosystem motifs: A synthetic commensalistic *E. coli*-CHO ecosystem was designed by engineering CHO-K1 for constitutive SEAP expression (used as survival marker) and for acetaldehyde-induced regulation-controlled transcription of the neomycin phosphotransferase ($_{AIR}$CHO-NEO-SEAP) (Fig. 3*A*). acetaldehyde-broadcasting *E. coli* cultivated proximate to $_{AIR}$CHO-NEO-SEAP enabled commensalistic survival of $_{AIR}$CHO-NEO-SEAP, whereas *E. coli* was unaffected by the growth of the mammalian cells in a separate culture dish (Fig.

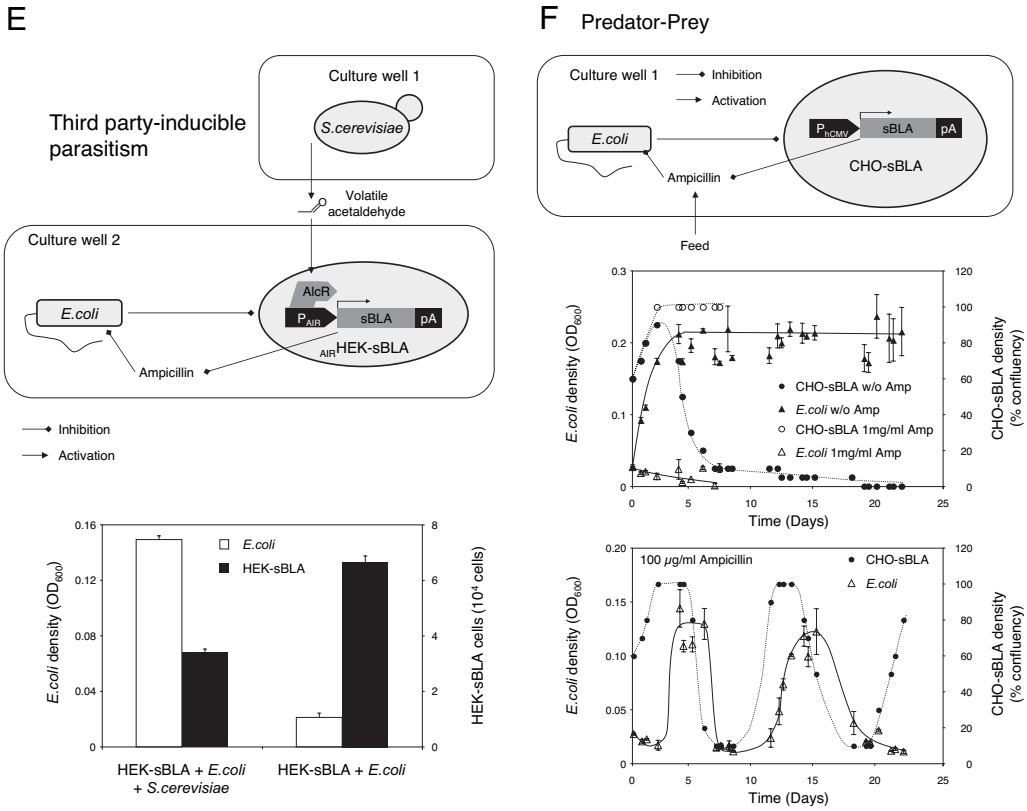

Fig. 3. Synthetic ecosystems. (*A*) Commensalism. Mammalian cells commensal to bacteria. When cultivating $_{AIR}$CHO-NEO-SEAP transgenic for AIR-controlled expression of the neomycin-resistance determinant (NEO) and constitutive SEAP expression in close proximity to *E. coli* metabolizing ethanol to volatile acetaldehyde, the mammalian cells survive and proliferate in neomycin-containing culture medium. $_{AIR}$CHO-NEO-SEAP (30,000) were cultivated for 48 h in the presence or absence of a Petri dish (10 cm in diameter) containing 20 ml of LB agar supplemented with 2.5% ethanol and inoculated with 10^5 *E. coli* DH5a (distance between the center of both culture dishes: 12 cm). Subsequently, the $_{AIR}$CHO-NEO-SEAP medium was supplemented with neomycin (1.6 mg/ml) for another 48 h before scoring SEAP production as an indicator of viability. (*B*) Amensalism. Mammalian cells amensal to bacteria. When cultivating $_{AIR}$CHO-RipDD transgenic for AIR-controlled expression of the death domain of the human receptor-interacting protein (RipDD) proximate to *E. coli* metabolizing ethanol to volatile acetaldehyde, the mammalian cells die by apoptosis. $_{AIR}$CHO-RipDD (30,000) were cultivated for 48 h in the presence or absence of a Petri dish (10 cm in diameter) containing 20 ml of LB agar supplemented with 2.5% ethanol and inoculated with 10^5 *E. coli* DH5a (distance between the center of both culture dishes: 12 cm) before scoring apoptosis. (*C*) Mutualism between mammalian cells and bacteria. When cultivating $_{AIR}$CHO-NEO-sBLA-SEAP transgenic for AIR-controlled neomycin resistance (NEO), sBLA, and constitutive SEAP expression proximate to *E. coli* metabolizing ethanol to volatile acetaldehyde, the mammalian cells survive and proliferate in neomycin-containing culture medium and secrete sBLA, which is transferred to the *E. coli* culture where it sustains bacterial growth in ampicillin-containing medium. $_{AIR}$CHO-NEO-sBLA-SEAP (30,000) were cultivated in medium supplemented with 5 μg/ml ampicillin for 48 h in the presence or absence of a Petri dish (10 cm in diameter) containing 20 ml of HTS medium supplemented with 2.5% ethanol and inoculated with *E. coli* DH5a [neo^R (0.05 OD_{600})] (distance between the center of both culture dishes: 12 cm). The cell culture medium was semicontinuously transferred to the *E. coli* population, and $_{AIR}$CHO-NEO-sBLA-SEAP were cultured in medium supplemented with 1.6 mg/ml neomycin for another 48 h with subsequent scoring of *E. coli* density and SEAP production as indicator of CHO viability. (*D*) Parasitism. Bacteria as parasites of mammalian cells. When cocultivating CHO-sBLA engineered for constitutive sBLA expression, ampicillin in the culture medium will be degraded, and bacteria will expand, thereby exhausting nutrients and impairing growth and survival of mammalian cells. In the absence of CHO-sBLA, *E. coli* fail to grow. CHO-sBLA (30,000) were cocultivated with *E. coli* DH5a (0.05 OD_{600}) for 48 h in cell culture medium containing 10 μg/ml ampicillin before assessment of *E. coli* and CHO-sBLA cell numbers. (*E*) Third party-inducible parasitism. Fungi inducing bacteria-triggered parasitism of mammalian cells. When cultivating *S. cerevisiae*, which naturally metabolizes glucose to volatile acetaldehyde and ethanol, in close proximity to cocultures consisting of wild-type *E. coli* and $_{AIR}$HEK-sBLA transgenic for AIR-controlled expression of sBLA, acetaldehyde-triggered sBLA production decreases ampicillin levels and promotes *E. coli* proliferation, which in turn exhausts nutrients and impairs growth and survival of mammalian cells. $_{AIR}$HEK-sBLA (30,000) were cocultivated with *E. coli* DH5a (0.05 OD_{600}) in medium containing 10 μg/ml ampicillin and incubated for 48 h in the presence or absence of a Petri dish (10 cm in diameter) containing 20 ml of YPD agar inoculated with 100 mg (dry weight) of *S. cerevisiae* (distance between the center of both culture dishes: 12 cm) before scoring of *E. coli* and $_{AIR}$HEK-sBLA cell numbers. (*F*) Interkingdom predator–prey-like ecosystem. Bacteria preying on mammalian cells. Cocultivation of wild-type *E. coli* with CHO-sBLA engineered for constitutive expression of sBLA in the presence of a continuous medium supply shows three distinct time courses; whereas the absence of ampicillin triggers rapid growth of *E. coli* and extinction of CHO-sBLA, high ampicillin concentrations (1 mg/ml) extinguish *E. coli* and enable rapid growth of CHO-sBLA. However, an intermediate ampicillin concentration (100 μg/ml) results in a predator–prey-like ecosystem in which *E. coli* and CHO-sBLA population size antagonistically oscillate. As the CHO-sBLA population increases and sBLA levels rise, ampicillin concentrations decrease and enable *E. coli* to grow more rapidly. With *E. coli* populations increasing, nutrients are rapidly depleted, which limits the growth of CHO-sBLA and decreases sBLA production. Continuous feeding of fresh ampicillin-containing medium results in elevated ampicillin concentrations that limit *E. coli* growth and promote expansion of the CHO-sBLA population, which initiates a new cycle. CHO-sBLA (150,000) were cocultivated with *E. coli* DH5a (starting density, 0.065 OD_{600}) in medium containing the indicated ampicillin concentrations with semicontinuous medium exchange (dilution rate, 0.25 day^{-1}). The *E. coli* population was scored by its optical density at 600 nm (OD_{600}), and the CHO-sBLA cell population was monitored by quantifying confluence. Data are represented as mean ± SD.

3*A*). The adverse amensalistic ecosystem, where mammalian cells survive only in the absence of acetaldehyde-broadcasting *E. coli*, was created by cultivating *E. coli* in close proximity to CHO-K1 engineered for AIR-controlled expression of the apoptosis-inducing human receptor interacting protein [RipDD (10)] ($_{AIR}$-CHO-RipDD) (Fig. 3*B*). Although apoptosis (programmed cell death) levels were low in the absence of *E. coli*, they significantly increased when acetaldehyde-producing *E. coli* were maintained in

the same culture atmosphere as $_{AIR}$CHO-RipDD (Fig. 3*B*). To enable feedback communication from mammalian cells to prokaryotic senders, we designed a secreted mammalian β-lactamase [sBLA; replacing the bacterial secretion signal by a mouse Ig-κ chain secretion signal (17)], which hydrolizes ampicillin in the culture medium and promotes survival of co-cultured *E. coli*. Combining AT&T-based acetaldehyde broadcasting with feedback BLA signaling, we designed ecosystem motifs displaying cross-talk among multiple species: Mutualism is a special form of symbiosis, in which both members of a different species benefit. Mutualism was designed by engineering CHO-K1 for constitutive SEAP expression (used as survival marker) and for AIR-controlled transcription of NEO and sBLA ($_{AIR}$CHO-NEO-sBLA-SEAP) and growing them in close proximity to an *E. coli* culture while establishing a unidirectional medium flux from $_{AIR}$CHO-NEO-sBLA-SEAP to *E. coli* (Fig. 3*C*). In this system, *E. coli* produces volatile acetaldehyde, which diffuses by gas phase to trigger sBLA and NEO expression in $_{AIR}$CHO-NEO-sBLA-SEAP, thereby enabling survival of these cells in the presence of neomycin. At the same time, sBLA hydrolyzes ampicillin and mediates rapid growth of *E. coli* after semicontinuous medium transfer (Fig. 3*C*). The absence of either *E. coli* or $_{AIR}$CHO-NEO-sBLA-SEAP prevented the growth of the other mutualistic partner (Fig. 3*C*). A parasitic bacteria-mammalian cell-based ecosystem was devised by cocultivating *E. coli* with CHO-K1 engineered for constitutive sBLA expression (CHO-sBLA). CHO-sBLA triggers sBLA-mediated ampicillin degradation in the culture medium thereby enabling rapid growth of parasitic *E. coli* in the mammalian cell culture, which ultimately exhausts nutrients resulting in impaired mammalian cell growth (Fig. 3*D*). However, although *E. coli* growth was compromised by ampicillin in the absence of CHO-sBLA, the mammalian cells grew well in the absence of parasitic bacteria (Fig. 3*D*). Based on this fundamental parasitic behavior, we developed a three-species synthetic ecosystem showing conditional parasitism, where the sensitivity of the target to the parasitic organism depends on the coexistence of a third species. Human embryonic kidney cells (HEK293-T), engineered for AIR-controlled sBLA production ($_{AIR}$HEK-sBLA) were cocultivated with *E. coli* in close proximity to *S. cerevisiae* (Fig. 3*E*). In the presence of acetaldehyde-broadcasting *S. cerevisiae* in the same atmosphere, $_{AIR}$HEK-sBLA produced sBLA, triggered ampicillin degradation in the medium, and enabled the growth of cocultured *E. coli*, ultimately killing the mammalian cells (Fig. 3*E*). However, in the absence of *S. cerevisiae*, $_{AIR}$HEK-sBLA production was repressed, which prevented *E. coli* growth and enabled survival of the mammalian cells (Fig. 3*E*).

When exposing the synthetic parasitic ecosystem to semicontinuous exchange with fresh ampicillin-containing media (dilution rate of 0.25·day^{-1}), *E. coli* exhibited the following population kinetics (Fig. 3*F*): (*i*) In the absence of ampicillin, *E. coli* density increased until it reached a steady-state and mammalian cells became extinct. (*ii*) At high ampicillin concentrations (1 mg/ml), the *E. coli* population declined rapidly until extinction, and CHO cells continued to grow. (*iii*) However, at intermediate ampicillin concentrations (100 μg/ml), the *E. coli* population first declined because of ampicillin-mediated killing but as CHO-sBLA continued to grow and secrete BLA, ampicillin levels decreased, and bacterial growth resumed. As the bacterial population increased, it inhibited expansion of the mammalian cell population, resulting in reduced BLA production, subsequent ampicillin accumulation in the ecosystem, and a decline in the *E. coli* population (Fig. 3*F*). The oscillating cross-talk controlling mutual population size in this synthetic interkingdom ecosystem is reminiscent of typical population time courses occurring in wildlife parasite–host or predator–prey interactions (18, 19).

The newly constructed AT&T interspecies communication system, with its biotin, antibiotic, and BLA extensions, is here shown to act as a universal data-transfer protocol for information perception, processing, and transduction both *in vitro* and *in vivo*. AT&T was successfully used for the design of autonomous production control in biopharmaceutical manufacturing processes and to establish a synthetic hormone-like communication center in mice (9, 20). The assembly of synthetic ecosystems could provide novel insight in the fundamental patterns orchestrating the complex coexistence of living systems.

Materials and Methods

Plasmids. A detailed description of plasmid construction is shown in supporting information (SI) *Text*. pWW993 (P_{ETR}-ADH-pA), erythromycin-regulated ADH (7, 13); pWW926 ($P_{hEF1\alpha}$-BTD-pA), constitutive human biotinidase (BTD); pWW811 (P_{SV40}-ES-pA), erythromycin repressor E fused to streptavidin (ES) (13, 14); pWW1015 (P_{AIR}-sBLA-pA), acetaldehyde-inducible sBLA (17, 21); pWW1019 (P_{hCMV}-sBLA-pA), constitutive sBLA (22); pWW1016 (P_{AIR}-NEO-pA), acetaldehyde-inducible neomycin resistance; and pWW1018 (P_{AIR}-RipDD-pA), acetaldehyde-inducible RipDD (14, 23).

Cultivation Conditions, Stable Cell Lines, Analytics, and Animal Experiments. CHO-K1 (American Type Culture Collection CCL-61), HEK293-T (24), *E. coli* DH5α, *S. erythraea*, *S. cerevisiae*, and *L. sativum* were cultivated as described in *SI Text* by using standard media. CHO-ADH and CHO-sBLA stably encode pIV-L2 (7) and pWW1019, respectively. SEAP and biotinidase were quantified as described (12, 17). For animal experiments, encapsulated $_{AIR}$CHO-SEAP were implanted into mice (13), and ethanol in the drinking water was applied (1.5 g/kg per 24 h). For details, see *SI Text*.

We thank Dahn L. Clemens for providing the ADH expression vector pIV-L2, Martine Gilet for skilled assistance with animal experimentation, and David Greber for critical comments on the manuscript. This work was supported by the Swiss National Science Foundation (Grant 3100A0-112549), the Swiss State Secretariat for Education and Research within EC Framework 6, and Cistronics Cell Technology GmbH.

1. You L, Cox RS, III, Weiss R, Arnold FH (2004) *Nature* 428:868–871.
2. Basu S, Gerchman Y, Collins CH, Arnold FH, Weiss R (2005) *Nature* 434:1130–1134.
3. Basu S, Mehreja R, Thiberge S, Chen MT, Weiss R (2004) *Proc Natl Acad Sci USA* 101:6355–6360.
4. Kobayashi H, Kaern M, Araki M, Chung K, Gardner TS, Cantor CR, Collins JJ (2004) *Proc Natl Acad Sci USA* 101:8414–8419.
5. Chen MT, Weiss R (2005) *Nat Biotechnol* 23:1551–1555.
6. Weber W, Rimann M, Spielmann M, Keller B, Daoud-El Baba M, Aubel D, Weber CC, Fussenegger M (2004) *Nat Biotechnol* 22:1440–1444.
7. Clemens DL, Forman A, Jerrells TR, Sorrell MF, Tuma DJ (2002) *Hepatology* 35:1196–1204.
8. Ganz T (1999) *Science* 286:420–421.
9. Friml J, Vieten A, Sauer M, Weijers D, Schwarz H, Hamann T, Offringa R, Jurgens G (2003) *Nature* 426:147–153.
10. Boorsma M, Nieba L, Koller D, Bachmann MF, Bailey JE, Renner WA (2000) *Nat Biotechnol* 18:429–432.
11. Potapov A, Liebich I, Donitz J, Schwarzer K, Sasse N, Schoeps T, Crass T, Wingender E (2006) *Nucleic Acids Res* 34:D540–D545.
12. Knight HC, Reynolds TR, Meyers GA, Pomponio RJ, Buck GA, Wolf B (1998) *Mamm Genome* 9:327–330.
13. Weber W, Fux C, Daoud-el Baba M, Keller B, Weber CC, Kramer BP, Heinzen C, Aubel D, Bailey JE, Fussenegger M (2002) *Nat Biotechnol* 20:901–907.
14. Weber W, Stelling J, Rimann M, Keller B, Daoud-El Baba M, Weber CC, Aubel D, Fussenegger M (2007) *Proc Natl Acad Sci USA* 104:2643–2648.
15. Montoya JM, Pimm SL, Sole RV (2006) *Nature* 442:259–264.
16. Bascompte J, Jordano P, Olesen JM (2006) *Science* 312:431–433.
17. Schlatter S, Rimann M, Kelm J, Fussenegger M (2002) *Gene* 282:19–31.
18. Bjornstad ON, Peltonen M, Liebhold AM, Baltensweiler W (2002) *Science* 298:1020–1023.
19. Yoshida T, Jones LE, Ellner SP, Fussmann GF, Hairston NG, Jr (2003) *Nature* 424:303–306.
20. Roth S, Stein D, Nusslein-Volhard C (1989) *Cell* 59:1189–1202.
21. Moore JT, Davis ST, Dev IK (1997) *Anal Biochem* 247:203–209.
22. Fussenegger M, Morris RP, Fux C, Rimann M, von Stockar B, Thompson CJ, Bailey JE (2000) *Nat Biotechnol* 18:1203–1208.
23. Hartenbach S, Fussenegger M (2005) *J Biotechnol* 120:83–98.
24. Mitta B, Rimann M, Ehrengruber MU, Ehrbar M, Djonov V, Kelm J, Fussenegger M (2002) *Nucleic Acids Res* 30:e113.