Synthetic ecosystems based on airborne inter- and intrakingdom communication

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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.

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 [PAIR (6)] (Fig. 1A). 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. 1A). 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/pineptone/dextrose (YPD) agar, and (iv) whole Lepidium sativum plants (garden cress) maintained in water containing 1% ethanol (Fig. 1A). 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 kingdoms 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.

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

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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., AIRCHO-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; PAIR, AlcR-specific acetaldehyde-responsive promoter). Inside receiver cells, acetaldehyde triggers AlcR-dependent PAIR-driven SEAP expression in a dose-dependent manner. AIRCHO-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], 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 AIRCHO-SEAP receiver cells, and the 96-well plate was incubated for 48 h before quantification of SEAP production. (B) Synthetic cell-to-cell communication in mice. Mice were i.p.-injected with microencapsulated AIRCHO-SEAP cells (200 cells per capsule, 2 x 10⁶ capsules per mouse) and, after 1 h, half of the mice were further injected with CHO-ADH (2 x 10⁶ 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 AIRCHO-SEAP (2 x 10⁶ capsules; 200 cells per capsule) populations were cultivated in 20 ml of medium in the presence and absence of CHO-ADH (2 x 10⁶ cells) and/or ethanol (1‰, vol/vol). (C) AT&T-based chronometry 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 AIRCHO-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 AIRCHO-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 AIRCHO-IFN were cocultured with differently sized CHO-ADH populations in 10-ml cultures containing 1‰ ethanol. IFN-β production and AIRCHO-IFN cell density were monitored for 72 h. Data are represented as mean ± SD.

Fig. 1.  

Cell number in adjacent well (ng)
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 heterogeneous protein levels (SEAP) in the bloodstream of these animals (Fig. 1A). 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 two wells also inoculated with AT&T sender cells (Fig. 1C). SEAP expression profiling after 48 h showed a precise inverse correlation between sender–receiver cell distance and SEAP production (Fig. 1H) 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. 1D). 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. 1E). 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(e)-(+)-biotinyl-L-lysine]), (ii) a central biotin-processing cell (BC 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_EFR-driven ADH and SEAP expression and triggers transmission of the AT&T broadcasting signal acetaldehyde (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. 2A and B). Bacteria can also communicate their presence by antibiotic-mediated signaling, as demonstrated by creation of a three-step
synthetic input–processing–output cascade consisting of (i) the erythromycin-producing Streptomyces Saccharopolyspora erythraea, (ii) a central erythromycin processing cell \[E_{\text{CHO-ADH-SEAP}}\] CHO-K1 engineered for macrolide-responsive \(P_{\text{PETR}}\)-driven ADH and SEAP expression (13), and (iii) a receiver cell line \(\text{AIRCHO-SEAP}\) CHO-K1 harboring a \(P_{\text{AIR}}\)-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 partners benefit 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 \(\text{AIRCHO-NEO-SEAP}\) (Fig. 3 A). Acetaldehyde-broadcasting \(E.\ coli\) cultivated proximate to \(\text{AIRCHO-NEO-SEAP}\) enabled commensalistic survival of \(\text{AIRCHO-NEO-SEAP}\), whereas \(E.\ coli\) was unaffected by the growth of the mammalian cells in a separate culture dish (Fig.
was created by cultivating *S. cerevisiae* parasitism. Fungi inducing bacteria-triggered parasitism of mammalian cells. When cultivating acetaldehyde and ethanol, in close proximity to cocultures consisting of wild-type *E. coli* expression proximate to and secrete sBLA, which is transferred to 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. *A*. 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 10⁵ *E. coli* DH5α (distance between the center of both culture dishes: 12 cm) before scoring apoptosis. (C) Mutualism between mammalian cells and bacteria. When cultivating *A*. 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. *A*. 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 HT5 medium supplemented with 2.5% ethanol and inoculated with *E. coli* DH5α (neos6 (0.05 OD₆₀₀)) (distance between the center of both culture dishes: 12 cm). The cell culture medium was semicontinuously transferred to the *E. coli* population, and *A*. 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) Parabiosis. 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* DH5α (0.05 OD₆₀₀) 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 *A*. 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. *A*. HEK-sBLA (30,000) were co-cultivated with *E. coli* DH5α (0.05 OD₆₀₀) 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 *A*. 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) extingish *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* DH5α (starting density: 0.065 OD₆₀₀) 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 (OD₆₀₀), and the CHO-sBLA cell population was monitored by quantifying confluence. Data are represented as mean ± SD.
the same culture atmosphere as \text{AIR}\text{CHO-RipDD} (Fig. 3B). To enable feedback communication from mammalian cells to prokaryotic senders, we designed a secreted mammalian \beta-lactamase [sBLA; replacing the bacterial secretion signal by a mouse Ig-\kappa chain secretion signal (17)], which hydrolyzes ampicillin in the culture medium and promotes survival of co-cultured \text{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 \text{CHO-K1} for constitutive SEAP expression (used as survival marker) and for \text{AIR}-controlled translocation of NEO and sBLA (\text{AIR}\text{CHO-NEO-sBLA-SEAP}) and growing them in close proximity to an \text{E. coli} culture while establishing a unidirectional medium flux from \text{AIR}\text{CHO-NEO-sBLA-SEAP} to \text{E. coli} (Fig. 3C). In this system, \text{E. coli} produces volatile acetaldehyde, which diffuses by gas phase to trigger sBLA and NEO expression in \text{AIR}\text{CHO-NEO-sBLA-SEAP}, thereby enabling survival of these cells in the presence of neomycin. At the same time, \text{BLA} hydrolyzes ampicillin and mediates rapid growth of \text{E. coli} after semicontinuous medium transfer (Fig. 3C). The absence of either \text{E. coli} or \text{AIR}\text{CHO-NEO-sBLA-SEAP} prevented the growth of the other mutualistic partner (Fig. 3C). A parasitic bacteria-mammalian cell-based ecosystem was devised by cocultivating \text{E. coli} with \text{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 \text{E. coli} in the mammalian cell culture, which ultimately exhausts nutrients resulting in impaired mammalian cell growth (Fig. 3D). However, although \text{E. coli} growth was compromised by ampicillin in the absence of CHO-sBLA, the mammalian cells grew well in the absence of bacterial \text{E. coli} (Fig. 3D). 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 \text{AIR}-controlled sBLA production (\text{AIR\text{HEK-sBLA}}) were cocultivated with \text{E. coli} in close proximity to \text{S. cerevisiae} (Fig. 3E). In the presence of acetaldehyde-broadcasting \text{S. cerevisiae} in the same atmosphere, \text{AIR\text{HEK-sBLA}} produced sBLA, triggered ampicillin degradation in the medium, and enabled the growth of cocultured \text{E. coli}, ultimately killing the mammalian cells (Fig. 3E). However, in the absence of \text{S. cerevisiae}, \text{AIR\text{HEK-sBLA}}-production was repressed, which prevented \text{E. coli} growth and enabled survival of the mammalian cells (Fig. 3E).

When exposing the synthetic parasitic ecosystem to semicontinuous exchange with fresh ampicillin-containing media (dilution rate of 0.25/day)\textsuperscript{-1}, \text{E. coli} exhibited the following population kinetics (Fig. 3F): (i) In the absence of ampicillin, \text{E. coli} density increased until it reached a steady-state and mammalian cells became extinct. (ii) At high ampicillin concentrations (1 mg/ml), the \text{E. coli} population declined rapidly until extinction, and CHO cells continued to grow. (iii) However, at intermediate ampicillin concentrations (100 \mu g/ml), the \text{E. coli} population first declined because of ampicillin-mediated killing but as \text{CHO-sBLA} continued to grow and secreted \text{BLA}, ampicillin levels decreased, and bacterial growth resumed. As the bacterial population increased, it inhibited expansion of the mammalian cell population, resulting in reduced \text{BLA} production, subsequent ampicillin accumulation in the ecosystem, and a decline in the \text{E. coli} population (Fig. 3F). 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 processing, 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\text{TRT-ADH}-pA), ethrythromycin-regulated ADH (7, 13); pWW926 (P\text{EFG1-\text{BTD-pA}}), constitutive human biotinidase (BTD); pWW811 (P\text{SV40-ES-pA}), erythromycin repressor E fused to streptavidin (ES) (13, 14); pWW1015 (P\text{AIR-sBLA-pA}), acetaldehyde-inducible sBLA (17, 21); pWW1019 (P\text{CHMV-sBLA-pA}), constitutive sBLA (22); pWW1016 (P\text{AIR-NEO-pA}), acetaldehyde-inducible neomycin resistance; and pWW1018 (P\text{AIR-RipDD-pA}), acetaldehyde-inducible RipDD (14, 23).

Cultivation Conditions, Stable Cell Lines, Analytics, and Animal Experiments. \text{CHO-K1} (American Type Culture Collection CCL-61), \text{HEK293-T} (24), \text{E. coli DH5}\text{\alpha}, \text{S. erythraea}, \text{S. cerevisiae}, and \text{L. sativum} were cultivated as described in SI Text by using standard media. \text{CHO-ADH} and \text{CHO-sBLA} stably encode pIV-L2 (7) and pWW1019, respectively. SEAP and biotinidase were quantified as described (12, 17). For animal experiments, encapsulated \text{AIRCHO-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.

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