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# Synthetic Biology of Multicellular Systems: New Platforms and Applications for Animal Cells and Organisms

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Like life itself, synthetic biology began with unicellular organisms. Early synthetic biologists built genetic circuits in model prokaryotes and yeast because of their relative biological simplicity and ease of genetic manipulation. With superior genetic tools, faster generation times, and better-understood endogenous gene expression machinery, prokaryotes and yeast were (and remain) appealing hosts for the engineering of synthetic systems. Now in its second decade, synthetic biology in unicellular organisms has produced myriad synthetic genetic circuits, a number of industrial applications, and fundamental new biological insights unlikely to have emerged from nonsynthetic approaches.

Yet, the biological complexity that made metazoans less attractive for early synthetic biology is precisely what makes these organisms exciting targets for a new generation. Multicellular phenomena such as development (with its linkage of growth, morphogenesis, and differentiation) seem dauntingly complex and have thus far evaded comprehensive understanding through biological approaches that rely on perturbations to natural systems. Synthetic approaches based on bottom-up reconstitution of these phenomena promise intimate insights, complementary to those obtained through traditional methods. At the same time, therapeutic applications of synthetic biology will have to function within, and ideally exploit, the many complex aspects of multicellular systems. For all these reasons, synthetic biology of animal cells has become an exciting, albeit challenging, frontier.

Today, engineering animal cells remains primitive, based on slow, imprecise, and generally clunky tools. How will we reach the point where we can routinely design, build, and analyze synthetic genetic circuits that operate within the inherently multicellular contexts of developing embryos and mature tissues? There is some hope: major new advances in DNA synthesis and assembly enable construction of increasingly elaborate DNA constructs. New tools for genome targeting, particularly site-specific recombinases and programmable systems such as CRISPRs and TALEs, greatly improve our control of genome editing.

Yet major technical challenges remain. The predictability and tunability of synthetic genetic elements remain poor. We often lack general strategies for isolating synthetic systems from


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the host cell and from each other. Locus-specific effects and clone-to-clone variation often limit systematic studies that require comparisons among different genotypes. Beyond these problems of implementation, our molecular toolbox remains limited. We do not have many regulatable transcription factors that work well in mammalian cells. We have few orthogonal signaling components that can link arbitrary intracellular pathways and enable cells to coordinate with one another in a controlled fashion; we also lack general and robust methods for integration of synthetic systems with the natural machinery of their hosts. At the end of the day, the time it takes to go from *in silico* design to stable cell lines containing full circuits, let alone whole animals, remains painfully long. In short, we need to better understand the components we do have, grow the number of components in our toolbox, tackle site-specific effects and reduce integration site variability, and speed the entire process up by an order of magnitude. Such advances will help us to tackle what is perhaps the biggest problem of all— our lack of understanding of fundamental circuit design principles. Those principles will emerge only through construction and rigorous analysis of new synthetic circuits, enabled by new technologies and an expanding synthetic biology community.

In this issue, we bring together four papers that bring the dream of synthetic multicellular biology closer. First, in order to increase the repertoire of regulatory components available, Stanton *et al*. identify homologues of the popular TetR repressor that target orthogonal binding sites; as with TetR, variants of these homologues can be deployed as repressors or activators *in vivo*. The activity of one such homologue, PhlF, can be controlled by the small molecule DAPG, analogously to the control of TetR by doxycycline, thus introducing a much-needed new inducible system for mammalian synthetic biology.

While new transcription factors are of great utility, coupling their activity to synthetic or natural environmental cues remains a major challenge for the field. Daringer *et al*. address this issue by developing a Modular Extracellular Sensor Architecture (MESA) that couples programmable detection of extracellular ligands to the induction of reporter gene expression through controlled protease recruitment, similarly to the Tango method previously demonstrated for detecting ligands of G-protein coupled receptors, receptor tyrosine kinases, and steroid hormone receptors.[1] MESA entails construction of two synthetic transmembrane fusion proteins with conditionally dimerizing extracellular sensor domains and an intracellular domain that is either a protease or a transcription factor with a linker that contains a protease recognition site; dimerization of the sensor domains brings the protease in proximity to the transcription factor domain, which it then cleaves off of the fusion protein, releasing it for transit to the nucleus. Alternatively, the two transmembrane proteins can carry separate N- and C-terminal domains of the protease, with dimerization reconstituting protease activity that releases a transcription factor tethered to a third transmembrane protein. The MESA system opens up new possibilities for creating synthetic intercellular signaling circuits in mammalian cells.

A third paper addresses some of the key challenges of genomic integration of synthetic circuits. We need the ability to stably integrate constructs into precisely defined sites, subject to minimal interference from the host genome. Human artificial chromosomes (HACs) represent promising vectors for constructing complex genetic circuits because they

are stably inherited (similarly to normal chromosomes), can accommodate large genetic cargo, minimize undesired interference with transgenes and endogenous genes, and, once constructed, can in principle be transferred among cell lines. However, the utility of HACs in creating transgenic mice is limited by variable retention rates in mice tissue. Here, Takiguchi *et al*. report the development of a mouse artificial chromosome (MAC) employing a native mouse centromere and show that this MAC is stably maintained in a variety of tissues in transgenic mice. The authors also develop a derivative of the MAC that contains five acceptor sites (landing pads) that can be targeted by corresponding recombinases for site-specific introduction of cargo. These MACs promise increased versatility and control, both in whole animals and in cell culture.

While these three papers represent advances at the cellular level, the fourth paper tackles synthetic biology at a totally different scale—entire populations of organisms—by expanding an elegant synthetic biology approach for controlling insect-borne disease. The Hay group previously developed a synthetic self-ish genetic element called *Medea* that can be used to drive population replacement in *Drosophila* by killing non-Medea-bearing progeny of *Medea*-bearing mothers.[2] Consequently, a minority of *Medea* flies is sufficient to rapidly spread the element through a larger wild-type population, enabling them to potentially spread a disease-inhibiting genetic cargo. The original *Medea* worked through microRNA-mediated silencing of a maternally expressed transcript, *myd88*, that was required for zygotic development, coupled with zygotic expression of a rescue transgene. Akbari *et al*. now report two novel *Medea* systems based on the same principle but targeting different pathways. An expanded *Medea* toolbox enables new strategies for mutational stability (*i.e*., dealing with inactivating mutations in any one *Medea* element) and for the controlled elimination of a given element from the population in case the whole enterprise goes awry. The authors also use mathematical modeling to demonstrate how specially designed *Medea* constructs could induce population suppression in response to an environmental cue, such as diapause-inducing drought, following initial replacement of the population with *Medea*-bearing animals. This work will provide a foundation for future efforts aimed at real-world disease vector control. It is also an extraordinary example of how a single synthetic biology project can cut across multiple levels of biological complexity, from a gene circuit to a developmental process to population dynamics.

Together, these papers exemplify a few of the remarkably diverse approaches currently being pursued to realize the many promises of multicellular synthetic biology. Looking ahead, there are a mind-boggling number of systems, approaches, and applications to explore. As we develop the field, we need to pursue new techniques and applications with equal parts imagination and rigor so that multicellular synthetic biology realizes it potential as both science and engineering—where the systematic construction of synthetic systems drives new biological understanding, which in turn advances our synthetic potential.

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