SYNTHETIC BIOLOGY

One cell, many fates

A synthetic gene circuit enables programming of many stable states in mammalian cells

By **Colin Kunze**[1,2] and **Ahmad S. Khalil**[1,2,3]

The ability of genetically identical cells to take on diverse and specialized roles, which are maintained over long time scales, underlies critical biological processes, including multicellular development. More than 60 years ago, Waddington invoked the concept of multistability, a property of dynamical systems, to rationalize how a cell progresses from an undifferentiated state to various distinct cell fates during development (*1*). It has since been revealed that even within a single tissue, there is extraordinary diversity of cell states; yet how they are generated and maintained remains unclear. On page 284 of this issue, Zhu *et al.* (*2*) describe MultiFate, a genetic circuit design that unlocks controllable and scalable multistability in mammalian cells. They generate seven stable cell states and transition cells between states or completely destabilize a once-stable state with exquisite control. This will enable the engineering of a range of multicellular behaviors in mammalian cells.

Bistability, the simplest form of multistability, has been studied extensively in natural contexts and synthetic systems. Bistability has been shown to underlie a host of biological processes, including cell fate decisions in frog oocyte maturation (*3*). The study of natural bistable systems has revealed important features such as the requirement for positive feedback. However, unraveling the main ingredients of multistable systems is challenging because of the complexity of regulatory networks and deep interconnectedness with auxiliary pathways. This difficulty can be overcome by using a synthetic biology approach, in which a minimal set of non-native genetic components are introduced into cells to recapitulate complex biological functions (*4*). In a pioneering study, a synthetic bistable "toggle switch" was constructed in bacteria that allowed cells to flip between two stable states (*5*). This study and others established a blueprint for how to investigate biological functions, such as bistability, from the bottom up and inspired potential applications of these synthetic circuits (*6*). Recently, the synthetic circuit toolbox has grown to include tristable and quadrastable systems, promising increased functionality of engineered cells (*7*, *8*). However, a clear procedure to expand multistability—and perhaps even exceeding the phenotypic capacity of natural systems—has remained elusive.

The design framework for MultiFate is inspired by natural transcription networks that regulate stem cell differentiation and development. These networks commonly feature positive feedback loops that involve master regulatory transcription factors (TFs) and promiscuous binding among the TFs, which have been implicated in generating multistability (*9*). Promiscuously dimerizing TFs are peculiar because they can have opposing cellular functions; for example, the pluripotency TF octamer-binding protein 4 (OCT4) can drive either pluripotency or endodermal differentiation depending on its dimerizing partner (*10*). MultiFate exploits this peculiarity, using autoactivating synthetic TFs with promiscuous interactivity to ultimately generate multistability (see the figure).

Zhu *et al.* first built MultiFate2, a circuit composed of two TFs. When activated by inducing dimerization, cells dispersed into one of three stable states, akin to inducing differentiation in a pluripotent cell. This tristable landscape is predicted by a mathematical model to transform into a bistable one with changes in TF stability (i.e., degradation rate). In reshaping the landscape, reduced TF stability generates irreversible transitions. Subsequent restoration of TF stability does not cause cells to spontaneously return to the previously destabilized state. This progression evokes comparisons to cellular differentiation and suggests how to maintain engineered multipotency in cells.

A major roadblock to implementing multistability thus far has been the increasing degree of design complexity required as more states are added. In MultiFate, increasing the number of states is straightforward: Expansion of MultiFate2 (tristability) to MultiFate3 (septastability) is achieved by adding another TF with the same design principles. With MultiFate3, Zhu *et al.* demonstrated several discrete changes in the potential landscape, reshaping septastability to hexastability to tristability with progressively diminishing levels of TF stability. Higher levels of synthetic multistability open the door to exploring these stepwise irreversible transitions. Through theoretical analysis of their system, Zhu *et al.* find that the number of stable steady states available increases rapidly with the number of TFs used, potentially attaining 256 distinct stable states with only nine TFs. With MultiFate, expandability is not hampered by complexity of circuit design but is instead restrained by the physical lim-

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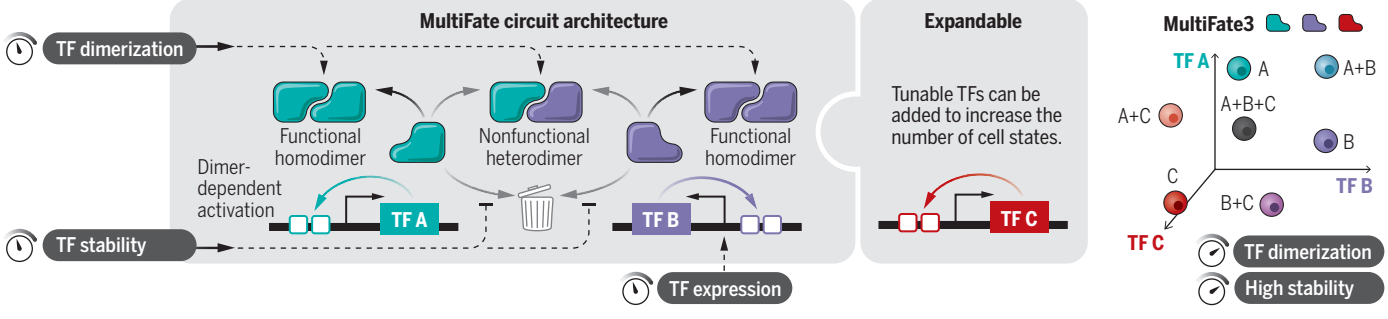
Synthetic multistability in mammalian cells

The MultiFate circuit uses synthetic transcription factors (TFs), each with a distinct zinc finger DNA binding domain but identical dimerization domains. This allows TFs to homodimerize (active) or heterodimerize (inactive). Small molecules control dimerization, independent activation of TFs, and TF stability, allowing controlled generation of multistable states. In MultiFate3, a three-TF circuit, up to seven stable cell states can be generated under high TF stability and dimerizing conditions.



GRAPHIC: KELLIE HOLLOWAY/SCIENCE

its of introducing additional highly expressed TFs. Exploration of these limits may offer clues about how organisms can generate morphological and phenotypic complexity with a relatively small number of conserved regulatory components (*11*).

The clever use of modular TF components with tunable molecular interactions (*12*, *13*) allows us to ask questions about the design and control of multistable landscapes. For example, Zhu *et al.* observed an asymmetrical population distribution of cell states upon initial activation of MultiFate due to differences in TF binding and transcriptional efficiencies. This could be advantageous for an organism whose development requires different abundances of cell types. MultiFate provides a foundational tool for exploring what system parameters control those ratios and how narrow or broad a regime can be to produce the desired distribution of fates. Furthermore, MultiFate adds to a growing set of new tools to investigate the roles various cells play in developmental biology. These bottom-up approaches to cell fate circuits complement top-down, single-cell sequencing techniques that provide high-resolution maps of developmental trajectories. Comparison of synthetic and natural trajectories could clarify the stability and functional relevance of the many cell states that have been identified across tissues and organisms.

MultiFate also provides a platform for exploring how transcriptional differentiation circuits interface with other controllers of cell state, such as cell signaling. MultiFate coupled with emerging synthetic cell-cell signaling systems, such as the SynNotch receptor (*14*), could produce more sophisticated developmental trajectories that provide insight into natural development (*15*). Finally, MultiFate may enable the engineering of a general cell therapy tool that encodes many potential therapeutic states and can be guided to individually tailored fates. ■

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ACKNOWLEDGMENTS

A.S.K. is a co-founder of K2 Biotechnologies and a scientific advisor for Senti Biosciences and Chroma Medicine.

10.1126/science.abn6548

MATERIALS SCIENCE

Many-particle electron states in graphene

Scanning tunneling microscopy probes ground state competition in a magnetic field

By **Markus Morgenstern**[1] *and* **Mark Goerbig**[2]

Two-dimensional electron systems in a magnetic field provide a paradigm for unraveling the complexity of electron-electron interactions. In particular, because of the large number of possible electron states at the same energy level, such a two-dimensional system provides a rich playground for studying the different ways electrons can be arranged. Consequently, there are a plethora of many-particle ground states with similar energies to be explored. However, these ground states are difficult to distinguish from each other without direct observation of the electron arrangements (*1*). On page 321 of this issue, Liu *et al.* (*2*) report that they have deciphered the secret of one of these ground states by imaging the electron distribution with atomic resolution using scanning tunneling microscopy.

Ever since the 2004 landmark paper by Novoselov and Geim that described how to probe the quantum properties of graphene (*3*), the material has been widely studied for the fundamental insights it offers into electronic systems (*4*). Graphene, defined as a single layer of carbon atoms arranged in a two-dimensional honeycomb lattice structure, is ideal for studying the intriguing ways that electrons interact with each other in a quantum-mechanical and relativistic manner.

Since early research into the material, one point of interest has been the arrangement of electrons in graphene under an external magnetic field (*5*). For neutral graphene under a magnetic field, the number of possible electron states with the same energy level is exactly twice the number of electrons. Thus, the electrons in this partially filled level arrange among themselves by each choosing between the possible states. The possible states are primarily distinguished by one label for each of two properties—for the spin (up or down) and for the so-called valley (K or K') that determines the possible positions of the electrons. For the K valley, the electrons of neutral graphene are located on carbon atoms with two carbon neighbors to the right and one neighbor to the left, whereas for the K' valley, the electrons occupy carbon atoms with one neighbor to the right and two neighbors to the left.

The exact combination of labels for any particular electron is determined by the exchange energy, which is a quantum-mechanical effect that occurs between identical particles—in this case, the electrons. The exchange energy plays a part in the repulsive electron-electron interaction by favoring a collective state where all electrons share the same labels to minimize their repulsion.

However, for graphene with an overall neutral charge in a magnetic field, it is impossible for all electrons to occupy states with the same label combination. Here, one-half of the states have to be occupied with electrons, whereas only one-quarter of the electron states have spin up and K as labels, one-quarter have spin up and K', one-quarter have spin down and K, and one-quarter have spin down and K'. Hence, the electrons must partially choose states with different labels. For example, if all electrons have the same valley label, half of their spins will be up and half of them down. In reality, the exact combinations of labels for the electrons are more complex and are very difficult to predict (*5*). One reason is that none of the labels is preselected by the mutual electron repulsion. One must also consider the quantum-mechanical superposition of label choices, meaning that each electron can simultaneously have the up and down spin label and also the K alongside the K' valley label. Moreover, as is usual for such superposition states, the different label choices are related to something known as the quantum-mechanical phase factor. These phase factors are necessary to describe the electrons as waves and as particles at the same time. In the superposition state, the wave of the K state and the wave of the K' state are overlapped. The phase factor of the superposition describes how the peaks of the two waves are positioned with respect to each other.

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Science, 375 (6578), • DOI: 10.1126/science.abn6548