efficiently inducing human glial progenitor cells and myelin-producing oligodendrocytes from pluripotent stem cells have proven more elusive (although our laboratory has recently achieved efficiencies that may be suitable for clinical consideration[8]). No surprise, then, that direct reprogramming of somatic cells to glial cells has taken much longer to accomplish compared with their neuronal neighbors.

Both Tesar and colleagues[2] and Wernig and colleagues[3] use murine rather than human fibroblasts as their starting material, taking advantage of the more rapid development of murine oligodendroglia to more quickly derive protocols for their production. Tesar's group employs a combination of eight transcription factor genes, from which a core set of three genes—*Sox10*, *Olig2* and *Nkx6.2*—is subsequently defined, to drive the production of induced oligodendrocyte progenitor cells. The study provides strong histological and ultrastructural evidence of efficient myelination by these cells in an elegant *in vitro* slice model of the congenitally hypomyelinated shiverer mouse. Remarkably, the induced progenitor cells not only are able to divide and expand for at least five passages but also appear to do so as oligodendrocyte-restricted progenitors. The authors report little evidence of astrocyte production, even though mammalian oligodendrocyte progenitor cells typically generate both astrocytes and oligodendrocytes.

Capitalizing upon an earlier assessment of differential gene expression by oligodendrocytes[9], Wernig and colleagues[3] screen a different set of factors than that tested by Tesar and colleagues[2]. They identify *Sox10*, *Olig2* and *Zfp536* as sufficient to direct oligodendrocyte progenitor cell phenotype. Yet when fibroblasts are transduced with this combination, which includes *Zfp536* in place of *Nkx6.2* used in the Tesar protocol, the cells develop as bipotential glial progenitors able to give rise to both astrocytes and oligodendrocytes. This bipotential phenotype is more similar to that seen in normal mammalian development, and seems directly analogous to the bipotential oligodendrocyte progenitor cells derived by my group from both human tissue and pluripotent cells[8,10]. However, whether the apparently distinct phenotypes observed in the two studies[2,3] merely represent differences in how the cells are assessed, or whether they indeed reflect a fundamental difference in the cells' respective lineage potentials and differentiated states, remains unclear.

If Tesar and colleagues[2] have indeed discovered a reprogramming protocol that avoids the bipotential glial progenitor stage and directly generates mitotic cells capable only of oligodendrocytic differentiation, then might these cells be developmentally downstream of the cells produced by Wernig and colleagues[3]? Allthough most studies have found oligodendrocyte progenitor cells to be bipotential until their terminal division, more phenotypically restricted populations of glial progenitors in rodents have been reported, suggesting that the Wernig and Tesar protocols might be generating different, hierarchically defined stages of a common lineage.

The distinction in lineage competence between the glial progenitors derived in the two studies[2,3] may have implications as this approach is translated to human cells, given the differences in oligodendrocyte biology between rodents and primates. Whereas rodents seem to harbor populations of lineage-restricted oligodendroglial progenitors, we and others have noted that human oligodendroglia are every bit as post-mitotic as neurons, and their progenitors are multilineage competent[11]. Therefore, might the protocol of Tesar and colleagues[2] yield direct oligodendroglial differentiation in human cells, bypassing the bipotential stage at which astrocytes are also generated? If so, would the resulting cells lose mitotic competence with oligodendrocytic fate restriction? This might be beneficial for directing terminal differentiation but would defeat the purpose of generating stable lines of mitotic glial progenitor cells able to be expanded for clinical transplantation. In fact, this issue cuts to the core of what constitutes direct reprogramming: will the derived glial cells be able to divide as phenotypically stable clones of mitotic, myelinogenic glial progenitor cells years after their introduced reprogramming genes have ceased transcription? As such questions are assessed, it is also important to recognize that the two protocols may not translate to human cells without modifications to the sets of transcription factors, given the differences in oligodendrocyte progenitor cell biology and fate restriction between rodents and humans.

Time and further modeling in human cells will allow these issues to be addressed and resolved, but the two groups have already made a giant leap forward in our ability to apply cellular reprogramming for direct oligodendrocytic induction and replacement. Thus, they have accomplished not just an especially challenging bit of cellular alchemy but have done so in the service of a patient population that may be especially likely to benefit from cell-based repair strategies, and especially grateful for the efforts of these pioneering investigators.

COMPETING FINANCIAL INTERESTS
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Endowing cells with logic and memory

Andre C Maranhao & Andrew D Ellington

Genetic circuits that process and permanently store information are created with recombinases that flip the orientation of DNA cassettes.

Every new technological development is accompanied by hype. In the early days of research on DNA computation and nanotechnology, proponents asserted that these technologies would become so powerful as to surpass the capabilities of silicon-based computers[1]. It didn't happen. Similarly, although synthetic biology is not really a new technology, being merely the logical extension of decades of biotechnological innovation, it has been claimed that standardized parts ganged together into circuits would lead to devices of immense complexity. It hasn't yet happened[2]. These two cases are more alike than they might seem in that both involve challenges to electronics by biotechnology. Ultimately, in the DNA computation field it was realized that computations where the desired output was the DNA itself, rather than the solution to a complex algorithm, were much more useful

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than the mimicry of electronics. Similarly, in synthetic biology, the work of Siuti *et al.*[3] in this issue now suggests that biological logic gates and memory can be best used to realize practical cellular programming goals rather than impossible electronic dreams[4].

Siuti *et al.*[3] have advanced the field by constructing genetic circuits that logically convert transient chemical inputs into permanent changes in DNA architecture. From a simplified view, each genetic circuit is composed of three subsystems: two recombinase expression cassettes acting on a logic-gate cassette. The logic-gate cassettes are assembled from various functional parts, such as reporter genes (e.g., *gfp*), promoters and terminators. When these DNA parts are flanked with sequence motifs recognized by the recombinases Bxb1 or phiC31, it becomes possible to carry out logic functions after induction of Bxb1 by the small molecule *N*-Acyl homoserine lactone (AHL) and/or phiC31 by anhydrotetracycline (aTc). The orientation of functional parts and the placement of recombinase recognition sites determine the logical function that is executed.

As an example, consider an AND gate (**Fig. 1**). A promoter and *gfp* gene exist in an inverted, inactive state flanked by phiC31 and Bxb1 recognition sites, respectively. A fluorescence output signal is activated in the presence of both input signals, AHL and aTc, which flip the promoter and GFP DNA cassettes to configurations that support gene expression. Overall, a chemical signal—a sufficient concentration of both transient inducers—is processed into a permanent change in DNA architecture, resulting in stable expression or repression of a GFP reporter. The genetic circuit also stores its computational output as a DNA configuration, in essence creating a heritable memory that is decoupled from the viability of the cell.

This innovation closely resembles natural inverters that determine cell state, such as the *Salmonella* phase variation system, in which reversible inversion of a promoter element alternates expression between two different flagellin proteins[5]. Through this mechanism, *Salmonella* can toggle between two antigenically distinct flagellin proteins and thereby evade the host immune response. However, in contrast to the two-state *Salmonella* system, which executes a single logic function, Siuti *et al.*[3] have engineered circuits representing all 16, two-input Boolean logic functions by having the orthogonal recombinases act on functional elements (promoters, terminators and reporters) from a separate logic cassette.

A similar genetic circuit was published almost simultaneously[6]. In this system, Bonnet *et al.*[6] assemble a circuit from recombinase

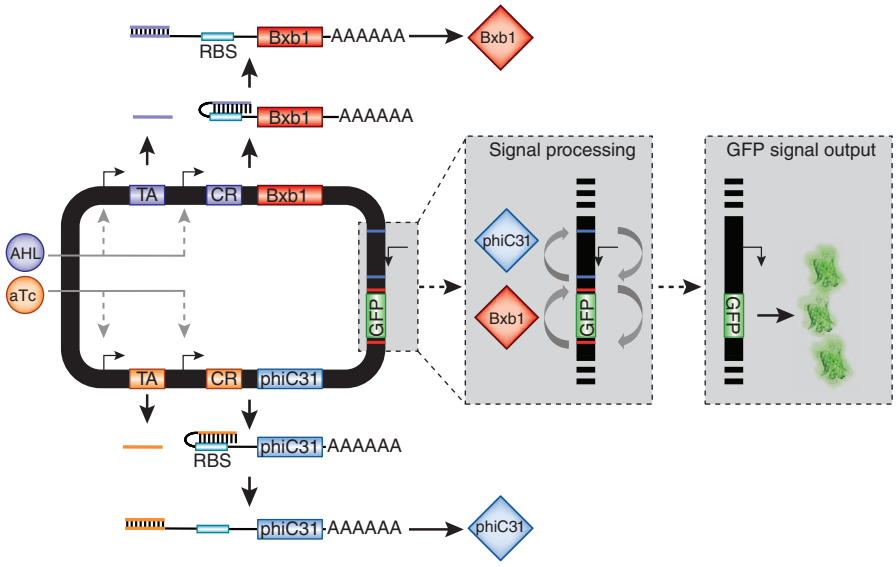

Figure 1 An integrated logic and memory device. Induction by the small molecules AHL and/or anhydrotetracycline (aTc) serves as logical input to the circuit. Two separate riboregulators induced by AHL and aTc provide tightly controlled expression of the recombinases, Bxb1 and phiC31, respectively. Each riboregulator consists of a *cis*-repressive (CR) mRNA sequence (which forms a hairpin structure with the ribosome binding site (RBS) blocking translation) and a small noncoding *trans*-activating (TA) RNA (which pairs with its complement *cis*-repressive sequence, thus permitting ribosome docking and translation). After induction and expression of Bxb1 and phiC31, each recombinase acts upon its cognate recognition sites by inverting the flanked DNA element. The circuit shown implements an AND gate: induction of AHL and aTc leads to inversion of both an inverted promoter sequence and an inverted *gfp* reporter gene. As a result, the circuit expresses GFP, the output signal.

and logic-gate cassettes but opt for the Bxb1 and TP901-1 recombinases under the control of arabinose and aTc, respectively. One major difference is that these authors use a two-plasmid system to segregate the invertible logic gate ('logic construct') from the two recombinase expression cassettes ('controller construct'). More importantly, the logic construct primarily consists of terminators sitting within nested, rather than serial, recombinase sites. This architecture has the advantage of layering logical control of transcription (does a transcript terminate, or not?) on top of the completely separate control of transcription initiation (does a transcript start, or not?). However, this streamlined design does not seem to have the same capacity for varied functionality as the work of Siuti *et al.*[3], in which both terminators and promoters are components of the logic gates. Notably, both attributes (the use of multiple functional elements and a layered control of read-through) were present in an earlier publication[7], in which inverters were used to create a cell-based counter.

The systems under discussion are, of course, similar to other innovations in synthetic biology. The brilliant Brainbow system used less-directed changes in inducible recombination to achieve individualized combinations of fluorescent proteins in neural cells[8]. Another previous study demonstrated more-directed changes in cell state by using dueling repressor interactions, rather than recombinases, as toggles for long-term shifts in gene expression[9]. The advantage of such toggles is that the sequence specificities of transcription factors are likely more programmable than those of recombinases.

What Siuti *et al.*[3] and Bonnet *et al.*[6] now accomplish is the addition of Boolean logical elements to the aforementioned advances in inversion and toggles. In particular, both studies continue efforts within the field of synthetic biology to instantiate digital programming in cells by showing thresholding effects of the logical gates, and how such thresholding could provide a means of reliably changing cell state. Whereas previous efforts in synthetic biology have led to proteins, antisense oligonucleotides, ribozymes, deoxyribozymes, riboswitches, ribosome:mRNA interactions, and other parts and interactions serving as Boolean logical gates[4], the current advances show that these various devices can actually change the state of the cell, rewriting the genome.

Interestingly, other examples of natural state changes seem to rely on more modular 'scripts' that are encoded more densely than are inversion-based logic gates. For example, the CRISPR-Cas system takes sequences from invading bacteriophage and converts them into small modules that act as a 'memory' of how

the bacteria has been challenged[10]. Given the recent development of Cas9 variants that can be programmed to act as repressors[11], networks of Cas9-based genetic toggles could generate more extensive programming of cell states than could recombinase-based technologies. At an even finer-grained level of encoding, epigenetic modifications to both DNA and nucleosomes in chromatin serve as reliable, heritable memory that guides gene expression[12]. Thus, the recombinase systems in Siuti *et al.*[3] and Bonnet *et al.*[6] can be seen as a wonderful recapitulation of nature itself (*Salmonella*), as a more rationally programmable version of the Brainbow system and counting technologies and, ultimately, as a strategy for moving toward the engineering of even denser and more extensive state changes in cells.

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Antibiotic and ROS linkage questioned

Ferric C Fang

New work fuels the controversy over the role of reactive oxygen species (ROS) in the activity of bactericidal antibiotics.

Emerging multidrug-resistant pathogens threaten to end the long era of the 'antibiotic miracle'. Clinicians fret over the meager number of novel antibiotics in development. In this context, a new paradigm for understanding the actions of bactericidal antibiotics, first proposed in 2007 (ref. 1), has generated a great deal of interest in the research community. Since Kohanski *et al.*[1] described the role of reactive oxygen species (ROS) in the actions of antibiotics, a number of papers have attempted to test, extend and apply the new model. Now two studies published in *Science*[2,3] are calling the linkage between antibiotics and ROS into serious question.

The ROS paradigm proposes that, as well as exerting their classic antimicrobial actions, bactericidal antibiotics kill bacteria by activating a final common pathway involving respiration, the tricarboxylic acid (TCA) cycle, NADH depletion, iron-catalyzed Fenton chemistry and hydroxyl radical production (**Fig. 1**). Considerable evidence has been presented to support this model. For example, antibiotics induce oxidation of hydroxyphenyl fluorescein (HPF)[1], a fluorescent sensor thought to be oxidized selectively by hydroxyl radicals and peroxynitrite. In addition, iron chelators (which inhibit Fenton reaction–mediated production of hydroxyl radicals by sequestering unbound iron) and thiourea (a ROS scavenger and antioxidant) each suppress antibiotic-mediated bacterial killing[1]. ROS formed in response to antibiotic treatment can inflict oxidative damage on DNA, resulting in lethal double-strand breaks[4] and mutations leading to the development of multidrug resistance[5]. Lastly, mutations inactivating bacterial antioxidant defenses can augment susceptibility to antibiotics[6], and ROS levels may determine whether subpopulations of bacterial cells persist despite antibiotic treatment[7].

At the same time, other work has raised questions about the new paradigm. For example, antibiotics kill bacteria under anaerobic conditions in which ROS are not formed[8]. In other studies, TCA cycle mutations have been shown to neither markedly affect bacterial susceptibility to fluoroquinolone antibiotics nor promote the emergence of multidrug resistance[9]. Moreover, a systematic screen of the Keio collection of *Escherichia coli* strains for targets that enhance antibiotic susceptibility has yielded few targets related to ROS scavenging or formation[10].

In a new report in *Science*, Liu and Imlay show that ampicillin, norfloxacin and kanamycin can kill bacteria in the absence of oxygen[2] (kanamycin's actions are influenced by oxygen, but this is attributable to the known effects of respiration and proton-motive force on aminoglycoside uptake). In addition, *E. coli* lacking endogenous catalase and peroxidase scavenging activity is only modestly more susceptible to norfloxacin and no more susceptible to ampicillin or kanamycin than wild-type bacteria[2]. Moreover, these antibiotics do not increase bacterial respiration or hydrogen peroxide formation[2]. The protective actions of iron chelators and thiourea, though confirmed

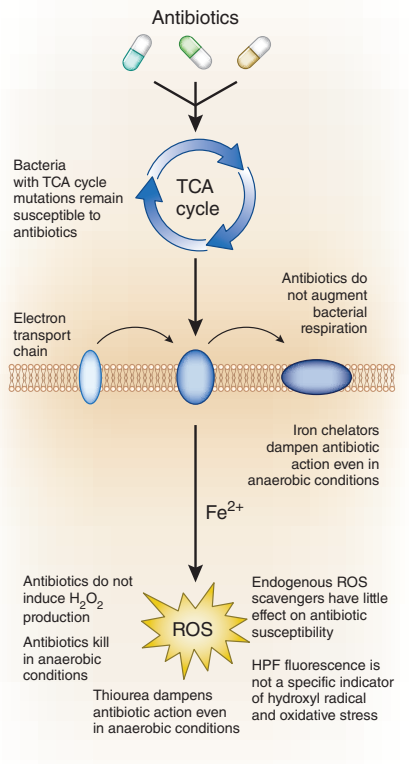

Figure 1 Divergent conclusions about several aspects of the antibiotic ROS paradigm. Evidence suggests that bactericidal antibiotics trigger a pathway involving the bacterial tricarboxylic acid (TCA) cycle, respiration, iron-catalyzed Fenton chemistry and production of reactive oxygen species (ROS). However, other studies present discrepant findings; these discrepant experimental results are described and placed next to the relevant point in the pathway that is proposed to lead from antibiotics to ROS production.

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