Recombinatorial Logic

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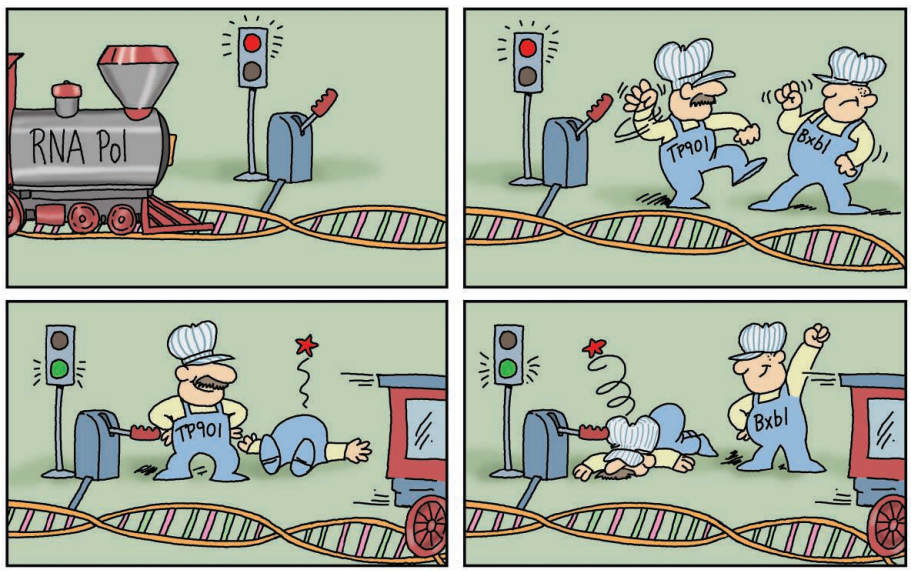
The use of sequence inversions allows all 16 possible logic gates for two inputs to be realized in bacterial cells.

Yaakov Benenson

Logic gates evoke images of circuit boards, but cells are arguably equally good in relying on logic computations. A classic example is the Lac operon, which activates itself upon the condition "lactose AND NOT glucose" (*1*). In recent years, there have been multiple reports on rationally designed, genetically encoded logic gates and circuits in living cells (*2*). Just like the Lac operon, these gates receive two or more molecular signals (inputs) and generate a product (output) whose level is logically linked to the inputs. Sixteen different logic connections are possible with two inputs and one output, but many of these operations have remained refractory to rational design. The trickiest of these gates usually make general statements about the inputs without referring to their exact values, such as "both inputs are the same" (an XNOR gate) or "two inputs are different" (an XOR gate). Two studies, one on page 599 of this issue by Bonnet *et al*. (*3*) and one by Siuti *et al*. (*4*), describe approaches that produce any of the 16 gates, including the notorious XNOR and XOR, in a compact manner by making relatively minor tweaks to the gates' genetic building blocks.



Controlling the signal. Transcription in a bacterial cell can be controlled by engineered logic gates. The approach taken by Bonnet *et al*. to create an XOR gate is illustrated by a cartoon that depicts RNA polymerase as a locomotive awaiting its go-ahead signal. A terminator sequence prevents transcription in two cases of activity by two recombinase enzymes, TP901 and Bxb1 if neither is active (**top left**), or if both TP901 and Bxb1 are active, effectively neutralizing each other's action (**top right**). When there is exactly one recombinase active (**bottom**), the go-ahead signal is given by inverting the terminator and allowing RNA to be synthesized.

The uneven progress in implementing synthetic gates has been mirrored by a recent argument that the natural evolution of some regulatory logic gates is much more difficult than for others (*5*). Indeed, the success of the approaches of Bonnet *et al*. and Siuti *et al*. resulted from repurposing phage-derived molecular tools whose natural role is not to control gene expression, but rather to insert phage DNA into host genomes: site-specific serine recombinases, or integrases. An integrase recognizes a pair of specific, nonidentical sequences called attP (on the phage) and attB (on the host) that, after recombination and phage insertion, are converted into a pair of sites called attL and attR that no longer serve as recombinase substrates. By intentionally framing an engineered DNA sequence by attP and attB sites, it is possible to trick the recombinase into inverting this sequence irreversibly. The "irreversibility" is not the whole story; an integrase can recognize attL and attR sequences in the presence of another protein called recombination directionality factor (RDF).

Using recombinases to control gene expression is not unheard of in nature: fimB/E recombinases flip a promoter controlling *Escherichia coli* virulence (*6*). In genetic engineering (*7*), recombinases have been used for insertion, excision, or inversion of DNA sequences to create either well-formed expression units or mutants incapable of gene expression. The devices in the recent reports deal exclusively in inversions because the underlying biochemical process is highly efficient and because it can be selectively reversed with the help of RDFs. The major advance comes from controlling the same gene with more than one recombinase, requiring certain activity patterns of multiple enzymes to create transcriptionally active configurations. This approach, together with ingenious design of the specific recombinase substrates, enabled the long-sought diversity of logical operations.

The gates built by Siuti *et al*. flipped gene promoters, a transcriptional terminator, and the gene-coding sequence. Bonnet *et al*. relied mostly on terminators and occasionally on promoters. For example, they implemented the XOR gate by placing a terminator in front of the gene-coding sequence and then flanking the terminator with two nested pairs of attP/attB sites for two different integrases. If either one of the enzymes was active, the terminator was flipped backward and out of context, allowing transcription to proceed. If both were present, the terminator would be inverted once and then again back into its blocking position (see the figure). The former study used a similar nested architecture but with a promoter instead of a terminator: A backward-facing promoter was restored into the correct orientation by either integrase, but was flipped back by the second one.

The irreversible nature of DNA modification sets recombinase-based gates apart from earlier work, where reversible input-output interactions caused the output to track changing inputs dynamically. In the new devices, integrase inputs perform irreversible modifications to the output DNA, so the logic conditions become more complex than simple "here and now." Thus, an AND gate generates the output if both inputs had been active in the gate's past, not necessarily at the same time.

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History-dependent operation invokes closely related concepts of sequential logic and finite-state machines. The latter can be described as a memory cell with an input receiver. The cell can store one piece of information (state) that changes when an input arrives. A number of studies explicitly explored recombinases as the tool for state machines and counters (*8*, *9*), and a rewritable memory unit had been implemented earlier using a bidirectional recombination process (*10*).

The integrase approach enables truly digital logic. In earlier work, a gene controlled by reversible inputs such as transcription factors (*11*, *12*) or microRNA (*13*) could be fully induced or repressed but could also exhibit intermediate expression levels. A single integrase-controlled gene can exist only in a finite number of states, and unless explicitly intended otherwise (*4*), it can be either fully active or completely inactive. The reported experiments were performed with multiple-copy plasmids, and the output level in each bacterium reflected intracellular ratios of different plasmid states, resulting in large output heterogeneity. However, each individual plasmid could only exist in a fully on or off state—a result that could be confirmed through careful single-cell measurements with single-copy plasmids or stable chromosomal integration.

How exactly the integrase affects the output on a single-cell, single-gene level is an open question. In both reports the integrases were controlled via inducible promoters, and simultaneous observation of the inputs (e.g., through fluorescent fusion) and the output in individual cells has not been made. These measurements are important because in principle, even a very small amount of integrase, given enough time, could induce switching and make the gates overly sensitive. Bonnet *et al*. made important first steps toward such characterization. They quantified integrase indirectly by substituting it with green fluorescent protein and inferred a population-averaged link between the integrase and the output. They observed signal attenuation and amplification at low and intermediate integrase levels, respectively. Both are highly desirable features in digital signal processing.

Recombinase gates represent a new paradigm in synthetic circuit design. The integration of logic, memory, input reset, and digital states makes the experimental systems reported by Bonnet *et al*. and Siuti *et al*. interesting test cases for the theory of biological networks. These gates are perhaps the farthest away from any natural counterpart and are thus likely to occupy synthetic and systems biologists in the years to come.

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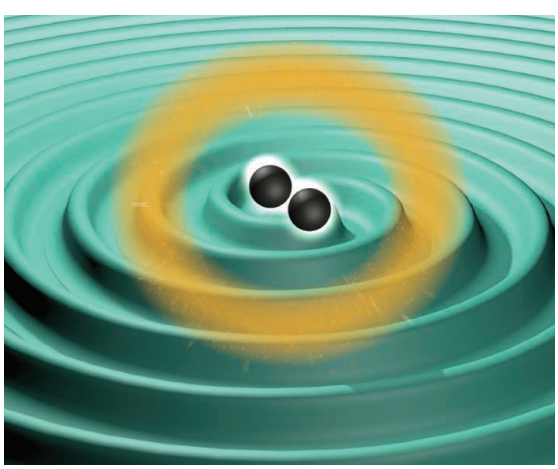
ASTROPHYSICS

Seeing Gravitational Waves

A suite of observatories will be needed to detect the visible electromagnetic counterpart of gravitational waves.

Mansi M. Kasliwal

Gravity is responsible for the long-range order of the universe. Using Einstein's general relativity, we now think of gravity as the geometrical curvature of the four-dimensional fabric of space-time (*1*). Extreme cosmological events such as the merging of neutron stars or black holes induce ripples in the fabric of space-time (see the figure). However, these ripples, or gravitational waves, are extremely weak, and their detection has remained elusive. To measure the small signal, an interferometric detector is required that can detect strain to one part in 10^{21} (that is, a billionth of a nanometer for a kilometer-length interferometer). Such extreme gravity events are also rare, occurring only once every 10,000 years per galaxy (*2*). An advanced version of such a detector is designed to find gravitational waves on a regular basis (roughly tens of events annually) beginning in 2017 (*3*). This heroic experiment alone will be somewhat unsatisfying—gravitational wave interferometers will only be able to hear the wave and detect when something happens (literally "hear" as the operational frequency of tens to thousands of Hertz overlaps with the human auditory range). The interferometers will be blind to exactly where the merger occurs. To locate the source of the gravitational waves, collaboration between the physics and the astronomy communities together with extensive simulations are under way (*4*).



Gravity wave detection. Merging neutron stars or black holes induce ripples in the fabric of space-time. The decade ahead promises to witness the first gravitational wave detections: to "hear" the sound waves with advanced gravitational wave interferometers and "see" the gold halo (produced via nucleosynthesis) with a slew of panchromatic telescopes.

There is a lot of activity in three complementary camps: instrumentalists, theorists, and observers. Instrumentalists are striving to set up a global network of advanced interferometers to localize the gravitational wave signals: the longer the baselines, the tighter the triangulation. The first such triangle to come online will be the Laser Interferometer Gravitational Wave Observatory at Hanford (LIGO-Hanford) (*5*), LIGO-Louisiana (*5*), and VIRGO-Italy

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