tors like adenovirus and herpes simplex virus. Furthermore, alongside transcriptome and proteome expression profiling techniques, AAV display might be employed for the identification and characterization of receptors whose expression is specific to a particular cell lineage, tissue or disease state. *In vivo* selection of targeted AAV variants in tumor patients might enable the identification of new diagnostic markers and targets for therapy, including vaccination and immunotherapy, reverse genetics and interference with function. *In vivo* application of AAV display, however, might face new challenges because viral particles could potentially interact with soluble blood or tissue factors that can serve as bridge(s) for cell uptake, or certain target tissues might not be accessible to the systemically applied virus owing to anatomical barriers. However, in the end, AAV display may be able to home in on quite a few new targets.

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Playing to win at DNA computation

Jeffrey J Tabor & Andrew D Ellington

An automaton built with DNA enzymes plays tic-tac-toe against human players.

Adleman's[1] seminal 1994 insight that computation could be encoded in DNA set off a raft of speculation on such topics as whether massively parallel DNA computations might one day break the data encryption standard[2,3]. However, the utility of DNA computation can be evaluated to some extent by the level of its adoption, and in the years following Adleman's brilliant insights, few if any applications for DNA computing have been realized. Accordingly, the development of a DNA machine that plays tic-tac-toe might be seen as the paramount impracticality in a field that has so far been more hype than substance[4]. To the contrary, the work by Stojanovic and Stefanovic[5] in this issue provides a fascinating new application for the computational abilities of DNA in the form of nucleic acid enzymes that can interpret multiple simultaneous substrates and perhaps eventually be used to reprogram cellular metabolism.

Stojanovic and Stefanovic taught DNA to play tic-tac-toe by designing a hierarchy of programmable deoxyribozyme cleavases. An RNA-cleaving deoxyribozyme (E6) originally selected by Breaker and Joyce[6] served as a starting point for engineering the automaton. The authors previously incorporated two modular allosteric domains into E6 that were capable of activating or inhibiting the enzyme in the presence of complementary oligonucleotide effectors, and they showed that through judicious engineering of these domains a series of enzymes capable of all possible Boolean logic operations could be generated[7]. Allosteric enzymatic reactions in which effectors indirectly transform substrates into products can be engineered to mimic traditional Boolean logic functions. A very simple example is the oligo 4-dependent cleavage reaction of well 1 (**Fig. 1**) which functions as a YES logic gate in which the presence of the allosteric effector is required by the logic gate (enzyme) to produce a specific output (fluorescent product).

In the current work, a series of such allosteric deoxyribozyme gates is engineered to incorporate a third allosteric domain, allowing higher-order logic functions to be performed with fewer enzymes. Different subsets of these enzymes are placed in each of nine wells representing the positions on a tic-tac-toe board (**Fig. 1**). For simplicity, the automaton goes first and plays in the center square (well), activating a deoxyribozyme that cleaves a fluorescent substrate and signals the play. There are now eight remaining plays, corresponding to eight different oligonucleotide effectors. Irrespective of which of the eight is added, the wells contain sets of deoxyribozymes that have been programmed to make the most competitive responses. For example, if a human player mistakenly puts his 'O' in a noncorner position (*e.g.*, well no. 4), deoxyribozymes will be activated to fluorescently signal an 'X' in the top, left corner (well no. 1). The automaton's subsequent moves are dictated by the human's responses, but because the game is 'hard-wired' the human has no chance to win (if he plays by the rules).

The effort required to assemble such a complex, functional group of molecular catalysts was extraordinary. Each enzyme had to be designed to interpret the same set of effectors differently. Effectors that might form stable secondary structures were excluded using computational methods, and the multiple deoxyribozymes were all engineered to preclude misfolding. Effector and enzyme concentrations were then empirically tweaked to differentiate signal from noise, and any designs that displayed nondigital behavior or cross-reactivity were further modified or replaced. Ultimately, these arduous efforts culminated in a tour-de-force implementation that included 23 different deoxyribozymes operating simultaneously in 9 different wells with 8 different possible oligonucleotide effectors.

The authors suggest that their biological logic gates might be refashioned as intracellular sentinels, capable of recognizing multiple disease signals and responding with a single functional output, such as the release of cytotoxic factors[5,7]. Indeed, the logic tree represented by the various allosteric deoxyribozymes resembles nothing so much as a natural signal transduction pathway or circuit. If the game of tic-tac-toe can be engineered by deoxyribozymes (rather than protein enzymes), then it may be possible to similarly reengineer signal transduction pathways using the same sorts of techniques and tools.

In this regard, researchers have begun to assemble synthetic genetic circuits from modular regulatory genes and proteins[8–10]. For example, even in the absence of a detailed biochemical understanding of the dynamics of gene regulation, it has proven possible to design an oscillatory genetic network and then optimize its function as a 'toggle switch' in *Escherichia coli*[8]. Random, as opposed to designed, arrays of genetic regulatory elements have been shown to give rise to a variety of dynamic cellular phenotypes[9], mimicking the multiple deoxyri-

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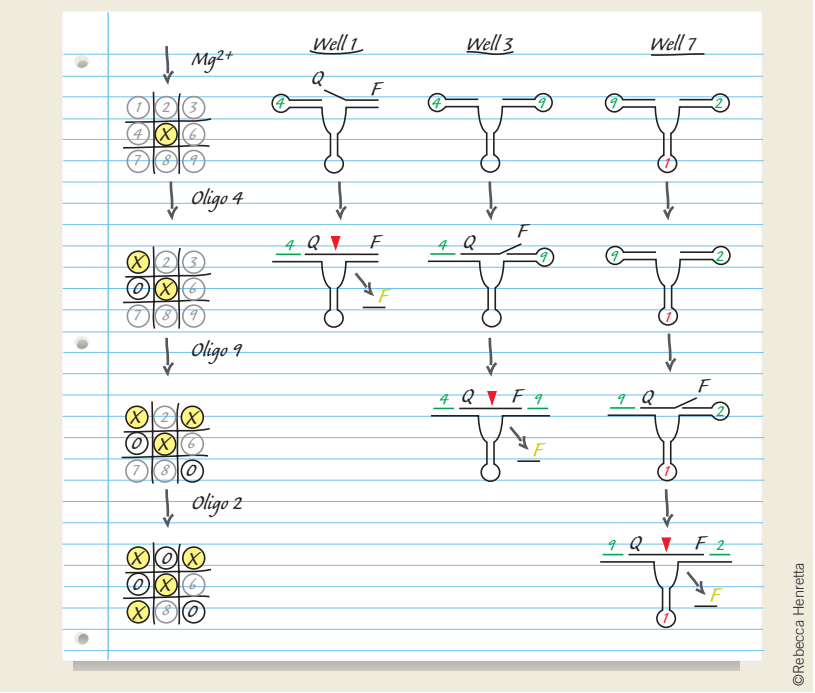

Figure 1 Representative game board and computational deoxyribozymes. Upon the addition of Mg^{2+}, a nonallosteric deoxyribozyme in square (well) 5 cleaves its substrate, yielding a fluorescent signal and signifying the automaton's first move. The human player responds by adding a DNA oligonucleotide effector to all nine wells, in this case indicating the choice of playing square 4. The effector signifying play in well 4 binds to and activates a preprogrammed, allosteric deoxyribozyme in square 1, which in turn cleaves (red triangle) the substrate and indicates the automaton's response: an 'X' in square 1. In all other wells, no activation occurs and no move is indicated. As the game continues, the addition of subsequent effectors activates specific deoxyribozymes in specific wells according to the underlying logic of the game. Deoxyribozymes with 1, 2 and 3 allosteric domains are shown in wells 1, 3 and 7, respectively. The unaffected domain of the deoxyribozyme in well 7 is an inhibitory domain that would participate in a different game than the one shown here. Should the player have chosen square 1, this deoxyribozyme would have been catalytically inactivated by oligonucleotide effector 1. Adapted from Stojanovic and Stefanovic.

boyzme logic gates that Stojanovic and Stefanovic have proofed *in vitro* (see also **Fig. 2**). Interestingly, some gates functioned similarly despite the fact that they had very different connectivities between component repressors and promoters, whereas others functioned differently even though they had the same connectivity. Finally, both approaches can be melded, in that the responsiveness of a designed genetic regulatory circuit can be further optimized by mutation and selection[10].

To date, such synthetic networks have by and large involved only transcription factors and promoters as regulatory elements. As the paper by Stojanovic and Stefanovic demonstrates, decision trees based on nucleic acids can be far more complex than those based on protein components, while at the same time maintaining an unparalleled (so to speak) control over the design and performance of the individual elements. As an extension of the insights that Adleman has previously applied to nucleic acid hybridization logic, the fact that the function of nucleic acid enzymes rests largely upon secondary structural interactions and Watson–Crick pairings implies that deoxyribozyme or other nucleic acid gates should be easier to program and have much better performance characteristics in synthetic networks than proteins (in fact, the activation parameters of designed and selected allosteric ribozymes have already proven to be much better than those of natural allosteric protein enzymes[11,12]). Functional nucleic acids are also more amenable than proteins to *in vitro* selection, and thus networks could potentially be optimized before introduction into a cell. For example, Stefanovic and Stojanovic envision that deoxyribozymes could be aligned in series rather than in parallel, such that the output of one logic gate would function as the input to another. This arrangement could in turn be readily adapted to a synthetic signal transduction network.

With tic-tac-toe conquered, will an 8 × 8 plate of DNAzymes ever defeat Kasparov in a

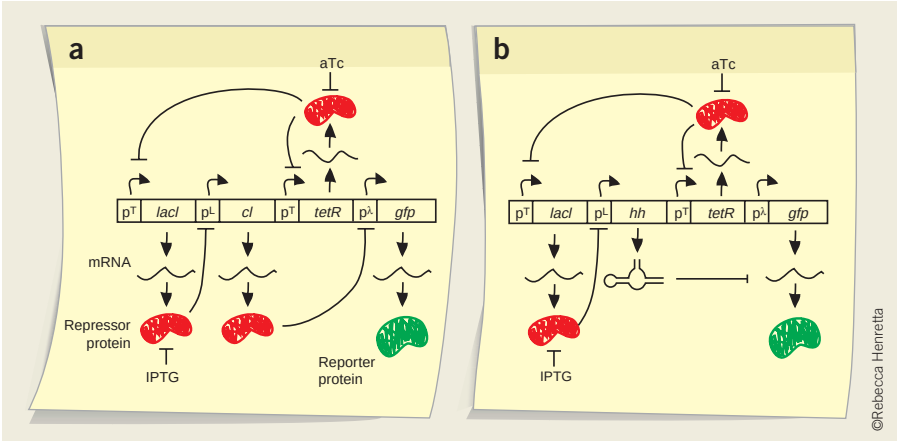

Figure 2 Signal tranduction pathway via protein or nucleic acid. (**a**) An *E. coli* genetic circuit built from three well-characterized eubacterial repressors (lac inhibitor (lacI), lambda inhibitor (cI) and tet repressor (tetR)), and their corresponding promoters and operators (P^L, P^λ and P^T, respectively). This circuit acts as a NOTIF logic gate, in which the gate is active in only one of four logical configurations. In the presence of aTc (anhydrotetracycline) alone, green fluorescent protein (GFP) is synthesized, whereas in the absence of aTC (without isopropylthio-beta-D-galactoside (IPTG)) or in the presence of IPTG (with or without IPTG), GFP is not synthesized. Adapted from ref. 9. (**b**) Proposed incorporation of a nucleic acid enzyme into the genetic circuit. Here, a hammerhead ribozyme targeted to the GFP mRNA might functionally replace the lambda cI repressor. Inhibition would occur posttranscriptionally as opposed to at the level of transcription initiation. Allosterically activated nucleic acid catalysts, such as those described by Stojanovic and Stefanovic, would provide an additional hierarchy of logical control over the performance of the circuit.

game of chess? Not likely. More complex problems are far more amenable to solution via traditional computation. However, the true power of DNA computation was never to mimic *in silico* accomplishments that have for many years outstripped biology (even savants manipulate large numbers much more slowly than the lowliest hand-held electronic calculator). Rather, molecular computers can be used to directly mimic and even solve problems that occur within the provenance of biology: that is, inside of a cell (**Fig. 2**). This application will lead to a more profound understanding of extant biological networks and will aid in the implementation of new cellular functions. What makes the automaton most interesting is not that schoolchildren the world over can now try their hand at outwitting DNA, but rather that it serves as the first use of programmable enzymes in a complex network.

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Corn with enhanced antioxidant potential

Peter Dörmann

Characterization and manipulation of a novel prenyltransferase from monocot plant seeds reveal its capacity to produce tocotrienols and increase this form of vitamin E in transgenic plants by 10- to 15-fold.

Vitamin E is a generic term describing a group of eight lipophilic compounds in the tocopherol and tocotrienol families. On the basis of the number and position of methyl groups on the chromanol ring, four different forms of both tocopherols and tocotrienols can be distinguished (α, β, γ or δ)[1]. Whereas tocopherols carry a saturated long-chain phytyl group, the side chain of tocotrienols includes three *trans* double bonds. Vitamin E is synthesized in plants, but cannot be produced in animals and thus represents an essential component of the human diet. It is a strong antioxidant, which protects polyunsaturated fatty acids in membranes against degradation by reactive oxygen species such as ozone, singlet oxygen, peroxides and hyperoxides. In this issue, Cahoon *et al.*[2] report the sequence of an enzyme specific for tocotrienol synthesis in monocots and overexpress it in transgenic plants achieving an increase in tocotrienols of 10- to 15-fold and 6-fold in *Arabidopsis thaliana* leaves and in corn seeds, respectively. This large increase in tocopherol/tocotrienol content is unprecedented, and emphasizes our capacity to alter metabolic pathways in transgenic plants.

In plants, biosynthesis of tocopherols and tocotrienols is localized to the plastids of seeds and the chloroplasts of leaves. Despite its protective characteristics, total loss of tocopherol in mutants of cyanobacteria or higher plants has no obvious effect on physiology and growth. This might be a result of overlapping activities of tocopherol with other antioxidants[3,4]. The gene encoding homogentisic acid phytyltransferase (HPT), the first step unique to tocopherol synthesis, has recently been isolated[3]. It was assumed that tocotrienols are produced from homogentisic acid and geranylgeranyl diphosphate, the unsaturated precursor of phytyl diphosphate, in an analogous reaction (see **Fig. 1**). On the basis of sequence similarities among the different prenyltransferases from plants, Cahoon and coworkers have now isolated a novel gene from monocot species and present compelling evidence that it encodes homogentisic acid geranylgeranyltransferase (HGGT), the only known enzyme specific for tocotrienol synthesis[2].

The precursors for tocopherol and tocotrienol synthesis in plants originate from two different pathways. Phytyl diphosphate and geranylgeranyl diphosphate are synthesized via the nonmevalonate pathway of plastid isoprenoid synthesis (**Fig. 1**). Homogentisic acid, the precursor of the vitamin E head group, is derived from shikimate. Biotechnological approaches to modify vitamin E content have to take into account the complexity of plastid prenyl lipid synthesis, because geranylgeranyl diphosphate, phytyl diphosphate and homogentisic acid are not only the substrates for vitamin E synthesis, but are also critical for the production of many other compounds important for plant development. Two additional substances with vitamin activity, β-carotene (provitamin A) and phylloquinone (vitamin K1), are synthesized from geranylgeranyl diphosphate and phytyl diphosphate, respectively. Furthermore, the synthesis of photosynthetic pigments and electron acceptors (chlorophyll, carotenoids, plastoquinone-9) and of two phytohormones (gibberellins, abscisic acid) depends on the plastid isoprenoid pathway (**Fig. 1**).

Previous approaches to alter vitamin E amounts in plants include overexpression of *p*-hydroxyphenyl-pyruvate dioxygenase and homogentisic acid phytyltransferase (HPT)[5,6]. However, the increases in total tocopherol/tocotrienol achieved in these studies were only 1.5- to 4-fold. Possible explanations for these observations are the restricted availability of the vitamin E precursors phytyl diphosphate, *p*-hydroxyphenyl-pyruvate or homogentisic acid, or their rapid consumption by competing pathways. Overexpression of 1-deoxy-D-xylulose-5-phosphate synthase with the aim to stimulate carbon flux into the plastid nonmevalonate pathway results in a moderate increase in tocopherol (about 1.4-fold) and a concomitant increase in carotenoids, chlorophyll, abscisic acid and gibberellins[7]. The fact that overexpression of phytoene synthase in transgenic rape seeds and in rice ('golden rice') results in a drastic increase in carotenoid synthesis (*e.g.*, β-carotene/provitamin A) suggests that geranylgeranyl diphosphate is not limiting for prenyl lipid synthesis in seeds[8,9]. In agreement with this, Cahoon *et al.* found that overexpression of HGGT in transgenic *Arabidopsis* and corn leads to a much stronger increase in tocotrienol synthesis than the increase

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