transport mechanism, the identification of CER5 sheds light on wax secretion in plants and may help to elucidate how the elaborate micro- and nanostructure of the wax layer is constructed. How did land plants invent wax secretion? The genomes of living land plants contain more than 100 ABC transporter genes (*12*). Because transporters seem to be sloppy with respect to their substrate specificity (*13*, *14*), it is feasible that when plants crept out of the water, they turned a member of the ABC transporter family into a lipid exporter by ensuring that it became localized to a different cellular compartment. Perhaps this is an example of an evolutionary principle in which sloppiness is transformed into flexibility.

Obviously, there is more work to be done to identify other components of the lipid export machinery. We need to define the exact export pathway and its components. The remaining *Arabidopsis cer* mutants provide an outstanding resource with which to fill in the gaps to obtain a more complete picture. Given that the reduced-wax phenotype of the *cer5* mutant is restricted to stems, the transporters involved in wax deposition on leaves and pollen will need to be identified. A comparative analysis of fatty acid transport in bacteria, plants, and animals, although likely to reveal variations as well as commonalities, will cross-fertilize research in these respective fields. Such an analysis will help to answer crucial questions, including whether the fatty acid substrates are free or bound and how the trilamellar inclusions form. The new insight provided by Pighin and colleagues into the ABC lipid transporter of plants has implications beyond understanding the lotus effect—given the multifunctional role of the wax cuticle, the new findings will be a boon to agriculture.

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CHEMISTRY

Redesigning Genetics

Steven A. Benner

A year has passed since the celebration of the 50th anniversary of the Watson-Crick model for the double-helical structure of DNA (*1*). Much of the celebration looked back at the marvelous advances that have emerged as genetics has come to resemble organic chemistry. Largely overlooked, however, is a new frontier in organic chemistry that has the goal of redesigning DNA to create artificial genetic systems. These artificial DNA-like molecules are providing deeper insight into how DNA works and are opening the door onto a new world of synthetic biology (*2*). They are also proving valuable for diagnostic testing of human diseases.

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According to the first-generation model of DNA, the DNA duplex is like a ladder, with the upright sections composed of pentose sugar molecules linked together by negatively charged phosphate groups (see the figure). According to the model, the uprights constrain the length of the base pairs that form the rungs of the ladder. This constraint, in turn, requires that the large purine bases, adenine (A) or guanine (G), pair with small pyrimidine bases, thymine (T) or cytosine (C)—a phenomenon known as size complementarity. According to the model, hydrogen bonds between purines and pyrimidines ensure that the correct large bases pair with the correct small bases. From this model arose the two principal rules ("A pairs with T, G pairs with C") that underlie all of molecular biology.

One motivation for redesigning DNA using organic chemistry came from a vision of therapeutic benefit. For example, an uncharged DNA analog might be able to pass through a cell membrane, bind to an unwanted RNA molecule according to Watson-Crick rules, and neutralize its activity (*3*). Many dozens of DNA analogs having uncharged scaffolds were made in pursuit of this vision (*4*). Remarkably, only one can be said to have been truly successful: the polyamide-linked nucleic acid analogs (PNA) made by Nielsen *et al*. (*5*).

We now know that the repeating negative charge of the DNA backbone is tightly tied to the rule-based molecular recognition needed for transmission of genetic information (*6*). The repeating negative charge keeps contacts between two complementary DNA strands as far away from the backbone as possible, enforcing Watson-Crick pairing. Without the repeating charge, DNA analogs bend, fold, aggregate, or precipitate. Even PNA does this, given sufficient length.

The repeating charge also dominates the physical properties of DNA. The charge allows the individual bases to be substituted by mutation to create new DNA molecules that behave physically like their parents, but carry different genetic information. The repeating negatively charged phosphates of the DNA and RNA backbone are therefore key to evolution. Hence, a repeating charge may be a universal structural feature of all molecules carrying genetic information in water, perhaps even those on alien planets circling stars in remote galaxies.

Other efforts to redesign DNA have asked simple questions about the architecture of base pairing. For example, Kool wondered how DNA might behave if one got rid of the hydrogen bonds entirely, and used size complementarity as the sole principle of pairing (*7*). Surprisingly, certain DNA polymerases are able to match size-complementary species without the benefit of hydrogen bonding. This result encouraged Goodman to comment that DNA has gone "on the wagon" to join "hydrogen bonds anonymous" (*8*). Schultz, Romesberg, and their colleagues have elaborated on Kool's general theme, generating base analogs that contact each other through unusual hydrophobic interactions (*9*). The latest products from the Kool laboratory are fluorinated bases that also pair using size complementarity in the absence of hydrogen bonds (*10*).

Things generally work out better, however, if the hydrogen bonds are retained. Hydrogen bonding might be important in size-expanded base pairs (*11*), something that has been seen previously in DNA backbones with both longer and shorter rungs (*12*). Carrying the theme further, Minakawa *et al*. asked what might happen if the hydrogen-bonding pattern were to be extended into the minor groove of the DNA backbone (*13*). With four hydrogen-bonding opportunities, we can imagine 16 different hydrogen-bonding patterns supporting 32 different nucleotide letters in an expanded genetic alphabet based on this architecture. The expanded scaffolding works well, and a new class of designer DNA molecules may emerge from this architecture.

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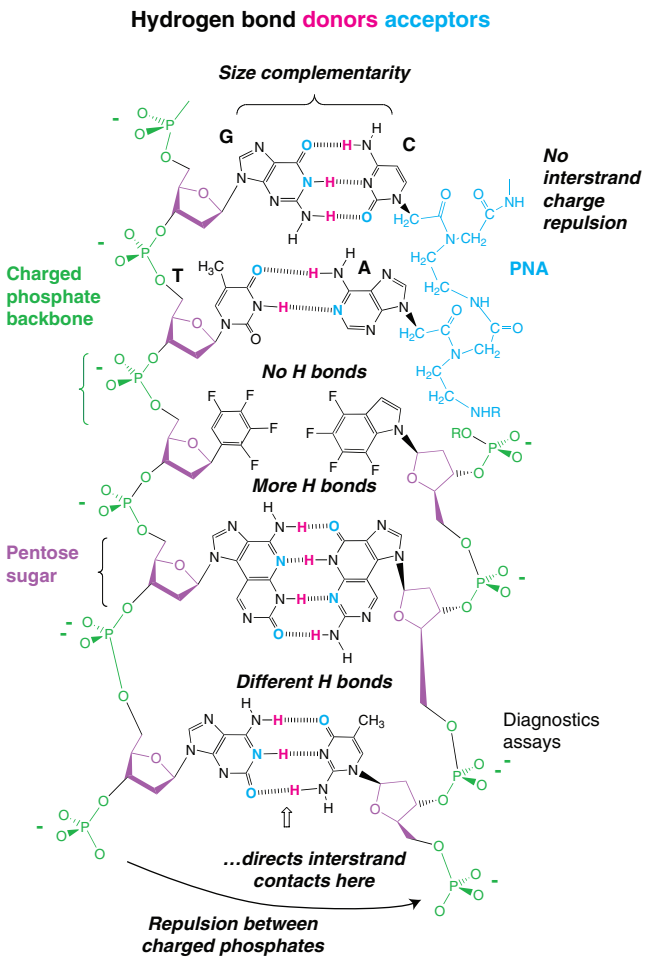

Tinkering with DNA. The standard DNA double helix has a scaffold of repeating negatively charged phosphate groups (green) that link together ribose sugars (purple). This scaffold supports size-complementary pyrimidine and purine bases (black) that present hydrogen bond donor (pink) and acceptor (blue) groups. Each nucleotide (sugar, phosphate, base) plays a role in transmitting genetic information. Attempts are under way to redesign DNA using organic chemistry for a variety of uses including diagnostic testing. For example, DNA molecules have been engineered to lack negatively charged phosphate groups (**upper right**) or hydrogen-bonding groups (**middle**), or have been made with an increased number of hydrogen bonds or rearrangements of these bonds (**bottom**). Redesigned DNA containing rearranged hydrogen bonds (branched DNA) enhances the medical care of about 400,000 patients annually through its use in diagnostic tests such as those detecting human immunodeficiency virus and human hepatitis C virus.

One need not extend the scaffold of the bases into the minor groove, however, to get extra bases (letters) into the genetic alphabet. More than a decade ago, Switzer *et al*. (*14*) and Piccirilli *et al*. (*15*) found that an additional eight letters can be added to the DNA alphabet if one simply shuffles the arrangement of hydrogen bond donating and accepting groups (see the figure). The physical properties of nonstandard bases have now been optimized. For example, tautomerism (unwanted movement of hydrogen atoms) that causes nonstandard bases to be lost during repeated copying has been fixed, undesirable acid-base properties of the artificial genetic components have been changed, and an annoying epimerization (unwanted change in the geometry of the molecule) displayed by some nonstandard nucleotides has been corrected (*16*).

The architecture of this artificially expanded information system is so reminiscent of the Watson-Crick architecture, and its properties are so similar to those found in standard DNA, that one may wonder why nature has not already exploited these extra DNA letters. Recent advances in our understanding of how the ribose sugar might have arisen prebiotically on early Earth (*17*) offer a clue. Ribose is stabilized by minerals containing borate, which might have allowed ribose to accumulate on early Earth. Attaching a heterocyclic ring to a ribose via a carbon-nitrogen bond, as in standard nucleotides, requires a dehydration event, certainly conceivable (although not trivial) prebiotically. Attaching a heterocyclic ring to a ribose via a carbon-carbon bond, as in some nonstandard nucleotides, appears to be far more difficult. The structure of our DNA may therefore reflect the minerals that were present in ancient deserts on early Earth.

Luckily, prebiotic chemistry does not constrain the application of expanded genetic alphabets to human health care. For example, the U.S. Food and Drug Administration recently approved a "branched DNA" assay developed by Urdea and Horn (*18*) that exploits nonstandard nucleotides. Incorporating extra letters into DNA speeds up hybridization and allows independent binding of two rule-based molecular systems: one based on the standard letters A, T, G, and C, and the other based on an artificial genetic alphabet. Currently, each year some 400,000 patients infected with the human immunodeficiency virus or the hepatitis B and C viruses have their care enhanced through diagnostic assays based on an expanded genetic alphabet (*19*). Expanded genetic alphabets are working their way into other preclinical assays that test for cystic fibrosis, SARS, and biohazards. They are also entering research, where nonstandard nucleotides underlie a large number of emerging tools for systems biology research and genome sequencing.

So what is next on the agenda as we redesign DNA? It is hard to say. Perhaps foreshadowing the future is the discussion of recent examples where artificial genetic systems have been copied, and the copies copied, using engineered polymerases (*20*). Although most polymerases will accept many nonstandard nucleotides with some degree of efficiency when given no other choice, polymerases have evolved for billions of years to efficiently accept only A, T, G, and C. Therefore, most polymerases wean unnatural nucleotides from a DNA molecule if given the chance.

Both the structure of the nucleotide and the structure of the polymerase can be changed to obtain a pair where this does not happen. Polymerase engineering is in its infancy, however, and most attempts at site-directed mutagenesis wreak site-directed damage on the enzyme. But with the advent of selection methods for polymerases (*21*), we can expect in the not-too-distant future fully artificial genetic systems that support a synthetic biology—a set of artificial chemical systems that can direct their own replication and evolve.

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