DNA Made of Purines Only

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The DNA double helix, containing both purine and pyrimidine bases, has evolved as the universal genetic system. In this issue of *Chemistry & Biology*, Battersby and colleagues describe the formation of double helix that is comprised solely of naturally occurring purine-nucleotides [1].

The double helical structure of DNA has fascinated researchers ever since its discovery [2]. Chemists, biologists, and physicists have used their repertoire of methods to answer key questions about the chemical and structural nature of the genetic system. However, it remains difficult to answer some fundamental questions. Why has nature chosen DNA as the universal genetic system and why have the bases adenine (A), thymine (T), cytosine (C), and guanine (G) evolved as the letters of the genetic alphabet? Why this particular double helix architecture? One way to approach these questions is to use our current knowledge in the design and synthesis of possible alternatives and to study whether DNA function can be emulated. Such studies put our understanding of DNA structure and function to the test. A particular challenge is the design of a completely new genetic system, in which the complete set of "natural" base pairs, A-T, T-A, G-C, and C-G, has been replaced by the alternatives. Battersby and colleagues have succeeded in demonstrating the function of a new base-pairing principle. According to their results, published in this issue, purine-purine pairing enables a new mode of DNA duplex association. Interestingly, the components of the new DNA duplex architectures all occur in natural nucleotides, which evokes the question about a possible intermediacy of purine-purine pairing in the early stages of molecular evolution.

The formation of base pairs is at the heart of DNA function. Thus, a considerable amount of research has been devoted to the exploration of alternative base pairs. Significant work has been invested in the design of orthogonal base pairs that may expand the repertoire of letters in the genetic alphabet [3–5]. Analogs of nucleobases have been introduced into DNA to probe interactions between DNA and proteins [6, 7]. However, these studies did not intend to change the double helix architecture of the DNA molecule. A drastic redesign of duplex architecture has been shown by Eschenmoser and colleagues [8]. They discovered a new base-pairing mode present in the structure termed homo-DNA, and demonstrated the occurrence of Watson-Crick pairs between guanine and isoguanine as well as 2,6-diaminopurine and xanthine. The backbone of homoDNA is comprised of a 6′→4′-linked hexopyranosyl sugar, which replaces the 5′→3′-linked 2′-deoxyribose unit in natural DNA. The distance between the two sugar C atoms that connect the nucleobases with the sugar phosphate backbone is larger in purine-purine than in purine-pyrimidine base pairs. It was reasoned that the nonhelical, ladder-type structure of homoDNA facilitated the accommodation of the size-expanded purine-purine pairs. Watson-Crick pairing between purines was expected to be hindered in DNA, due to the constraints induced by the double helical structure. Similar opinion was also adopted by Diederichsen and coworkers, who demonstrated Watson-Crick interactions between purines in linear alanyl-peptide nucleic acids [9]. However, a seminal paper from Kool and colleagues revealed that size-expanded base pairs can be incorporated also into DNA double helical structures, termed xDNA (Figure 1) [10]. They synthesized four new DNA nucleotides, each of them differing from the native ones by an extra aromatic ring [11]. In double helical xDNA each of the enlarged bases is pairing with a natural nucleobase. Thus, xDNA has eight bases instead of the four bases in natural DNA. A central feature of xDNA is the enlarged distance between the two phosphate backbones of the double helix. Interestingly, the extended DNA duplex was found to be more stable than the natural Watson-Crick structure, presumably due to the contribution of π-stacking.

Kool's version of a size-expanded DNA relied on the incorporation of artificial nucleobases. In this issue of *Chemistry & Biology*, Battersby and colleagues provide ample evidence for the existence of a size-expanded duplex structure that is made of components that can be found in the pool of the naturally occurring ribonucleosides [1]. They introduce an all-purine DNA in which the natural pyrimidine bases thymidine and cytosine have been replaced by hypoxanthine (H) and isoguanine (J) (Figure 1). Thermal denaturation studies, that involved the use of fluorescent probes, suggested a specific base-pairing between adenine and hypoxanthine and between guanine and isoguanine. More sophisticated hybridization experiments that employed 7-deazaadenine instead of adenine and hypoxanthine instead of guanine revealed that the interaction between the noncanonical base pairs was likely to proceed in a Watson-Crick analog manner. Prerequisite for this kind of interaction is the N_3-H-tautomer of isoguanine, which is less preferred in aqueous solution but obviously stabilized upon double strand formation.

Although it can be expected that the all-purine DNA duplex has a widened

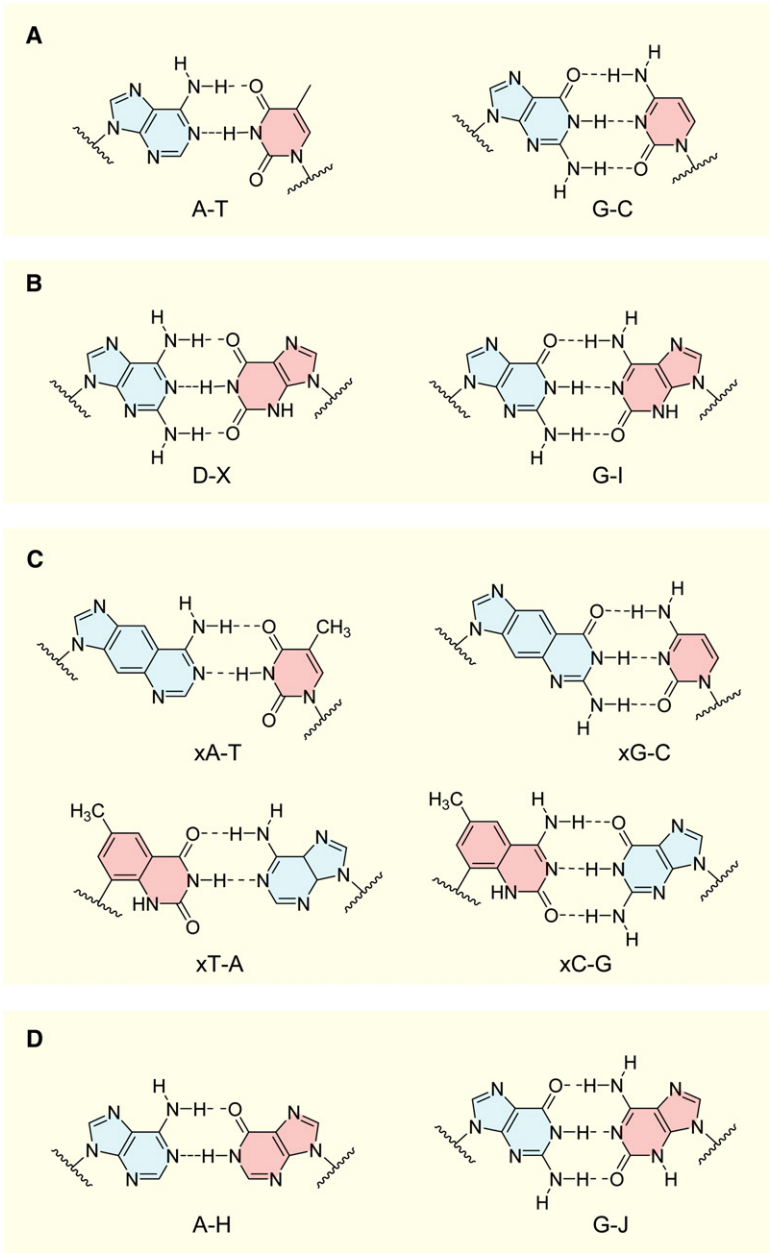

Figure 1. Enlarged Watson-Crick Base Pairs
(A) In naturally occurring DNA, purines form Watson-Crick base pairs with pyrimidines.
(B) In Eschenmoser's linear homoDNA [8] the purines 2,6-diaminopurine (D) and guanine (G) can pair with the purines xanthine (X) and isoguanine (I) in a Watson-Crick fashion.
(C) Size-expanded Watson-Crick base pairs in helical DNA duplexes have been shown by Kool [10].
(D) In this issue, Battersby and colleagues introduce purine-purine pairing as an alternative mode of DNA duplex association between components that all occur in nature.

diameter resembling Kool's xDNA [10] and should therefore be similarly stabilized by an additional contribution of base stacking, all-purine DNA duplex was found to be substantially less stable than canonical DNA duplexes. This may be due to a reduced hydration and a missing electrostatic interaction between A and H when compared to the canonical A–T interaction.

In contrast to the canonical duplexes, the all-purine DNA duplexes, although they may carry the same content of information, are probably not recognized by DNA-directed enzymes or small molecules targeting natural DNA. Thus, the all-purine DNA may be of utility as an orthogonal base-pairing system in the design of probes for applications in biotechnology and materials science. A hallmark of Battersby's all-purine DNA is the fact that all of its building blocks occur in nature, which inevitably raises the question whether the Watson-Crick interaction between A and H and G and J has played a role in an early progenitor self-replicating system. Since RNA is not only able to act as a genetic storage molecule but can also act as a biocatalyst, the hypothesis of the "RNA world" as the progenitor of life as we know it has found wide acceptance [12]. However, since experimental attempts to demonstrate the ability of pyrimidine nucleotides to oligomerize onto a complementary template have run into difficulties, a simpler ancestral nucleic acid preceding RNA has been suggested [13]. Nearly twenty years ago, Wächtershäuser speculated that such an ancestral nucleic acid might be an all-purine nucleic acid [14]. Battersby and colleagues now show that chimeric structures composed of purine-pyrimidine and purine-purine base pairs are possible, which could reflect a hypothetic evolutionary transition between an ancestral and the modern genetic system. At least this article in *Chemistry & Biology* provides some experimental data that might lead us to re-engage in this old debate. Why is our modern genetic system still seemingly superior to all other possible systems? This question remains wide open, regardless of the putative role proposed for an all-purine ancestral genetic system.

REFERENCES

1. Battersby, T.R., Albalos, M., and Friesenhahn, M.J. (2007). Chem. Biol. *14*, this issue, 525–531.
2. Watson, J.D., and Crick, F.H. (1953). Nature *171*, 737–738.
3. Piccirilli, J.A., Krauch, T., Moroney, S.E., and Benner, S.A. (1990). Nature *343*, 33–37.
4. Abou-Zied, O.K., Jimenez, R., and Romesberg, F.E. (2001). J. Am. Chem. Soc. *123*, 4613–4614.
5. Hirao, I., Harada, Y., Kimoto, M., Mitsui, T., Fujiwara, T., and Yokoyama, S. (2004). J. Am. Chem. Soc. *126*, 13298–13305.

6. Jiang, Y.L., Stivers, J.T., and Song, F. (2002). Biochemistry *41*, 11248–11254.

7. Beuck, C., Singh, I., Bhattacharya, A., Hecker, W., Parmar, V.S., Seitz, O., and Weinhold, E. (2003). Angew. Chem. Int. Ed. Engl. *42*, 3958–3960.

8. Groebke, K., Hunziker, J., Fraser, W., Peng, L., Diederichsen, U., Zimmermann, K., Holzner, A., Leumann, C., and Eschenmoser, A. (1998). Helv. Chim. Acta *81*, 375–474.

9. Hoffmann, M.F.H., Bruckner, A.M., Hupp, T., Engels, B., and Diederichsen, U. (2000). Helv. Chim. Acta *83*, 2580–2593.

10. Liu, H., Gao, J., Lynch, S.R., Saito, Y.D., Maynard, L., and Kool, E.T. (2003). Science *302*, 868–871.

11. Gao, J., Liu, H., and Kool, E.T. (2005). Angew. Chem. Int. Ed. Engl. *44*, 3118–3122.

12. Orgel, L.E. (1968). J. Mol. Biol. *38*, 381–393.

13. Orgel, L.E. (1986). Orig. Life Evol. Biosph. *17*, 27–34.

14. Wächtershäuser, G. (1988). Proc. Natl. Acad. Sci. USA *85*, 1134–1135.

Exploiting Green Treasures

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In this issue of *Chemistry & Biology*, Ishida and colleagues [1] report on the characterization of new aeruginoside metabolites and the respective NRPS gene cluster in the cyanobacterium *Planktothrix agardhii*.

Cyanobacteria are among the most talented, culturable micro-organisms from which novel, structurally diverse, biochemically active natural products have been isolated. Many of the compounds are small nontoxic or toxic peptides with unusual amino acids, polyketides, or hybrids of different biosynthetic pathways [2, 3].

Cyanobacteria are structurally diverse, geographically widespread in freshwater, marine, and terrestrial habitats, and some genera are nitrogen fixing and are therefore of great importance for the natural balance of the ecology. Due to the production of highly active hepatotoxins and neurotoxins, increasingly common cyanobacterial water blooms are of serious concern in freshwater reservoirs or lakes [2, 3, 4]. Investigating these toxins and other bioproducts with a focus on cancer drug discovery and chemical ecology is a promising research field where culturable cyanobacteria play an outstanding role. Diverse metabolites with relevant pharmaceutical activities have been isolated from cyanobacteria. However, the true relevance of these highly active agents for the producer strains remains elusive at present.

In this issue of *Chemistry & Biology* [1], two analogs of cyanobacterial aeruginosides, produced by the genus *Oscillatoria* (syn. *Planktothrix*), are reported. More than 200 compounds originate from different species of the order *Oscillatoriales* [2]. In the last decade, the toxic *Planktothrix agardhii* received considerable attention, and was recognized as a prolific source of novel metabolites [5]. Several compounds from different chemical classes are currently known. These include the first aeruginosins (205A and 205B) reported from *P. agardhii* that were highlighted as glycopeptides inhibiting serine proteases [6], several variants of the hepatotoxin complex microcystin isolated from a single strain [7], and the multicyclic microviridins that are the largest known cyanobacterial oligopeptides with characteristic ester and secondary amino bonds [3]. However, the full spectrum of chemical compounds produced by different isolates of the *P. agardhii* species has not been cataloged. Perhaps more interestingly, the whole metabolite pattern of a single strain as a distinct cocktail of bioactive agents has not been investigated in detail with respect to its ecological and physiological relevance; although, Fujii and coworkers [5] gave some initial insights.

The approach taken by Ishida and colleagues [1] is a sophisticated application of the general knowledge about the organization of nonribosomal peptide synthetase (NRPS) biosynthetic gene clusters in cyanobacteria to probe a yet unexplored part of the metabolite pattern of *P. agardhii* strain CYA 126/8. The method used elegantly turns around a classic concept in natural product research. Typically, a biosynthetic gene cluster is identified long after the respective metabolite is identified and fully characterized in a chemical-pharmaceutical screening program [8]. In the new report, a NRPS gene sequence was identified by a degenerate PCR approach in *Planktothrix* and used for insertional mutagenesis with a chloramphenicol resistence cassette to give evidence for the involvement of the genes within the cluster in putative peptide biosynthesis. Importantly, parallel cultivation of wild-type cells and mutant cells called attention to previously unknown metabolites. Subsequent careful chemical analysis identified two new