Crossover Reactions Between Synthetic Replicators Yield Active and Inactive Recombinants

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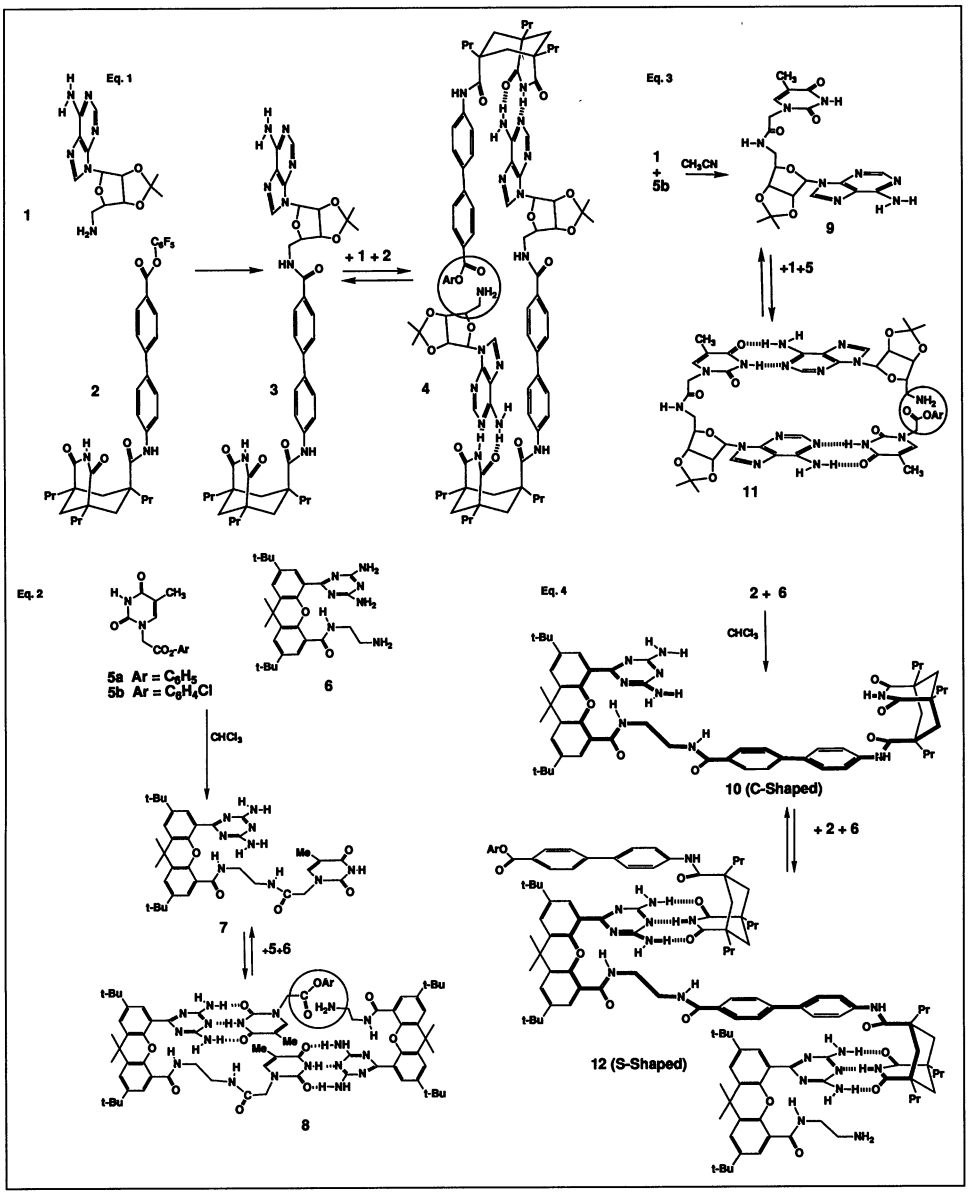
Self-replicating molecules can be synthesized through the covalent linkage of two complementary subunits to give a self-complementary structure. Complementarity refers to sizes, shapes, and the weak intermolecular forces involved in molecular recognition between the two subunits. In order to provide a model system for evolution at the molecular level, "crossover" or recombination experiments were staged with synthetic replicators. These reactions gave rise to new structural types. The ability (or inability) of the new recombinants to catalyze their own formation is shown to be a consequence of their molecular shapes.

Self-replicating molecules (replicators) lie at the interface of chemistry with biology. Synthetic replicators provide a means by which evolutionary phenomena such as competition, reciprocity, and mutation can be expressed at the molecular level (*1*). We describe new self-replicating structures arising from recombination of other replicators. Two such recombinations were synthesized. One forms hydrogen-bonded dimers readily and shows autocatalytic behavior, whereas the other features mismatched binding surfaces that diverge; it is unable to act as a template for its own formation.



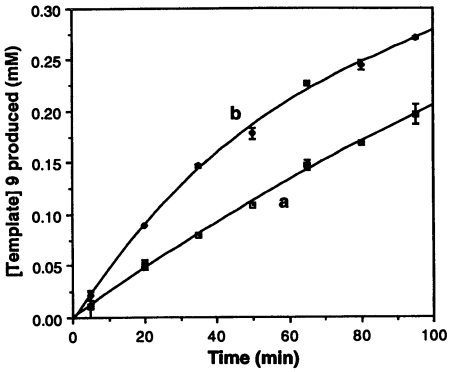


Fig. 1. Appearance of **9** as a function of time. Initial concentrations are [**1**] = [**5b**] = 3.0 mM in CH_3CN. Triethylamine (18 equivalents) was present in all of the reaction solutions. Error bars represent standard deviations of multiple independent runs. (a) Reaction of **1** and **5b** without additive. (b) Reaction of **1** and **5b** with 0.07 equivalent of **9** added.

The structural requirement for replicators is self-complementarity (*2*), and earlier we described two such minimalist systems. The first involved adenine-imide hydrogen bonding (*3*) and aryl stacking as the intermolecular forces that lead to molecular self-recognition (Eq. 1). The second features thymine-diaminotriazine hydrogen bonding (*4*) (Eq. 2) in the coupling reaction of **5a** with **6**. Both reaction products **3** and **7** are replicators: they catalyze their own formation through the template effects depicted in structures **4** and **8**.

A competition experiment was carried out in which recombination (crossover) products could be produced. Specifically, coupling of the adenine **1** with the thymine *o*-chloro-phenylester **5b** gave the dinucleotide analog **9** with an amide linkage (*5*) (Eq. 3), whereas the corresponding reaction of **2** with **6** gave **10** (Eq. 4).

At first glance, both recombinants might be expected to replicate. They bear self-complementary recognition surfaces and can gather their respective reaction components in termolecular complexes. In fact, the adenine-thymine hybrid **9** does act as such a template. Addition of **9** to mixtures of **1** and **5b** in CH_3CN led to the increased coupling rates (Fig. 1) characteristic of autocatalytic systems (*6*). It is a riotously fertile hybrid. Compared with previous synthetic replicators, it shows the largest autocatalytic effects observed to date (*7*). The situation is quite different for the recombinant **10**. No increase in initial coupling rate for **2** with **6** was

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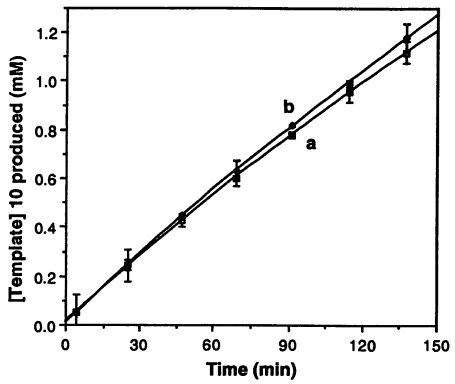

Fig. 2. Appearance of **10** as a function of time. Initial concentrations are [**2**] = [**6**] = 8.0 mM in $CHCl_3$. Triethylamine (12 equivalents) was present in all of the reaction solutions. Error bars represent standard deviations of multiple independent runs. (a) Reaction of **2** and **6** without additive. (b) Reaction of **2** and **6** with 0.28 equivalent of **10** added.

observed on adding 0.28 equivalent of the product **10** (Fig. 2). It is an inactive recombinant.

The differences in reactivity of **9** and **10** can be related to the orientations of their respective recognition surfaces. In **9** these can achieve a parallel arrangement. The molecule behaves as a template and can attract **1** and **5** in a productive termolecular complex **11**, poised for intramolecular coupling (Eq. 3). The initial reaction product is the hydrogen-bonded cyclic dimer of **9**. Dissociation of this dimer then results in the increasing concentrations of template that provide the autocatalytic effect.

The hybrid **10** is composed of two **U**-shaped modules—the Kemp triacid (*8*) and the xanthene diacid (*9*). Its overall configuration is either **C**-shaped, in which its recognition surfaces converge (**10**, Eq. 4), or **S**-shaped, in which its recognition surfaces diverge (as in **12**). Neither case allows a productive termolecular complex to be assembled. Nor can the molecule form a hydrogen-bonded cyclic dimer; instead, its self-complementarity is expressed by forming chains.

Self-complementarity is also a common feature of macromolecules, and the orientation of the recognition surfaces within these structures determines the nature of the assemblies that are formed. When these surfaces permit the formation of a dimer, replication is possible (*2*). Even dynamic systems such as micelles are capable of self-assembly and replication (*10*). With carefully fixed recognition elements, the assembly of synthetic self-complementary structures into predictable, closed surfaces that encapsulate molecules—or reaction events—of an appropriate scale should be possible (*11*).

REFERENCES AND NOTES

1. J. I. Hong, Q. Feng, V. Rotello, J. Rebek, Jr., *Science* **255**, 848 (1992).
2. J. Nowick, Q. Feng, T. Tjivikua, P. Ballester, J. Rebek, Jr., *J. Am. Chem. Soc.* **113**, 8831 (1991).
3. V. Rotello *et al.*, *ibid.*, p. 9422.
4. T. K. Park, Q. Feng, J. Rebek, Jr., *ibid.*, in press.
5. All new compounds were characterized by high-resolution mass spectrometry and infrared and nuclear magnetic resonance spectroscopy. Details will be published elsewhere.
6. For autocatalytic effects in the coupling of nucleic acid analogs, see G. von Kiedrowski, *Angew. Chem. Int. Ed. Engl.* **98**, 932 (1986); W. S. Zielinski and L. E. Orgel, *Nature* **327**, 346 (1987); G. von Kiedrowski, B. Wlotzka, J. Helbing, M. Matzen, S. Jordan, *Angew. Chem. Int. Ed. Engl.* **30**, 423 (1991).
7. For example, the acceleration of the initial coupling rate for the case shown in Fig. 1 is 35 ± 5% for only 0.07 equivalent added product and 35% for 0.05 equivalent added product at 4 mM reactants. For replicators **3** and **7** in $CHCl_3$ (a solvent in which template effects are enhanced), three to five times as much product was required before comparable rate increases were observed.
8. D. S. Kemp and K. S. Petrakis, *J. Org. Chem.* **46**, 5140 (1981).
9. J. Nowick, P. Ballester, F. Ebmeyer, J. Rebek, Jr., *J. Am. Chem. Soc.* **112**, 8902 (1990).
10. P. A. Bachmann, P. Walde, P. L. Luisi, J. Lang, *ibid.* **113**, 8204 (1991).
11. The dimer **9** is such a structure. For progress elsewhere on this front, see R. G. Barr and T. J. Pinnavaia, *J. Chem. Phys.* **90**, 328 (1986); M. C. Etter, Z. Urbanczyk-Lipkowska, D. A. Jahn, J. S. Frye, *J. Am. Chem. Soc.* **108**, 5871 (1986); M. Simard, D. Su, J. D. Wuest, *ibid.* **113**, 4696 (1991).

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Reproducible Imaging and Dissection of Plasmid DNA Under Liquid with the Atomic Force Microscope

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Reproducible images of uncoated DNA in the atomic force microscope (AFM) have been obtained by imaging plasmid DNA on mica in *n*-propanol. Specially sharpened AFM tips give images with reproducible features several nanometers in size along the DNA. Plasmids can be dissected in propanol by increasing the force applied by the AFM tip at selected locations.

Scanning probe microscopes such as the AFM (*1*, *2*) can be used at near-ambient conditions and can yield even atomic resolution on some surfaces (*3*). If this high resolution can be obtained on DNA there could be many benefits, including the potential for sequencing DNA. Until now, however, high-resolution AFM images of DNA have been difficult to reproduce.

Recently Vesenka *et al.* (*4*) and Bustamante *et al.* (*5*) developed a method for anchoring and imaging plasmids that gives reproducible images with mean apparent plasmid widths on the order of 10 to 15 nm. We report here an improvement of this technique that shows reproducible structure along the DNA strands and can resolve detail that is in some cases the size of the double helix. This method may have applications in diverse fields ranging from protein–nucleic acid interactions to chromosome mapping.

Double-stranded plasmids [BlueScript II from Stratagene, La Jolla, California and pSK 31, a gift from W. Rees at the University of Oregon, Eugene, Oregon (*5*)] were attached to mica treated with magnesium acetate. This method (*6*) builds on earlier methods for imaging DNA on mica in the electron microscope (*7*, *8*). DNA on mica was stored over desiccant and then imaged under *n*-propanol in a Nanoscope II AFM (*9*) at constant force mode by using narrow (120- or 200-μm) silicon nitride cantilevers with integrated tips (*10*). The scan speed was typically 9 Hz, or 1 min per image. Good DNA images were easily obtained, although it was sometimes a challenge to get a plasmid distribution over the sample that was neither too sparse nor too dense.

Imaging plasmids under propanol (Fig. 1, A to E) gives better resolution of detail along the strands and narrower apparent widths than imaging in air (Fig. 1F). Propanol was chosen as a medium for imaging based on previous results in air and ethanol. Imaging forces in air are typically on the order of ten times greater than imaging forces in liquids such as ethanol (*11*, *12*). Therefore it is desirable to image DNA under liquid to obtain images under the most gentle conditions. Ethanol had been

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