Synthetic Self-Replicating Molecules

Molecules crafted in the laboratory can make copies of themselves, "mutate," compete for resources and assemble, giving a paradigm for life

by Julius Rebek, Jr.

Imagine a molecule that likes its own shape: finding a copy of itself, it will fit neatly with its twin, forming for a while a complete entity. If the original molecule is presented with the component parts of itself, it will assemble these into additional replicas. The process will continue as long as the supply of components lasts.

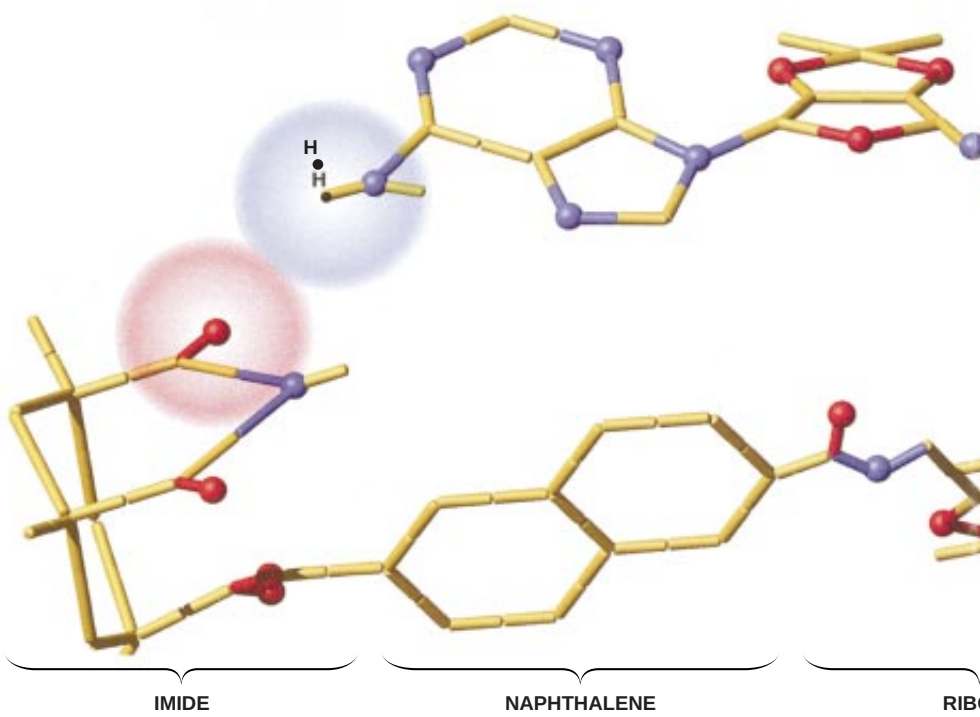
My colleagues and I at the Massachusetts Institute of Technology have designed such self-assembling molecules and crafted them in the laboratory. Our efforts are intended to illuminate the ways in which life might have arisen. Probably it began when molecules came into existence that were capable of reproducing themselves. Our organic molecules, although they operate outside of living systems, help to elucidate some of the essential principles of self-replication.

Attempts to imitate life are still very young compared to the beginnings of life on the earth perhaps three and a half billion years ago. No one can say for sure what the atmospheric or terrestrial conditions were at the time or which molecule crossed the critical frontier between organic chemistry and biology.

In 1953 Stanley L. Miller, then at the University of Chicago, made one of the first—if not the first—attempts to recreate this transition. He formed amino acids in a mixture of water, methane, ammonia and hydrogen—substances thought to have been present on the primitive earth—by subjecting the chemicals to an electric discharge. James D. Watson and Francis Crick's unraveling of the structure of DNA—also in 1953—further fueled this quest.

For some decades now, the most widely accepted recipe for the origin of life has specified DNA or RNA in lukewarm water, neither acidic nor alkaline, plus only those reagents that were presumably part of the primitive earth's atmosphere. Practitioners of prebiotic chemistry such as Miller and Leslie E. Orgel of the Salk Institute for Biological Studies in San Diego have provided deep insights into molecular replication under these constraints. Indeed, countless metric tons of DNA replicate every day in living creatures in precisely this manner, under the surveillance of a host of enzymes.

But recent findings indicate that the primitive earth was perhaps not quite as hospitable as the "warm pond" paradigm would have [see "In the Beginning…," by John Horgan; SCIENTIFIC AMERICAN, February 1991]. Such suspicions, coupled with discoveries of organisms living at (literally) blood-curdling temperatures or near sulfurous volcanic vents at the bottom of the ocean, have led scientists to suggest that life possibly arose by some very different route. Perhaps the only qualities essential to the molecules that become live are detailed in two remark-



ARNI, a self-complementary molecule (*bottom*), gathers the components it needs—an adenine ribose (*top left*) and a naphthalene imide (*top right*)—to replicate. Blue balls indicate nitrogen atoms and red balls, oxygen. Halos surround atoms involved in hydrogen bonding.

able books by Richard Dawkins, *The Blind Watchmaker* and *The Selfish Gene.* Although written 15 years ago, Dawkins's sketches astonishingly prefigure the results of the past four years of my work on self-replication.

Molecules, natural or synthetic, are able to replicate when their shapes and chemistry have a feature called complementarity. By virtue of the way a molecule occupies space and the way its attracting atoms or groups of atoms are distributed along its arms, one molecule may fit snugly into the chemical nooks and crannies of another. The "goodness of fit" between two such complementary molecules thus depends not only on their spatial structure but also on the different kinds of chemical bonds that hold them together in groups. Such groups, or "complexes," form and dissipate rapidly in microseconds or nanoseconds—times that are very short, yet long enough for chemical reactions to take place.

The forces holding complexes together are many times weaker than the covalent bonds binding atoms into molecules. One kind of force—important in complexes—is called a hydrogen bond. This bond comes about when a hydrogen atom possessing a partial positive charge is attracted to, for instance, an oxygen atom that has a partial negative charge. More general attractions of this class go by the name of polar interactions.

Another kind of force, the van der Waals force, is more subtle: if correctly positioned, electrons of one molecule can jostle away those of another, creating a charge imbalance that results in attraction. Yet a third kind of attraction is "aromatic stacking"—an arrangement that flat organic molecules (often having a pleasant odor; hence the name) sometimes assume when they do not like the solvent they find themselves in. By sidling up to one another, flat surface to flat surface, they can squeeze out all the solvent molecules between them and achieve a more stable, stacked configuration.

Once a complex forms, the molecular surfaces that match up with one another are relatively protected. Destructive solvents, dissolved acids, bases or oxidants cannot get to them. Strong covalent bonds then have time to join the complementary parts. Sometimes two of three molecules in a complex link together; the third merely serves to ease the process.

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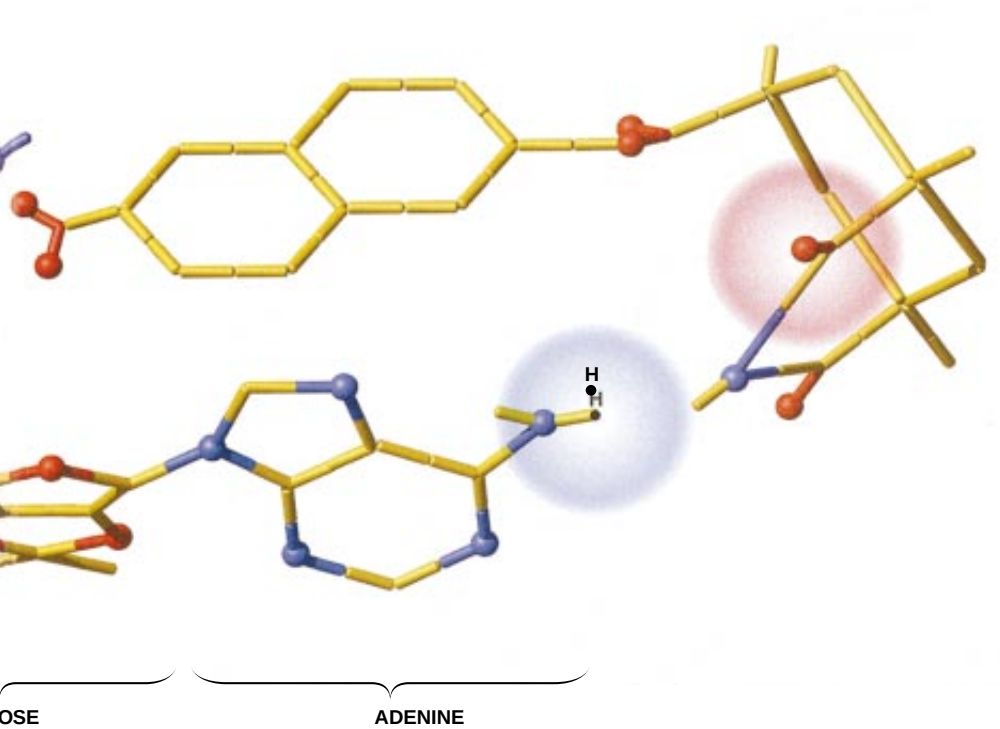
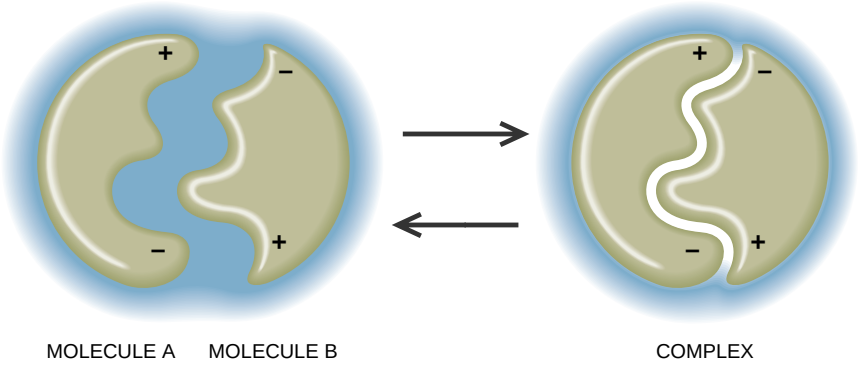
Such a coupling gives rise to a rather popular scheme for replication—the one preferred by DNA. A simple depiction of this scheme uses concave and convex shapes. A concave molecular surface—lined with appropriately enticing atoms—can recognize and surround its convex complement. Further, it can act as a mold for assembling the convex molecule from its component parts. In turn, the convex molecule serves as a template for gathering and fusing the component parts of the concave one. These two replication events—each molecule forming the other—establish what is called a bi-cycle [*see bottom illustration on next page*]. Our recent experiments indicate that a bi-cycle can be extremely efficient.

There is an alternative paradigm of replication: two complementary molecules in a complex can join at some site that is not on the recognition surface. They form a single molecule, one end of which is complementary to the other—and the whole is complementary to itself [*see bottom illustration on page 51*]. The recognition surfaces at the ends of this new, self-complementary molecule are still accessible to other molecules. The ends can each gather a fragment identical to that at the other end.

Once gathered, the two new components cannot move freely and travel through space in tandem; the chances of their becoming linked to each other are greatly enhanced. Thus, the self-complementary entity makes a copy—and in similar manner, many copies—of itself. No enzymes are needed: the molecule catalyzes its own formation.

This is the method we have used in the laboratory to make molecules capable of reacting with one another in ways reminiscent of life. Among them are molecules that bear a passing resemblance to genetic materials—specifically, to nucleic acid components known as adenines. Adenines are flat; besides, they have hydrogen and nitrogen atoms that can form hydrogen bonds with the oxygen and hydrogen atoms of their complementary molecules, called imides. Our imides are con-





MOLECULAR RECOGNITION occurs when two fragments whose geometric and chemical properties complement one another form a complex. The + and – signs indicate electrostatic attractions. Moreover, the solvent is squeezed out between the molecules, helping to stabilize the short-lived complex.

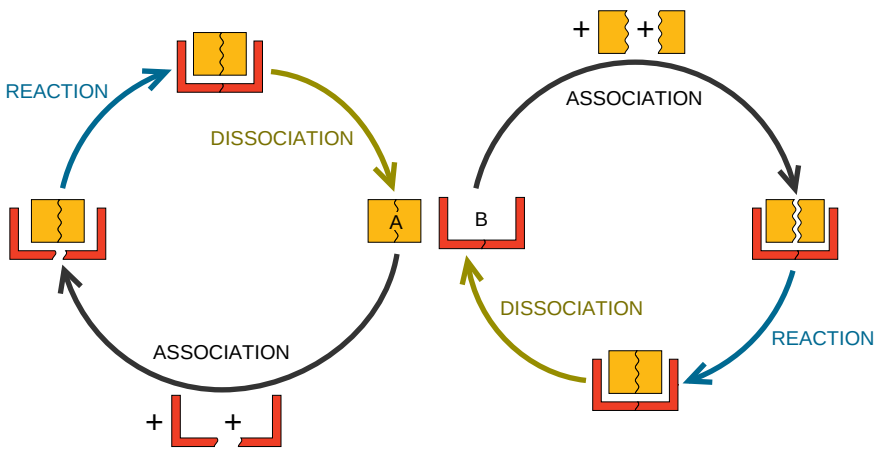
structed from a humpbacked molecule, Kemp's triacid, the skeleton of which folds over in such a way that large, concave structures can easily be fashioned from it. So the imide features a hydrogen bonding site crookedly attached to an aromatic stacking surface; these fit perfectly with the hydrogen bonding site and the flat stacking surface of adenine.

When associated together in a complex, the adenine and the imide become covalently attached, forming a self-complementary molecule. Our early attempts to get this molecule to self-replicate were thwarted by its unforeseen floppiness. Although some flexibility is helpful for molecular recognition—a leather boot is easier to slip on than a wooden one—a lot of flexibility can make fitting very difficult—try slipping on a sock without using your hands.

Molecules become floppy if they have single bonds, involving only two electrons each. Such a bond allows the parts it joins to rotate with respect to each other, giving rise to many different shapes. When Tjama Tjivikua, my graduate student from Namibia, linked the adenine to the imide by a covalent bond, he had to work with a chain of carbon atoms. The chain was so long and flexible that the resulting self-complementary structure doubled over on itself, rather like a jackknife folding shut. So snugly did the adenine fit into the imide that the self-satisfied molecule no longer associated with other molecules or replicated.

Happily, this situation was curable. The remedy called for inserting a larger and more rigid molecule in place of the single chain to prevent folding. Our choice was a larger stacking surface, a naphthalene, bolstered by a less flexible link between the two components, a cyclic ribose group.

This new J-shaped molecule, adenine ribose naphthalene imide (ARNI for short), provided us with our first instance of replication. Using high-performance liquid chromatography to detect minute changes in chemical concentrations, Tjivikua and Pablo Ballester, a postdoctoral visitor from Majorca, achieved the result. They compared the rate of formation of ARNI in a solution that contained only its components with the rate of formation when some ARNI was added. The presence of ARNI increased the rate of formation, clear evidence of the presence of a self-replicating system.



REPLICATION BI-CYCLE involves two molecules of complementary shapes, represented by block A and sleeve B, into which it fits. In the left cycle, the block (*middle*) collects the two parts of the sleeve (*bottom*) around it to form a complex (*left*); the parts then react to form a whole sleeve (*top*). The block and sleeve quickly dissociate. In the right cycle, it is the sleeve that assembles the fragments of the block. Thus, the two complementary molecules catalyze each other's formation.

If one plots the progress of a reaction through time, one generally derives a curve that assumes the shape of a reclining parabola. The product forms fastest at the beginning, when the reactants are at their highest concentrations; the rate of formation slows down as the reactants are consumed. For an autocatalytic reaction—one in which the product, like our ARNI, catalyzes its own formation—the growth curve should be S-shaped, or "sigmoidal" [*see top illustration on opposite page*]. The reaction begins slowly. As the product appears and begins to act as a catalyst, the reaction accelerates. An upward curve results. Finally, as the materials are consumed, the reaction grinds to a halt.

The degree of sigmoidal curvature depends on several factors, the most important of which is the efficiency of the autocatalytic step. If the background reaction—in which the components combine by themselves, without getting help from the self-replicating molecule—is too strong, it can swamp the signal from the self-replicating process. In 1990 Günther von Kiedrowski and his co-workers in Göttingen showed that a self-replicating nucleic acid could exhibit such sigmoidal growth—proving that the autocatalyzed synthesis is in this case more efficient than the random one.

Although ARNI did not show sigmoidal growth, our next attempt, ARBI, did. We slowed the background reaction rates by giving ARBI a slightly longer stacking element, a biphenyl instead of naphthalene. We now had proof of a bona fide synthetic self-replicating molecule.

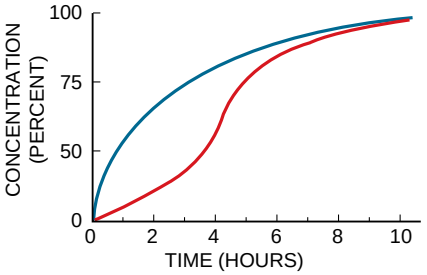
Is it alive? Not by most current defi-

nitions. Our (or its) critics were quick to point out that as a life-form, ARBI had severe limitations: the molecule would make copies only of itself. To allow evolution, a self-replicating molecule has to be capable of "making mistakes": occasionally synthesizing other molecules that can perhaps be better replicators. Unlike art and music critics, those in science at least indicate in which direction improvements may lie. We responded by devising molecules that were capable of making—indeed, that were incapable of not making—mistakes.

In organic chemistry, a "mistake" is made when there is a lack of selectivity between reaction partners. We needed a molecule that would catalyze not only its own formation but also that of a molecule of similar shape. Besides, at least one of these two molecules had to be able to change into a more efficient replicator.

A molecule can readily be manipulated into making replicas of a competitor. Instilling the capacity to "evolve" takes more planning. In the course of our search for a solution, we tapped into some earlier findings on the hydrogen bonding sites of adenine. There are two ways in which an imide can attach to an adenine. It can find a site along the Watson-Crick edge, which is involved in the replication of DNA, or it can dock along the Hoogsteen edge, a region normally exposed in DNA (though sometimes joined in such exotic forms as triple helices).

We had already shown that simple adenines can attach to our imides along either edge. For example, roughly equal amounts of Watson-Crick and Hoogsteen complexes are formed with an imide attached to a naphthalene surface. But if one of the hydrogens of the amino (NH_2) group of adenine—involved in hydrogen bonding—is replaced by a larger group of molecules, the situation changes. This new group positions itself in such a way as to block access to the Watson-Crick edge while leaving the Hoogsteen edge largely open. For example, when a small methyl group joins the adenine, more than 85 percent of our synthetic imide receptors are found to bind along the Hoogsteen edge.



S CURVE (*red*) is the signature of an autocatalytic reaction when the concentration of the reaction product is plotted against time. At first, the molecule forms slowly. As it catalyzes its own synthesis, the reaction suddenly accelerates, only to slow down as the reactants are consumed. Conventional reactions (*blue*) simply have a parabolic shape.

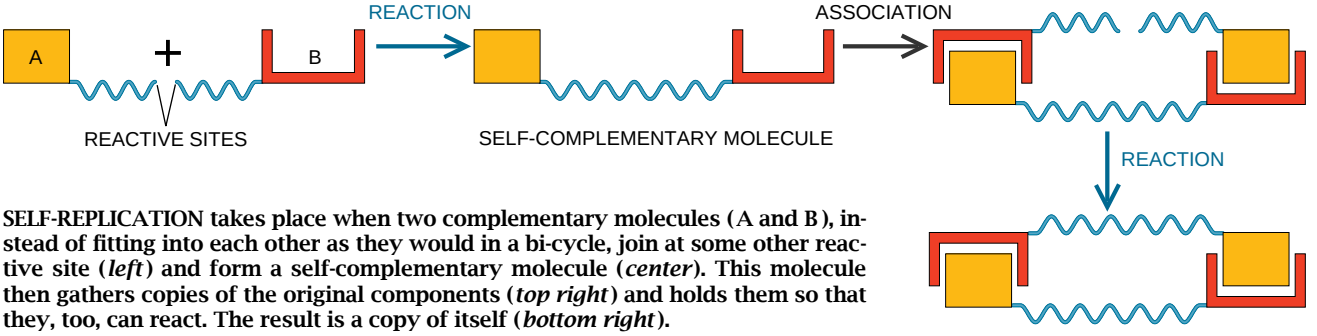
We decided to exploit the change in the rate of replication that comes from blocking the Watson-Crick edge. Accordingly, we prepared two different adenines, one bearing a benzyloxycarbonyl, or "Z," group (a popular blocking group in protein synthesis) and another bearing a Z group with an additional nitrogen group: Z-NO_2. The plan was this: an altered adenine and the imide would assemble on the product template as before. But the blocking groups would be dangling off the molecule in sites quite far from where the covalent bond forms. The Z group at one end would not know if the blocking group at the other end was a Z or Z-NO_2; synthesis should take place regardless of the groups' identities.

Moreover, blockade of the Watson-Crick edge would limit hydrogen bonding to the Hoogsteen edge. Thus, the modified replicators ZARBI and ZNARBI—made from adenines containing Z and Z-NO_2, respectively—would have to replicate slowly. At this point, the choice of blocking group becomes critical. Although many attached groups can lead to the formation of replicators that make mistakes, the nitro group (NO_2) is somewhat special. Investigators have known for 30 years that they can remove the group easily by irradiating it with particular wavelengths of ultraviolet light. Once the Z-NO_2 is excised, the Watson-Crick edge is freed. The new, lighter molecule can now match up along this edge as well as the Hoogsteen edge, making it, we hoped, twice as efficient as the molecules from which it is derived.

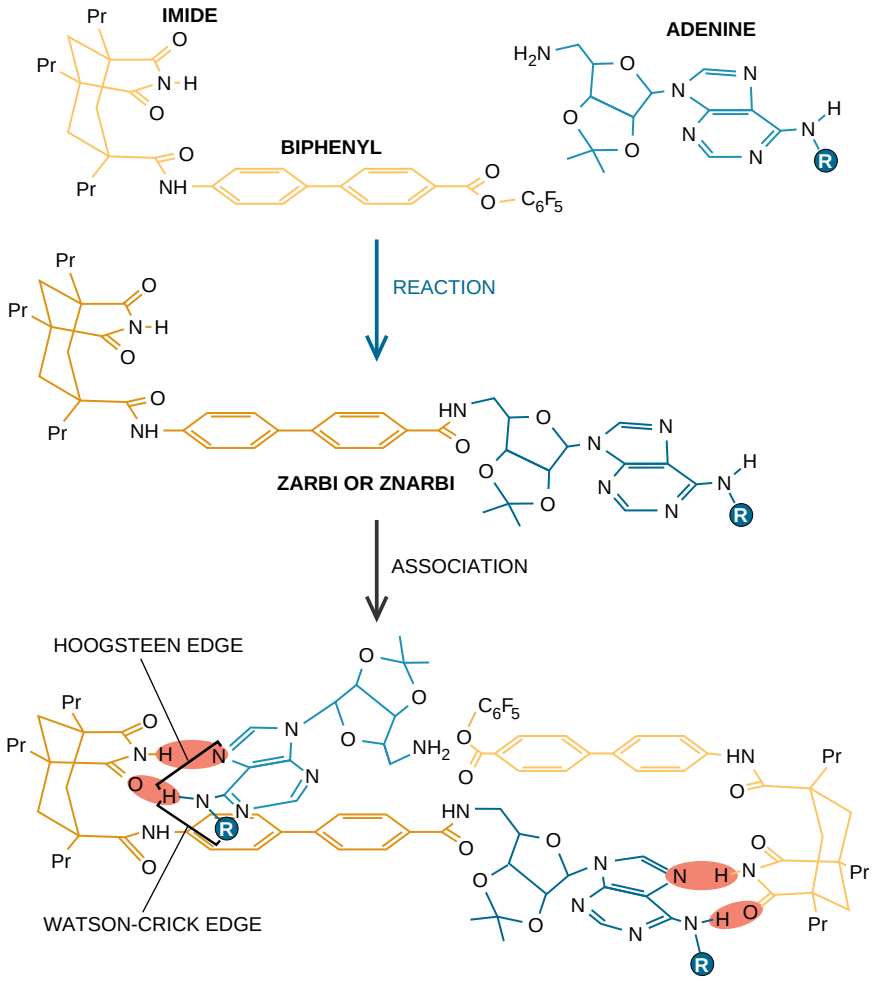
We subjected our adenines bearing Z or Z-NO_2 groups to our now standard tests for self-replication. When the adenines joined with the biphenyl receptors to produce ZARBI and ZNARBI, the new molecules did indeed behave as replicators—though admittedly not very efficient ones. The replicators compensated for their clumsiness with their ability to make mistakes. ZARBI would catalyze its own formation and also act as a template for assembling its rival, ZNARBI. The latter reciprocated, catalyzing its own formation and also that of ZARBI.

Now came the challenge of demonstrating a chemical version of mutation: a permanent, heritable structural change affecting the survival capacity of an organism—or in our case its analogue, a self-replicating molecule. Changes in a molecular replicator's structure might be caused by shifts in temperature, acidity, salinity or many other factors. We chose irradiation with light.

We first placed ZARBI and ZNARBI in direct competition for reproductive resources. Qing Feng, then my graduate student, and Jong-In Hong, a postdoctoral fellow, allowed the adenine derivatives bearing the Z and Z-NO_2 groups to compete for a limited amount of the complementary biphenyl receptor.



SELF-REPLICATION takes place when two complementary molecules (A and B), instead of fitting into each other as they would in a bi-cycle, join at some other reactive site (*left*) and form a self-complementary molecule (*center*). This molecule then gathers copies of the original components (*top right*) and holds them so that they, too, can react. The result is a copy of itself (*bottom right*).



"MUTANT MOLECULES" are produced when an adenine with an extra group R joins with a biphenyl imide to form a self-complementary molecule. The R can be a Z (benzyloxycarbonyl) or a Z-NO_2 group, giving, respectively, a ZARBI or a ZNARBI molecule. The latter can collect an adenine along the Hoogsteen edge (the R blocks the Watson-Crick edge) and fuse it with a biphenyl imide, catalyzing its own formation and also that of its competitor. The red ovals indicate hydrogen bonding.

ZNARBI proved to be a slightly better replicator. When all the receptor was used up, we irradiated the reaction vessel with ultraviolet light having a wavelength of 350 nanometers. After a few hours of irradiation, the Z-NO_2 blocking groups had all been removed from both the ZNARBI replicators as well as from the adenine progenitors. That is, the ZNARBI molecules had all been converted to ARBIs and the Z-NO_2-bearing adenines to plain adenines. A "mutation" had occurred, prompted by a change in the environment. ZARBI and the Z-adenine remained unaltered.

Next we added more biphenyl receptor. ZARBI found the radiation product, ARBI, to be its competitor. The sleeker ARBI, having the advantage of replicating in either the Hoogsteen or the Watson-Crick mode, rapidly took over the resources of the system.

A simple evolutionary interpretation can be sketched for this experiment. Think of ZARBI as the original in this sequence; its replication requires the presence of Z-adenine and the biphenyl receptor. If nitric acid is added, then some of the Z-adenines are used up to form Z-NO_2 adenines; the latter give rise to ZNARBIs. ZNARBI is a better replicator compared with its ancestor ZARBI. When the system is irradiated, a second change takes place. ZNARBI converts to the simpler and more efficient ARBI. This last molecule proves to be the best replicator of the three.

Although mutation is considered to be the driving force for most evolutionary alterations, another significant paradigm for change is recombination. Two chromosomes can split, exchange strings of DNA and rejoin, thus combining their characteristics. Also, certain computer programs attempt to "teach" strings of information to solve a problem. If the strings are allowed to split and recombine at random, they soon give rise to much better problem solvers. Mutation allows for single, small changes; recombination, on the other hand, allows the creation of hybrids that are very different from the progenitors.

Our interest in demonstrating recombination at the molecular level led us to develop an entirely new set of self-replicating molecules. The principle was the same: two complementary molecules were joined by a covalent bond to give a single, self-complementary whole that could aid its own synthesis. Feng and another student, Tae Kwo Park, devised a replicating system based on a different component of nucleic acids, thymine. Some time earlier Park had developed a synthetic receptor that would recognize thymine's imide nucleus and also lie on thymine's flat aromatic surface. This receptor featured a U-shaped molecular skeleton. The bottom of the U was a large, rigid aromatic spacer known as xanthene; one arm of the U featured an amine and the other arm a diaminotriazine, the receptor for thymine. When the latter two became joined by a covalent bond, a self-complementary unit was generated, called diaminotriazine xanthene thymine, or DIXT. We were able to show that DIXT was also self-replicating.

The stage was now set for a recombination experiment. Could the adenine-based replicators and the thymine-based replicators, when placed in the same vessel, shuffle their components into new combinations? They did indeed. Even so, we were surprised by the results. One of the new recombinants, ART (adenine ribose thymine), was the most prolific replicator we had yet encountered, whereas the other one, DIXBI (diaminotriazine xanthene biphenyl imide), was unable to replicate at all—it was "sterile."

How did this difference in the ability to replicate come about? The efficiency of the ART replicator is easily rationalized. ART looks a good deal like a piece of DNA, possibly the best replicator in existence. Furthermore, its ribose piece makes the recognition surfaces parallel to one another, a very helpful configuration. This and the high affinity of adenine for its complement thymine make for an easily assembled complex—the intermediate stage in replication.

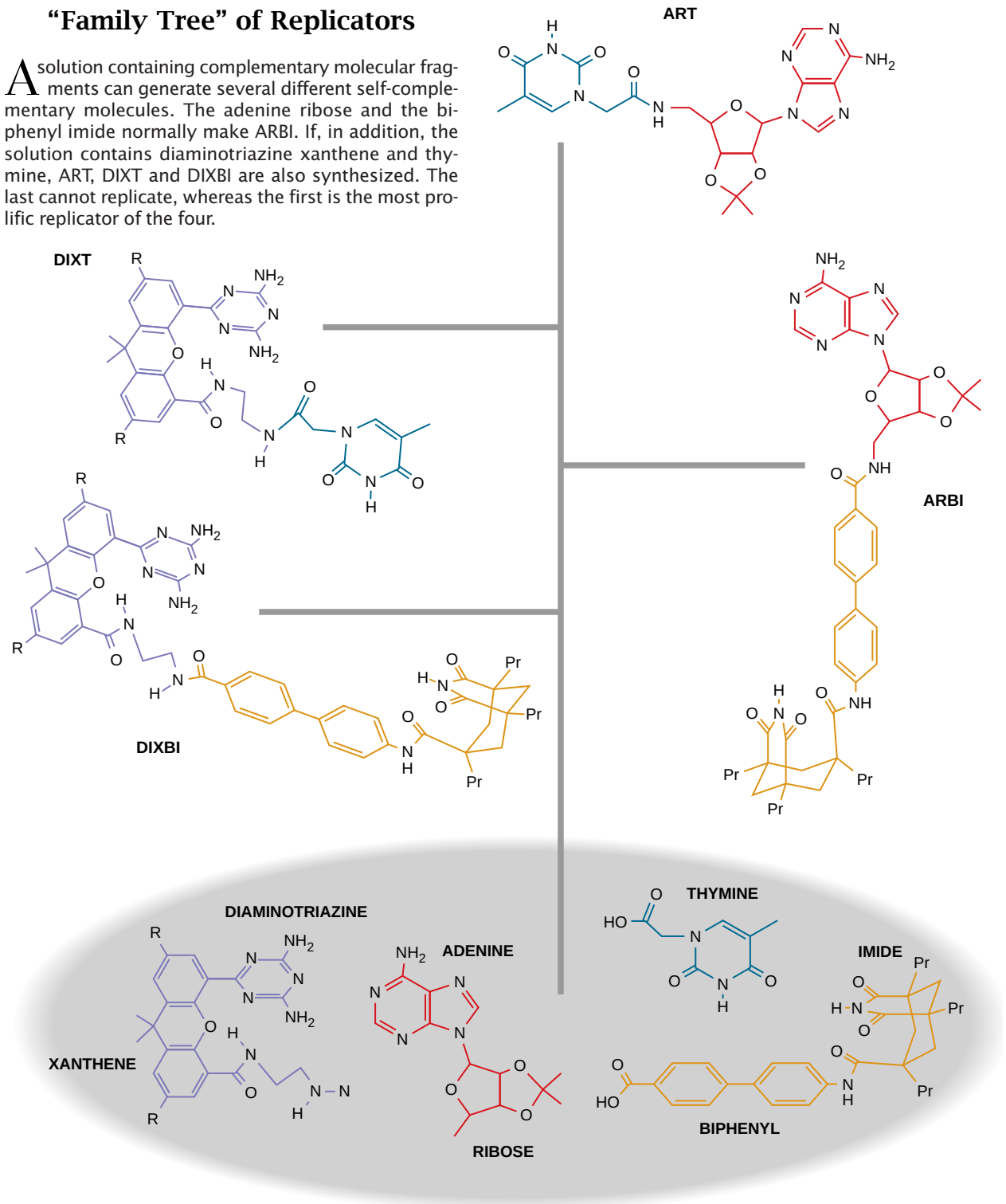
The inefficiency of DIXBI can also be traced to its overall molecular shape.

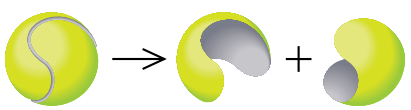
DIXBI is composed of two U-shaped molecules connected by a rigid biphenyl spacer; its overall structure can adopt a C or an S shape. The recognition surfaces are exposed inside the C shape, where there is not enough room for a replicating complex to form. In the S shape the recognition surfaces are far apart, so that when a complex forms, the reactive pieces are too far away from one another to bond covalently. Thus, even though DIXBI is self-complementary, it cannot achieve replication.

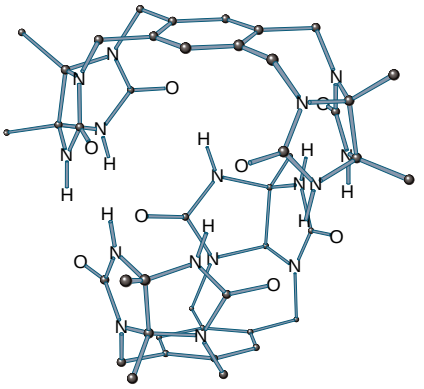
With this experiment we were able to show that a relatively small pool of components can give rise to a "family tree" of replicators. Three of these are effective at self-replication, but one branch of the tree dies out. To push this analogy further, it would be appropriate for the sterile molecule to be

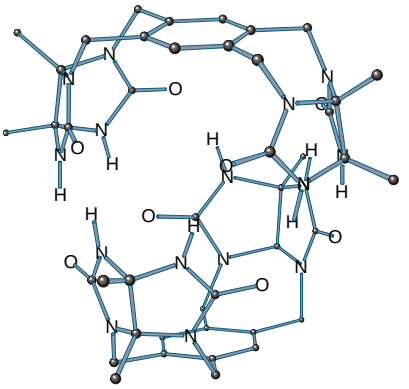
"Family Tree" of Replicators

A solution containing complementary molecular fragments can generate several different self-complementary molecules. The adenine ribose and the biphenyl imide normally make ARBI. If, in addition, the solution contains diaminotriazine xanthene and thymine, ART, DIXT and DIXBI are also synthesized. The last cannot replicate, whereas the first is the most prolific replicator of the four.









TENNIS BALL cut along its seam yields two self-complementary shapes that inspire a design for a cell wall. To the right is a molecule that can assemble with its twin into a hollow sphere. The drawings are stereoscopic; if you can cross your eyes enough to overlap the images, you can see a three-dimensional molecule.

chopped up and converted into pieces that the effective replicators could use for themselves. We have made some progress in this direction. It requires equipping our molecules with acids and bases that can manipulate other molecules more actively than simple recognition will allow.

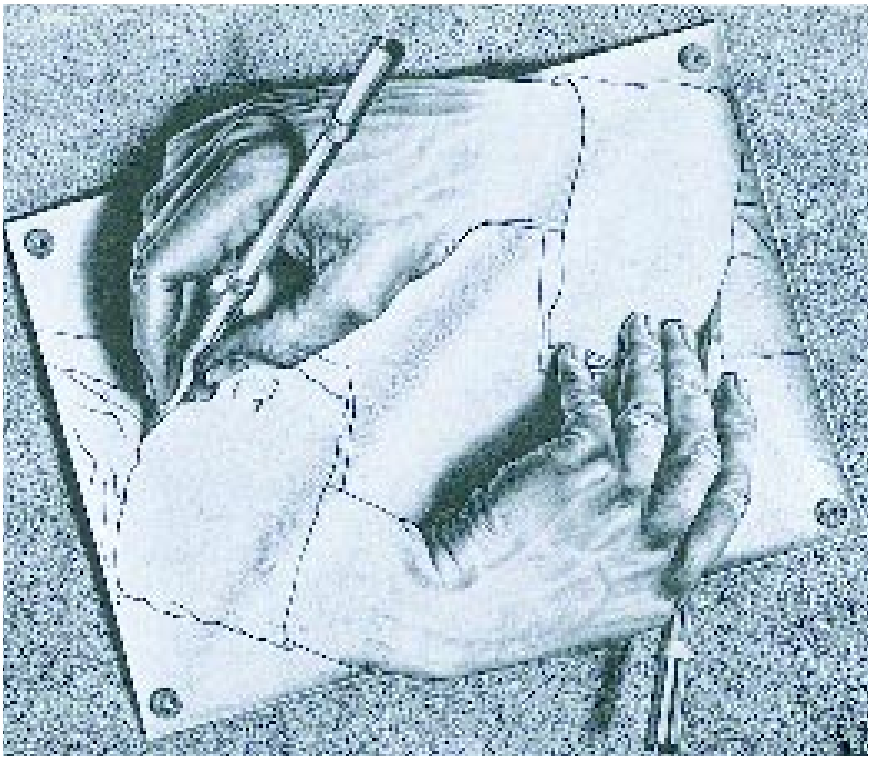
Although it has been enjoyable to pursue replication and even evolution with synthetic molecules, we have been looking to the next step in expressing life as a series of molecular reactions. We feel, as do other workers, that a key attribute of life is a boundary: a container or a cell wall that separates inside from outside and prevents desirable molecules from floating away—while keeping undesirable ones at bay.

Inspired by a naturally occurring membrane, we have made some small, initial steps toward this goal. Viruses use a protein shell as a container; the shell is made up of many identical copies of a single protein unit. The units are also self-complementary—but the recognition surfaces are oriented so that they automatically assemble into a closed shell. Indeed, Crick had predicted that many identical copies of proteins would compose the viral coat, since there is not enough information in the viral genome for many different molecules to be involved.

When we used self-complementarity as our guide, a minimalist design struck us, based on the structure of a tennis ball. Cut along its seam, a tennis ball gives two identical pieces, the convex ends of which are complementary in shape to the concave middles. René Wyler, a Swiss postdoctoral fellow, has now synthesized a structure that mimics the shape of the tennis-ball pieces, while adding chemical complementarity. The units fit together with hydrogen bonds along the seam.

There are good indications that a smaller molecule, such as a solvent chloroform molecule, can fit within our molecular tennis ball. But it is too small to accommodate even our most minimal replicators. We are now working with Javier de Mendoza of the Free University of Madrid to develop a larger molecule—a softball—that may have an interior roomy enough to hold some of our replicators.

Once we have made it past the problem of containment, the biggest obstacle to the molecular life agenda will be these questions: How can our fledgling organism harness energy? From sunlight or from other molecules? How can the component pieces of the replicators and their containers be replenished? These are the challenges of the next decade. Whether they are met or not, the efforts of chemists to answer them will surely provide insight into the organic chemistry of life—how it came about and how it continues to flourish.



***DRAWING HANDS,* a 1948 lithograph by M. C. Escher, illustrates the principles of self-complementarity and self-replication.**

FURTHER READING

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