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Synthesis as a Route to Knowledge

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Abstract A science is an intellectual activity defined by its mechanisms that prevent its scientists from always reaching the conclusions that they set out to reach. Such mechanisms are needed because, if scientists are given full control over what hypotheses they select, what data they discard, and what results they publish, they can communicate any conclusion that they desire. Synthesis, by setting a grand challenge, forces scientists across uncharted territory where they encounter and solve unscripted problems. When theory is inadequate, the synthesis fails, and fails in a way that cannot be ignored. Therefore, synthesis drives discovery and paradigm change in ways that simple hypothesis testing cannot. Here, we describe the discoveries that emerged when synthetic biologists were challenged to create an alternative genetic system that has different molecular structures than DNA and RNA. In pursuit of this particular "grand challenge," synthesis forced the recognition that the community did not know as much about the double helix as it had constructively assumed. In general, a field of science having access to synthesis as a research strategy can create knowledge even if its practitioners do not fit the ideal of a dispassionate, advocacy-free scientist.

Keywords Advocacy · DNA · Double helix · Knowledge · Neutralism · Non-classical carbocations · RNA · Selectionism · Synthesis · Synthetic biology

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The Scientific Enterprise Clearly Delivers the Empowerment that One Expects from Knowledge

Discussions by members of the community of thinkers who call themselves "philosophers of science" often ask what knowledge is, and how scientific communities create it (Cover and Curd 1998). Over the past century, these discussions have been broad in scope, including attempts to formally describe the process by which knowledge is developed, as well as denials that knowledge exists at all. In between, the discussions often emphasize the sociological, political, and irrational aspects of the scientific enterprise (Kuhn 1962; Feyerabend 1975).

These discussions have served to show how difficult it is to find rules and criteria that capture the essence of "the scientific process" and distinguish "science" from other activities (Suppé 1977). However, simply denying the existence of something special in the scientific enterprise provides no easy way out. Those who do so simply trade one problem for another, struggling to account for the fact that scientists produce something that is empowering, at least in the material and manipulative senses of this term. Similar empowerment seems not to emerge from law, advertising, and politics (to choose other intellectual activities).

Whatever science is, it certainly produces the empowerment that is expected to be a feature of "knowledge." A few examples illustrate this. From physics, empowerment is illustrated by nuclear power plants, spacecraft that land on the Moon, and television sets. Empowerment from chemistry is illustrated by colorful fabrics, medicines that cure diseases, and Polaroid photography. Empowerment from biology is illustrated by its identification of genes that cause cancer, viruses that cause AIDS, and vaccines. Indeed, biological research has empowered the human

species almost to the point where we might soon be relieved of the limitations of the Darwinian evolution that is responsible for our existence. We are not far from being empowered to correct genetic diseases before they occur, without needing to watch our children die as the only natural way to remove defective genes from our gene pool.

We may not agree that these sciences produce "knowledge." We may find ourselves unable to define "knowledge" to the satisfaction of our philosopher colleagues. Nevertheless, we must agree that science has produced something that behaves as knowledge is expected to behave. Whatever knowledge is, it should confer the ability to manipulate and control upon those who possess it, and that control should be transferable to other practitioners. Physics, chemistry, and biology seem to have done just that.

But how?

Practicing Scientists and Their Training

Scientists themselves largely ignore philosophical discussions about their professional activities as they go about their business of creating "that which behaves like knowledge." However, these discussions must, at least occasionally, percolate into the education of scientists. After all, to be successful, future scientists will need intellectual skills far beyond what is taught in middle school as "the" scientific method. If the education of scientists about "method" stopped here, scientists would all think that knowledge is the simple outcome of making observations, formulating explanatory hypotheses, and testing those hypotheses through deftly constructed experiments.[1] In fact, this process rarely leads to the discoveries and paradigm changes that we value most as the product of scientific activity.

At the very least, this education must teach another fact: if a scientist is given full discretionary control over which observations to remark (and which to ignore), which hypotheses to formulate (and which to neglect), which data to consider (and which to discard), and which results to publish (and which to hold private), she or he can arrive at *any* conclusion (Benner et al. 2013). Indeed, this is what advocates do in other fields. Lawyers present only the data that show the client to be innocent. The marketer mentions only the features of the product that make it a desirable purchase. The politician's argument makes clear that he or she is the only worthy candidate. As Feyerabend (1975) was fond of remarking, science as a community activity is based on rhetoric. The successful scientists are the ones who persuade the community. The persuasive tools used by advocates in general are usually just as effective on human scientists as on humans in general.

Science cannot, however, be associated with a requirement to forbid advocacy. At the very least, scientists must advocate when they want their peers to "fund my grant application" or "hire my graduate student." It is, however, a small step from these (presumably innocuous) exercises in advocacy to the desire to advocate that the community "accept my theory." And it is easy to "believe one's own marketing." Deeply rooted in advocacy is the transformation of an advocate aware of what she or he is doing to a true believer. A committed advocate finds it very hard to step back from advocacy to discover that the advocacy is wrong, no matter how wrong it is.

Therefore, when we train scientists, we often mention aphorisms that suggest principles that might help our students avoid being captured by their own advocacy. The physicist Richard Feynman, for example, cautioned that, "The first principle is that you must not fool yourself—and you are the easiest person to fool" (Feynman 1974). Indeed, Popper remains pervasive in science education in part because his demarcation criterion (a scientific proposition is one that is falsifiable) easily morphs into a prescription (actively work to falsify your favorite hypothesis). This prescription is intended to temper scientists' desire to prove (as least to their peers) that they are right (and, even better, that they are right because they are better scientists, smarter individuals, or cleverer human beings).

But aphorisms and a culture that values self-falsification is clearly inadequate to prevent scientists from arriving at conclusions they wish to advocate. The literature is replete with examples where scientists, having become advocates for their theory, lose all ability to use whatever power "scientific method" provides to discover "knowledge."

Indeed, many scientific disputes easily develop to appear more like disputes in law, marketing, and politics than the disinterested search for truth that we teach as an ideal. For example, for over a half century, organic chemists disputed whether a simple organic molecule (the "norbornyl cation," Fig. 1) is correctly described as a molecule having classical rules of bonding (four bonds to each carbon atom, one bond to each hydrogen atom), where each bond placed two electrons between two atoms, or whether the molecule is correctly described by a model where two electrons might be distributed between three atoms in a "non-classical" bonding arrangement (Scholz et al. 2013). For a half-century, dueling schools published advocacy papers, each selecting data to support the side that they advocated. In population biology, the dispute

[1] *Pace*, much of the peer review of applications seeking funds to support scientific research appears to accept this thought. For example, proposals to the US National Science Foundation are routinely declined because the work proposed is not "hypothesis based."

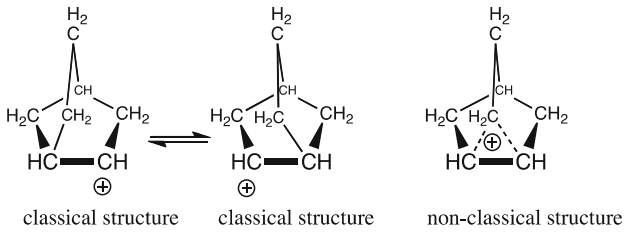

Fig. 1 A half century of advocacy characterized two schools of chemistry, one that insisted that the "norbornyl cation" ($C_7H_{11}^+$) was better described as two species having classical bonding (on the *left*, joined by the *arrows*), and the other insisting that it was better described as a single species with non-classical bonding, indicated by *dotted lines* (Scholz et al. 2013)

between "neutralists" and "selectionists" had the same character (Hey 1999).

Each Science Needs a Field-Appropriate Way to Prevent Its Scientists from Always Reaching the Conclusion That They Set Out to Reach

Clearly, scientists must have discretion over what observations to record; observations are simply too many to do otherwise. Further, no matter how they are trained, scientists (like humans) will always filter observations through a series of expectations that are governed by a "world view." The only observations that need explanations are those that are unexpected under that view.

Likewise, scientists must have discretion over what hypotheses to test, what system to test them on, and what data to credit. Much of the intuition that drives science is based on these choices. Further, as Duhem and Quine pointed out (Quine 1953), data that contradict expectations based on a hypothesis need not falsify that hypothesis. Sometimes, instruments *are* defective. Some reagents *are* contaminated. As a result, no scientist who follows without discretion the "Popperian script" can hope to do any but trivial science. They must use their intuition about which data are falsifying, and which can be justifiably managed through some ad hoc auxiliary hypothesis.

The juxtaposition of the need to be selective in observation, hypothesis formulation, data presentation, and publication presents a conundrum for even "ideal scientists," fictional entities who consciously attempt to discredit their favorite hypotheses, who balance each slip in the direction of advocacy by an experiment designed to undo decades of their own work, and who include in their grant applications phrases like "we really may be wrong about all of this" and "you should also consider funding our competitors." At the very least, the bias to publish experiments that work over experiments that fail will generate a literature that gives the impression that theory in a field is more successful than it really is. And the advocacy model of science, where each school of scientists acts like lawyers, cherry picking data that support their theory, cannot not work in a world that has no judge-enforced rules of procedure, and no carefully selected juries that serve as the final arbiters of fact.

Rather, each field of science needs some other method to prevent scientists from always reaching the conclusions that they set out to reach. Indeed, some have defined science as an intellectual enterprise that has field-appropriate procedures to do this (Benner 2009). Here, it is best if such a procedure is followed within a laboratory, allowing "knowledge" to emerge without the need to require the literature of advocates to sort out the true from the expected. However, even when the science collapses into the advocacy mode, the procedure will work to prevent the advocacy from being captured by the most powerful scientists at the most prestigious universities supported by the most powerful politicians.

For Chemistry and, Now, Biology, Synthesis Offers a Mechanism to Prevent Its Scientists from Always Reaching the Conclusion That They Set Out to Reach

In fields where technology enables it, synthesis has been suggested as a procedure that prevents scientists from always reaching the conclusions that they set out to reach (Woodward 1968; Benner et al. 2010). Here, "synthesis" means the physical act of creating something by the deliberate and rational assembly of parts. The distinction between rational synthesis and (for example) accidental synthesis might be illustrated by the difference between the construction of a new living entity by animal husbandry (conception, implantation, and so on) and the construction of a new living entity by the chemical synthesis of a DNA chromosome that is then transplanted into a cell whose DNA had been functionally removed (for example, Gibson et al. 2010).

Even with the rational assembly of parts, synthesis comes in various forms. For example, the cell whose DNA was synthesized by Gibson et al. (2010) was hardly designed at all; the functional aspects of the sequence of nucleotides in the synthetic DNA were taken entirely from the natural world. The team at the J. Craig Venter Institute could no more design that information than they could conjure a baby out of carbon, oxygen, nitrogen, hydrogen, sulfur, and phosphorus atoms. Accordingly, that widely publicized exercise in "synthetic biology" taught nothing about the information required for a living cell; it just showed that a scientist fully instructed by Nature could follow Nature's instructions.

Instead, to be effective at directing scientists away from the conclusions that they desire, synthesis is best when it sets a "grand challenge" at the boundaries of what theory is able to design. If any part of the required theory is inadequate, the synthesis fails, and fails in a way that cannot be ignored. Thus, in this view, synthesis drives discovery and paradigm change in ways that hypothesis-directed research cannot.

Synthesis has various mechanisms for doing so. First, at the very least, the failure of a rationally designed synthetic scheme alerts the scientists that they do not understand something that they might have thought they understood. Further, the problems that a synthesis must solve are often unscripted. Therefore, synthesis manages the natural human propensity to prefer only easily testable hypotheses chosen to confirm a bias. Finally, once failure is encountered, the goal is not to confirm a favored hypothesis. Rather, the goal is to meet the grand challenge by getting the synthesis to work. This is the reward, and is largely independent of what the synthetic scientists, or their community, desire.

The most aggressive proponents of synthesis go a step further. More than a recipe for discovery via the solution of unscripted problems, these proponents *define* understanding in terms of synthesis. For example, Feynman was quoted approvingly by another physicist, Stephen Hawking (2001, p. 83), as having written: "What I cannot create I do not understand." If we are permitted reasonable word replacements and "modus tollens," Feynman's "If I cannot create X, then I do not understand X" might be transformed into an operational test (of sorts) for "understanding": "If I understand X, then I can create X." This is a fundamentally different proposition from other concepts of understanding. Further, it leaves open the possibility that a practitioner can create X without actually understanding X.

But Can Synthesis Generate Knowledge?

Many different types of "knowledge" are distinguishable, including knowledge of things, knowledge by acquaintance, and knowledge by description. Also different are knowledge "that," knowledge "why," knowledge "where," knowledge "when," and "knowledge how," inter alia.

At the very least, a successful synthesis provides "knowledge how to," in the same sense as a chef provides a recipe to bake a culinary object. If one mixes the eggs, flour, and sugar correctly, an object having the properties of a soufflé emerges. If the mixing is adequately recorded in a publication, that knowledge is transferable. The recipe behaves as "knowledge how to" is supposed to behave, conferring upon an independent individual the power to create another soufflé.

Few modern practitioners of the synthetic arts would be satisfied by this, however. For example, synthetic chemists would note that Wöhler (1828) did indeed synthesize urea by heating ammonium cyanate ($NH_4^+ + CNO^- \rightarrow H_2NCONH_2$). Further, he transferred his "knowledge how to" to others by providing a recipe for the synthesis of urea that remains entirely reproducible, even today.

However, Wöhler's synthesis was done without theory-based design. Further, benefiting from the subsequent two centuries of description of this reaction, Wöhler had none of the modern understanding of this reaction, including a recognition that the reaction proceeds in two steps ($NH_4^+ + CNO^-$ à $NH_3 + HCNO \rightarrow H_2NCONH_2$). Absent a modern understanding of chemical reactions, Wöhler could not have realized that his reaction is generalizable to some other molecular systems (for example, $CH_3NH_3^+ + CNO^- \rightarrow CH_3NH_2 + HCNO \rightarrow CH_3HNCONH_2$), but not to all other molecular systems.

This is hardly surprising. In 1828, even the atomic and molecular theories of matter were not particularly well defined. Wöhler's synthesis of urea can be contrasted with the Woodward–Eschenmoser synthesis of vitamin B12 (Woodward 1968), set in the 1960s after modern chemical theory had achieved essentially its modern form. Instead of one visible step, the synthesis required dozens of steps. Each step was rationally designed based on then-accepted theory. During the process of the synthesis, the synthetic team observed unexpected outcomes of specific reactions. This led them to propose a role of "orbital symmetry" in guiding the outcome of a class of chemical reactions (Woodward and Hoffmann 1970). While Woodward did not live to receive the Nobel Prize for this discovery, his collaborator (Roald Hoffmann) and a scientist working independently in the same area (Kenichi Fukui) did.

Woodward has written about the importance of the unscripted efforts directed towards the grand challenge (B12 synthesis) in leading to the discovery of orbital history. Woodward also specifically denied that the discovery would have been made by a standard set of inquiries into molecular reactivity (Woodward 1968). Whether or not a role for orbital symmetry would have eventually emerged using standard inquiries, absent the pursuit of the synthesis of vitamin B12 as a grand challenge, it is clear that this synthetic effort gave more than a recipe to make B12. What emerged was something that has been empowering across chemistry, behaving as knowledge is supposed to behave.

Similar arguments were also presented by Jack Szostak, who used theory-driven deliberate synthesis to construct an artificial chromosome, an accomplishment that was recognized by a Nobel Prize in Physiology or Medicine (Szostak and Blackburn 1982). Here again, efforts based on

existing theory failed to generate a functioning artificial chromosome in yeast. This alerted the synthetic scientists to the fact that their theories concerning chromosomal architecture were inadequate. Attempting to meet the grand challenge, they refined their theories, used the refined theories to redesign their experiments, and eventually achieved success. Again, the outcome was something more than a recipe for making yeast chromosomes, although a straight line connects their work to the construction of large DNA molecules at the J. Craig Venter Institute (Gibson et al. 2010). What they generated was empowering, worked across a range of structures other than the yeast chromosome, and was transferable, all attributes expected from knowledge.

The Role of Failure as a Constructive Tool to Generate Knowledge

This discussion of synthesis can be taken to imply that failure is more important than success. Indeed, if a synthesis proceeds uneventfully based on existing theory, one might argue that the theory is correct, including those parts that were not explicitly understood to be important to the synthetic effort. With clean success, a synthesis has failed to discover anything new. Conversely, one might argue that the synthetic scientist did not set the challenge to be "grand" enough.

Examples help us understand the importance of failure in synthesis. Here we choose examples from the field of chemistry that concerns nucleic acids, DNA, and RNA. These examples illustrate how a community can be convinced that it has knowledge when, in fact, it lacks knowledge. Further, it shows how synthesis can dislodge this conviction, and deliver that knowledge, as measured by manipulative power.

At the center of these examples is the double helix structure of DNA. The double helix is known to every middle school student in the form of a model produced by James Watson and Francis Crick (1953). In cartoon structure of the double helix (Fig. 2),

(a) Two DNA strands align in an antiparallel fashion.
(b) The strands are held together by nucleobase pairing that follows simple rules: A pairs with T and G pairs with C (the "Watson–Crick pairing rules").
(c) Two molecule-based rules for molecular complementarity explicate the pairing rules. The first, size complementarity, pairs large purines with small pyrimidines. The second, hydrogen bonding complementarity, matches hydrogen bond donors from one nucleobase with hydrogen bond acceptors from the other.

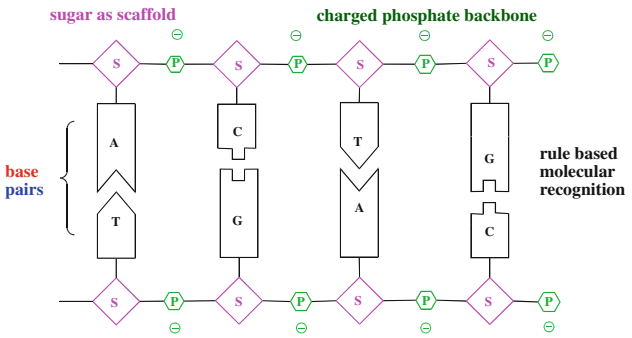

Fig. 2 The rules governing the molecular recognition and self-assembly of DNA duplexes seemed, in the Watson–Crick "first generation" model for the double helix, so simple that the community came to believe that they had full knowledge of the structure; nothing was known to be unknown

Failure in Attempts to Synthesize a Replacement DNA with a Different Sugar

In the Watson–Crick "first generation" model for the double helix, the nucleobase pairs were central. In contrast, the sugar and negatively charged phosphate groups in the backbone were viewed as being largely incidental to the molecular recognition event at the center of natural genetics and Darwinian evolution.

If this simple model for the double helix were correct and complete, then we should be able to synthesize a different molecular system with different sugars and/or phosphates (but the same nucleobases) to get an unnatural synthetic system that could mimic the molecular recognition displayed by natural DNA and RNA. We might even be able to get this artificial genetic system to direct its own replication and, possibly, evolve.

Efforts to meet this grand challenge based on the Watson–Crick "first generation" theory for the double helix met with repeated failure. For example, Schneider and Benner (1990) attempted to replace the ribose sugars by flexible glycerol units to give a "flexible" kind of synthetic DNA (Fig. 3). This followed a suggestion of Joyce et al. (1987), who had noted some of the difficulties in identifying processes that, on Earth before biology, might have generated ribose and 2′-deoxyribose, the "R" and the "D" in RNA and DNA respectively.

Unfortunately, the system failed to deliver quality rule-based molecular recognition; the double helix failed to form. Synthetic DNA that replaced one ribose by a glycerol bound to its complementary DNA strand less tightly. Putting in two flexible glycerols lowered the binding even more. Faced with this failure, we went further, synthesizing DNA analogs where *all* of the sugar units were replaced by glycerol units. This replacement destroyed the ability of the molecule to form a double helix entirely.

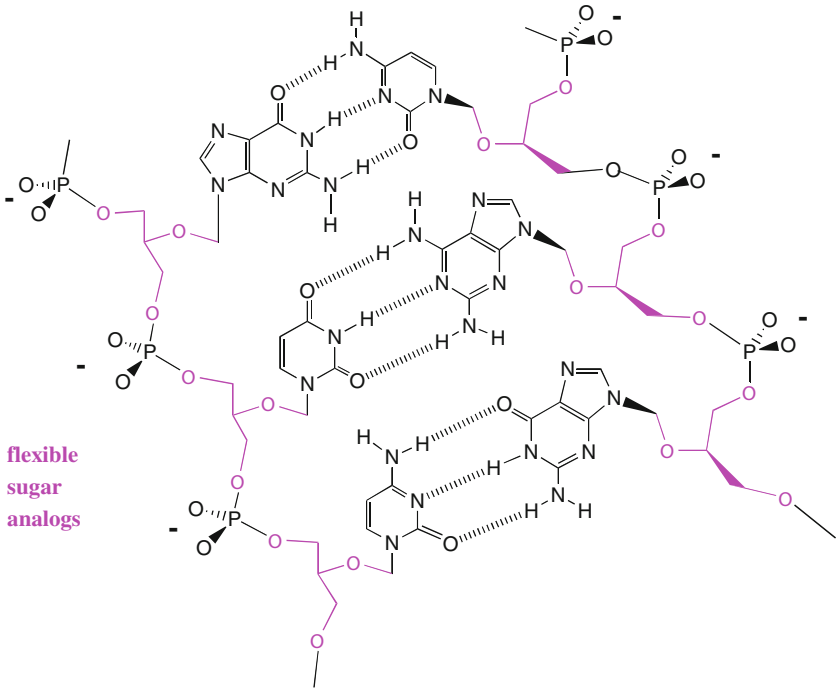

Fig. 3 The failure of these flexible glycerol DNA molecules forced synthetic biologists to reevaluate the theory that held that the backbone sugars in DNA contributed little to the formation of the double helix (Schneider and Benner 1990)

This failure taught us the inadequacy of our then-existing theory to account for genetics. The synthetic effort taught us that we did not know something. The theory that guided the synthetic effort taught that the structures of the backbone sugars of DNA were incidental to the formation of the double helix and, thereby, to Darwinian evolution and biology. The theory, confidently believed, failed to support a synthetic endeavor. This failure forced the theory to advance. The synthesis of *unnatural* genetic systems taught us something about *natural* genetic systems. This drove the synthesis of more unnatural systems that replaced failure by success.

Was synthesis necessary? It is hard to say, but this part of the "first generation" theory for the double helix had remained largely unchallenged in the three decades since it was first proposed in 1953. It had appeared in textbooks and television series. These facts all support the notion that without synthesis, this feature of DNA would *never* have been recognized. Synthesis drove discovery and paradigm change in ways that analysis cannot. And this came about only because failure was encountered in synthesis efforts not scripted to test the theory, analyzed, and pursued.

Failure Changing the Phosphates

Failure was also encountered when we attempted to replace the charged phosphates in the backbone of DNA by a linker that had about the same size as phosphate, but that lacked charges. The phosphate linkers were also viewed in the first generation Watson–Crick theory as being largely incidental to the molecular recognition that is central to genetics and Darwinian evolution.

In fact, the repeating negative charges carried by the phosphate groups in the DNA backbone appeared to be downright undesirable. The repeating charges on the phosphate linkages prevented DNA from getting into cells. The charged phosphate linkers were sites of nuclease attack. The repulsion between two negatively charged backbones of two DNA strands seemed to weaken undesirably their association to form a double helix. DNA molecules without the negative charges in their backbone were expected to form *better* duplexes.

If, it was thought, we could get rid of the charges without disrupting the rules for Watson–Crick pairing (A pairs with T, G pairs with C), we might be able to create a new class of therapeutic molecules with an entirely new mechanism for biological activity. These were called "antisense drugs" (Burgess et al. 1994). The idea was simple. If we could synthesize an uncharged analog of DNA that could enter a cell by passive diffusion, it would survive degradation by nuclease attack. If the charges were indeed incidental to genetics, this neutral synthetic DNA analog would still bind to complementary DNA molecules inside a cell following Watson–Crick rules. The anti-sense DNA analog would therefore target, with sequence specificity, only the unwanted DNA, perhaps from a virus or a mutated cancer gene. Antisense DNA might be a magic bullet for diseases associated with undesired DNA or RNA.

Following this theory, Zhen Huang, Christian Schneider, Clemens Richert, and others in my group synthesized an uncharged unnatural DNA-like molecule that replaced the

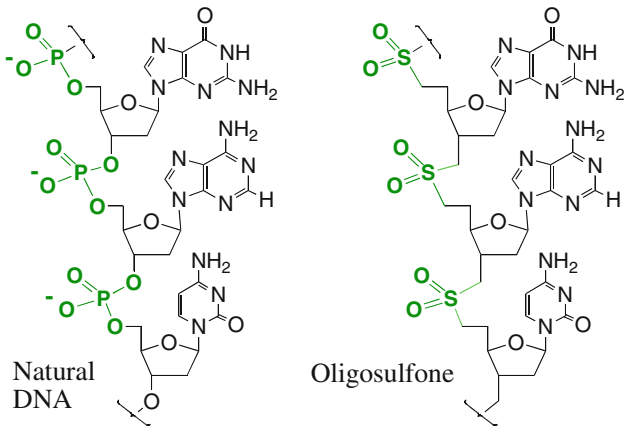

Fig. 4 Replacing phosphates ($-PO_2^-$ units, each having a negative charge, *left*) in DNA by dimethylenesulfone linkers (the $-SO_2^-$ units, *right*, each lacking a negative charge) gave an uncharged analog of DNA

anionic phosphate diester linker in natural DNA and RNA with uncharged dimethylenesulfone linkers (Fig. 4) (Huang et al. 1991). This gave DNA and RNA analogs that have roughly the same geometry as the natural molecules. Indeed, Martin Egli solved a crystal structure of a short GSO_2C dinucleotide duplex. He found that the uncharged duplex was held together by G:C and C:G pairs in a mini helix just like its RNA analog, whose crystal structure had been solved by Alex Rich two decades earlier (Roughton et al. 1995).

This appeared to validate the first generation Watson–Crick theory for the double helix. It appeared that one *could* replace the charged phosphate linkers with uncharged linkers of approximately the same shape, and still form G:C and C:G pairs.

As noted above, a successful synthesis may mean only that one has erred a bit on the safe side in selecting a challenge. To be consequential in driving discovery and paradigm change, if a theory seems to work, the challenge should be deepened until the theory fails.

Accordingly, we synthesized longer DNA and RNA analogs having more sulfone linkers. Instead of molecules with just one uncharged linker, we made molecules with two uncharged sulfone linkers to see how they worked. We then made molecules with three, five, and then seven uncharged sulfone linking bricks.

It was not long before the theory that we were using to guide the synthesis broke down. Longer oligosulfones folded on themselves (Richert et al. 1996). Folding prevented them from pairing with *any* second strand, even one that was perfectly complementary in the Watson–Crick sense of the term. This failure led to a thought that should have been obvious, but was not in our culture (we too had been trained to view the DNA double helix as an unchallengeably elegant structure): pairing between two strands *requires* that neither strand fold on itself.

Another failure was then encountered. Different oligosulfones differing by only one nucleobase in their structure were found to display different levels of solubility, aggregation, folding, and chemical reactivity. This prompted another thought that, in retrospect, should have been obvious. To support Darwinian evolution, a genetic molecule must have features that allow it to change its detailed structure, the details that encode genetic information. But the changes must be possible *without changing the overall properties of the system*. In particular, the changes in structure that correspond to changes in genetic information cannot change the rules by which the genetic molecules template the formation of their descendents. Changes do not do this in DNA and (in general) RNA. As we learned by synthesis, they do so in oligosulfones.

These results further drove the development of a second-generation model for the DNA double helix and the relation between its structure and Darwinian evolution (Benner and Hutter 2002). In this model, the phosphate linkers and the repeating backbone charge become quite important for four reasons.

First and trivially, a polyanion is likely to be soluble in water. This was appreciated by Watson and Crick already in 1953, although less so by Linus Pauling. Pauling had proposed an incorrect model for DNA where the phosphates did not point out into solvent, but rather (and paradoxically given their negative charges) interacted with each other (Olby 1994). When Watson and Crick first learned about the structure for DNA assemblies that Pauling was proposing, this feature immediately let them conclude that Pauling's model must be wrong.

Less trivially, the repeating charges in the backbone of natural polyanionic DNA repel each other. Within a strand, this repulsion helps keep DNA strands from folding on themselves. A polyanion is more likely to adopt an extended conformation suitable for templating than a neutral polymer, which is more likely to fold. As "not folding" is a property needed for a strand to bind to its complement, the repeating charges were proposed in the second-generation model to be important for the ability of DNA to support Darwinian evolution for this reason, as well as for solubility reasons.

The anion–anion repulsion between phosphates on two different strands is also important. When two strands approach each other, the repulsion forces inter-strand interactions away from the backbone. This drives the contact between two strands to occur at the Watson–Crick edge of the nucleobases (Fig. 5). Without the polyanionic backbone, inter-strand contacts can be anywhere (Steinbeck and Richert 1998). Thus, the second-generation model views as naïve the assumption that this repulsion is bad. In fact, the repulsion moderates and controls the natural propensity of biomolecules to associate with other

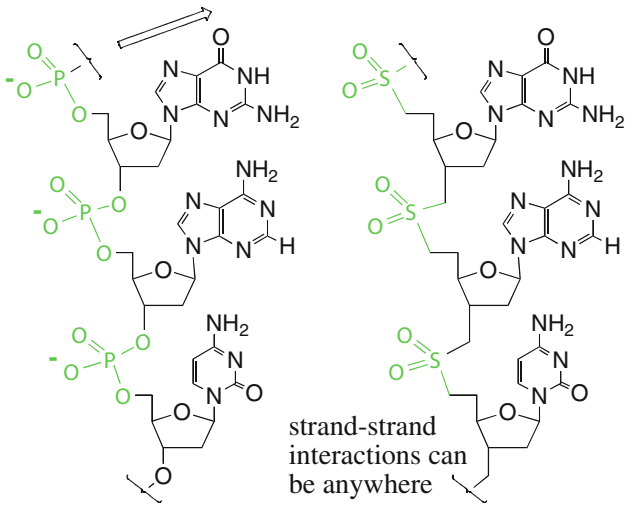

Fig. 5 The repeating backbone anion drives the interaction between two strands as far from the backbone as possible. This guides strand–strand interactions, and forms the basis for Watson–Crick pairing rules (Benner and Hutter 2002)

biomolecules, and directs in DNA that association to the part of the molecule where information is contained, the Watson–Crick edges of the nucleobases.

In light of failure in a synthetic effort, the inter-strand repulsion between two strands that both have repeating charges on their backbones is also seen to be important for pairing rules essential for Darwinian evolution. Without the repulsion from two backbones, both negatively charged, base pairing would not occur at the site where hydrogen bonding was needed. It would occur at other sites, including the Hoogsteen site, and not obey the simple rules required for genetics.

But the failure of the synthesis yielded a still more fundamental role for the repeating charge in a DNA molecule, one that suggested that repeating backbone charges were necessary for any biopolymer to support Darwinian evolution. Here, the argument is more subtle, and begins with the realization that replication alone is not sufficient for a genetic molecule to support Darwinian evolution. A Darwinian system must generate *inexact* replicates, descendants whose chemical structures are different from those of their parents. Further, these differences must then be replicable themselves. It does no good if the mutant has changed its biophysical properties so dramatically that the mutant genetic molecule precipitates, folds, or otherwise loses the ability to encode selectable information.

While self-replicating systems are well known in chemistry, those that generate inexact replicas with the inexactness itself being replicable are not. As a rule, changing the structure of a molecule changes its physical behavior. Indeed, it is quite common in chemistry for *small* changes in molecular structure to lead to large changes in physical properties. This is certainly true in proteins, where a single amino acid replacement can cause the protein molecule to precipitate (the archetypal example of this is sickle cell hemoglobin). This means that inexact replicates need not retain the general physico-chemical properties of their ancestors, in particular, properties that are essential for replication.

This thought, again arising through the analysis of a failed synthesis, prompted the thought that a repeating backbone charge might be universal for *all* genetic molecules that work in water, on Earth, Mars, and Titan, but also for alien life throughout the cosmos. The polyanionic backbone dominates the physical properties of DNA. Replacing one nucleobase in the sequence of a DNA molecule by another therefore has only a second order impact on the physical behavior of the molecule. This allows nucleobases to be replaced during Darwinian evolution without losing properties essential for replication.

In the language of modern engineering synthetic biology, the repeating charge in the DNA backbone allows nucleotides to behave largely as *interchangeable parts*. It allows the whole to be the sum of its parts. It allows engineers, even those totally unfamiliar with Structure Theory, to design DNA molecules that pair with other DNA molecules according to simple rules. Because of this repeating backbone charge, and *only* because of this repeating backbone charge, is it possible to make "tiles" or biobricks from DNA, for example.

And *only* because of this repeating backbone charge can DNA and RNA support Darwinian evolution. The sequence ATCCGTTA behaves in most respects the same way as the sequence GCATGACA, even though these have very different molecular structures. This is because in both cases, the molecules are polyanions. These differences hold the genetic information. Were it otherwise, we could not mutate ATCCGTTA to give GCATGACA, even if GCATGACA better allowed us to survive, get married, and have children.

For this reason, the second-generation model for DNA proposed that a repeating charge should be a universal structural feature of *any* genetic molecule that supports Darwinian evolution in water, regardless of where it is found on Earth (Benner and Hutter 2002). Polycationic backbones are also predicted to be satisfactory under what is now called the "polyelectrolyte theory of the gene" (Benner and Hutter 2002). Thus, if NASA missions *do* detect life in water on other planets, their genetics are likely to be based on polyanionic or polycationic backbones, even if their nucleobases and sugars differ from those found on Earth.

Again, it is hard to believe that these insights would have emerged without synthetic biology. After all, first generation Watson–Crick theory had been in textbooks for three

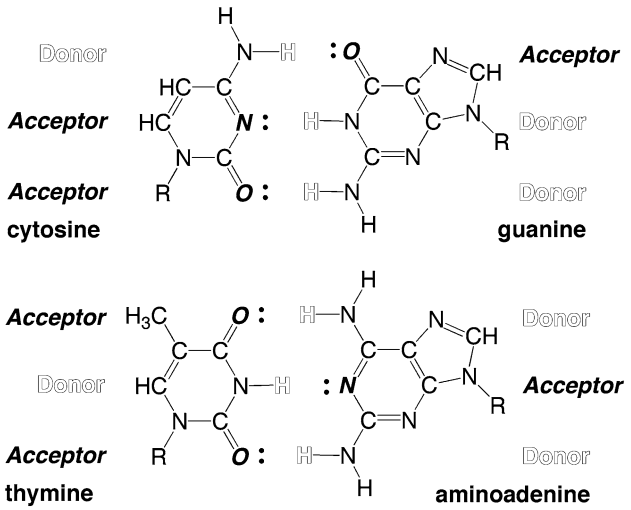

Fig. 6 The two standard Watson–Crick pairs, idealized by replacing natural adenine (which lacks the bottom NH_2 group) with amino adenine

decades without recognizing the fundamental role of the repeating charge to the ability of DNA strands to bind their complements and support Darwinian evolution. Lacking that recognition, venture capitalists and other investors had bet billions of dollars on "antisense" drugs that required that molecular recognition remain in DNA analogs after the repeating charge was removed. Had they had the polyelectrolyte theory of the gene at their disposal, they would not have lost so much money. Synthesis drives discovery and paradigm changes in ways that analysis cannot.

Could Base Pairing Behind Darwinian Evolution be so Simple?

But what about the nucleobases, which had long been understood to be critical to the biological properties of DNA? And what about the simple rules that were proposed by Watson and Crick to account for genetics and Darwinian evolution: big pairs with small and hydrogen bond donors pair with hydrogen bond acceptors? (See Fig. 6; donors are in white type, acceptors in italics.)

Could things be so simple? Again, if they were, then the synthetic biology paradigm suggested a grand challenge. If the hydrogen bond donor and hydrogen bond acceptor groups in the A:T and G:C pairs were shuffled, eight new nucleobases could be conjectured that fit together to give four new base pairs having the same geometry as the A:T and G:C pairs (Fig. 7). As with the four standard nucleobases examined by Watson and Crick, the new nucleobases were predicted to pair with size complementarity (large with small) and hydrogen bond complementarity (hydrogen bond donors with acceptors), *if the theory behind the pairing were so simple*.

As before, it was not enough to model the design on paper. Or even by computer. We needed to use synthetic technology from organic chemistry to *constructively create* (in Malaterre's sense of the term; see Fig. 1 in Malaterre 2013, this issue) these new forms of matter, put them into DNA molecules, and see whether they worked as part of an artificially expanded genetic information system.

Thus all of the synthetic components of our artificial genetic alphabet worked. We were then able to put these synthetic nucleotides into synthetic DNA and RNA strands, and do all of the characterization of these that chemists do.

Once the synthetic task was complete, we observed that our artificial synthetic genetic system worked, and worked well. Artificial synthetic DNA sequences containing the eight new synthetic nucleotides formed double helices with their complementary synthetic DNA sequences. Complementation followed simple rules; just as A pairs with T and G pairs with C, P pairs with Z, V pairs with J, X pairs with K, and isoG pairs with isoC. The synthetic large nucleotides paired only with the correct synthetic small nucleotide. The

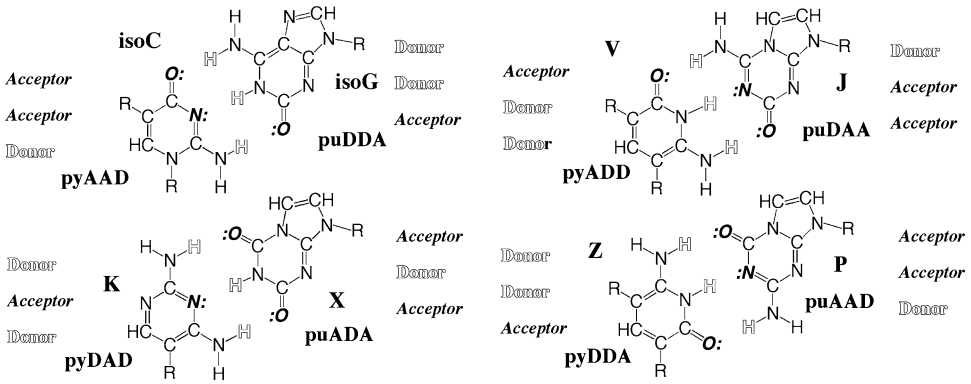

Fig. 7 Shuffling hydrogen bond donor and acceptor groups in the standard nucleobase pairs generated eight additional heterocycles that, according to simple theory, should form four new, mutually independent, base pairs. (Donors are in *white type*, acceptors in *italics*.) This is called an "artificially expanded genetic information system" (AEGIS). Could molecular behavior at the center of genetics and Darwinian evolution be so simple? Synthesis was used to decide

artificial synthetic DNA worked as well as natural DNA, at least in its ability to pair following simple rules. Indeed, it worked so well that it is part of a series of diagnostic tools that have over $100 million in annual sales.

A Synthesis Cannot be Gamed

Robert Hutchins, an early president of the University of Chicago, is said to have remarked that he believed that "Luck plays an important role in science. That is why I only hire lucky scientists." We have noted that Wöhler's synthesis of urea was "lucky," in the sense that it was not supported by rational expectations based on an existing theory. It was, in the modern sense of the term, a discovery.

In contrast, the synthesis of vitamin B12, the construction of an artificial chromosome in yeast, and the redesigning of DNA to create diagnostics tools worth hundreds of millions of dollars and models for universal genetic systems of alien life, are all examples of theory-driven synthesis. Following Malaterre, we find within these examples detailed illustrations of the cycle of failure, learning, new theorizing, redesign, and follow-on synthetic efforts that lead not only to recipes about the specific systems, but also general models about how systems outside of the purview of the original system behave. These models are transferable to other systems and other scientists, as expected for knowledge. They are empowering, as expected for knowledge. And they have elements of universality as expected for knowledge.

They also meet other criteria set forth by Malaterre. Because of their success in semi-universal application to other systems, they provide confidence to the practicing scientist (if not to the philosopher of science) that all causally relevant variables have been identified. Further, the combination of failure and success makes confident the practicing scientists (if not the philosopher of science) that the activity has explored effectively the Duhem–Quine space of auxiliary hypotheses, and has found all of those that are relevant.

On these grounds lies the argument of practicing synthetic scientists that the synthesis approach generates knowledge, and does so in ways that alternative scientific methods do not, and possibly cannot. The argument is, however, not entirely formal. In fact, it captures certain elements of various "radical" philosophies of science (Kuhn 1962; Feyerabend 1975), in that it constructs the synthetic "method" in part to overcome features intrinsic to human sociology and human psychology that might otherwise prevent scientific discovery.

However, synthesis may in part provide a way to allow science to provide knowledge even if we accept certain of these "radical" positions. Within the realms of science advocacy, science publication, and science funding, anything might indeed "go" (Feyerabend 1975). In these realms, scientists might indeed cherry-pick hypotheses to test that do not truly challenge the underlying theory. They might indeed discard data that are inconvenient for the conclusion that they want to reach. They may very well use "any trick, rational, rhetorical or ribald" to induce their communities to accept their theory.

However, when that theory is used to guide synthesis, the tests of the theory are unscripted. The science cannot be gamed. And this allows fields having access to synthesis to advance regardless of the failure of its scientists to emulate the "ideal" dispassionate individual.

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