PERSPECTIVE

Translation: In retrospect and prospect

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ABSTRACT

This review is occasioned by the fact that the problem of translation, which has simmered on the biological sidelines for the last 40 years, is about to erupt center stage—thanks to the recent spectacular advances in ribosome structure. This most complex, beautiful, and fascinating of cellular mechanisms, the translation apparatus, is also the most important. Translation not only defines gene expression, but it is the *sine qua non* without which modern (protein-based) cells would not have come into existence. Yet from the start, the problem of translation has been misunderstood—a reflection of the molecular perspective that dominated Biology of the last century. In that the our conception of translation will play a significant role in creating the structure that is 21st century Biology, it is critical that our current (and fundamentally flawed) view of translation be understood for what it is and be reformulated to become an all-embracing perspective about which 21st century Biology can develop. Therefore, the present review is both a retrospective and a plea to biologists to establish a new evolutionary, RNA-World-centered concept of translation. What is needed is an evolutionarily oriented perspective that, first and foremost, focuses on the nature (and origin) of a primitive translation apparatus, the apparatus that transformed an ancient evolutionary era of nucleic acid life, the RNA World, into the world of modern cells.

Keywords: adaptor; A-site-P-site; evolution; paradigm shift; RNA World; templating

THE DARK SIDE OF MOLECULAR BIOLOGY

Where to start with an issue as big as molecular biology? In the middle of the last century the Watson–Crick structure of DNA and the deciphering of the genetic code solved one of Biology's great problems, "the gene"—ending one of the most exciting eras in scientific history. Biology's focus now shifted to other major challenges posed by the cell and by multicellular organisms (see Stent, 1968). However, something was amiss here.

What bothers me is that I don't think the problem of the gene was ever fully solved, and nobody seems aware of this "oversight"—because from the molecular point of view, the problem *was* solved! So, what's the point at this late date of quibbling? There are two very good reasons: (1) This quibble is the tip of an iceberg, an iceberg I have come to call the dark side or Achilles heel of molecular biology—that is, its failure to embrace evolution. And, (2) Biology today is at a crossroads. A decision has to be made as to where that

science is going in this new century, and if we biologists don't make it, the decision will be made for us (which ultimately benefits no one). Yet it's hard to know where you are going if you don't understand where you came from. And, that is why molecular biology's dark side has to be understood. The truth, the very powerful truth, of the molecular approach to biology certainly cannot be denied. But we can and must ask whether the molecular perspective sees biology in its wholeness. I am writing this piece because I feel I have something useful to say about the road traveled and the choice of roads ahead.

At the heart of this matter lies the "problem of the gene," about which 20th century Biology pivoted. What exactly is the "problem of the gene" and what does it mean to solve it? Details aside, the gene is defined (as it has always been) in terms of the genotype-phenotype relationship—which is basically to say that the gene is defined by the dual processes of gene replication and gene expression. Solving the problem of the gene, then, amounts to understanding each of these processes in some fundamental way. However, what serves as fundamental understanding in the two cases is different—and that difference is the key to our discussion.

The processes that define the gene each have three major facets, one informational (the coding, or information transfer rules), another mechanistic (how the process works), the third evolutionary (how the process came to be). In one fell swoop the Watson–Crick structure revealed the coding rules (the 1:1 structural correspondence between A and T, and so on), the underlying reason for them, and provided a physical-chemical foundation (i.e., base pairing) for the replication mechanism. While the DNA structure strictly says nothing about the evolution of gene replication, the obviously central role of base pairing therein makes that evolution appear straightforward. Evolution seems merely to have provided a proteinaceous chassis for the physical-chemical interaction that preceded and now underlies and defines gene replication. For this reason alone, the evolutionary aspect of gene replication has always been seen as incidental to the fundamental explanation of gene replication, and so, could in effect be ignored. The Watson–Crick structure per se provides that fundamental understanding!

Would that all biology could be so explained. Gene expression (translation) surely cannot. Only one of the three facets of translation has been "solved," that is, its coding aspect. Yet what kind of explanation have we here? The coding rules (the dictionary of codon assignments) are known. Yet they provide no clue as to why the code exists and why the mechanism of translation is what it is. The genetic code today stands apart from the rest of translation. There was a time, however when this was not the perception: The problem of the genetic code *was* the problem of translation (as we shall see). Biologists greeted its solving with the same universal acclaim, the same sense of major accomplishment, that had accompanied Watson and Crick's solution to gene replication.

The problematic nature of our view of translation continues. Its mechanical aspect remains unsolved—totally unsolved despite *half a century* of work by a cadre of dedicated molecular biologists. Fortunately, the recent spectacular advances in ribosome structure have finally opened the door here (Gutell et al., 1994; Cate et al., 1999; Ban et al., 2000; Frank & Agrawal, 2000; Schluenzen et al., 2000; Wimberly et al., 2000; Yusypov et al., 2001). Yet, I am willing to bet that when all is said and done, the mechanism we eventually adduce from complete ribosome structures will provide no understanding of translation that any biologist would consider fundamental: Translation will appear a Rube Goldberg machine, a complex and fascinating, but so-what molecular toy (Woese, 1980). (I also hope I lose that bet—as you will see.)

The evolutionary aspect of gene expression would not appear to offer any hope of a basic understanding of the process either—at least from a molecular perspective. Molecular biology inherently views the evolution of biological entities as meaningless historical accidents—a strong undercurrent throughout the history of the problem of the gene. Is translation, then, not amenable to fundamental explanation? Is it just one of those historical accidents, not understandable in terms of some (preexisting) underlying physical-chemical mechanism? That, certainly, is the conclusion to which the molecular perspective leads—a conclusion that implies that gene replication is somehow basic to biology whereas gene expression is not! You can sense this in the way gene replication is venerated while the problem of gene expression is found merely "interesting": In the 1960s, when, having "solved" the problem of the gene, the great molecular biologists of the day turned to Biology's remaining major problems, the unsolved problem of translation was not on their agenda.

I find it unthinkable that in any real biological sense, gene expression is anything but a fundamental problem. After all, without translation, without the genotype-phenotype relationship, biology as we know it would never exist! Translation *demands* fundamental explanation. The difficulty, however, does not lie with translation. It lies with what modern biologists consider "fundamental." The physicist David Bohm knew this when he long ago wrote: "It does seem odd … that just when physics is … moving away from mechanism, biology and psychology are moving closer to it. If the trend continues … scientists will be regarding living and intelligent beings as mechanical, while they suppose that inanimate matter is too complex and subtle to fit into the limited categories of mechanism" (Bohm, 1969).

Evolution cannot be dismissed as mere historical accident: It is obviously part and parcel of biology. As Dobzhansky (1963) succinctly put it: "[N]othing in biology makes sense except in the light of evolution." Molecular biology has to bring evolution to the fore and integrate it fully—not hold it at arm's length.

Twentieth century molecular biology operated from the world view of its intellectual progenitor, 19th century physics: All is mechanism. Yet mechanism doesn't know evolution. The understanding that is 20th century Biology represents, if you will, a time slice through biology—one temporal glimpse of the evolutionary process. What is fundamental from such a molecular "Flatland" perspective would seem only a subset of what Biology is, were it appreciated in its fullness. Translation is the quintessential biological phenomenon. It's not the coding rules; not how this incredible translation mechanism works; not whether translation is or is not the direct expression of some preexisting and underlying physical-chemical principle, that is the real problem. It is how translation *evolved*.

THE POWER OF THE PARADIGM

I don't much like the term "paradigm." It is one of those for-want-of-a-better-word words. The concept has be-

come a playground for pedants. Yet a term is needed to help biologists appreciate the fact that a science (almost always) progresses within an organizational framework that is overarching, underlying, all-inclusive, and totally pervasive of that science. In other words, a "paradigm" (as I am forced to call it) strongly influences how any science is done—what experimental directions are worth pursuing, the interpretation and significance of results, what theories (conjectures, hypotheses, and the like) are worthy of consideration or test, where the science is headed (its goals). In my experience, few biologists understand (or care) that their science is so strongly influenced—defined and delimited—by a paradigm, by conventional wisdom and custom. And even fewer of those working in translation recognize that their field has suffered under the hegemony of an inadequate and misleading paradigm for over three decades. This has to be changed if there is to be genuine progress. (Any direction, any real goal given the problem of translation today comes not from its antiquated paradigm, but from the general mechanistic orientation of molecular biology.)

I have run contrary to two major paradigms in my career and, so, fully appreciate their power. Lest you doubt this power, just look at the way Biology is structured today—how biology departments are defined and what their curricula are, what the content of biology text books is, how biological funding agencies are organized, and so forth. All this largely reflects the conceptual power of one paradigm, the eukaryote-prokaryote dichotomy—an anthropocentric and false taxonomic conjecture that was, from the start, accepted dogmatically, as truth.

For the last three decades (until relatively recently) conventional wisdom held that ribosome function is defined by proteins; the RNA component of the ribosome served simply as the chassis upon which the function-defining proteins were mounted. It is within this framework that the experiments attempting to define ribosome function through reconstitution of a subunit from isolated ribosomal proteins and rRNA were conceived and executed (Hosokawa et al., 1966; Nomura & Erdmann, 1970)—experiments that in the end revealed nothing substantial about how translation works. In those days, no one believed that ribosomal RNA itself had any functional role to play in translation. What a contrast to today: "The ribosome is a ribozyme" is the new mantra (Ban et al., 2000; Cech, 2000; Nissen et al., 2000).

This is only a small beginning, however. The old protein-based translation paradigm is on its way out, a new RNA-World-centered paradigm is emerging. Translation is not just another molecular structure to be solved. It represents, it *is*, the evolutionary transition from some kind of nucleic acid-based world to the protein-based world of modern cells (Woese, 1972; Yarus, 2000). Herein lies the basis for a new and productive translation paradigm. But before glorying in any bright future, we need to explore how we came to this point.

THE YEAR OF THE DRAGON

Our story begins in 1940. Biologists of the '30s and '40s were fascinated by the specificity exhibited by enzymes and antibodies, the fact that they could distinguish sharply among small molecules whose structures were very similar. These impressive, chemically unexpected discriminations were explained by the now familiar notion of a complementary, "lock and key," fit between enzyme and substrate, or between an antibody and some hapten (Landsteiner, 1936).

In 1940, Pauling and Delbruck (1940) made what in retrospect is a truly radical proposal; that the notion of molecular complementarity (previously seen only in terms of enzyme and antibody specificities) be expanded to include the macromolecular interactions involved in gene replication and gene expression (as well as molecular folding). This seminal article was, in the first instance, a reaction to an argument by an eminent theoretical physicist, Jordan, to the effect that macromolecular replication could involve *like* specifying *like* via a quantum mechanical resonance mechanism. It was complementarity, not identity, Pauling and Delbruck asserted, that underlay such processes as gene replication and gene expression; and in these cases that meant "templating," a process whereby a preexisting macromolecule would provide an extensive surface upon which to fashion, to direct the synthesis of its "complement." What particularly intrigued the authors was that in some cases complementarity and identity could be the same thing (Pauling & Delbruck, 1940):

It is our opinion that the processes of synthesis and folding of highly complex molecules in the living cell involve, in addition to covalent-bond formation, only the intermolecular interactions of van der Waals attraction and repulsion, electrostatic interactions, hydrogen-bond formation, etc.,… These interactions are such as to give stability to a system of two molecules with *complementary* structures in juxtaposition, rather than of two molecules with necessarily identical structures; we accordingly feel that complementariness should be given primary consideration in the discussion of the specific attraction between molecules and the enzymatic synthesis of molecules.…

[M]aximum stability of a complex is achieved by bringing the molecules as close together as possible, in such a way that positively charged groups are brought near to negatively charged groups, electric dipoles are brought into suitable mutual orientations, etc.… [I]n order to achieve the maximum stability, the two molecules must have complementary surfaces, like die and coin, and also a complementary distribution of active groups.

The case might occur in which the two complementary structures happen to be identical; however,…

the stability of [such a] complex of two molecules would be due to their complementariness rather than their identity. When speculating about possible mechanisms of autocatalysis it would therefore seem to be most rational from the point of view of the structural chemist to analyze the conditions under which complementariness and identity might coincide.

From the biological side it would seem most rational to postulate the possibility of both processes; *viz.* formation of complementary non-identical structures and formation of complementary identical structures . . .

From this time on, what became known as "templating" pervaded discussions of macromolecular synthesis.

SOMETHING TO BRAGG ABOUT

The existing historical record is not totally clear as to whether Pauling and Delbruck's templating notion played an explicit role in Watson and Crick's derivation of DNA structure (see Judson, 1996). However, there can be no doubt about templating's role in the *interpretation* thereof. The mechanism of gene replication inferred from the double-stranded DNA structure is per se the greatest paean to templating imaginable; the echos of Pauling and Delbruck reverberate throughout the second of Watson and Crick's two great papers (Watson & Crick, 1953):

> Previous discussions of self-duplication have usually involved the concept of a template, or mould. Either the template was supposed to copy itself directly or it was to produce a "negative," which in its turn was to act as a template and produce the original "positive" once again. In no case has it been explained in detail how it would do this in terms of atoms and molecules.

> Now our model for deoxyribonucleic acid is, in effect, a *pair* of templates, each of which is complementary to the other. We imagine that prior to duplication the hydrogen bonds are broken, and the two chains unwind and separate. Each chain then acts as a template for the formation on to itself of a new companion chain....

> [O]ur model suggests that this duplication could be done most simply if the single chain (or the relevant portion of it) takes up the helical configuration. We imagine that ... polynucleotide precursors ... are available in quantity. From time to time the base of a free nucleotide will join up by hydrogen bonds to one of the bases on the chain already formed.... [T]he polymerization of these monomers ... is only possible if the resulting chain can form the proposed structure ... [S]teric reasons would not allow nucleotides "crystallized" on to the first chain to approach one another

> in such a way that they could be joined together into a new chain, unless they were those nucleotides which were necessary to form our structure. Whether a special enzyme is required to carry out the polymerization, or whether the single helical chain already formed acts effectively as an enzyme, remains to be seen.

> Our structure … is an open one. There is room between the pair of polynucleotide chains … for a polypeptide chain to wind around the same helical axis.… The function of [such a polypeptide] might well be to control the coiling and uncoiling [or] to assist in holding a single polynucleotide chain in a helical configuration.…

> Despite [the] uncertainties we feel that our proposed structure for deoxyribonucleic acid may help to solve one of the fundamental biological problems—the molecular basis of the template needed for genetic replication. The hypothesis we are suggesting is that the template is the pattern of bases formed by one chain of the deoxyribonucleic acid and that the gene contains a complementary pair of such templates.

With the Watson–Crick structure, templating had finally moved from hand waving generality to concrete, detailed expression. (Note in passing how trivial the evolutionary aspect of gene replication seemed to Watson and Crick: Whether an enzyme needed to evolve to carry out the polymerization was even open to question!)

NATURAL LOGIC: TEMPLATING AND THE GENETIC CODE

When it came to gene expression, the templating concept was certainly front and center from the start. The physicist George Gamow was the first and prominent on the scene. Within a year of the publication of Watson and Crick's seminal papers, Gamow followed with a theory for how a specific protein could be synthesized from its corresponding gene through templating (Gamow, 1954). The details of Gamow's coding theories (there was more than one) are no longer of interest, for in their specifics his models were wrong. However, his Occam's razor approach and the impact his thinking had on his contemporaries was a major factor in moulding how gene expression was perceived. For Gamow, as for Pauling and Delbruck before him, gene replication and gene expression (translation) were basically similar processes: Both reflected molecular complementarity and templating. In such a context, the genetic code is just as important, just as revealing vis-à-vis translation, as the Watson–Crick pairing rules are with regard to the replication of the gene: the genetic code is fixed, predetermined by specific complementary (stereochemical) interactions between the codons in the template and their corresponding amino acids,

and, therefore, the code reflects the interactions that define the nature of translation. Only later would biologists recognize that the genetic code was, at best, peripheral to the working of translation.

Gamow also contributed to the concept of translation in lesser, though still important ways (Gamow, 1954). He took the simplest possible view of the codon-amino acid correspondences, one that was integral to his initial model for protein synthesis. The codons comprised contiguous bases (or base pairs) and the template, in turn, contiguous codons. The codons were uniform in size, namely three nucleotides, which is the minimum number compatible with the fact that 20 amino acids had to be distinguished. As three bases (or base pairs) allowed for 64 different combinations, Gamow saw the code as degenerate, some amino acids, at least, being recognizable by two or more different nucleotide triplets. [Gamow also initially assumed the codons to overlap one another (in order to properly space the amino acids on the nucleic acid template), an assumption that facts later forced him to abandon, and that—unnoticed at the time—undermined his whole approach].

As a physicist, Gamow tended to see a natural logic to the genetic code. To him, there being precisely 20 encoded amino acids was no happenstance. This number had to be the product of simple underlying physical (chemical) principles. Herein lay a challenge worthy of great minds—solving this code from first principles, devising a theory that would automatically place the 64 possible nucleotide triplets into 20 classes (each member of a class recognizing the same amino acid). This grand challenge, cracking the code, had biology in its grip until the mid 1960s, when the code was finally deciphered—by experimentalists, not theoreticians (Nirenberg et al., 1965; Söll et al., 1965).

THE UNRAVELING

Gamow's approach to the code (and that of others at the time) had a strange quality. The concern was not with stereochemistry, that is, the essence of templating, but with the "magic number" 20, how it could be generated from "first principles." However, Francis Crick did not see it that way. He found the very idea that nucleic acids could specifically recognize amino acids anathema. Crick's historic words in his famous (but never published) "Letter to the RNA Tie Club" put it elegantly (see Judson, 1996, pp. 292–293):

I cannot conceive of *any* structure (for either nucleic acid) acting as a direct template for amino acids, or at least as a specific template … If one considers the physico-chemical nature of the amino acid side chains we do not find complimentary features on the nucleic acids. Where are the knobby hydrophobic … surfaces to distinguish valine from leucine and isoleu-

cine? Where are the charged groups, in specific positions, to go with acidic and basic amino acids?…

I don't think that anybody looking at DNA or RNA would think of them as templates for amino acids.…

He then proposed two solutions to the problem thereby created (Judson, 1996, pp. 292–293). (The preferred one only is remembered.)

What the DNA structure *does* show (and probably RNA will do the same) is a specific pattern of *hydrogen bonds*, and very little else. It seems to me, therefore, that we should widen our thinking to embrace this obvious fact.… [I would propose that] each amino acid … combine chemically, at a special enzyme, with a small molecule which, having a specific hydrogen-bonding surface, would combine specifically with the nucleic acid template.… [In this way] each amino acid is fitted with an adaptor to go on to the template.

Somewhat later Crick fleshed out his idea (Crick, 1958, pp. 155–156):

… [T]he template would consist of perhaps a single chain of RNA.… [A]ssume that the backbone [of the RNA chain] is supported in a helix of the usual type by … structural protein … Each adaptor molecule containing, say, a di- or trinucleotide would … be joined to its own amino acid by a special enzyme. These molecules would then diffuse to the [RNA] template and attach to the proper place on the bases of the RNA by base-pairing so that they would then be in a position for polymerization to take place.

Here was Crick's iconoclasm triumphant. The problem of the genetic code and its relationship to translation would need complete reformulation—though not quite. What had the adaptor hypothesis done to Gamow's original concept? The short answer is that it had essentially shattered it. No longer were the codon assignments absolute, predetermined, which meant that the code did not represent the essence of translation: The problems of the code and the nature of the translation mechanism had become separate issues. And gene expression, therefore, could not be analogous to gene replication in having some physical-chemical mechanism that underlay and gave rise to the whole.

However, the adaptor hypothesis left one element of the original conception intact, and that was templating—the bedrock upon which everything rested. All the adaptor hypothesis had done here was to replace *direct* templating with *indirect* templating (of *adapted* amino acids). This change was given little or no serious thought at the time (or later). But it was far from minor. By postulating indirect templating, the adaptor hypothesis had arguably brought the templating notion itself into question: It certainly wasn't the *original* templating notion any more. Yet, so great was our faith in its central

role in biology, that we were not about to question the templating notion at this point—or later, as we shall see.

The disjoining of the code from the mechanism of translation seemed not to dim enthusiasm for the former or diminish the sense of importance surrounding it—and that, in one sense, surprises me, for the code could no longer be seen as the key to translation: The code was a king deposed. But by that time, Biology had become infected with the problem. We had to solve it because it was there. The code had taken on a life of its own.

What does a theoretician do now?

For many theoreticians (the physicists in particular), the code became a purely cryptographic puzzle, which resulted in sophisticated searches for subtle correspondences (Kay, 2000). But without doubt, the most memorable and influential theory to emerge from this new chapter in the code's history (in that it retained a biological semblance and theoretical panache) was Crick's famous "comma-free code"—one of those wonderful, but ephemeral triumphs of intellect over reality (to which theoreticians are predisposed). The comma-free code remained based upon the hopeful presumption that the code might be inferred from first principles of some kind. And because it came out of the adaptor revolution, it implicitly took (indirect) templating for granted.

The comma-free code addressed one of the new class of problems created by the adaptor hypothesis: When the words in a sentence are all run together (spacings deleted), how do you read it? What distinguishes the intended (meaningful) words from "overlap" words, that is, those formed from contiguous letters that span adjacent intended words? Since a templating process was seen as starting at many places in a message, the correct reading isn't established by a fixed start point, from which the read then serially progresses (following some rule) until the end. The correct reading of a message has to be inherent in the nature of the message itself. Crick and coworkers solved this problem by postulating two categories of words, those with and those without meaning, requiring that the set of meaningful words be defined so as to contain no words that could be formed as "overlap" words (Crick et al., 1957). Thus, the message contained meaningful words when read in one way only. Astoundingly, when all words comprise three letters (and are formed from a four letter alphabet), the set of meaningful words can be no larger than 20 (Crick et al., 1957). What a coincidence. What a marvelous and compelling coincidence! (An enjoyable account of this era can be found in Judson, 1996.)

The comma-free code is a perfect example in capsule of the power of the paradigm, how an idea, a theory, right or wrong, can control thinking in an area.

For a short and exciting period around 1960, the comma-free code held Biology in thrall. Crick et al. had found the secret of the code—and with it a whole new playground for theoreticians. Accordingly, more sophisticated variants of the original comma-free code were devised—for example, a code designed so that only one of the *six* reading frames in a double-stranded gene would contain meaningful words (Golomb et al., 1958); or a comma-free code that was both error detecting and error correcting (Golomb, 1962). Of course, the magic number 20 was now gone, and the size of the codon now exceeded three.

This was also a time when in vitro systems for determining the codon assignments were getting off the ground, and the influence of the comma-free code was felt here too. From what I have heard, some experimentalists did not use poly U as the informational input in their early in vitro systems precisely because of the comma-free code (UUU is obviously not a comma-free codon). If poly U were to be used at all, it was suggested, it should be as a *negative* control in these protein synthesizing systems; that is, it would not lead to any protein synthesis! Fortunately, experimentalists do not always heed the dictates of theory.

It's all in the timing

The *timing* of the adaptor hypothesis was a crucial factor in the development of our concept of translation. The adaptor hypothesis entered the scene in the mid 1950s—just as Hoagland, in Zamecnik's laboratory, was trying to work out the role of his newly discovered "soluble RNAs" (sRNAs) in translation. According to Hoagland's own account, Watson one day visited his laboratory, and upon being told the sRNA story, he (Watson) responded that the molecule's role in translation had already been predicted (though not formally published) by Francis Crick: sRNA was Crick's postulated adaptor! Hoagland seems to have been crushed by this, because thereafter he took the adaptor explanation of sRNA for granted (Hoagland, 1959)—and appears to have stopped his own search for the function of sRNAs in translation. In retrospect Hoagland (1996, p. 79) admitted to being "deflated and miffed at having the theoretical framework of our discovery foisted on us by an outsider.…" And that was that! The deference shown by experimental biochemists to the molecular theoreticians, which this episode epitomizes, cleared the way for the adaptor interpretation of tRNA to be imprinted deep into the fabric of Biology.

Crick, on the other hand, was not ready at that point to equate his postulated adaptors with sRNAs. The latter were much larger than the adaptors he pictured: "[sRNA] appears too short to code for a complete polypeptide chain, and yet too long to join on to template RNA (in the microsomal particles) by base pairing …" (Crick, 1958, p. 156). As mentioned above, Crick pic-

tured his adaptors as being in the size range of tri-nucleotides, but he also thought that a metabolic (dynamic) connection might exist between adaptors and sRNAs: "… the twenty different adaptors may be synthesized by the *breakdown* of RNA, probably the 'soluble' RNA, [thereby making sRNAs] a half-way step in this process of breaking the RNA down to trinucleo-tides and joining on the amino acids" (Crick, 1958, p. 156). It would seem that only by popular demand, as it were, did Crick ultimately rationalize the equiv-alence between sRNAs and adaptors. And after that, neither he nor anyone else seriously questioned why tRNA is so large, why a molecule of this size is needed to be a mere adaptor—not to mention whether tRNA actually is in essence an adaptor.

A gedankenexperiment of sorts helps one to appre-ciate why the timing of the adaptor hypothesis was so critical. Assume the adaptor hypothesis had never ex-isted. How then might our concept of translation have developed? Mahlon Hoagland, in the Zamecnik labo-ratory, had been part of an aggressive, intellectually free-ranging, and experimentally creative effort to un-cover the role of sRNA in protein synthesis. That lab had entertained a number of diverse ideas as to the role sRNAs might play in translation (and/or other as-pects of metabolism), ideas they would put to test (Zamecnik, 1969; Hoagland, 1996). Do you think that these investigators (and then the wider Biology com-munity once it had become familiar with sRNAs) would have settled *ab initio* for an adaptor-like interpretation of tRNA's role in protein synthesis? What seems far more likely is that the existence of an unanticipated soluble RNA component in protein synthesis would have triggered a flurry of discussion and speculation as to what these RNAs were doing. And some of these spec-ulations would have envisioned quite different roles for sRNA in protein synthesis. Absent the adaptor hypoth-esis at this critical juncture, it is by no means a fore-gone conclusion that tRNA's role in translation would be pictured as it is today.

[Before continuing, I want it made clear that my crit-icisms of the adaptor hypothesis, made necessary by the negative effect that hypothesis has had on the de-velopment of the translation paradigm, are not to be taken as an indictment of Francis Crick. The adaptor hypothesis is just that, a hypothesis. Any halfway de-cent theory needs to be thoroughly examined, alterna-tives considered (if they are conceivable), and it and any alternatives subject to experimental test (if possi-ble). This is precisely what did not happen with the adaptor hypothesis: Biologists accepted tRNA the adap-tor, made dogma of it, right from the start. In light of the complexity of tRNA, at least, the universal acceptance of its adaptor role seems to me terribly naive. Thus, if there is blame to be laid here, it should be on us biol-ogists, for our intellectually weak-kneed response to this theory. Crick is not responsible for that.]

In summary

The adaptor hypothesis tore apart the original mono-lithic fabric of the concept of translation. The coding, mechanistic, and evolutionary facets of the problem now became separate issues. The idea that gene ex-pression, like gene replication, was underlain by some fundamental physical principle was gone. Gone too was the notion that nucleic acids can in any way recognize amino acids—thereby effectively shutting the door to imagining an origin for translation in some long-gone RNA World, which certainly didn't encourage one to think that there might have been such an evolutionary era (Woese, 1967, 1972; Gilbert, 1986). All that was left of the original concept was templating, now indirect rather than direct.

NOT BURNING THE LAST BRIDGE

Things had changed greatly on the experimental front by the mid-1960s. Not only were in vitro protein syn-thesizing systems up and running, but mRNA had been discovered, and the nature of the ribosome was emerg-ing. Most importantly from the present perspective, pro-tein had been shown to be synthesized and the gene replicated, not through templating, but by tape reading processes—processes that start at a fixed point and proceed sequentially therefrom, recognizing one input molecule (nucleotide or tRNA) at a time. The Delbruck–Pauling, Watson–Crick image of a string of monomers aligned on the template as a prerequisite to their being joined into a polymer was not in keeping with the facts. Something was wrong with classical templating, the foundation for our view of translation. Now it was cer-tainly time to burn that last bridge, to rethink our per-spective from the ground up. In demolishing the basis for the old view of translation, tape reading had fortu-nately offered the basis for a new one, more dynamic and productive.

So, what then happened? Nothing. The small boats had not noticed the sea change. To the extent that we thought about the matter, it seems only to have been to rationalize tape reading as a form of templating: tape reading was "local" templating, that is, the alignment of a single monomer unit (or adapted monomer unit) on a "template" as a precondition to its incorporation into a growing polymer chain—not the sort of templating en-visioned by Pauling and Delbruck at all. Since it is ac-complished entirely within the context of an enzyme, tape reading could more profitably have been viewed as an example of the notion that antedated and in a general sense inspired Pauling and Delbruck's grand templating vision, namely, lock-and-key recognition of substrate(s) by an enzyme.

One of the reasons the classical templating perspec-tive was never seriously questioned, of course, was that it is not unreasonable to invoke templating in the

context of primitive nucleic acid replication. Indeed, the fundamental premise of origin-of-life experiments almost from the beginning has been strings of (activated) nucleotides that align on a template prior to their polymerization. And classical templating can be demonstrated experimentally in vitro too, at least for the special case where purine nucleotide monomers align on poly-pyrimidine templates (Orgel, 1973).

Although the templating notion is no longer front and center, it is still there, which is worrisome in that the character of paradigms turns on the appeal of their grand images—and templating is surely one of these. The templating notion had informed us that gene replication and gene expression (translation) are of a kind; both reflected an underlying templating. That is why, in my opinion, Crick's argument for the adaptor did not really get to the heart of the issue—translation not based on templating was unthinkable. But tape reading is not templating—from which the disinterested mind can easily infer that, regardless of how gene replication evolved, translation did not arise as templating, and so cannot be properly conceptualized in these terms.

A CARTOON GUIDE TO TRANSLATION: THE A-SITE–P-SITE THEORY

As familiarity with the ribosome and the process of translation grew in the 1960s, a need arose for a theory of how the process worked. That need was fulfilled by the A-site–P-site model (Watson, 1964, 1976), which quickly became the most powerful shaping influence of all on the developing paradigm. The theory's power stems largely from its being pictorial, a cartoon that has appeared in all biology texts for the last several decades. Such a cartoon is easy to remember, almost impossible to forget—and, most importantly, it is impossible to think around. Virtually all of the many new findings concerning translation over the last several decades have been interpreted in terms of this model. And through what amounts to circular reasoning, we have come to see A-site–P-site as a powerful theory that explains almost everything about translation—with the result that the model has been accorded a sacrosanct status within the translation paradigm. There seems no reason to question or test it; no need to conceptualize translation in any other than this simplistic pictorial way.

I will spare the reader yet another rendering of the A-site–P-site model, and launch directly into a discussion of the model's nature—its roots, what it means to our concept of translation, how we use it, and the need today to move beyond it. The first thing to notice is that the A-site–P-site model *explicitly* assumes tRNA to function as an adaptor (Watson, 1964). This is the main reason why almost all biologists see the ribosome—and the ribosome alone—as the mechanism that performs translation. tRNA is merely a "substrate."

Second, the A-site–P-site model is framed in terms of (ribosomal) sites. This may be because so little was known about translation in the 1960s that it was not feasible to conceptualize it any other way (Watson, 1964). However, that in itself would not explain why biologists have remained content with such a formulation. But the paradigmatic nature of this mesmerizing little cartoon does. I think there is more to it than that however. Molecular biologists were conditioned by the templating and adaptor notions to think statically; and if the imagery of the A-site–P-site model is anything, it is static. It frames the initial and final states of a process—and really says nothing about the process itself.

Third is the model's value/role as theory. A theory's function is to explain, to predict, and to focus the field. A good explanation integrates a finding into a broader context, gives it a significance it would otherwise not have. (The double stranded structure of DNA does this exquisitely with the "Chargaff rules.") If you think about it, you will discover that most new findings regarding translation are outside the purview of the A-site–P-site model, and if not, they tend to be "explained" by their incorporation into the model. Rarely, if ever, does the model provide eye-opening explanation. Facts are not enriched by the model. The model is "enriched" (made more complex, that is) by the facts.

The finding of Moazed and Noller (1989) that "translocation" from the A- to the P-site does not occur simultaneously in the large and small ribosomal subunits is a perfect example here. The A-site–P-site model does not explain this finding; rather, the model has been changed to accommodate it: The resulting "hybrid state" cartoon first shows a stick-figure tRNA residing in both the large and small subunit portions of the A-site (as usual). The tRNA then "tilts" so that its anticodon half remains in the small subunit portion of the A-site but its peptide-carrying half resides in the large subunit portion of the P-site, before finally changing to the classical P-site positioning in both subunits (Moazed & Noller, 1989).

The fact that an experimental result is not inconsistent with a theory does not necessarily speak to the theory's predictivity or to its validity. To be meaningful, predictions need to be specific in a way that the theory can stand or fall with the experimental result. The A-site–P-site model does not predict experiments by which it can be critically tested, and the reason the model is not contradicted by facts is simply because it is so ill-defined and general that results it can't somehow "accommodate" are hard to find. Nowadays the model does not even pertain to the majority of experimental findings.

In focusing the field, however, the A-site–P-site model had, and continues to have its most powerful impact. While such conceptual power is welcome with a good theory such as the Watson–Crick model for gene replication, that power can be counterproductive (stifling, misdirecting) in the case of weak theories such as A-site–P-site and the adaptor hypothesis.

What the A-site–P-site model really is is our attempt to construct a model of translation on the fly, starting from certain simple and quite constraining ideas that we consider inviolable. Thus, A-site–P-site is not a full-fledged theory and should not be treated as such (which, unfortunately, it is). Our attempt to construct a useful translation paradigm will be successful only to the extent that our starting assumptions are valid and the focus they provide productive. A-site–P-site fails in both respects. We need to critically reexamine its underlying assumptions (and the effect these have had on the development of our view of translation).

TABULA RASA

Purging past prejudices is a necessary step in establishing any new paradigm. Template thinking, adaptor thinking, and A-site–P-site imagery must be put aside if we are to genuinely understand translation. Looking back on the historical meanderings of the translation concept, it becomes evident "how little theory was able to contribute" (Crick, 1966). Yet, it is precisely the attempt to theorize our way to an understanding that created the (conceptual) bind translation is in today. We would have been better off, in my opinion, had experimentalists like Zamecnik, Hoagland, Lipmann, and others been left to their own devices. They were explorers wandering in an uncharted experimental wilderness, and so, open to all manner of possibilities and ideas. This is precisely what was called for at that particular juncture. A field tends to start with a simple mapping of the territory and identification of its "inhabitants." This is best accomplished with a minimum of intellectual fetters. The theoretical hoopla that initially surrounded translation surely aroused scientific interest, but it was needlessly prejudicing and turns out at very least to have misplaced the emphasis. Our concept of translation today would have been quite different than it is, had we proceeded more innocently.

Not surprisingly, the templating, adaptor, and A-site–P-site notions suffer from the same basic defect: They are by nature overly static, and so influence us to use the wrong type of imagery in conceptualizing translation, and this, at best, amounts to emphasizing the wrong aspects of the problem. Look at the templating notion. Picture I: monomers aligned and oriented. Picture II: monomers still in their places, but now chemically joined. What could be more static? The adaptor hypothesis is based on templating and the A-site–P-site model on the adaptor hypothesis. Seen through such eyes, the *workings* of this incredibly dynamic translation machine are lost.

Contrast imagery of this sort to that which stems from a tape reading perspective. Tape reading is per se dynamic. Process, not position, is primary; tape reading invites you to understand mechanism. A true tape reading perspective would not settle for tRNA, the adap-tor. It demands to know what tRNA is doing during translation. A tape reading perspective would not see translation solely in terms of sites. Its focus would be on the changes that occur during the process, on states of the system and transitions among them. This is the imagery for a dynamic concept of translation.

A second, particularly pernicious characteristic of the templating notion is that it overly reduces biology to chemistry, to static stereochemistry, and, thus, implies that the essence of a biological entity or process resides in some particular physical or chemical interaction. This makes the evolutionary transition from a preexisting physical/chemical entity, process, and so forth, into a biological one simple and straightforward—leaving the original physical-chemical basis transparently displayed in the biological entity. This may be true in some cases, say gene replication, but it certainly does not hold for translation and many other biological entities/processes. Here the extensive evolution undergone obscures the entity's origin, and becomes its essence.

Let's face it. Biologists have in effect been experimenting outside the purview of the conceptual trinity (templating, the adaptor hypothesis, and the A-site-site model) for a long time now, but they are still hidebound to the classical paradigm when it comes to putting the picture together. Something has to give. Isn't it time to say: "No, tRNA is not an 'adaptor'—a 'substrate' for the translation mechanism. tRNA is probably a 'motor'—a central functioning part of the translation mechanism." And, "No, it is not the sacrosanct 'A' and 'P' sites that are important, it is the molecular movements underlying and defining them that should be the focus of attention." We are trying to reconstruct a machine here, not paint a still life.

TOWARDS AN RNA WORLD VIEW OF TRANSLATION

For me "RNA World" represents an ancient evolutionary era when biopolymers, including simple polypeptides, flourished; and, because translationally produced proteins had yet to arise, it was an era dominated by nucleic acids. The whole was the evolutionary product of and was sustained by some sort of metabolic network, which had come into existence prior to enzymes as we know them. The saga of what I (1972) like to call this "era of nucleic acid life" involves the gradual emergence of programmed polypeptide synthesis. The protagonist in the saga, of course, is the translation apparatus, which came from obscure beginnings to develop into an enormous and powerful molecular mechanism that eventually completely changed the nature of the living world.

An RNA World perspective is fundamentally at odds with the way molecular biology perceives the living world. The molecular perception takes cells and organisms as

givens and asks how (in molecular terms) these entities work. It is inherently mechanistic. The RNA World represents one of the great transitions in the history of life on this planet. A primary concern of an RNA World perspective, then, is how molecular biology's "givens" came into being, how modern protein-based life arose from a world of nucleic acid life. Such a perspective is not fundamentally mechanistic; it is evolutionary.

The modern translation apparatus obviously cannot be viewed as one of the molecular givens, as some sort of "*machina ex deus.*" Translation is complex enough that: (1) it had to have evolved through numerous stages, from far simpler beginnings (Woese & Fox, 1977); and (2) any true understanding of the mechanism will require an understanding of the way it evolved. The origins of translation, that is, before it became a true decoding mechanism, are for now lost in the dimness of the past, and I don't wish to engage here in hand waving speculations as to what polymerization processes might have preceded and given rise to it, or to speculate on the origins of tRNA, tRNA charging systems, or the genetic code. Yet, there is merit at this juncture in trying to picture what a primitive translation apparatus was like, for this would provide a theoretical superstructure helpful in the interpretation of the accumulating wealth of detailed structural data concerning translation, and it would provide future experimentation a needed focus.

The ground for developing an RNA World view of translation lies in a few very simple (to me self-evident) assumptions: (1) The primitive translation apparatus had to have been far simpler than its modern counterpart; (2) The primitive apparatus was based upon RNA and RNA interactions; (3) The *essence* of the primitive apparatus remains at the heart of modern translation: Modern translation is no Rube Goldberg machine, but rather a simple primitive mechanism that has been highly embellished and refined over the evolutionary course; and this means that understanding the primitive mechanism is part and parcel of understanding its modern counterpart; and (4) Any role that protein components come ultimately to play in translation is confined to facilitating, refining, or enabling key steps in the process, steps that are, however, *defined* by RNAs and their interactions.

The more we come to know about translation, the more it seems an RNA-based mechanism. tRNA's central role in codon recognition has never been in doubt. But now peptidyl transfer is seen to be mediated by RNA as well (Nissen et al., 2000). When it comes to mRNA movement, however, biologists are still uncertain. They tend to see protein at work, principally in the form of elongation factor EF-G (Lewin, 2000). Yet neither EF-G nor any other translation factor is *required* for translation. As shown long ago, translation can occur without any factors and without GTP—so long as ribosomal protein S12 (the "streptomycin protein") is

not present (Gavrilova et al., 1974). So, even here, a defining role for RNA cannot be ruled out—and should be actively sought.

Given the above assumptions, the first order of business is to develop a useful picture (theory) of a simple primitive RNA-based translating mechanism. Unfortunately, the ribosome today is not simple, and the more we study it, the more complex it becomes in both structure and function—the more it seems a molecular Rube Goldberg machine (Frank & Agrawal, 2000; Yusypov et al., 2001). The ribosome is the quintessence of evolutionary elaboration. However, tRNA is different. A simple molecule today, it has to have had simple ancestry. Yet, tRNA is not so simple structurally that one cannot envision its undergoing conformational change. And, after all, tRNA is the molecule most intimately associated with the growing peptide—bringing the amino acid in, shepherding the peptide (covalently) throughout the process, and releasing a finished protein at the end. For me, there is little doubt that translation began with simple tRNA-like entities, ancestors to the modern tRNAs (Woese, 1970).

Going back to the future

With almost all effort and attention over the last several decades focused on finding the translation mechanism in the ribosome per se, we seem to have overlooked some tantalizing hints of mechanism in tRNA. Seven-membered hairpin loops are not all that common in RNA structure in general (Gutell et al., 1994); yet two such hairpins occur in tRNA, namely the anticodon and common arms. The similarity between them is quite remarkable: not only are their loops of the same size, but both loops are underlain by stalks of five base pairs, and they show compositional similarity (see below). This close resemblance cannot be explained by overall tRNA structure, for the common and anticodon arms are incorporated very differently into that structure (Kim et al., 1974; Ladner et al., 1975). I feel there to be some deep and ancient functional parallelism underlying all this. And, for unknown reasons evidence of that relationship is preserved in modern tRNA.

The configuration of the anticodon loop suggests mechanism in its own right. In its canonical (crystal structure) form, the loop is structured so that the 3′ five of its seven nucleotides (nt 34–38) form a single-stranded coaxial helical extension of the 3′ strand of the underlying (double-stranded) stalk (Kim et al., 1974; Ladner et al., 1975), a conformation that places the anticodon at the top of the extension, with the nucleotide that reads the last base of the codon outermost. This coaxial arrangement could be further extended by interaction with mRNA, in which case, not only the codon that pairs to the anticodon but the following codon too, could coaxially stack about the same helical axis (Woese, 1970).

I have long been attracted to the old idea that the two codon–anticodon couples involved in a decoding event are coaxially stacked together. (However, I do not see this as more than a transitory state.) Were such a sextuplet duplex stack to form, the anticodon loop of an incoming (A-site) tRNA could not, for steric reasons, be in the above-described canonical conformation. Rather than rejecting the codon–anticodon sextuplet duplex because of this, I take it as an indication that tRNA undergoes conformational change during translation. Indeed, it seems reasonable that cyclic conformational changes in tRNA anticodon arms might define the actual mechanism of mRNA movement. Several decades ago I made such a proposal, a molecular "reciprocating ratchet," in which the anticodon arms of two adjacent tRNAs jointly underwent conformational changes that pulled the mRNA through the ribosome (Woese, 1970). However, focusing on some particular tRNA-driven mechanism is not important at this juncture. First it is necessary for biologists simply to recognize the *possibility* that tRNAs can undergo conformational changes and, therefore, play an active role in translation.

[For those of you who are interested, the proposed ratcheting mechanism (Woese, 1970) involved alternating conformational changes in the anticodon loop, between the above canonical conformation (in which the 3′ five nucleotides are coaxially stacked on the 3′ strand of the underlying double-stranded stalk) and what can be called an "anticanonical" conformation (in which the 5′ five nucleotides of the loop are so stacked, in this case on the 5′ strand of the underlying stalk). As described above, the canonical conformation allows a given codon and its successor to stack along the same helical axis, whereas the anticanonical conformation allows that codon and its *predecessor* to do so. The alternation between canonical and anticanonical conformations would then serve to "ratchet" the mRNA through the translation machine. (But note that the anticanonical canonical conformation is less stable energetically than its canonical counterpart. To begin with, weakly stacking pyrimidines underlie the anticodon in this conformation. And then, the tilt of the base pairs in an (RNA) A-form helix makes the distance between the top of the anticodon and the innermost base pair of the stalk greater in this case than it is in the canonical conformation. For these reasons, I think that if the anticanonical form exists, it probably does so only transiently and within the context of the ribosome.]

The degree to which the tRNA molecule is evolutionarily constrained, in structure, in sequence, and in modification, is telling us something. The two residues in the anticodon loop preceding the anticodon are generally pyrimidines (which is true for the initial two residues in the common arm as well), and the two residues following the anticodon are generally purines. The purine immediately adjacent to the anticodon is almost always an A residue that is hypermodified. A purely

structural rational might be given to some of the compositional constraints: strongly stacking purines underlying the anticodon would tend to strengthen the canonical conformation. However, the modifications of residue 37 are not so simply explained. These can prevent normal base pairing by this anticodon-adjacent residue (an A), but the modifications surely need not be so elaborate to do this. Similarly, the fact that these modifications can differ greatly in size would argue against their acting in some purely steric capacity. Interestingly, the nature of these modifications correlates strongly with the composition of the first base of the codon being read, which could suggest a role for the modifications in fine tuning the energetics/accuracy of translation. In any case, these features are consistent with, if not suggestive of, a central role for tRNA in the actual mechanism of translation.

The role of the ribosome

If the mechanism of primitive translation centers about tRNA-like entities, how does the ribosome fit into the picture, and when? A primitive translation apparatus based solely upon tRNA-like entities should be highly imprecise. Codon recognition, reading frame maintenance, and perhaps even the direction in which the next codon in mRNA is (defined and) read, would not be as accurate as it is today. This imprecision is a function of the simple nature of the primitive mechanism given ambient thermal noise. To pass beyond this stage, the performance level of the simple primitive machine has to be improved and the process generally facilitated. That is where the ribosome comes in (Woese, 1973). The evolving ribosome could make the conformational shifts that occur in the simple mechanism better defined and more precisely executed by incorporating them into programed cascades of conformational shifting, for example. In providing an environment for the primitive mechanism that may be inaccessible to water (see Huttenhofer & Noller, 1992) and is, in effect, quasi-solid state, the ribosome could constrain translational and some rotational degrees of freedom, which could have an effect similar to lowering the temperature, that is, reduction of thermal noise (Woese, 1973).

WRAPUP

I began this piece with the assertion that Biology today is at a crossroads. There are two reasons for this, one technological, the other conceptual. The capacity to sequence genomes has opened a new world to biologists. But sequencing provides only the means; it doesn't define the ends. Biology of the last century was shaped by the molecular paradigm. Now that paradigm has effectively run its course; it no longer provides a fresh vision of the future. And it has a dark side, a flaw, which is now surfacing.

The foundations for 20th century Biology were laid in the 19th century, with cell theory, Mendelian genetics, and Darwinian evolution. However, the edifice that became 20th century Biology, reductionist and materialistic to the core, was built upon only the first two of these. Mechanism does not know evolution. Yet comparative analysis of sequence data, a major preoccupation of biologists today, is inherently evolutionary—no matter how hard we try to avoid the fact. Sanger's sequencing revolution, which began as a technological exercise within a strictly molecular, mechanistic context, has proven itself an evolutionary Trojan Horse. As a result biologists today are looking in the mirror and asking themselves what Biology is and where it is to go in this new century.

Today our science in under tremendous formative pressure from the society at large—from the medical-industrial complex in particular—to become a discipline of applications, to merely be society's handmaiden. Is Biology's future simply in creating Man the medical miracle? Or is it, as I and certain others feel, in returning to and expanding our science's roots—in finally embracing full out a Darwinian perspective. This is the real Biology, the Biology that addresses "the most challenging intellectual problem of all time" (Brenner, 1998), that is, Mankind's eternal question, how we came to be. And at the center of it all sits the problem of translation—how it works, how it arose, and how its evolution transmogrified an ancient RNA World.

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