CLEMENS RICHERT

THE NEW WORLD

Thoughts of a nucleic acid chemist who finds himself in the postgenomic era

"So, what do you want to work on?" The question still rang in my head long after my interview for graduate school in chemistry was over. Nine years ago, I was not so sure whether I should become a protein biochemist or a synthetic nucleic acid chemist. Those days of uncertainty are over.

Even though the antisense community went from euphoric to underfunded, even though I had to weather the tough days of being junior faculty in a competitive field, and even though colleagues hinted that repetitive biopolymers could be synthetically boring, I still feel that few fields offer as many challenges (and rewards) as synthetic and biological nucleic acid chemistry.

Consider the challenge associated with using the genetic information provided by the Human Genome Project. Mining this information—for example, in the form of genotyping single-nucleotide polymorphisms (where C is cytosine, G is guanine, A is adenine, and T is thymine), determining the function of new genes, and measuring the expression patterns of entire genomes—simultaneously calls for experimental techniques that could only be imagined when the intention to chemically synthesize a gene was announced in 1967. Today, any of 100,000 different sequences can be detected with a DNA chip smaller than the palm of a newborn's hand. That these chips can be prepared efficiently via chemical syntheses attests to the ingenuity and persistence of nucleic acid chemists. So does the fact that sequencing no longer requires radioactive materials.

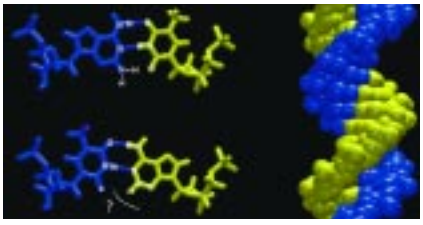
We owe much to new technologies, often developed by physicists and engineers working side by side with chemists. Matrix-assisted laser desorption and ionization/time-of-flight (MALDI/TOF) mass spectrometry, for example, allows detection of femtomole quantities of oligonucleotides, often in the form of a single, sharp, and accurately predictable peak. Many stimuli came from molecular biologists and physicians who discovered exciting new applications for nucleic acids, which include but are not limited to RNA interference, chimeraplasty, and immunostimulation with "CpG" oligonucleotides. Without them, fewer chemists would have pursued compounds that turned into successful drugs or physiologically relevant targets whose true roles in cells have to be uncovered.

Not all work in nucleic acid chemistry is as applied. Quite a few fundamental questions remain largely unanswered to this day. How important, for example, is hydration for the stability of Watson-Crick duplexes of DNA and RNA? Is the low base-pairing fidelity at the termini of duplexes (often cited as the cause for the degeneracy of the genetic code) the result of frequent breathing and wobbling, or do matched and "mismatched" base pairs provide a similar enthalpic stabilization when stacking partners are lacking? Do polymerases refuse to elongate after the incorporation of most unnatural nucleotides because the hydroxyl group to attack the next incoming nucleotide triphosphate is poorly positioned or because some as yet unknown allosteric effects prevent the activation of the a-phosphate of just these nucleotide triphosphates?

Perhaps an even simpler question: How does one generate oligonucleotide probes whose affinity for their respective target sequences is independent of the G/C content? Nature evolved DNA to contain weak A:T and T:A base pairs, probably to give cells TATA boxes and telomer sequences, to name only two examples. As a result, G/C-rich DNA probes (including those on DNA chips) tolerate mismatches in their duplexes before the A/T-rich sequences even begin to bind to their targets. Tolerated mismatches, however, can give false positives in hybridization experiments with chips. When developing improved hybridization probes, a first level of design could call for analogs of A and T that form three hydrogen bonds with their respective Watson-Crick partners. How they might be designed is not as clear for thymidine residues. How to design (and synthesize) nucleobase analogs on a second, more subtle level to obtain A:T and T:A base pairs stabilized through stacking interactions (with modifications other than propynyl groups) is currently unknown. After all, it is still difficult to design complexes of molecules with molecular weights of several thousand daltons.

Here, synthesis and experimental biophysical evaluation come into their own, sending us back to our benches to find new, functional nucleic acid analogs. For my part, there is nothing that I would rather be than a nucleic acid chemist in the postgenomic era.

PROBING NUCLEIC ACIDS
Richert (seated) with graduate student Jan Rojas in his lab at the University of Constance.



FORTIFICATION **Because A/T-rich hybridization probes fail to bind target strands under conditions where G/C-rich counterparts begin to form stable duplexes (right), high-fidelity DNA chips call for chemically modified DNA whose adenosine and thymidine residues have a high affinity for their Watson-Crick pairing partners. Shown on left are fortified A:T base pair (top) and a T:A base pair whose fortified analog has yet to be developed (bottom).**

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