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Nucleic acids continue to surprise

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Nucleic acids and their interaction with proteins remain at the center of molecular biology. Despite decades of structural, biophysical and biochemical investigation, both RNA and DNA are still providing surprises, as shown by the reviews in this issue of *Current Opinion in Structural Biology*. The diverse topics spanned by these reviews include the use of nucleic acids to make new materials, recent detailed structures of small nucleic acids-ligand complexes to large assemblies like the ribosome, novel in vivo methods to probe RNA structure, and proteins that remodel nucleic acid structures (helicases), fix chemical damage to DNA, and modulate gene expression by binding to RNAs. The structural biology of nucleic acids stands at a fertile crossroads of disciplines, integrating concepts from genomics, dynamics, and traditional molecular biology.

Since the determination of the B-form DNA duplex structure over fifty years ago, DNA has continued to surprise us with new and polymorphic structures. The review by Egli and Pallan provides a sweeping look at the diverse structures of DNA and the diverse interactions with its many ligands. New G-quadruplex structures continue to emerge, and there are several new complexes with quadruplex-binding small molecules. Other ligand complexes with DNA include a structure of a minor-groove binding polyamide that sequence-specifically recognizes the minor groove. Duplex DNA continues to exhibit polymorphism, and certain sequences have the propensity to form unusual base pairs, which may facilitate sequence-specific protein recognition. In addition, a number of structures have emerged of chemically modified DNA, which is important for understanding the effects of mutagenic lesions and how they are recognized by the DNA repair machinery. Finally, a look at the ever-increasing repertoire of non-natural DNA analogs is given, including both non-native backbone chemistry and nonstandard bases. This review provides a concise summary of recent results in this diverse area.

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In addition to the inherent structural variability seen in DNA crystal structures, DNA has taken on a second career as a material for the creation of novel nanotechnologies. The review of Shih outlines the approach of building complex DNA architecture. The linear structure of DNA, and the abilities of multiple strands to come together at junctions, like the 4-way holiday junction in DNA recombination, allows construction of complex higher order DNA structures. The DNA strands in a 4-way junction can lie parallel to each other, driven by coaxial stacking of the parent helices. By starting with a long, single strand of DNA (such as M13 phage DNA), short oligonucleotides can be designed to assemble onto the DNA template, folding and bending the DNA into complex higher order shapes. This “DNA origami” has led to creation of DNA structures that are simple shapes such as stars, to complex images, such as a map of the United States. By clever choice of oligonucleotides and hybridization, these architectures can be extended in 3-dimensions, creating boxes, tubes, and higher-order structures. The simple base-pairing rules and helical structure of DNA may allow a revolution in nanotechnology, especially when DNA structures are grafted onto other materials such as semiconductors.

DNA is under a constant chemical assault that undermines the genetic code; toxic metabolites and foreign agents can react with DNA bases or lead to strand scission. Cells have evolved a complex machinery to repair damage to DNA. The review of Perry et al. presents the spectacular recent advances in structural biology of DNA damage response. The authors cover both nucleotide and base excision repair mechanisms, through which damaged sites are recognized through sensing along the minor groove, with base flipping into an active site to perform repair. Combinations of structural and dynamic results have revealed how structural specificity in repair is achieved, and is coupled to processes such as DNA replication. Conformational plasticity and control of protein interface geometry are common themes in these systems. Structures of larger DNA repair systems, like the DNA-PK damage-sensing kinase, have been recently solved using combinations of cryoelectron microscopy (cryoEM) and crystallographic approaches. The rich deluge of structural understanding in DNA damage repair has been driven by multiple approaches, from chemical biology, to dynamic spectroscopy and deuterium exchange, through structural method such as NMR, crystallography and cryoEM. The complexity of these systems demands such an approach.

Progress on structural biology of RNA has lagged that of DNA, although crystal structures have now been solved for most of the large primordial RNAs, including the ribosome, Group I and Group II self splicing introns, and RNase P. Many functional RNAs are very large or flexible, making crystallization difficult, and the review by Weeks describes a series of advances in the area of chemical footprinting to handle these cases. The advent of sequence independent chemistry for RNA footprinting makes it now possible to deduce secondary structures, in terms of helices and loops, on even very large RNA molecules. This process has been facilitated by the development of quantitative analytical tools to handle the cleavage data, and inclusion of footprinting data dramatically improves secondary structure prediction algorithms. Finally, advances in determining tertiary structures have been made, and methods for time-resolved footprinting are now enabling folding kinetics and pathways to be observed directly.

RNA binding proteins control the expression of genetic information by regulating transcription, splicing, translation and RNA metabolism. Genomics has revealed the deep richness of RNA-binding proteins in different organisms. The review of Kaymak et al. focuses on nematodes, highlighting recent structural studies of different classes of RNA binding proteins. Nematodes are unusual, in that they have an expansion in the number of RNA-binding proteins compared to other organisms. The authors focus on 3 major classes of RNA binding proteins: the pumilio-like proteins (PUF family), the CCH-like tandem zinc finger proteins (TZF family) and the argonaute families (AGO family). Crystal structures have revealed the distinct architectures of these proteins, and coupled with biochemical experiments, have lead to an understanding of their RNA specificity. The distinct structures of PUF, TZF and AGO proteins provide different strategies for RNA recognition (PUF, extended surface, TZF, zinc fingers, and AGO, double-stranded RNA recognition and cleavage). The structural biology of these RNA-binding proteins is clearly linked with the evolving understanding of nematode genetics and development.

Cellular processes that involve transactions with nucleic acids indubitably involve helicases. These proteins couple ATP binding and hydrolysis with unwinding of nucleic acid structures, disruption of protein-nucleic acid complexes, or directional movement along a nucleic acid. Helicases can act monomerically or as higher order toroids. In their review, Fairman-Williams et al. outline comprehensive sequence analysis of non-toroidal helicases, showing that they divide into two separate superfamilies (SF1 and SF2). The two superfamilies are united by a similar nucleotide-binding core, but have distinct sequence motifs that couple this cleft to nucleic acid binding domains. These sequence differences lead to different structures and mechanistic features of how ATP binding and hydrolysis couples to nucleic acid manipulation. In addition to core features, helicases also can have accessory domains that modulate the biological activities of the helicases. The understanding of helicase biology will further require a convergence of structural, dynamic and functional analyses to build on the sequence families outlined here.

The final review chapter by Demeshkina et al. provides a detailed look at the interactions of the ribosome with the mRNA and tRNA ligands. Over the past decade, numerous structures of the ribosome have now been solved with various combinations of ligands corresponding to different state in the cycle of translation. The ribosome must both firmly grasp the mRNA and the codon-anticodon minihelix in the P-site, and release it during translocation. Similarly, decoding requires dynamic discrimination of cognate and noncognate minihelices at the A-site, but this interaction must be stably maintained during translocation after peptide bond formation. These interactions are maintained by an extensive network of intermolecular interactions between the tRNA:mRNA complex and the ribosome. In particular, the role of the tRNA modifications on this recognition is discussed.

The field of nucleic acid structure and nucleic acid-protein interactions has exploded in recent years, driven by our expanding view of RNA in biology. Structural studies have come in rapid succession, and have revealed the basic architectures of many key systems, including the ribosome and transcription machinery. Basic understanding of nucleic acids from a structural, biophysical and biochemical standpoint has enhanced our foundation to probe novel biological functions. The future will require complementary dynamic studies to

merge structure and mechanism, and the patience to grasp the breathless pace of biological discovery. The reviews in this issue provide a snapshot of the current state of affairs in several key areas, and they chronicle the recent and rapid progress in this exciting field.

Acknowledgments

The Puglisi lab studies the role of RNA structure and dynamics in cellular processes and disease.

The Williamson lab uses biophysical approaches to study RNA structure and RNA-protein interactions, including ribosome assembly.