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Battle for the Bulge: Directing Small Molecules to DNA and RNA Defects

Small molecules were tailored to specifically bind bulged DNA by complementing the geometry and nucleotide size of the bulge site. The prospect of generating small molecules that influence the secondary structure of DNA and RNA holds great promise for clinical applications.

Nucleic acids can fold into a variety of complex three-dimensional structures, a characteristic best exemplified by the ribosome [1–3]. Like their peptide counterparts, folded DNA and RNA can have diverse functions, including catalytic activity [4, 5] . However, unlike proteins, nucleic acids are known to adopt especially stable secondary structures that often assemble into tertiary structures in a hierarchical fashion [6]. Nucleic acid secondary structures themselves can be quite diverse and include helices, stem-loops, bulges, internal loops, and multibranched junctions. Helical defects have been more extensively described in RNA, where the single-stranded nucleic acid folds back on itself, generating an essentially unlimited combination of secondary structural elements. DNA, however, can also produce complex secondary structures during replication and recombination, leading to the formation of lesions and triplet repeats, which in turn can result in a variety of diseases and cancers. The paper by Xi and coworkers in this issue of *Chemistry & Biology*, therefore, addresses a critically important issue, that of selectively targeting specific DNA secondary structures with synthetic compounds [11]. If this strategy proves to be successful, it could ultimately generate powerful drug leads that could target diseases at the source of the problem, the genome itself.

The authors synthesized a variety of compounds and measured their various affinities for binding to both bulged and perfect DNA stem-loops. They begin with the compound NCSi-gb, which is the product of a general base-catalyzed intramolecular cyclization of the antitumor natural product, neocarzinostatin chromophore (NCS-chrom). NCSi-gb is composed of a spirocyclic motif, two aromatic ring systems that can stack between nucleobases, and a pendant aminosugar. NCSi-gb can bind to bulged DNA and RNA and can also form an isostructural molecule capable of cleaving bulged DNA. In addition, NCSi-gb is able to induce formation of a DNA bulge by stacking between the base pairs that flank the bulge site [7]. In an effort to extend their repertoire of bulge binding compounds, the authors synthesized and measured the binding abilities and specificities of NCSi-gb-related compounds. A pair of enantiomers was studied, which, like NCSi-gb, contained a spirocyclic motif, two aromatic ring systems, and a pendant aminosugar, but lacked the steric bulk of a 5-membered cyclic carbonate. Interestingly, the enantiomers have binding affinities for bulged DNA molecules that are predictably different from the behavior of the natural product. For example, the smaller compounds bind more readily to DNA hairpins with one-nucleotide bulges, presumably due to absence of steric interference within the environment of the bulge itself. The two enantiomers also behave in unique ways, as expected from the chiral nature of the DNA target.

The authors have identified a complex relationship that exists between the size and flanking nucleotide sequence of bulged DNA and the affinity of the natural products and derivatives for the DNA targets. This effect is especially pronounced in some cases. For example, a two-nucleotide (GT) bulge within a DNA sequence binds NCSi-gb with variable binding affinity that differs in strength by up to 350-fold, solely as a result of variations in the flanking sequence. That observation suggests that the helical context of the bulge is a critical determinant of binding affinity. Therefore, it may be possible to rationally design and synthesize compounds with the ability to discriminate for or against these defining features of the DNA bulge, as has been done in some cases for helical DNA [8]. However, generating such compounds for use in the clinic may prove to be especially difficult, since the direct effects of flanking base pair changes on the structure of helical defects are not systematically known. Alternatively, the authors also mention that it may be possible to take a combinatorial approach to the synthesis of future compounds. Desired compounds could be isolated using a two-tiered selection strategy; first, in a round of *positive* selection, a pool of compounds would be isolated that bound to a particular secondary structure, and second, this pool would be narrowed by *negative* selection, where compounds that bound related secondary structures would be discarded. Such a process might lead to compounds with high discrimination for certain DNA targets. It might also prove interesting to approach the combinatorial synthesis from the perspective of the nucleic acid, using in vitro selection or SELEX [9, 10] . If the synthetic compounds could be tethered to a solid support, then one could screen for DNA secondary structures that bind to the compound, revealing all secondary structures that bind tightly. From this, one could see if the expected target were present or not and any related, and perhaps unexpected, DNA secondary structures with high binding affinity.

The prospect of developing small molecules with specificity for certain nucleic acid secondary structures is certainly an exciting one. With the vast array of DNA and RNA secondary structures possible and their occurrence in many biologically critical processes, there are sure to be many important therapeutic applications. The authors comment that compounds could be engineered to have reactive functionalities. These molecules could be especially useful for mapping the secondary and tertiary structures of nucleic acids and for probing DNA

secondary structural defects or rare non-Watson Crick conformations. In addition, in the case of RNA, these compounds could be used to lock secondary structures and prevent their folding up into functional tertiary structures.

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Selected Reading

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