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Previews

Site-Selective DNA Binding Drugs

Last month in *Chemistry & Biology*, Gottesfeld and colleagues demonstrated that designer DNA binding drugs could inhibit the expression of target genes in cultured human cells. This landmark observation provides proof-of-concept for the artificial control of gene expression in living cells.

At the nexus of chemistry and biology, one area that has immense potential impact for the treatment of human disease is the development of DNA binding drugs that attach to specific target sequences to regulate gene expression. The first synthetic agents capable of selectively recognizing double-stranded DNA took the form of two linked DNA intercalators, such as methidium, that bound cooperatively and caused an increase in the length of the bound nucleotide sequence by stretching [1]. In the 1980s, the "rediscovery" of triplex DNA, initially observed in synthetic polynucleotides in 1957 [2], arose from a search for constrained DNA cleavage agents that cut only certain restricted sites [3, 4]. The identification and characterization of such tightly regulated compounds sparked the idea that compounds could be designed to recognize a single DNA target site in the context of megabase [5] and gigabase [6] eukaryotic genomes. In the ensuing decade, a number of groups put forth a large body of work that explored the chemical and biological properties of synthetic triplex-forming oligonucleotides in the "antigene" application of triplex DNA [7]. Their research established a number of critical parameters that have since become touchstones for current research: double-stranded DNA can be targeted with exquisite specificity, with good binding affinity in most cases, targeting can take place under physiological conditions, targeting with an oligonucleotide can direct a variety of nonspecific DNA active agents to specific locations, the triple helix is a substrate for DNA enzymes (e.g., helicase, DNA repair), and triplex formation can mediate important biological functions such as inhibition of target gene expression and site-directed mutagenesis in cell-free systems, cells, and whole organisms in some cases [8].

Despite these successes, cell culture experiments with triplex-forming oligonucleotides have been difficult to interpret because of the pleiotropic effects of using nucleic acids as drugs in complex biological systems and the difficulty of demonstrating in situ occupancy of the target DNA binding site within the chromatin of live cells. Several groups have now unambiguously reported either in situ occupancy of DNA target sites by triplex-forming oligonucleotides [9–12] or the capacity of triplex-forming oligonucleotides to mediate site-specific mutagenesis in eukaryotic cells [13, 14] and mice [15], but no one has shown both site occupancy by triplex-forming oligonucleotides and an effect on gene expression simultaneously in intact living mammalian cells. Furthermore, to date, site-specific mutagenesis had been reported to occur only at low frequency. Importantly, in addition to providing supporting evidence for the principle of designing site-specific DNA binding agents, studies of triplex-forming oligonucleotides necessitated generation of several molecular methods to detect DNA-bound compounds at a single target site in the endogenous chromatin of treated cells [9, 12, 16, 17]. While investigation of antigene triplex-forming oligonucleotides continues in several laboratories [12, 18, 19], many investigators have turned their attention to other approaches to site-selective DNA recognition to circumvent some of the unfavorable side effects, particularly inefficient cellular transport and trafficking, of synthetic nucleic acids as developmental therapeutic agents and biological tools.

Alternative tools employed to target specific regions of double-stranded DNA include designer peptide DNA binding domains, usually built from a combination of zinc finger peptide subunits selected by phage display, to create artificial transcription factors and other useful molecules [20, 21] and cytotoxic DNA interactive small molecules, which have been made more potent by linking combinations of minor groove binding pharmacophores, yielding such compounds as pyrrolo[1,4]benzodiazepine (PBD) dimers [22], bis-benzimidazole dimers [23], and cyclopropabenzindole (CBI)-PBD combinations [24] with recognition sequences of 6–9 base pairs.

The promising new strategy of synthetic polyamide DNA binding agents arose from the examination of DNA binding by natural products such as netropsin and distamycin, composed of 2 or 3 methylpyrrole groups (Py) coupled by peptide bonds and with selectivity for DNA tracts rich in adenine-thymine (AT) base pairs. This observation inspired the concept of using the Py subunit as a building block to recognize the AT base pair in synthetic minor groove DNA binding agents. Replacing a pyrrole with an imidazole (Im) ring allowed for the recognition of guanine-cytosine (GC) base pairs by synthetic polyamides. The discovery of a 5 base pair recognition sequence and 2:1 polyamide:DNA stoichiometry in a short ImPyPy trimer led to the deduction of Py and Im subunit pairing rules for recognition of base pairs in the minor groove [1]. Incremental improvements in synthetic polyamides have included linking the two polyamide chains chemically in a hairpin dimer with an aliphatic amino acid (abbreviated γ; maintains pairing alignment and prevents slipped dimers), the substitution of the Py or Im rings with β-alanine (abbreviated β; adds flexibility to the polyamide chain to match the curvature of DNA), and the substitution of the Py ring with hydroxypyrrole (abbreviated Hp; discriminates TA from AT base pairs), thus providing a specific polyamide pair to recognize each of the 4 base pairs of double-stranded DNA ([25]; Table 1).

The goal of this extensive and elegant series of studies was to control the expression of a target gene with a small molecule [26]. To this end, several studies have demonstrated that pyrrole-imidazole polyamides can localize to the nucleus in cultured cells, and in some in-

Table 1. Minor Groove Recognition Code

Base Pair	Polyamide Pair
GC	Im/Py
CG	Py/Im
AT	Py/Hp (Py/Py or β/β)
TA	Hp/Py (Py/Py or β/β)

Im, imidazole; Py, pyrrole; Hp, hydroxypyrrole; β, β-alanine.

stances a measurable change in target gene expression provides indirect evidence of target site binding by the small molecule [27–29]. Conversely, well-designed studies have been performed on nucleosomal binding by polyamides, demonstrating that the nucleosome presents a barrier to the binding of the polyamide in the minor groove of DNA only at specific contact points with histone proteins [30]. However, as with triplex-forming oligonucleotide "drugs," direct demonstration of chromatin binding in the nucleus of living cells and correlation with suppression of gene expression by a site-specific or site-selective DNA binding agent has been an elusive goal until now. In the September issue of *Chemistry & Biology*, Gottesfeld and coworkers provided the first direct evidence for polyamide-DNA binding in the nuclear chromatin of living cells in culture [33]. The major findings of this paper are that a fluorescently labeled polyamide is taken into the nucleus of four different cells lines of lymphoid or myeloid lineage. The movie accompanying this report (provided in the Supplemental Data) graphically demonstrates the rapid uptake and nuclear localization of bodipy-labeled polyamide. The polyamide in this paper is capable of targeting the human immunodeficiency virus-1 long terminal repeat (HIV-1 LTR) with a remarkable binding affinity of about 100 pM, but the target sequence is not unique to HIV-1; thus, site-specific binding in the presence of total genomic DNA may be reduced as a function of the number of competing binding sites. The authors use a competitive binding assay to calculate that one binding event occurs in every 2 kb of genomic DNA, in agreement with the theoretical number of binding sites based on the size of the recognition sequence. The polyamide can direct DNA alkylation by a nitrogen mustard, chlorambucil, to specific target bases in the minor groove adjacent to the polyamide binding site. By converting sites of DNA alkylation to single strand breaks, the polyamide binding sites can be identified by ligation mediated polymerase chain reaction (LM-PCR). Thus, the polyamide-chlorambucil conjugate serves as a probe to determine the accessibility of nuclear chromatin in living cells after addition of the polyamide to the cell culture media. The authors directly demonstrate the binding of the polyamide to chromatinized DNA within the HIV-1 LTR, a major contribution of this paper. Finally, using gene expression analysis the authors demonstrate that expression of a remarkably limited number of genes is suppressed by treating cells with the polyamide. In spite of a fairly ubiquitous DNA recognition sequence occurring 1.3 million times in the human genome, only 21 genes are significantly suppressed or activated by polyamide treatment. Of these, heat shock protein 70 (HSP70) was the most dramatically suppressed, and sequence analysis revealed four putative binding sites for the polyamide.

Like all good research, this paper raises a number of interesting questions. A key issue for future endeavors will be elucidating the mechanism of inhibiting gene expression in cells. To this end, one must be mindful that cell treatments can have a multitude of effects, and separating the primary effects from the secondary ones can be challenging. It will be of particular interest to understand why some genes can be switched off by polyamide treatment, while the expression of other genes that also bear the polyamide binding site are not affected. Is this a consequence of DNA structure, occupancy of the binding site by transcription factors, or unstable polyamide binding in some actively transcribed genes? Ideally, DNA binding at the limited number of genes affected by polyamide treatment, such as HSP70, would be quantitatively demonstrated using LM-PCR and compared to the occupancy of target sites in genes that are not suppressed by the polyamide treatment. Several DNA interactive compounds have now been appended to minor groove binding polyamides; these agents include chlorambucil, used in this report, as well as cyclopropapyrroloindole (CPI) [31], PBD, and camptothecin [32]. It will be interesting to learn the gain in biological activity afforded by these molecules compared to the already high-affinity polyamides. While we await further experiments with great interest, the studies described by Gottesfeld et al. represent an important contribution to the field of designed site-selective DNA binding agents, demonstrating that these agents can and do bind to their intended target sequences in living cells.

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Vaccine Delivery by Carbon Nanotubes

Novel nanomaterials, such as carbon nanotubes, are under active investigation for potential use in biomedical applications. In this issue of *Chemistry & Biology*, researchers describe antigen-antibody interactions and immune responses using peptide-carbon nanotube conjugates.

The potential of novel nanomaterials, such as carbon nanotubes, is enormous. These tubular arrangements of sp2 hybridized carbon atoms are under active investigation due to their phenomenal physical properties [1]. For example, they can function as semiconductors in nanoscale devices, be spun into the toughest material (man-made or natural [2]), or act as actuators with a force generation 100 times larger than that of mammalian muscles.

Carbon nanotubes (CNT) exist in two types, single wall (SWNT) and multi wall (MWNT). SWNT have lengths of up to 10 μm and diameter of up to 2 nm, depending on the production process. MWNT are thicker and longer. CNT are not easily processed due to their lack of solubility in many solvents. However, the CNT carbon atoms present an excellent platform for chemical functionalization. Noncovalent and covalent functionalization has been utilized to overcome the problem of processability (see [3–6] and references therein).

In the last five years, inorganic nanomaterials such as nanocrystals, nanowires, and nanotubes have been receiving an increasing amount of attention for potential biomedical applications. Nature has spent billions of years assembling nanoscale building blocks (such as lipids, peptides, and nucleic acids) into complex and functional structures. For example, selectivity and recognition at the molecular scale, such as antibody-antigen interactions, is a critical feature of living systems. However, nature has not had the opportunity to produce biomolecular interactions with the desired nanoscale materials [7]. It has been shown that selective design of peptides can be used to control interactions between the biological and nonbiological world. Peptide sequences have been used to bind to metal particles [8] and carbon nanotubes [4, 9, 10].

The interactions between carbon nanotubes and biological materials are mainly being investigated for biosensing (see [11, 12] and references therein). The basic concept for utilizing carbon nanotubes as transducers in biosensing applications is the ability to enable specific interactions with the analyte through functionalization of the nanotube surface and characterization of specific interactions (i.e., sensing) and reducing nonspecific interactions.

In an important new development, the work by Bianco, Prato, and collaborators [13] published in this issue demonstrates the potential use of carbon nanotubes in vaccine delivery. The basic concept for utilizing carbon nanotubes in vaccine delivery is to link the antigen to carbon nanotubes while retaining its conformation and thereby inducing antibody response with the right specificity. In addition, carbon nanotubes should not trigger a response by the immune system, i.e., they should not possess intrinsic immunogenicity.

In previous work, the Bianco and Prato research group's carbon nanotubes were covalently functionalized with a pyrrolidine ring through the 1,3-dipolar cycloaddition