Point of VIEW

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The New Biomimetic Chemistry: Artificial Transcription Factors

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ABSTRACT While many research programs have focused on the challenge of developing small molecules that can inhibit protein–protein interactions, some researchers have taken the problem one step further by attempting to develop small molecules that mimic the essential features of an entire protein. An area of particular interest has been in the field of artificial transcription factors (ATFs), where the essential function of some transcription factors is to recruit and promote the assembly of a larger transcription complex, leading to the expression of a gene of interest. The goal of synthesizing small-molecule ATFs holds promise as a means to independently control the expression of genes such as those that are misregulated in cancer and disease.

Because early studies demonstrated that short peptide sequences (~12–24 amino acids) from the activation domain (AD) of transcription factors were by themselves able to act as ADs, researchers envisioned that ADs, and conceivably the entire transcription factor, could be mimicked by small molecules with drug-like properties (*1, 2*). Mapp *et al.* (*3*) now report a significant advance toward this goal through the discovery and demonstration of a highly efficacious small-molecule artificial transcription factor (ATF), which functions at nanomolar potencies in intact cells. This study breaks new records in size and potency in the steady progression of studies by several groups over the past decade.

The first study in this area is credited to Verdine and coworkers (*4*). They demonstrated that when a 29-amino-acid peptide fragment from VP16 is conjugated to the small-molecule drug FK506, GAL4-reporter gene expression could be observed in cell-free *in vitro* transcription assays, which included a GAL4 DNA-binding domain/FKBP12 protein chimera. The high-affinity association of FK506 for FKBP allowed the Gal4/FKBP chimera to create a physical bridge between the target DNA and the peptide AD, which responds analogously to a yeast two-hybrid assay (Figure 1). The original FK506–peptide conjugate worked in cell-free assays but did not work in cells (see below). Since then, studies have focused on identifying more potent and more active artificial ADs with cellular stability and bioavailability as well as different mechanisms to target their actions to DNA.

Verdine and coworkers (*4*) were able to overcome the problem of cellular stability by demonstrating that the more protease-resistant, non-natural D-form of the VP16 peptide was able to activate transcription in cells. However, because this study apparently required liposomes to facilitate cell entry of the peptide, it has not found general use. Uesugi's group (*5*) identified a small-molecule protein–protein interaction inhibitor that blocks interactions between ESX, a DNA-binding transcription factor, and Sur-2/DRIP130. They proposed and later demonstrated that a related compound, wrenchnolol, could function as an AD in cell-free transcription assays (*6, 7*).

The Kodadek laboratory (*8*) has used a combinatorial approach to screen peptoid libraries for sequences that bind to the kinase-inducible interaction domain (KIX) domain of CREB binding protein (CBP) and thus serve as ADs. When the peptoid AD (KBPo2) is presented as a dexamethasone conjugate, Dex–KBPo2 could penetrate cells to provide a stunning 900-fold induction of reporter gene expression only in cells expressing a GAL4–glucocorticoid receptor (GR) chimera. Although Dex binds GR with nanomolar affinity, the observed potency of this Dex–peptoid conjugate ($EC_{50} = 10\ \mu M$) suggests that cellular availability of the conjugate, or other aspects of the conjugate structure, may limit its potency in cellular assays.

Is smaller better? It is generally held that molecules above a certain size are no longer

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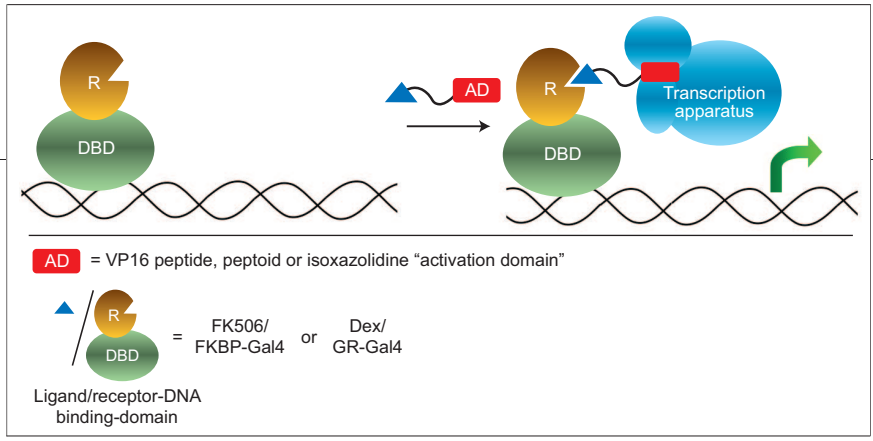

Figure 1. ATFs based on receptor–DNA-binding chimeras.

able to passively enter cells, an indication that a smaller AD would have better bioavailability. On the other hand, small molecules intuitively appear less likely to be able to mimic the protein–protein interaction properties of ADs that can be orders of magnitude larger. These are the principal challenges between which researchers must navigate. Recognizing that cellular bioavailability may be the limiting factor, rather than activity or *in vitro* potency, the Kodadek laboratory has adopted a strategy to directly screen molecules by using mammalian cell-based assays (*9, 10*). Although peptoids are not always "small molecules", a recently reported second-generation peptoid AD (MW = 794) is actually smaller than wrenchnolol (MW = 802) and has low micromolar potency in cells (Figure 2).

The Mapp group (*11*) was the first to report a small molecule with demonstrated AD properties. It remains the smallest AD reported to date and is also the most potent. This isoxazolidine-based AD (MGAD; Mapp group AD) was created by presenting functional groups common to natural ADs in a radial fashion from a heterocyclic scaffold. Weighing in at <300 g mol^{-1}, MGAD provides a robust transcriptional response when conjugated to methotrexate in cell-free transcription assays containing a LexA/dihydrofolate reductase chimera. The much smaller size of MGAD, along with its neutral amphiphilic character, makes it appear ideally suited for penetrating intact cells. Now, a publication by Rowe *et al.* (*3*) demonstrates that this is indeed the case. With the obvious caveats of comparing assay data obtained from different laboratories, the Mapp laboratory constructed a Dex–MGAD conjugate and evaluated its activity in cellular assays by following essentially the same method used by the Kodadek group to evaluate their peptoid ADs. The much smaller Dex–isoxazolidine conjugate provided a very strong 80-fold induction of reporter gene expression with a remarkable 33 nM (EC_{50}) potency. This extraordinary combination of potency, activity, and size makes a significant step toward the goal of creating pharmacological agents capable of acting as transcription factors.

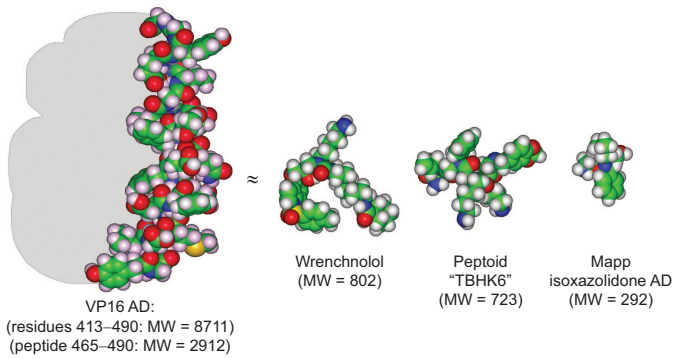

Figure 2. Synthetic ADs mimic the function of much larger protein domains.

Not all molecules that bind components of the transcription apparatus serve as ADs. Whereas the KIX-CBP binding peptoid KBPo2 acts as an AD, a different peptoid that binds KIX-CBP was not an effective AD, an indication that not all coactivator binding agents are ADs (*8*). On the other hand, evidence suggests that some artificial ADs can bind to sites on coactivators that are distinct from the natural site of coactivator binding and still function. Clearly, many aspects of artificial ADs remain to be explored. Large differences remain in transcriptional activities in cell-free systems *versus* cellular systems, or even between cellular systems, that will require further investigation.

A clear limitation in using synthetic ADs is the need to express chimeric ligand-binding/DNA-binding proteins to target their actions to the gene of interest. However, can we really expect a synthetic molecule to be able to mimic the functional properties of an entire protein? Researchers are working on doing just that. An early example in this area involved appending a peptide AD to a triple-helix-forming oligonucleotide (*12*). This study demonstrated the principle of directly targeting DNA with a synthetic molecule but suffered from the obvious problems of cellular bioavailability. Today, the first choice for programmable sequence-specific DNA-binding molecules is the DNA-binding polyamides developed by the Dervan laboratory (*13*). The Dervan laboratory was the first to demonstrate that hairpin polyamides fused to peptide ADs could function as ATFs with DNA-binding and AD functions (*14, 15*). Fusing a hairpin polyamide sequence to their artificial AD wrenchnolol, Uesugi *et al.* (*7*) were able to modestly up-regulate gene transcription (3.5-fold) in a cell-free assay; however, this "all-in-one" molecule did not function in cell-based assays, presumably because of poor cell permeability (Figure 3). Recently, the Kodadek laboratory demonstrated that a peptoid AD conjugated to a different polyamide sequence was able to provide 5-fold up-regulation of reporter gene transcription (at 5 μM) in cells, demonstrating that the DNA-binding and the AD functions of transcription factors can be mimicked by a syn-

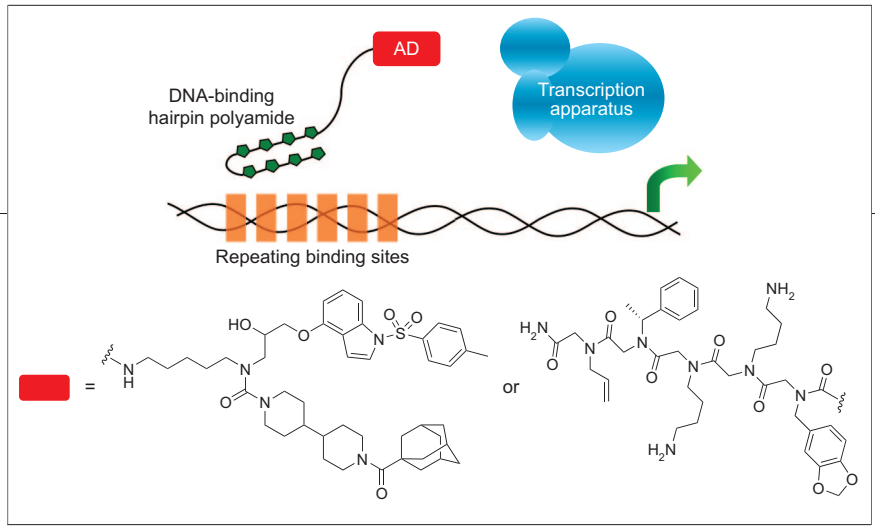

Figure 3. Polyamide-linked ADs serve as biomimetics of DNA-binding transcription factors.

thetic molecule capable of directly entering cells (*10*). Though a modest beginning, these studies represent a critical proof of concept of mimicking an entire DNA-binding transcription factor with synthetic bioavailable mimetics.

Twenty years ago, it may have seemed unimaginable that transcription factors with molecular weights ranging in the tens of kilodaltons could be mimicked by much smaller bioavailable synthetic compounds. The studies performed to date now make this notion quite reasonable. Will ATFs allow us to someday pharmacologically regulate the genome to compensate for errors in expression associated with cancer or other diseases? Will we be able to reversibly enhance or alter phenotypes by regulating genes on demand? Although numerous challenges must still be addressed, including increasing potency, cellular bioavailability, and target gene specificity, as well as the myriad additional challenges associated with any *in vivo* applications, the field is aglow with excitement for the potential applications this technology may someday enable.

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