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Toward Synthetic Transcription Activators: Recruitment of Transcription Factors to DNA by a PNA-Peptide Chimera

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Small molecules that mimic the activity of gene-specific activators would be valuable research tools. Native activators minimally contain a sequence-specific DNA-binding domain (DBD) to direct the factor to the appropriate promoter and an activation domain (AD) to recruit transcription complexes to the target gene[1] (Figure 1). Most activators are thought to bind one or more coactivator proteins that reside in the RNA polymerase II holoenzyme[2] and thus recruit this complex to the promoter. Therefore, we proposed that a minimal synthetic transcription activator could be constructed by linking molecules with the appropriate DNA- and protein-binding characteristics.[3] We report here an important step toward this goal: the synthesis and characterization of a compound able to mediate the recruitment of a coactivator to a specific DNA site.

To mimic the activity of a native DBD, we chose to employ a peptide nucleic acid[4] (PNA) of the "clamp" or "bis-PNA" variety[5] (Figure 2). Bis-PNAs that connect two similar homopyrimidine PNAs via a suitable linker bind to double-stranded DNAs with high affinity and selectivity via a strand invasion mechanism. As an artificial AD, we employed a peptide (Figure 2) that has been shown previously to support robust activation in yeast when fused to an appropriate sequence-specific DBD.[6] This peptide was isolated via phage display simply on the basis of its ability to bind the yeast Gal80 protein, a repressor that binds tightly to the native AD of the Gal4 transactivator.[7] It was reasoned, correctly, that Gal80-binding peptides might serve as Gal4 AD mimics.

A Bis-PNA designed to bind the DNA sequence 5′-AAGGAG-GAGA-3′ was synthesized on an automated system by standard solid phase methods, and the 20 residue Gal80-binding peptide (Gal80BP) was added to the PNA subsequently by manual solid-phase peptide synthesis (see Supporting Information for details). The final product (Figure 2) was purified by reverse phase HPLC and analyzed by MALDI-TOF mass spectroscopy.

To assess the DNA-binding properties of the PNA-peptide chimera, double-stranded DNAs of about 100 base pairs were produced by PCR. These DNAs contained either 0, 1, or 5 binding sites (DNAs **1**−**3**, respectively) for the PNA-peptide chimera. A 300-fold molar excess of PNA-Gal80BP was incubated with DNA at room temperature for 16 h. This large excess of PNA-peptide conjugate and the extended incubation are necessary for efficient binding since the strand invasion reaction is slow when using linear DNAs in physiologically relevant buffers. Fortunately, we found that the DNA·PNA-peptide complex could be easily separated from excess unbound chimera by using a small silica gel column (QIAGEN, Inc.) in the presence of a high concentration of chaotropic salts. Formation of the DNA·PNA-Gal80BP complex

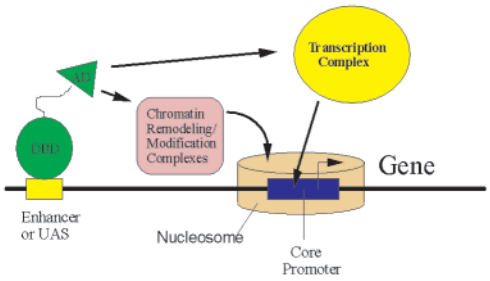

Figure 1. Cartoon of the mechanism of action of activators. UAS = upstream activation sequence.

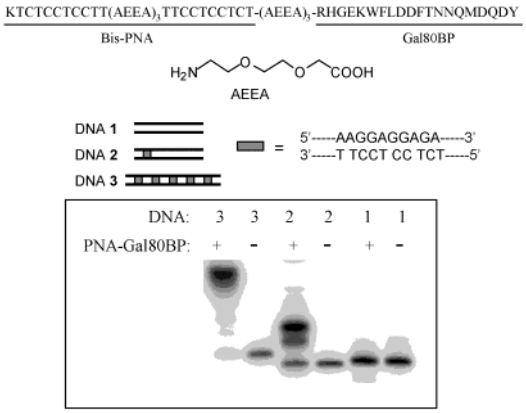

Figure 2. Schematic representation of the structure of the molecules used in this study and an analysis of the DNA-binding properties of the PNA-peptide chimera. The PNA-Gal80BP is depicted with the C-terminus (an amide) on the left and the N-terminus on the right. A gel mobility shift assay (boxed section) shows that the PNA-peptide chimera binds to DNAs **2** and **3**, which contain one and five binding sites, respectively, but not to DNA **1**, which lacks the PNA binding site.

was dependent on the presence of the target DNA sequence, as demonstrated by a gel mobility shift assay using ^{32}P-labeled DNAs **1**−**3** (Figure 2). The presence of two electrophoretically distinguishable bands in the solution containing PNA-Gal80BP and the single site PCR product may reflect parallel and antiparallel DNA-PNA binding,[8] though this point has not been addressed rigorously. The DNA·bisPNA-Gal80BP complexes were very stable. No significant dissociation was observed even after a week.

A DNA·Gal4·Gal80 complex maintains silencing of the *GAL* genes in yeast under noninducing conditions. To ask if this type of DNA·activator·repressor complex could be mimicked by using the PNA-Gal80BP model compound, the experiment shown in Figure 3A was performed.

Six histidine-tagged Gal80 protein (His_6-Gal80) was incubated with PNA-Gal80BP, DNA **1**, **2**, or **3** and nickel-saturated NTA-

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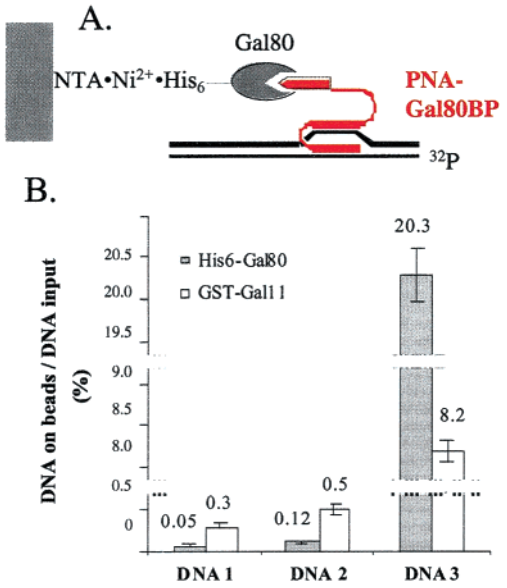

Figure 3. Recruitment of transcription factors to DNA by the PNA-peptide chimera. (A) Cartoon of the assay employed for Gal80 recruitment. The bead-bound transcription factor was incubated with the preformed and column-purified DNA·PNA-peptide complex. The beads were pelleted and washed and the radiolabeled DNA retained on the beads was determined by scintillation counting. For the experiments employing GST-Gal11(1−350), glutathione agarose beads were used in place of NTA-agarose beads. (B) Results of the recruitment assay. Note the breaks in the *y* axis scale. When the PNA-peptide was omitted, no retention of labeled DNA above the background was observed (data not shown).

agarose beads, to which the His_6 tag binds tightly. A 1000-fold excess of unlabeled carrier DNA was also included to block nonspecific binding of the labeled DNA to the beads. After a 1 h incubation, the beads were pelleted and washed several times. The level of radioactive DNA retained on the beads was then analyzed by scintillation counting (Figure 3B). Only a small percentage of the labeled DNA **1**, which lacks a PNA-binding site, was retained by the beads in the presence of His_6-Gal80. For the **2**·PNA-Gal80BP complex, the percentage of labeled complex retained was also low, but was reproducibly greater (≈2-fold) than that observed in the control experiment with DNA **1**. When the DNA contained five PNA-binding sites (**3**), a much greater fraction of the complex was retained by the immobilized His_6-Gal80. No DNA was retained if either His_6-Gal80 or the PNA-peptide conjugate was absent. Furthermore, when another His_6-tagged protein (His6-Gal4 AD[9]) was used in place of the Gal80 derivative, the level of the PNA-Gal80BP complexes of DNAs **2** and **3** retained on the beads was equivalent to the background. It is not surprising that the single site containing DNA **2** is "pulled down" far less efficiently than the multiple site-containing DNA **3**. The K_D of the Gal80-peptide complex is only approximately 0.2 μM[6] and has a correspondingly fast off rate. Therefore, most of the Gal80-peptide complex will dissociate during the washing steps in the case of the complex containing DNA **2**. However, because Gal80 protein is a native dimer that can also tetramerize,[9] the complex including DNA **3**, which includes five copies of the Gal80-binding peptide, could bind the repressor much more tightly through multiple contacts.

To ask if the PNA-Gal80BP chimera is capable of recruiting a coactivator to DNA, a similar experiment was conducted with use of a GST fusion protein containing the N-terminal domain (residues 1−350) of the yeast Gal11 protein in place of Gal80. This fragment of the Gal11 coactivator has been shown by us[10] and others[11] to be one of the targets of the native Gal4 AD in the yeast RNA polymerase II holoenzyme and the G80BP has been shown to also recognize this coactivator.[6] As is also shown in Figure 3, results very similar to those observed in the Gal80 experiments were observed. That is, glutathione agarose bead-mediated "pull-down" of GST-Gal11(1−350) in the presence of the PNA-peptide chimera and labeled DNAs **1**−**3** resulted in little retention of DNA **1**, slightly higher levels of the single site-containing DNA **2**, and much higher levels of DNA **3**. Again, in all cases, retention of the labeled DNA was completely dependent on the presence of the GST-Gal11(1−350) fusion protein and the PNA-peptide conjugate. Since GST is a native dimer,[12] the same avidity-based argument explains the large difference in retention of the multiple site-containing DNA **3** and the single site-containing DNA **2**.

We conclude that the PNA-Gal80BP molecule can indeed recruit transcription factors to a specific DNA site, thus mimicking a fundamental property of native activators. The activity of this molecule in transcription assays in vitro and in cells is under investigation. In this vein, it is important to point out that Dervan and co-workers have demonstrated hairpin polyamide−peptide conjugates can activate transcription in vitro,[13] a major milestone in the field. However, the peptides employed in these experiments were derived from native ADs or functional genetic screens.[14] This work demonstrates than a peptide derived from a simple protein-binding assay, which could be applied to libraries of molecules other than peptides, can reconstitute an important function of a native AD in the appropriate fusion construct.

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Supporting Information Available: Experimental procedures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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