Invasion of the CAG Triplet Repeats by a Complementary Peptide Nucleic Acid Inhibits Transcription of the Androgen Receptor and TATA-binding Protein Genes and Correlates with Refolding of an Active Nucleosome Containing a Unique AR Gene Sequence*

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Lidia C. Boffa†, Patricia L. Morris§, Elisabetta M. Carpaneto‡, Marjorie Louissaint¶, and Vincent G. Allfrey†

From the †Department of Experimental Oncology, Istituto Nazionale per la Ricerca, sul Cancro IST, Genoa 16132, Italy and the §Population Council and the ¶Laboratory of Cell Biology, Rockefeller University, New York, New York 10021

The DNA sequence of the genes for the androgen receptor (AR) and TATA-binding protein (TBP), like many other genes encoding transcription factors, contains a series of tandem CAG repeats. Here we explore the capacity of complementary peptide nucleic acids (PNAs) to invade the CAG triplets of the AR and TBP genes in human prostatic cancer cells and show that the PNAs readily entered the nuclei of lysolecithin-permeabilized cells and effectively inhibited sense transcription of unique AR and TBP DNA sequences downstream of the site of PNA-DNA hybridization, but not upstream of that site. These PNAs had little or no effect on transcription of the c-myc gene, which lacks a CAG triplet domain. Conversely, a PNA complementary to a unique sequence of the c-myc gene did not inhibit transcription of the AR or TBP genes but did inhibit c-myc transcription. Comparisons of PNA effects on sense and antisense transcription of the AR, TBP, and c-myc genes confirm that progression of the RNA polymerase complex beyond the site of PNA-DNA hybridization is impaired in both directions. Suppression of the AR gene results in refolding of a transcriptionally active nucleosome containing a unique 17-mer AR DNA sequence.

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†To whom correspondence should be addressed. Tel.: 212-327-8770; Fax: 212-327-8771; E-mail: allfrey@rockvax.rockefeller.edu.

§The abbreviations used are: PNA, peptide nucleic acids; TBP, TATA-binding protein; AR, androgen receptor; TES, 2-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]aminoëthanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; EPNP, 1,2-epoxy-3-(p-nitrophenoxy)propane.
PNA Effects on Sense and Antisense Transcription

Materials and Methods

Cell Culture—Human prostatic carcinoma cell lines LNCaP and DU-145 were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The LNCaP cells were cultured in RPMI 1640 medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (JRH Biosciences, Lenexa, KS) and 20 ng/ml gentamicin (Life Technologies, Inc.) at 37°C in an atmosphere of 5% CO₂ in air. DU-145 prostatic cancer cells were cultured in Eagle's minimal essential medium (Sigma) supplemented with 10% fetal bovine serum and gentamicin. In all cases, cells in log phase growth were harvested at 5 × 10⁶ cells/ml.

PNA Effects on Transcription—An 18-mer PNA, 5'-CAGCACGACGGCACGACG-3', complementary to the transcribed strand of the AR triplet repeat domain was synthesized and linked to biotin at its amino-terminal through two 8-amino-3,6-dioxaoctanoyl linkers to create a distance between the PNA and the biotin label. A second PNA, with the same sequence, but lacking the biotinylated linker was also tested, with identical results on transcription of the AR and TBPA genes (custom synthesis by Per-Septive Biosystems, Framingham, MA). The purity of the biotinylated PNAS was confirmed by reverse phase chromatography and mass spectrophotographic analysis (M, 5,568 and 6,325.5, respectively). The PNA used for strand invasion of the unique AR sequence (163–185) upstream of the PNA was the 17-mer biotinylated sequence 5'-Bio-O-O-CAGGTTAGCTCACC-3', complementary to the sense strand of the unique sequence (4528–4544) in the second exon.

LNCaP and DU-145 cells were harvested and washed three times with Dulbecco's phosphate-buffered saline (Life Technologies, Inc.) containing 5 mM Na⁺ butyrate. The cells were suspended in 100 μl/ml of buffer A (35 mM Hepes, pH 7.4, 80 mM KCl, 5 mM MgCl₂, 0.5 mM CaCl₂, 5 mM 2-mercaptoethanol), and the hundredths of the nuclei were homogenized in a glass teflon–gloved homogenizer with a type B pestle (Kontes, Inc., Vineland, NJ). The nuclei were suspended in buffer F at a concentration of 1 mg/ml solution of lysosederin in buffer A. The cells were collected by centrifugation at 2000 × g for 1 min and resuspended in sufficient buffer B (30 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 1 mM Na⁺ butyrate, 0.1 mM Na₃EDTA, 0.25 mM sucrose) to provide four or five 200-μl aliquots of cell suspension in 2-ml microcentrifuge tubes, and the cells were then permeabilized to nucleotides by treatment for 1 min at 0°C with one-third volume of a 1 mg/ml solution of lysolecithin in buffer A. The cells were collected by centrifugation at 500 × g for 10 min and washed three times in calcium-free Dulbecco's phosphate-buffered saline (Life Technologies, Inc.) containing 5 mM Na⁺ butyrate to suppress histone deacetylase activity during cell lysis and isolation of the nuclei (26, 27). To lysate the cells, they were suspended in buffer E (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl₂, 0.5% Nonidet P-40, 5 mM Na⁺ butyrate, 0.1 mM PMSF, and 0.1 mM 1,2-epoxy-3-(p-nitrophenyl) (EPNP, Kodak, Rochester, NY) for 5 min at 0°C, with gentle mixing by pipetting. The cells were collected by centrifugation at 500 × g for 10 min, resuspended in buffer B, and centrifuged for 1 hr at 37°C. The PNA stock solution to bring the final PNA concentration to 1 mg/ml solution of lysosederin in buffer A. The cells were collected by centrifugation at 500 × g for 10 min, resuspended in buffer B, and centrifuged for 1 hr at 37°C. The DNA probes for sense and antisense transcripts of the unique downstream AR sequence (2303–2333) were 5'-dGGGGTTCCT-CAGCGTGTCAGCTGCATTTG-TCT-3' and 5'-dAATGCGAGGCTC-GAGCGCTAGGACCC-3', respectively. In tests for the effects of PNAS targeting the CAG domain (270–371) of the human TBP gene, the probes for sense and antisense transcripts of the unique downstream sequence (905–932) were 5'-dACTAATTTGTTGAGGATAGCAC-3' and 5'-dATGTCAGCCTTGGCAG-3', respectively. The corresponding probes for transcripts of the unique TBPA sequence (99–126) of the human TBP gene were 5'-dTAAGTGG-GGAAGTTGTTGCTCTGAC-3' and 5'-dTGATGACAGACA-3'. The autoradiograms were scanned with a laser densitometer and peak areas corresponding to each slot-blot were integrated. In all experiments, the relationship between 32P activity in the RNA and the intensity of the corresponding autoradiogram was determined by comparison with standard curves in which photographic density is plotted for a series of dilutions of a standard 32P-labeled salmon sperm DNA solution applied to the same membrane as the experimental samples. This established the range of linear response and clearly indicated where reblotting or correction for overexposure was necessary.

Isolation of Nuclei and Separation of Transcriptionally Active and Inactive Nucleosomes—All procedures were carried out at 4°C unless otherwise specified. Cells were collected by centrifugation at 500 × g for 10 min and washed three times in calcium-free Dulbecco's phosphate-buffered saline (Life Technologies, Inc.) containing 5 mM Na⁺ butyrate to suppress histone deacetylase activity during cell lysis and isolation of the nuclei (26, 27). To isolate the cells, they were suspended in buffer E (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl₂, 0.5% Nonidet P-40, 5 mM Na⁺ butyrate, 0.1 mM PMSF, and 0.1 mM 1,2-epoxy-3-(p-nitrophenyl)propane (EPNP, Kodak, Rochester, NY) for 5 min at 0°C, with gentle mixing by pipetting. The cells were collected by centrifugation at 500 × g for 10 min, resuspended in buffer B, and centrifuged for 1 hr at 0°C. The nuclei were suspended in buffer B and broken by shearing in a glass Dounce-type homogenizer with a type B pestle (Kontes, Inc., Vineland, NJ). The homogenate was centrifuged at 500 × g for 10 min, and the nuclear pellet was washed three times by resuspension and centrifugation in buffer B. The nuclei were suspended in buffer B at a concentration of 1 mg DNA/ml and equilibrated at 37°C for 10 min. Micrococal nuclease (Boehringer Mannheim) was added at 10 units/ml, and the reaction was started by the addition of CaCl₂ to 0.5 mM. After incubation at 37°C for 7 min, endonuclease action was halted by rapid chilling and addition of EDTA to a final concentration of 100 mM. The DNA was centrifuged at 10,000 × g for 20 min. The resulting supernatant, containing the nucleosomes released by endonuclease digestion, was analyzed for its DNA content by measuring the As₂₆₀ of the DNA and by the Hoechst dye-binding assay (28). Under these conditions, 11% of the total DNA of the LNCaP nuclei was released into the supernatant (average of 4 experiments).

To separate the transcriptionally active from inactive nucleosomes, the supernatant was brought to 5 mM in Na₃EDTA, and applied to an organoammonium-aragose column (Affi-Gel 501, Bio-Rad), Elution of the nucleosomes bound in the DNA elution peak was carried out in buffer G (10 mM Tris-HCl, pH 7.4, 25 mM NaCl, 25 mM KCl, 5 mM Na⁺ butyrate, 5 mM Na₃EDTA, 0.1 mM PMSF, 0.1 mM EPNP, 2% sucrose) until the A₂₆₀ of the eluate decreased to a steady baseline. The resulting nucleosomal DNA was bound to the Hg⁺ column through their accessible SH groups, were released by elution in buffer containing 20 mM dithiothreitol. DNA was purified from the bound and Hg⁺-bound nucleosome fractions and analyzed for AR gene content by slot-blot hybridizations (29) to a 32P-labeled DNA oligonucleotide probe for the unique downstream sequence 5'-dCTTTTGAAGAAGACCTTG-3' and analyzed for AR gene content by slot-blot hybridizations (29) to a 32P-labeled DNA oligonucleotide probe for the unique downstream sequence 5'-dCTTTTGAAGAAGACCTTG-3'.

RESULTS

Inhibition of AR Gene Transcription by PNA Invasion of CAG Triplet Repeats—The mRNA of the androgen receptor gene is characterized by a series of tandem CAG triplets that encode a tract of glutamine residues in the receptor protein. To test whether a PNA targeted to the sense strand of the CAG domain (nucleotides 334–390) (i.e., the tandem CAG sequence serving the unique CAG upstream sequence (2303–2333) were 5'-dGGGGTTCCT-CAGCGTGTCAGCTGCATTTG-TCT-3' and 5'-dAATGCGAGGCTC-GAGCGCTAGGACCC-3', respectively. In tests for the effects of PNAS targeting the CAG domain (270–371) of the human TBP gene, the probes for sense and antisense transcripts of the unique downstream sequence (905–932) were 5'-dACTAATTTGTTGAGGATAGCAC-3' and 5'-dATGTCAGCCTTGGCAG-3', respectively.
as template for the CAG repeats in AR mRNA) would inhibit transcription beyond the site of PNA-DNA hybridization, we selected a prostatic cancer cell line, LNCaP, which had been shown to contain both the androgen receptor protein and its mRNA (30). Run-on transcription experiments were carried out on cells that were gently permeabilized with lysolipid under conditions shown to preserve nuclear RNA synthetic activity during nuclear transplantation experiments (31). The permeabilized cells were exposed to increasing concentrations of an 18-mer biotin-free PNA complementary to the CAG-encoding domain of the AR gene, which lacks a domain of CAG triplet repeats.

Other genes containing CAG triplet repeats were also affected by the same PNA. For example, PNA invasion of the tandem CAG triplets at nucleotides 270–371 of the human TATA-binding protein gene inhibited transcription of a unique TBP DNA sequence (nucleotides 905–932) downstream of the hybridization site by 80% at 10 μM and by 88% at 20 μM PNA (Fig. 1B).

The specificity of PNA targeting to different genes was confirmed in two ways. First, it was shown that sense transcription of the c-myc gene, which lacks a domain of CAG triplet repeats, was not inhibited in the same cells under identical conditions, only minor variations (±7%) were observed in four determinations of ([α-32P]UTP incorporation into transcriptions of two unique c-myc DNA sequences (nucleotides 2303–2333 and 7177–7213). In similar experiments, using both LNCaP and DU-145 prostatic cancer cells, a 21-mer PNA targeted to the antisense strand of the AR CAG-repeat domain was found to have no inhibitory effect on c-myc transcription (Fig. 3A). Conversely, exposure of the LNCaP cells to a PNA complementary to a unique sequence in the second exon of the c-myc gene had little or no effect on transcription of a unique sequence (nucleotides 1890–1912) of the AR gene but did inhibit transcription of a unique DNA sequence in the third c-myc exon (Fig. 2C).

Such specificity in PNA interactions with intact chromatin is in accord with earlier results on the selective binding and recovery of PNA-DNA hybrids of the TBP gene in colon cancer cells (21) (see “Discussion”).

To determine whether PNA binding to the sense strand of the AR gene also affected antisense transcription, we compared the synthesis of sense and antisense transcripts at unique AR DNA sequences located downstream and upstream of the CAG-repeat domain. The results confirmed that 20 μM PNA inhibited transcription of the downstream sense strand by 88%, but they also revealed that PNA binding to the sense strand led to an increase in 32P-labeled transcripts of a unique AR sequence upstream of the PNA binding site (Fig. 2A). The opposite results were obtained for antisense transcription in which PNA binding to the CAG triplet domain inhibited transcription of the upstream AR sequence by 82–86% but stimulated downstream transcription (Fig. 2B). Simultaneous assays for transcripts of the CAG triplet domain show that 20 μM PNA effectively inhibited both sense and antisense transcription of the tandem repeats by 93% (average of nine determinations).

The contrasting effects of PNAs on sense and antisense transcription were also evident in the TBP gene, where PNA invasion of the CAG triplet domain inhibited downstream transcription of the sense strand by 88% (average of seven determinations) but stimulated transcription of a unique TBP sequence upstream of the PNA-DNA hybrid (Fig. 2B). These results are fully compatible with a model in which PNA binding to the CAG triplet domain impedes progression of the RNA polymerase complex in both directions. Further support for this view is provided by experiments using a 17-mer complementary PNA to target the sense strand of the unique sequence (nucleotides 4528–4544) of the human c-myc gene, which is known to be transcribed in both sense and antisense directions (32). At a PNA concentration of 20 μM, transcription of a unique c-myc downstream sequence (nucleotides 7177–7213) was inhibited by 87% in the sense direction and stimulated in the antisense direction. Conversely, sense transcription of a unique upstream sequence (nucleotides 2303–2333) was stimulated by the PNA, whereas antisense transcription was inhibited by 94% (Fig. 2C).

Distribution of a Unique Sequence of the AR Gene in Transcriptionally Active and Inactive Chromatin—The human prostatic cancer cell lines LNCaP and DU-145 have been shown to...
After run-on transcription experiments, the $^{32}$P-labeled RNA samples were isolated and slot-bloted to appropriate oligo-DNA probes for sense upstream transcription was inhibited. For antisense transcription, in which progression of the RNA polymerase complex was not impaired until it reached the PNA binding site, and strand downstream of its binding site on the gene, while transcription was stimulated upstream of the PNA block. The opposite effect was observed for antisense transcription, in which progression of the RNA polymerase complex was not impaired until it reached the PNA binding site, and strand downstream of its binding site on the gene, while transcription was stimulated upstream of the PNA block. The opposite effect was observed.

Active transcription of the c-myc gene, which lacks a CAG-specific PNA on transcription in the same defined region of the gene. To test whether this difference in AR mRNA content in the two cell lines is attributed to a general loss of transcriptional activity of the DU-145 cells because the same RNA preparations gave a strong hybridization signal when probe with human c-myc DNA (Fig. 3A). Active transcription of the c-myc gene was also noted in the LNCaP cells (Fig. 3A). As both types of prostatic cancer cells actively transcribe the c-myc gene, which lacks a domain of CAG triplet repeats, a full-length c-myc cDNA probe was used as a negative control in hybridization experiments to test the effects of the CAG-specific PNA on AR transcription in the two cell lines. The results for LNCaP cells show an 83% reduction in AR unique transcripts at 10 $\mu$M PNA and 89% reduction at 20 $\mu$M (average of three experiments) (Fig. 3A). Under the same conditions, 10 $\mu$M PNA inhibited AR gene transcription in the DU-145 cells to the same extent as in the LNCaP cells (Fig. 3A).

The disparity in the intensity of the hybridization signals of LNCaP and DU-145 cells also offers an opportunity to correlate the transcriptional activity with alterations in chromatin structure in the same defined region of the gene. To test whether differences in AR mRNA content in the two cell lines is attributed to a general loss of transcriptional activity of the DU-145 cells because the same RNA preparations gave a strong hybridization signal when probe with human c-myc DNA (Fig. 3A). As both types of prostatic cancer cells actively transcribe the c-myc gene, which lacks a domain of CAG triplet repeats, a full-length c-myc cDNA probe was used as a negative control in hybridization experiments to test the effects of the CAG-specific PNA on AR transcription in the two cell lines. The results for LNCaP cells show an 83% reduction in AR unique transcripts at 10 $\mu$M PNA and 89% reduction at 20 $\mu$M (average of three experiments) (Fig. 3A). Under the same conditions, 10 $\mu$M PNA inhibited AR gene transcription in the DU-145 cells to the same extent as in the LNCaP cells (Fig. 3A).

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Fig. 2. Contrasting effects of PNAs specifically targeted to the sense strands of the AR, TBP, and c-myc genes on sense and antisense transcription downstream and upstream of the PNA binding sites. Equal numbers of LNCaP cells were exposed to one of three PNAs. Two were 18-mer CAG PNAs with the same base sequence, but differing in their modification by biotin, are shown. Both were targeted to the CAG triplet domains of the AR gene (A) and TBP gene (B). The third PNA was complementary to a unique DNA sequence of the c-myc gene (C). After run-on transcription experiments, the $^{32}$P-labeled RNA samples were isolated and slot-bloted to appropriate oligo-DNA probes for sense and antisense transcripts of unique sequences downstream and upstream of their PNA binding sites. Intensities of the hybridization signals are compared for control cells (solid bars), for cells treated with the unmodified 18-mer CAG PNA (lightly striped bars), or its biotinylated form (darkly striped bars), and for cells treated with the 17-mer c-myc PNA (stippled bars). Note that in every case, the PNA inhibited transcription of the sense strand downstream of its binding site on the gene, while transcription was stimulated upstream of the PNA block. The opposite effect was observed for antisense transcription, in which progression of the RNA polymerase complex was not impaired until it reached the PNA binding site, and upstream transcription was inhibited.

Fig. 3. Correlation between rates of transcription and the unfolded state of nucleosomes of the AR gene in LNCaP and DU-145 prostatic cancer cells. A, slot-blot hybridizations of $^{32}$P-labeled RNAs from control cells to a DNA-oligonucleotide probe for a unique AR gene sequence downstream of the CAG triplet domain. Note the contrast in levels of $^{32}$P-labeled AR transcripts in these two cell lines. Exposure of each cell line to a PNA targeted to the CAG triplet repeats strongly inhibited sense transcription of the AR gene downstream of the PNA binding site but had no effect on c-myc transcription in the same cells. B, distribution of AR DNA in transcriptionally active and inactive nucleosomes of LNCaP and DU-145 cells. Nucleosomes released in a limited micrococcal nuclease digestion of isolated nuclei were fractionated by affinity chromatography on mercurated agarose columns. DNA was isolated from each nucleosome fraction and probed for its AR gene content by slot-blot hybridizations to a $^{32}$P-labeled 17-mer DNA probe for the same unique AR sequence. 1, DNA of unbound nucleosomes of LNCaP cells; 2, DNA of the Hg$^{2+}$-bound nucleosomes of LNCaP cells; 3, DNA of unbound nucleosomes of DU-145 cells; 4, DNA of the Hg$^{2+}$-bound nucleosomes of DU-145 cells. Note the extremely low content of the AR sequence in the transcriptionally active nucleosomes of DU-145 cells, as compared with that of the corresponding fraction from LNCaP cells.
ally active and inactive nucleosomes of each cell type.

The chromatographic separation of active and inactive nucleosomes is based on chemical and electron microscopic evidence that the nucleosome core unbinds during transcription to reveal the previously inaccessible cysteiny1-SH groups of histone H3 molecules located at the center of the core (33). Consequently, mixtures of active and inactive nucleosomes, released from isolated nuclei by micrococcal nuclease digestion, can be separated by entrapping the unfolded, SH-reactive nucleosomes on an organomercurial-agarose column. After washing the column to remove the unbound nucleosomes, the SH-reactive nucleosomes are rapidly released from the mercurated support by elution in dithiothreitol (35, 36). The success of mercury-affinity chromatography in separating active from inactive nucleosomes has been demonstrated in organisms as diverse as humans, rodents, yeast, and Physarum (35–39). Of particular significance are observations showing that the nucleosome core is a dynamic structure and subject to rapid and reversible changes. For example, in the early response of quiescent 3T3 fibroblasts to growth factors, the capture of nucleosomes containing the c-fos and c-myc genes on mercurated-agarose columns reflects, with accuracy, both the timing and extent of transcription of each gene, as monitored by run-on transcription experiments (37). Other experiments have shown that the active nucleosomes refold within minutes and are no longer retained by the Hg⁺ column when transcription is blocked by α-amanitin (38, 39).

The same chromatographic procedure was used to fractionate the nucleosomes of DU-145 cells. Nucleosomes released during a limited micrococcal nuclease digestion of the isolated nuclei were applied to an organomercurial-agarose column. The unbound nucleosomes were washed from the column and collected by centrifugation, whereas the Hg⁺-bound nucleosomes were subsequently eluted in one step with 20 mM dithiothreitol (34, 35). DNA analyses of the unbound and Hg⁺-bound fractions indicated that the bound nucleosomes contained approximately 20% of the total nucleosomes applied to the column, in agreement with many earlier experiments on other cell types (34–39). Total DNA was purified from each nucleosome fraction and analyzed for its content of AR DNA by Southern blot hybridizations to the 32P-labeled oligonucleotide probe for the unique AR sequence, 5’-dCTTTTTTAAGAGACCTT (25). The results show only a very faint hybridization signal for the DNA of the dithiothreitol-eluted nucleosomes of DU-145 cells (Fig. 3B), amounting to less than 3% of the signal from the unbound nucleosomes. This paucity of AR DNA in the unbound, transcriptionally active nucleosome fraction of DU-145 cells is in agreement with earlier observations on the deficiency of AR mRNA in this cell line (30), and with the results of run-on transcription experiments (Fig. 3A), all findings indicate that a block in transcription is the basis for the absence of the androgen receptor in DU-145 cells.

A similar fractionation of the nucleosomes of LNCaP cells by mercury-affinity chromatography also resulted in a distribution of 80% unbound versus 20% Hg⁺-bound nucleosomes, but when equal amounts of DNA from each fraction were analyzed for AR DNA content by hybridization to the 32P-labeled oligonucleotide probe for the unique AR sequence, a very substantial signal for the AR gene was seen in the Hg⁺-bound nucleosomes (Fig. 3B). Densitometric comparisons of the slot-blot indicated a 154% enrichment of the AR gene in the Hg⁺-bound nucleosomes of the LNCaP cells, as compared with the unbound nucleosomes. (The strong hybridization signal for the AR gene in the unbound fraction of the LNCaP nucleosomes is consistent with the fact that the AR gene is expressed at a relatively low level in the prostate (40).) The result for the Hg⁺-bound nucleosomes of LNCaP cells contrasts with the 3% recovery observed in the Hg⁺-bound nucleosomes of DU-145 cells (Fig. 3B). Thus, the differences in the recovery of this specific sequence of the AR gene in the thiol-reactive nucleosomes of these two cell types provide further evidence that unfolding of the nucleosome cores accurately reflects the transcriptional state of the gene. It should be pointed out that the recovery of the AR gene in the Hg⁺-bound, transcriptionally active nucleosome fraction was detected by hybridization to a specific AR DNA sequence, and it follows that the unique 17-mer AR DNA sequence occurs within a nucleosome which unfolds during transcription to reveal the histone H3-thiol groups at the center of the core.

**DISCUSSION**

Over 33 transcription factors are characterized by domains of tandem glutamine residues encoded by CAG triplet repeats. Expansion of the CAG repeats of the androgen receptor gene has been correlated with the incidence and severity of the Kennedy disease (spinal and bulbar muscular atrophy). Recent studies have shown that changes in the size and position of the polyglutamine tracts of the androgen receptor strongly influence its transactivation functions. For example, progressive expansion of the CAG repeats in human AR DNA diminishes transcription from androgen-responsive reporter genes (41, 42). Conversely, elimination of the CAG tracts from human and rat androgen receptor genes results in an elevation of transcriptional activation (41). How such differences in the number of glutamine repeats affect the functions of the androgen receptor and other transcription factors is not known, but recent x-ray and molecular modeling studies by Perutz et al. (43) point out the potential of paired polyglutamine tracts to act as “polar zippers,” possibly joining transcription regulatory factors on separate DNA segments (43).

Mutations affecting the size of the CAG domains of the androgen receptor gene have been noted in prostatic cancer (44). The present experiments have focused on the AR gene in prostatic cancer cell lines as a model for further analysis of the potential role of PNAs in the control of gene expression in malignant cells. We have shown that CAG-specific PNAs can penetrate the chromatin and inhibit transcription of the AR and TBP genes beyond the site of PNA-DNA hybridization, without a corresponding effect on transcription of the c-myc gene, while PNA targeted to a unique sequence of the c-myc gene inhibits its transcription without affecting the synthesis of AR or TBP mRNAs. The PNA effect depends on the direction of movement of the RNA polymerase complex. Targeting of the sense strand of the AR, TBP, and c-myc genes inhibited transcription of downstream DNA sequences, but it had no inhibitory effect on RNA polymerase transit through DNA sequences upstream of the PNA binding site. Instead, upstream transcription of the sense strand was stimulated in all three genes (Fig. 2), possibly due to continued recruitment and accumulation of active RNA polymerase complexes ahead of the PNA-DNA block. This could also explain the opposite results obtained for antisense transcription, in which RNA synthesis was stimulated below the site of PNA-DNA hybridization and inhibited above it (Fig. 2).

All our results show that PNA binding to the sense strand of the AR, TBP, and c-myc genes can inhibit transcription equally well on both strands of the DNA template. Such parity was not observed in studies of PNA effects on transcript elongation in vitro, in which PNA bound to the nontranscribed strand of a plasmid was only half as effective in chain termination (12). We attribute the stronger PNA response in our experiments to the 20-fold higher PNA concentrations used in the permeabilized cells, to the probability that the RNA polymerase II elongation...
complex in vivo is much larger than that of a reconstituted Pol II complex and less likely to pass by the PNA block, and to the additional constraints imposed by the nucleosomal structure of the chromatin templates.

The present results on the specificity of PNA effects on transcription of the AR, TBP, and c-myc genes are in accord with the observation that a PNA targeted to the CAG triplet repeats can enter and specifically capture chromatin restriction fragments containing the TBP gene (21), and they lend further support to the view that appropriately designed PNAs have great potential as antisense chemotherapeutic agents, especially in view of the fact that PNAs are extremely refractory to digestion by nucleases and proteases (45). However, it should be noted that PNAs targeted to transcription factors with multiple transactivation functions, such as TBP, could have deleterious effects throughout the genome.

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Lidia C. Boffa, Patricia L. Morris, Elisabetta M. Carpaneto, Marjorie Louissaint and Vincent G. Allfrey

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