Anti-repression of RNA Polymerase II Transcription by Pyrrole−Imidazole Polyamides†

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ABSTRACT: Pyrrole−imidazole polyamides are ligands that bind in the minor groove of DNA with high affinity and sequence selectivity. Molecules of this class have been shown to disrupt specific transcription factor−DNA interactions and to inhibit basal and activated transcription from various RNA polymerase II and III promoters. A set of eight-ring hairpin-motif pyrrole−imidazole polyamides has been designed to bind within the binding site for the human cytomegalovirus (CMV) UL122 immediate early protein 2 (IE86). IE86 represses transcription of the CMV major immediate early promoter (MIEP) through its cognate cis recognition sequence (crs) located between the TATA box and the transcription initiation site. The designed polyamides bind to their target DNA sequence with nanomolar affinities and with a high degree of sequence selectivity. The polyamides effectively block binding of IE86 protein to the crs in DNase I footprinting experiments. A mismatch polyamide, containing a single imidazole to pyrrole substitution, and also a polyamide binding to a site located 14 base pairs upstream of the repressor binding site, do not prevent IE86 binding to the crs. IE86-mediated transcriptional repression in vitro is relieved by a match polyamide but not by a mismatch polyamide. Transcription from a DNA template harboring a mutation in the crs is not affected either by IE86 protein or by the match polyamides. These results demonstrate that this new class of small molecules, the pyrrole−imidazole polyamides, are not only effective inhibitors of basal and activated transcription, but also can be used to activate transcription by blocking the DNA-binding activity of a repressor protein.

The pyrrole−imidazole polyamides are a class of synthetic small molecules that can be designed to bind predetermined DNA sequences (*1*, *2*). These molecules, containing *N*-methylpyrrole and *N*-methylimidazole amino acids, bind their target sequences with affinities comparable to those of natural DNA-binding transcriptional regulatory proteins (*1*). Base pair specificity depends on side-by-side pairing of aromatic pyrrole and imidazole amino acids in the minor groove of DNA (*2*). A pairing of an imidazole (Im) opposite a pyrrole (Py) targets a G·C base pair, whereas a Py opposite an Im targets a C·G base pair. The Py/Py pair is degenerate and targets both A·T and T·A base pairs. Recent studies have shown that the A·T degeneracy can be overcome by replacing one pyrrole ring of the Py/Py pair with 3-hydroxypyrrole (Hp), with the result that Hp/Py preferentially binds T·A pairs (*3*).

We have examined whether pyrrole−imidazole polyamides can be used to block the binding of well-characterized eukaryotic transcription factors to their target DNA sites and whether such compounds would inhibit transcription, both in vitro and in vivo. We have demonstrated that a high-affinity polyamide, which was designed to bind to a region within the internal control element of the *Xenopus* 5S RNA gene, effectively inhibits the binding of the 5S gene-specific transcription factor TFIIIA to this sequence (*4*). Inhibition of TFIIIA binding in vitro was shown to block 5S gene transcription by RNA polymerase III. Moreover, the polyamides selectively disrupt transcription complexes on the chromosomal 5S RNA genes in *Xenopus* kidney-derived fibroblasts grown in culture (*4*). These results suggest that the pyrrole−imidazole polyamides are cell-permeable and can inhibit transcription of target genes in living cells. These studies have now been extended to transcription factors utilized by messenger RNA-coding genes transcribed by RNA polymerase II (*5*). We find that polyamides are effective inhibitors of both tissue-specific and general transcription factors (*5*). Since multiple genes utilize common general and tissue-specific transcription factors, polyamides were synthesized to bind sequences adjacent to the binding sites for required transcription factors (*5*). A polyamide was synthesized to target sequences adjacent to the TATA-box element of the HIV-1 promoter: this polyamide inhibits TBP binding and basal transcription by RNA polymerase II. A

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polyamide which targets the DNA sequence adjacent to the binding site for lymphoid enhancer factor 1 (LEF-1) inhibits LEF-1 binding and is an effective inhibitor of activated transcription in vitro. Additionally, a polyamide bound to a site overlapping the binding site for the winged-helix-turn-helix transcription factor ets-1 blocks the DNA-binding activity of this factor. Most importantly, when used in combination, these polyamides were found to be effective inhibitors of HIV-1 replication in isolated human lymphoid cells (*5*).

In the present study, we have examined whether the pyrrole−imidazole polyamides can exert a positive effect on transcription by interfering with the activity of a specific repressor protein. The human cytomegalovirus (HCMV) IE86 repressor protein is well suited for this study, because it is a minor groove DNA-binding protein and transcriptional repression is dependent on IE86 binding to its DNA target site (*6, 7*). IE86 negatively regulates the major immediate early promoter (MIEP) of HCMV by binding to a sequence element (the cis repression signal, crs) located between the TATA box and the start of transcription (see ref *6* and references cited therein). It was shown that IE86 exerts its negative effect on transcription by blocking recruitment of RNA polymerase II to the preinitiation complex (*7, 8*). We find that polyamides that specifically recognize the crs prevent IE86 from binding and relieve transcriptional repression, while a mismatch polyamide or a polyamide which binds to a nearby site has no effect. Furthermore, occupancy of the crs element by a small polyamide is not sufficient to repress transcription, supporting the model that IE86-mediated repression results from steric interference with the recruitment of RNA polymerase II to the preinitiation complex (*7, 8*).

MATERIALS AND METHODS

Polyamide Syntheses. Synthesis of the hairpin pyrrole−imidazole polyamides shown in Figure 1 was by solid-phase methods as described (*9*). The purity and identity of each compound were verified by analytical HPLC, ^{1}H NMR, and MALDI-TOF mass spectroscopy (*9*). The polyamides were dissolved in H_2O and maintained as frozen stock solutions at 250 μM concentration. Appropriate dilutions of the polyamides were made in H_2O prior to each experiment.

IE86 Protein Expression and Purification. The full-length IE86 protein was cloned in the vector pDS56, 6His. Protein induction and purification were as described (*10*). Briefly, *E. coli* cells harboring the IE86 expression plasmid were grown to an optical density of 0.7−0.8, measured at 550 nm, prior to protein induction with 200 μg/mL IPTG. Cells were harvested after 90−120 min, and stored frozen at −80 °C. Cells were lysed by incubation in 50 mM sodium phosphate, pH 7.8, 1 mM PMSF, 1% Tween 20, 1 M NaCl, and 1 mg/mL lysozyme for 20 min on ice, followed by sonication. The extract was centrifuged for 30 min at 16 000 rpm in a Sorvall SS34 rotor, and the cleared supernatant was subjected to nickel chelate chromatography as described by the manufacturer (Qiagen). The equilibration and wash buffer contained 50 mM sodium phosphate, pH 7.8, 500 mM NaCl, and 10% glycerol. The final wash buffer contained 75 mM imidazole, pH 6.0, and the elution buffer contained 500 mM imidazole. Fractions containing the eluted protein were pooled and dialyzed against 50 mM sodium phosphate, pH 7.8, 250 mM NaCl, 1 mM β-mercaptoethanol, and 30% glycerol. In some cases, histidine-tagged IE86 was further purified on a Mono Q column (HR5/5, Pharmacia).

DNase I Footprint Titrations. A singly end-labeled DNA restriction fragment was derived from the plasmid pMIEP(−54/+7)CAT (*7*). The probe was labeled at the 3′ end of the bottom strand at the *Hin*dIII site located at nucleotide position −66 upstream of the MIEP TATA box. Labeling with [α-^{32}P]dATP and Klenow DNA polymerase was carried out according to standard techniques. After digestion with *Eco*RI, the labeled 300 bp fragment was recovered from a nondenaturing polyacrylamide gel and purified by Elutip-D chromatography (Schleicher & Schuell). Footprinting reactions contained approximately 0.5 ng of the labeled fragment in a 50 μL of binding reaction, yielding a DNA concentration of approximately 50 pM. Reactions also contained 400 ng of poly(dG)-poly(dC)-nonspecific DNA in a buffer containing 25 mM Tris-HCl, pH 8, 0.5 mM dithiothreitol, 0.5 mM EDTA, 6.25 mM $MgCl_2$, and 10% glycerol. Binding reactions contained IE86 protein and polyamides as indicated in the figure legends. The labeled DNA was preincubated with polyamides for 15 min at ambient temperature before addition of IE86, where indicated, and a further 15 min incubation at ambient temperature. $CaCl_2$ and $MgCl_2$ were added to a final concentrations of 2.5 and 5 mM, respectively, and the reaction volume was increased to 100 μL. Digestion was allowed to proceed for 30 s at 23 °C with 3.3×10^{-5} unit of DNase I (Boehringer Mannheim), diluted in 10 mM Tris-HCl, pH 8. Reactions were stopped by the addition of SDS (0.2%, w/v, final concentration) and EDTA to 10 mM. After extraction with phenol/chloroform and ethanol precipitation, in the presence of 20 μg of glycogen as a carrier, the samples were analyzed by electrophoresis on a 6% or 8% sequencing polyacrylamide gel containing 8.3 M urea and 88 mM Tris−borate, pH 8.3, 2 mM EDTA. The dried gels were exposed to Kodak Bio-Max film with DuPont Cronex Lightning Plus intensifying screens at −80 °C. Quantitation of the footprint titrations was by storage phosphorimage analysis utilizing Kodak Storage Phosphor Screens (SO 230) and a Molecular Dynamics SF PhosphorImager. The data were analyzed using the ImageQuant software from Molecular Dynamics.

In Vitro Transcription Reactions. Transcription reactions were performed with nuclear extract prepared either from cultured CEM cells, prepared by a modification of the protocol of Dignam et al. (*11*), or from HeLa cells (purchased from Promega). The wild-type DNA template was plasmid pMIEP(−1145/+112)CAT, containing the major immediate early promoter (MIEP) element of human cytomegalovirus (*12*). A control DNA template contained a mutation in the crs sequence (*8*) in which the first four nucleotides of the IE86 binding site were changed from 5′-CGTT-3′ to 5′-GCAA-3′. This mutation did not affect the sequences of the polyamide binding sites (see Figure 1B). pMIEP(−1145/+112)CAT was digested with *Pvu*II to yield a 1.6 kb fragment, and pMIEP(mcrs)CAT with *Eco*RI/*Eco*RV, yielding a 1.3 kb fragment. Restriction fragments were purified from agarose gels using a Qiagen gel extraction kit and used in transcription reactions at final concentrations of 8 μg/mL for pMIEP(−1145/+112)CAT and 4 μg/mL for pMIEP(mcrs)CAT. The DNA template was preincubated with

polyamides for 15 min at ambient temperature, followed by an additional 30 min incubation with IE86 where indicated, in a buffer containing 10 mM Hepes, pH 7.9, 50 mM KCl, 0.1 mM EDTA, 2.5 mM DTT, 10% glycerol, 3 mM $MgCl_2$, and 20 units of RNasin (Promega) in a total volume of 25 μL. Nuclear extract was then added (17 μg of protein/reaction), and transcription complexes were allowed to form for 1 h at 30 °C prior to a transcription step with 10 μCi of [α-^{32}P]UTP, 600 μM of the remaining unlabeled nucleoside triphosphates, and 10 μM UTP for 1 h at 30 °C. The reaction was stopped by adding 175 μL of a solution containing 0.3 M Tris-HCl, pH 7.4, 0.3 M sodium acetate, 0.5% SDS, and 2 mM EDTA. RNA was purified by extraction with phenol/chloroform, precipitated with ethanol in the presence of glycogen as a carrier, and analyzed by electrophoresis on a denaturing (8.3 M urea) 6% polyacrylamide gel. Autoradiograms were obtained by exposure of the dried gel to BioMax film with DuPont Cronex Lightning Plus intensifying screens for 15−18 h at −80 °C. Relative levels of transcription were quantitated using a Phosphorimager (Molecular Dynamics). Poly(U) polymerase activity present in the nuclear extract was used as an internal standard to account for variability between each sample (*7*).

RESULTS

Polyamide Binding Sites within the CMV Promoter. Five pyrrole−imidazole polyamides (**1**−**5**) were synthesized by solid-phase methods (Figure 1A). Binding affinities for each of these compounds with their predicted target sites and with mismatch sites were determined by quantitative DNase I footprint titrations (*1*). Binding models are shown in Figure 1B. The eight-ring polyamide **1**, ImPyImPy-γ-PyPyPyPy-β-Dp (where γ denotes γ-aminobutyric acid, β denotes β-alanine, and Dp denotes dimethylaminopropylamide), binds the six base pair sequence 5′-AGTGAA-3′ within the IE86 binding site with an apparent dissociation constant of 1.4 nM. An additional polyamide was synthesized that binds the same site, but with a higher affinity: polyamide Im-β-ImPy-D-PyPyPyPy-β-Dp (polyamide **2**, where D represents diaminobutyric acid) binds the sequence 5′-AGTGAA-3′ with a K_d of 0.25 nM. In this latter compound, the β-alanine/pyrrole pair recognizes the T·A base pair at the second position of the recognition site (*13*). Changing a single imidazole to a pyrrole yields ImPyPyPy-γ-PyPyPyPy-β-Dp (polyamide **3**), which reduces the affinity of polyamide **3** by ∼75-fold relative to polyamide **1** for the target site (*1*). A second control polyamide, ImImPyPy-γ-ImPyPyPy-β-Dp (polyamide **4**), binds the sequence 5′-AGGTCT-3′ adjacent to the TATA box of the MIEP with a K_d of 1 nM. A double mismatch polyamide, ImPyPyPy-γ-ImPyPyPy-β-Dp (polyamide **5**), has the same composition of pyrrole and imidazole rings as polyamide **1** but differs from polyamide **1** in ring sequence (Figure 1A). Previous studies have shown that the control polyamides **3** and **5** bind the sequences 5′-WGWWWW-3′ and 5′-WGWWCW′3′, where W = A or T (*1*). No match sites for these polyamides are present in the proximal HCMV promoter (Figure 1B, top).

Polyamide Inhibition of IE86 Binding. We used the polyamides described above together with purified recombinant IE86 protein in DNase I footprint experiments with a singly end-labeled restriction fragment derived from the CMV major immediate early promoter. As predicted, polyamide **1** protects the sequence 5′-AGTGAA-3′ in the center of the IE86 binding site. Approximately 50% protection is seen with 2 nM polyamide, and complete protection is obtained with 200 nM polyamide **1** (Figure 2, lanes 3−5). Recombinant protein IE86 protects a region extending between positions −24 and +3. Approximately 250 nM IE86 is required to give complete protection (Figure 2, lanes 6 and 14). When polyamide **1** is preincubated with DNA for 15 min prior to addition of protein, the IE86 footprint is partially replaced by the polyamide **1** specific footprint at a concentration of 2 nM polyamide, and is completely replaced at a concentration of 200 nM polyamide (Figure 2, lanes 7−9). The control polyamide **4**, which protects a site 5′-AGGTCT-3′ immediately upstream of the TATA box, does not interfere with IE86 binding in a concentration range of 2−200 nM. Both the IE86-specific footprint and the polyamide **4** footprint coexist, even though the boundaries of these footprints are only 9 base pairs apart (Figure 2, lanes 14−17).

The same experiment was repeated with the higher affinity polyamide **2**, which binds the same site 5′-AGTGAA-3′. Complete protection is seen with 5 and 10 nM polyamide **2** (Figure 3, lanes 4 and 5), which are also the concentrations required to completely inhibit IE86 from binding (Figure 3, lanes 8 and 9). A partial inhibition of IE86 binding is detected at 1 nM polyamide **2** (Figure 3, lane 7). The mismatch polyamide **3** did not give a footprint on the CMV fragment in the concentration range used in these experiments (1−200 nM), and it did not interfere with IE86 binding (Figure 3, lanes 11−13 and 15−17).

Effect of Polyamides on IE86-Mediated Transcriptional Repression in Vitro. We next tested the effects of the polyamides on CMV MIEP transcription in an in vitro system consisting of a nuclear extract prepared from cultured human CEM cells (Figure 4A,B) or with a HeLa cell nuclear extract (Figure 4C). It is known from previous studies that IE86 specifically inhibits transcription by binding to the cis repression sequence (crs), and physically blocks the access of RNA polymerase II to the preinitiation complex (*7, 8*). We tested whether polyamide **1**, which has a binding affinity comparable to that of IE86, could counteract the negative effect of IE86 on transcription. For these experiments, we chose a concentration of IE86 protein that would give approximately 75% inhibition of transcription (Figure 4A, lane 2). We next preincubated various concentrations of polyamide **1** with the DNA template prior to addition of IE86 protein (lanes 3−8). At 1 μM polyamide **1**, the level of transcription is restored to approximately that of the control reaction in the absence of IE86 or polyamide (lane 1). The concentration of polyamide required for blocking IE86 in this experiment is significantly higher than that required in the footprinting experiments (Figure 2). This difference reflects the 150-fold higher concentration of specific polyamide binding sites in the transcription experiments compared to the concentration of sites in the radiolabeled restriction fragment in the footprinting experiments. Interestingly, polyamide **1** bound to the crs in the absence of IE86 has no effect on transcription, even at a concentration of 1 μM (Figure 4A, lane 9). The double mismatch polyamide (polyamide **5**, Figure 1), which has no binding site in the proximal CMV promoter, has no effect on IE86-mediated

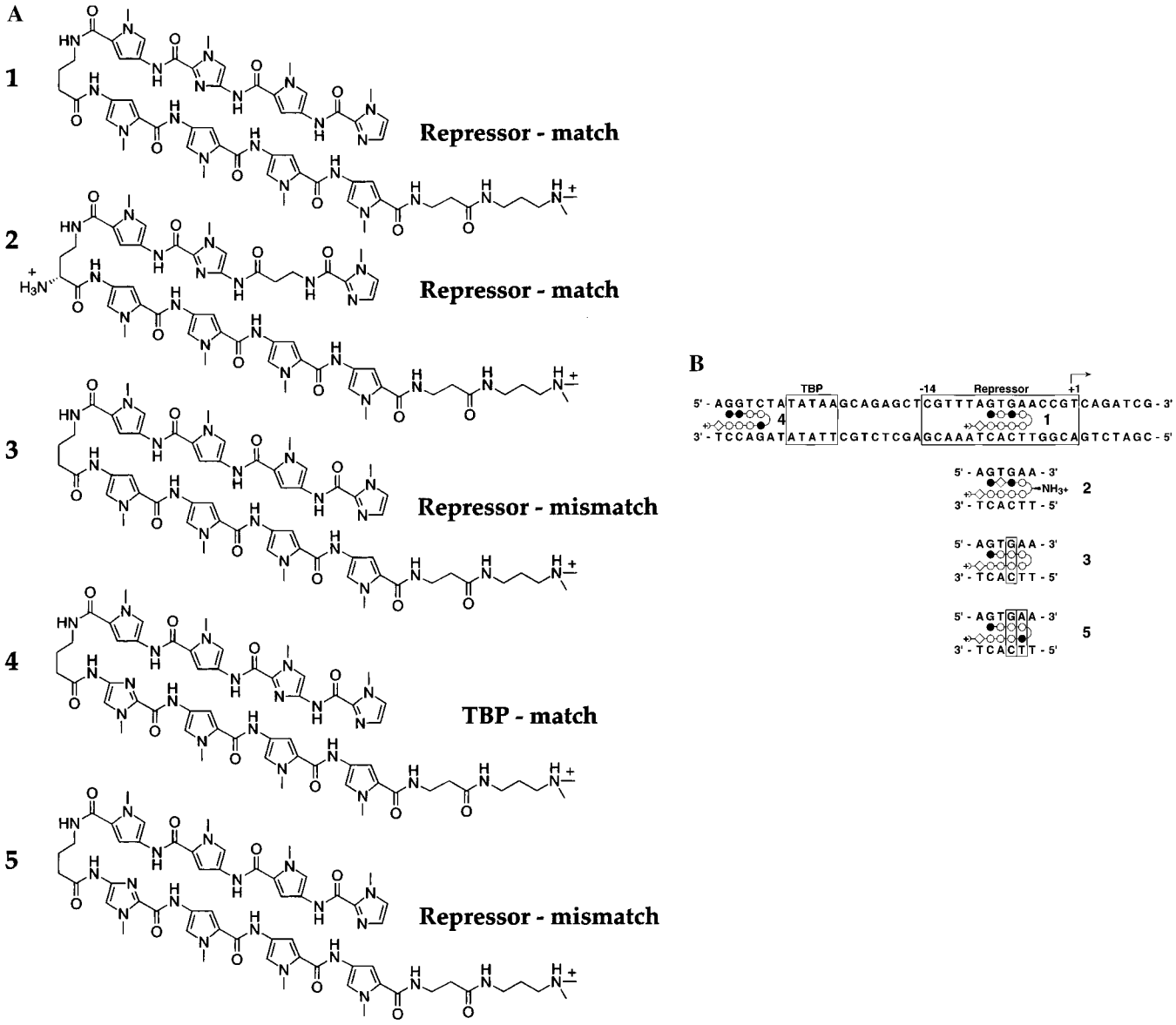

FIGURE 1: Structures and DNA-binding sites for synthetic polyamides designed to target the HCMV repressor binding site. (A) Structures of the polyamides ImPyImPy-γ-PyPyPyPy-β-Dp (**1**), Im-β-ImPy-D-PyPyPyPy-β-Dp (**2**), ImPyPyPy-γ-PyPyPyPy-β-Dp (**3**), ImImPyPy-γ-ImPyPyPy-β-Dp (**4**), and ImPyPyPy-γ-ImPyPyPy-β-Dp (**5**) [where Im = imidazole, Py = pyrrole, γ = γ-aminobutyric acid, β = β-alanine, D(Daba) = diaminobutyric acid, and Dp = dimethylaminopropylamide]. Polyamide **2** contains an additional amino group at the hairpin junction (diaminobutryic acid). Polyamides **1** and **2** are match polyamides for the HCMV repressor binding site. Polyamides **3** and **5** are mismatch polyamides. Polyamide **4** is a match polyamide for the sequence adjacent to the TBP binding site (TATA box). (B) Sequence of the promoter region from position -34 to position $+8$. The TATA box and the repressor binding site (located at -14 to $+1$ relative to the start-site of transcription) are boxed. The polyamides are schematically represented at their respective DNA-binding sites. The black and white circles represent imidazole and pyrrole rings, respectively; the hairpin junction (curved line) is formed with γ-aminobutyric acid (or with diaminobutyric acid in polyamide **2**), and the diamond represents β-alanine. The mismatches with polyamides **3** and **5** are boxed. Polyamide **4** binds the sequence 5′-AGGTCT-3′ adjacent to the TATA box.

repression (Figure 4B, lane 5) under conditions where the match polyamide **1** restores activity (lane 3).

To demonstrate the specificity of the effect of polyamide **1** on IE86-mediated transcriptional repression, we used a second CMV MIEP DNA template with a mutation in the crs sequence that prevents IE86 from binding (*8*). In this template, the first four nucleotides of the IE86 binding site were changed from 5′-CGTT-3′ to 5′-GCAA-3′, with no changes in the DNA sequence recognized by the match polyamides **1** and **2** (Figure 1B). This template and the wild-type MIEP were used in separate transcription reactions, and the effects of IE86 protein and polyamides on transcriptional activities were normalized to the levels of transcription in control reactions lacking IE86 or polyamides (as described under Materials and Methods). Addition of IE86 to the mutant template has no significant effect on transcription compared with 80% inhibition from the wild-type template (Figure 4C). The match polyamides **1** and **2** (Figure 1A) partially relieve IE86-mediated repression from the wild-type MIEP, but have no significant effect on transcription from the crs mutant promoter (Figure 4C). These results again demonstrate that a polyamide bound between the TATA element and the transcription start-site has no pronounced effect on transcription. Moreover, the combination of footprinting and transcription experiments demonstrates that reversal of IE86-mediated inhibition by poly-

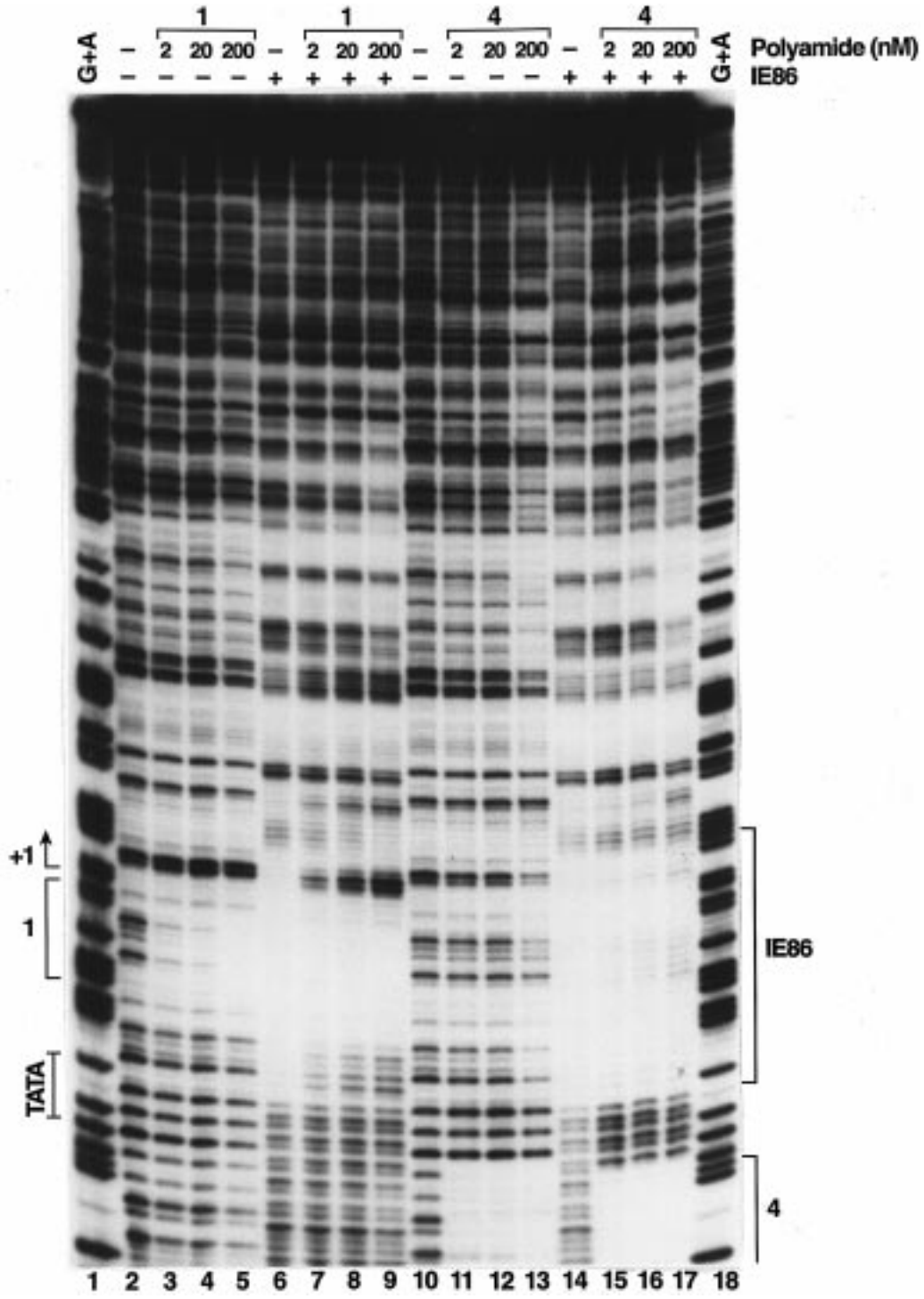

FIGURE 2: Inhibition of IE86 binding to the crs element by polyamide **1**. DNase I footprinting reactions contained approximately 250 nM his-tagged IE86, where indicated, and polyamide **1** in the concentrations indicated at the top of the lanes. Lanes 1 and 18 represent G+A sequencing ladders. The extents of the footprints generated by polyamide **1**, IE86, and the control polyamide **4** are indicated by brackets alongside the autoradiogram. The location of the TATA box and the start of transcription (+1) are indicated.

amides **1** and **2** is due to blockage of IE86 binding by these polyamides and not to general stimulation of transcription.

DISCUSSION

Pyrrole−imidazole polyamides have been designed to bind specific DNA sequences with high affinities and to serve as reagents to manipulate expression of specific genes. So far, members of this new class of DNA-binding ligands have proven to be effective inhibitors in vitro and in vivo of transcription of RNA polymerase III promoters (*4*) as well as RNA polymerase II-dependent promoters, and polyamides have been shown to inhibit both basal and activated transcription (*5*). In each of these cases, polyamides were designed to bind with high affinity to six or seven base pair sequences either within or adjacent to the DNA recognition sites for transcription factors, thereby preventing the transcription factors from binding to their target site. The generality of this approach has now been extended in the present study. Polyamides that bind within the IE86 repressor protein binding site in the CMV major immediate early

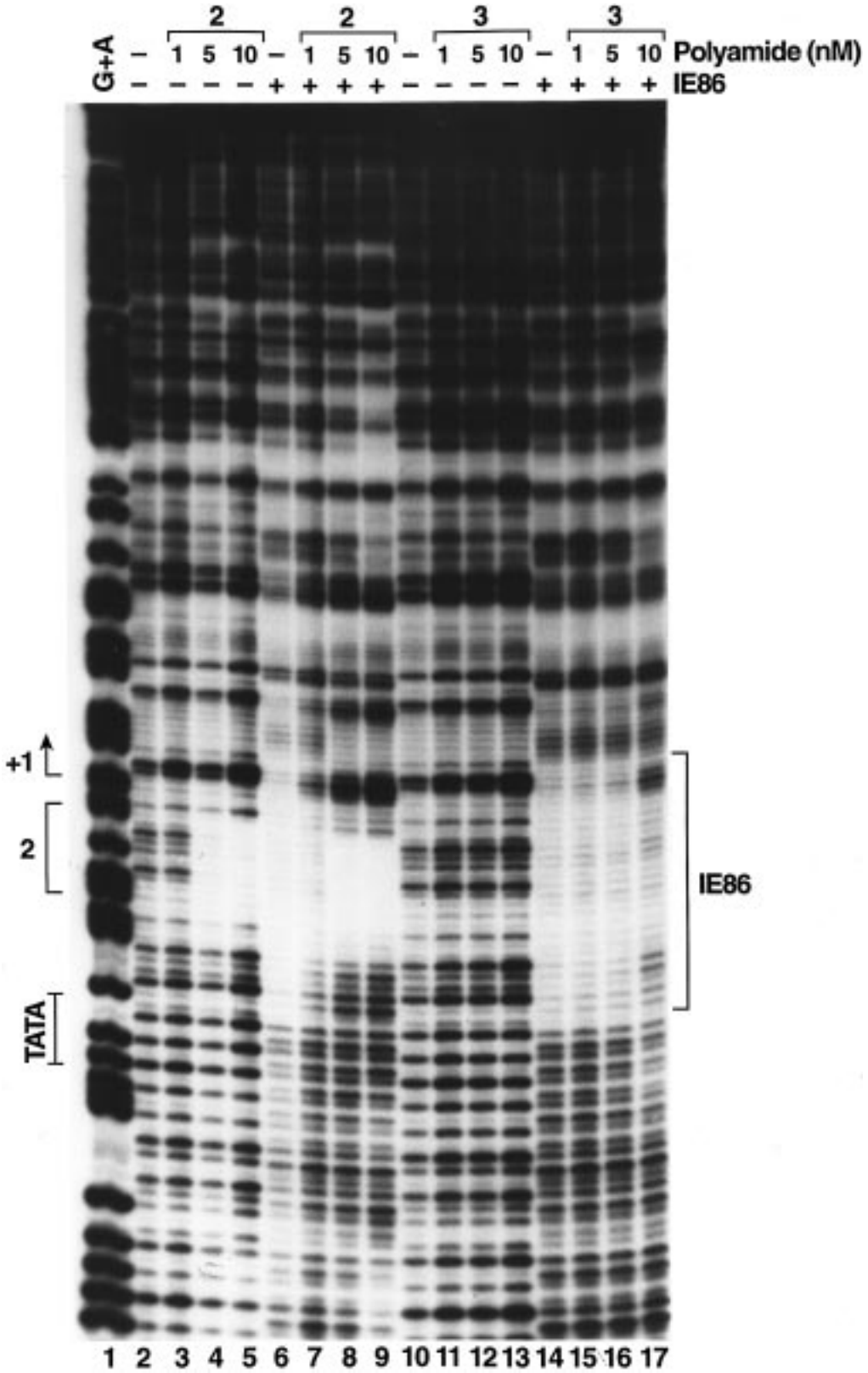

FIGURE 3: Inhibition of IE86 binding to the crs element by polyamide **2**. A similar DNase I footprint experiment as in Figure 2 was performed with the higher affinity polyamide **2** and the mismatch polyamide **3**. The concentrations of the polyamide are indicated in nanomolar at the top of the lanes. The extent of the footprints for polyamide **2** and IE86 protein and also the TATA box and transcription start-site are shown at the side of the autoradiogram.

promoter prevent IE86 from binding to the DNA, thereby preventing transcriptional repression. Thus, polyamides can also exert a positive effect on transcription by interfering with a repressor DNA-binding protein.

IE86 represses transcription from the major immediate early promoter of CMV by binding to a cis repression signal (crs), located between the TATA box and the start of transcription, and selectively blocks recruitment of RNA

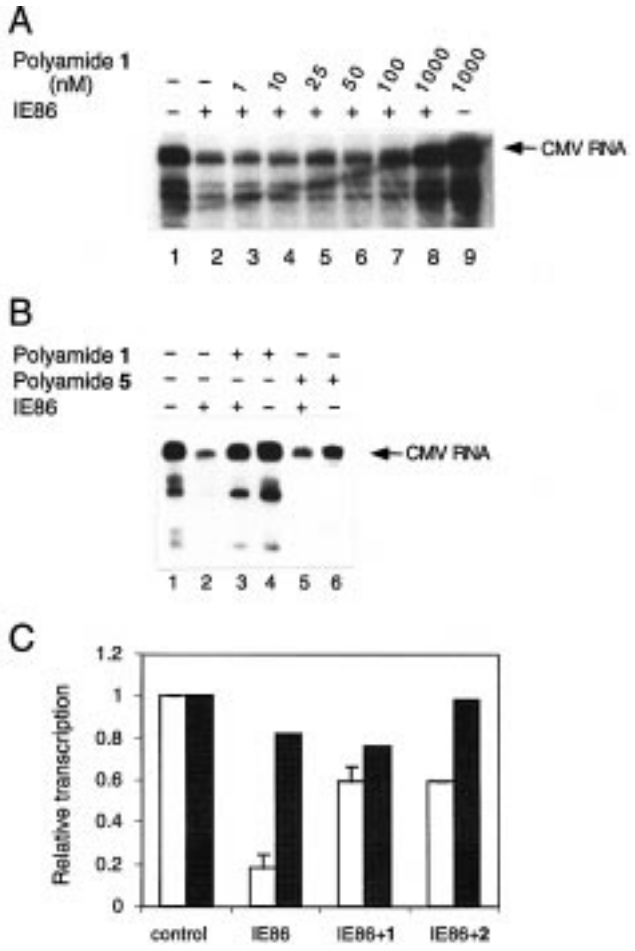

FIGURE 4: (A) Polyamide **1** prevents IE86-mediated transcriptional repression. In vitro transcription reactions were performed with linearized CMV MIEP plasmid DNA and CEM extract as described under Materials and Methods. The concentration of IE86 that gave approximately 75% inhibition of transcription was empirically determined and used in the reactions shown in lanes 2−8. Lanes 3−8 contained the indicated concentrations of polyamide **1**. Lane 9 contained polyamide **1** at 1 μM, but lacked IE86 protein. The bands below the major RNA transcript are present in each lane in levels proportional to the overall level of transcription and likely represent premature termination products. (B) Mismatch polyamide **5** does not counter the inhibitory effect of IE86. The concentration of IE86 protein that gave 75% inhibition of MIEP transcription was added to the reactions in the indicated lanes. Match (**1**) and mismatch (**5**) polyamides were added at concentrations of 1 μM, respectively, where indicated. Polyamides were preincubated with the DNA for 15 min prior to the addition of IE86. (C) Polyamides **1** and **2** have no effect on transcription from a template harboring a mutation in the crs. Transcription reactions were performed with HeLa nuclear extract and either wild-type MIEP promoter (open bars) or mutant crs MIEP promoter (closed bars) as described under Materials and Methods. Phosphorimage analysis was used to quantitate levels of transcription, which were normalized for recovery and expressed relative to the control reactions for each template lacking polyamides or IE86. IE86 protein was added in the indicated reactions along with polyamides **1** or **2** at 1 μM, where indicated. Data shown are the average of three determinations (and standard deviations) for the wild-type promoter.

polymerase II to the transcription complex (see refs *7*, *8* and references cited therein). Repression can be mediated through heterologous proteins as well, if the crs is replaced by their respective DNA recognition sequence (*8*). However, occupancy of the crs by a polyamide is not sufficient to interfere with the transcription machinery. A similar situation was found for the HIV-1 promoter where a polyamide binding near the transcription start-site and within the transcribed DNA did not interfere with basal transcription by RNA polymerase II (*5*). Therefore, it can be concluded that polyamides are effective modulators of transcription provided that they directly interfere with a DNA-binding regulatory protein, which can be an activator or a repressor protein. Polyamides bind in the minor groove of DNA, and polyamides were shown to inhibit DNA-binding proteins that contact the minor groove (*4*, *5*, *14*). Polyamide inhibition of IE86 is consistent with biochemical data showing that IE86 interacts primarily in the minor groove of the DNA helix (*6*). The specificity of this inhibition was demonstrated through the use of match polyamides **1** and **2** and mismatch polyamides **3** and **5** and with a control DNA template containing a mutation in the crs which prevents IE86 from binding.

In summary, the results presented here provide additional evidence for the effectiveness of the novel class of pyrrole−imidazole polyamides as modulators of transcription of specific genes. Polyamides designed to block DNA binding of activator proteins inhibit transcription, and polyamides designed to interfere with a repressor protein activate transcription.

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