Inhibition of RNA Polymerase III Elongation by a T_{10} Peptide Nucleic Acid

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Received for publication, October 13, 2000, and in revised form, November 2, 2000
Published, JBC Papers in Press, November 2, 2000, DOI 10.1074/jbc.M009367200

The terminator elements of eukaryotic class III genes strongly contribute to overall transcription efficiency by allowing fast RNA polymerase III (pol III) recycling. Being constituted by a run of thymidine residues on the coding strand (a poly(dA) tract on the transcribed strand), pol III terminators are expected to form highly stable triple-helix complexes with oligothymine peptide nucleic acids (PNAs). We analyzed the effect of a T_{10} PNA on in vitro transcription of three yeast class III genes (coding for two different tRNAs and the U6 small nuclear RNA) having termination signals of at least ten T residues. At nanomolar concentrations, the PNA almost completely inhibited transcription of supercoiled, but not linearized, templates in a sequence-specific manner. The total RNA output of the first transcription cycle was not affected by PNA concentrations strongly inhibiting multiple round transcription. Thus, an impairment of pol III recycling fully accounts for the observed inhibition. As revealed by the size and the state (free or transcription complex-associated) of the RNAs produced in PNA-inhibited reactions, pol III is "roadblocked" by the DNA-PNA adduct before reaching the terminator region. On different templates, the distance between the active site and the leading edge of the arrested polymerase ranged from 10 to 20 base pairs. Given their ability to efficiently block pol III elongation, oligothymine PNAs lend themselves as potential cell growth inhibitors interfering with eukaryotic class III gene transcription.

High throughput transcription of eukaryotic genes encoding small RNAs involved in protein synthesis (i.e. the tRNAs and the 5 S rRNA) is one of the main tasks of RNA polymerase III (pol III)1 and its associated factors (TFIIB, TFIIC, and TFIIB). TFIIB (a 5 S-specific component) and TFIIC are sequence-specific DNA-binding proteins that recognize the internal control regions of class II genes. Once bound, they direct the assembly of TFIIB upstream to the transcription start site; TFIIB then recruits pol III and actively participates in the transcription initiation step (1, 2). To satisfy the high cellular need for its RNA products, the pol III transcription machinery maximizes the efficiency of reinitiation in two ways: once formed, a functional preinitiation complex is stably maintained at the promoter for multiple rounds of pol III recruitment by the initiation factor TFIIB (1); in addition, a promoter-engaged polymerase initiates new transcription cycles more rapidly than the first one by means of a terminator-dependent, fast recycling pathway (3). The terminator of pol III-transcribed genes, generally a run of 5 or more thymidine residues on the coding strand, can thus be viewed as a key promoter element that, by allowing pol III to enter a facilitated initiation pathway, greatly increases the overall transcription output. Shortening or deleting the terminator element causes a transcription impairment due to pol III readthrough (4) and also precludes the entrapment of terminating pol III molecules for structural and functional studies. Therefore, a nondestructive approach, such as the protein roadblock strategy utilized for Escherichia coli RNA polymerase (5), has to be used for the functional dissection of the coupled termination/reinitiation processes. Peptide nucleic acids (PNAs), recently developed nucleic acid mimics in which the nucleobases are attached to a pseudopeptide backbone, can form extremely stable complexes with complementary RNA and DNA targets and have thus the potential to interfere with key steps of gene expression (6). In particular, oligopyrimidine PNAs bind with high affinity to double-stranded DNA targets through a strand displacement mechanism, in which two invading PNAs form a Watson-Crick-Hogness triple-helix with the complementary DNA strand, leaving the other strand unpaired (7). The potential of oligopyrimidine PNAs for gene-targeting strategies has originally been documented in studies demonstrating the PNA-dependent arrest of transcription elongation by phage RNA polymerases and human RNA polymerase II on artificial DNA templates bearing an oligopurine cassette on the transcribed strand (8–11). We reasoned that the poly(dA) tract present on the transcribed strand of class III gene terminators represents a unique natural case of a widespread eukaryotic control element that can be targeted by oligothymine PNA molecules. To gain insight into the structural and functional features of a late elongating pol III and to lay the grounds for a detailed understanding of the anti-gene properties of PNAs, we thus set out to analyze the transcriptional effect of a terminator-bound oligothymine PNA in a well-defined in vitro pol III system from the yeast Saccharomyces cerevisiae.

EXPERIMENTAL PROCEDURES

PNAs—The H-T_{10}-Lys-NH₂ PNA was synthesized manually, according to published procedures (12–14), on a (4-methylbenzhydryl)amino resin (Novabiochem) in a 8-μmol scale. Boc-t-Lys(2-Cl-Z) (Novabiochem) and Boc-T PNA monomers (Perseptive Biosoystems) were used. Coupling reactions were carried out using O-(1H-benzotria-
The minator element made up of 12 T residues, instead of the expected 10. The product was precipitated with diethyl ether and dissolved in double-distilled water. PNA binding to the tDNAIle terminator was analyzed by permanganate probing. Multiple round or single round transcription experiments were carried out as described previously (16). Sequence analysis of the tRNA^{16S} gene revealed a terminator element located 12 T residues downstream of the terminator element. Primer extension reactions initiated from a32P-labeled primer annealed to the coding strand starting from the D-lysine at the C terminus is known to favor DNA binding. The tRNA{16S} (UAC) gene, in the pRS316 vector, was a gift of Salam Shaaban. The tRNA Glu(UUC) gene, in the pUCGlu plasmid (17), is identified at MIPS as E{TTC}ER2. The yeast tRNA{16S} gene (4), in the pRS316 vector, was a gift of Salam Shaaban. The tRNA^{16S}(UUC) gene, in the pUCGlu plasmid (17), is identified at MIPS as E{TTC}ER2. The yeast tRNA{16S} gene was carried by plasmid pB6 (18). The tRNA^{16S}(GCU) gene (referred to as N(GTTT)CR at MIPS), with 65 bp of 5'- and 84 bp of 3'-flanking regions, was amplified from yeast genomic DNA using Deep Vent DNA polymerase (New England BioLabs) and the following oligonucleotide primers: Asn_gtt19 (forward), 5'-CATACTGAAAGGTTAGGG; 5'-GATTTCATCCATGCCAGTG; the resulting amplification product (235 bp) was sequence-verified and inserted into the Smal site of the pBlueScript KS(+) vector.

Permanganate Probing—The pBlueScript-DNA^{16S}(ATG) plasmid (100 ng) was preincubated with the T10 PNA in 1 ml Tris/HCl (pH 7.8) at 37 °C for 60 min in a volume of 11 μl, then the unincorporated amionophore, piperidine cleavage, and cleaved DNA purification were performed as described (19). Purified DNA samples were analyzed by primer extension in reaction mixtures containing 0.25 mM each of dATP, dCTP, dGTP, and dTTP, 25 mM Tris/HCl, pH 9.5, 5 mM MgCl2, 8 units of ThermoSequenase DNA polymerase (Amersham Pharmacia Biotech), and 1 pmol of a 5'-end-labeled oligonucleotide primer (5'-CAACTCA-CAAAAGATAGTTAGG; 5'-GATTTCATCCATGCCAGTG) complementary to the coding strand starting at 67 bp downstream of the terminator element. Primer extension reactions consisted of 31 cycles of denaturation (94 °C, 4 min in cycle 1, 45 min in cycles 2–31), annealing (50 °C, 45 min), and elongation (72 °C, 45 min); the resulting samples were ethanol-precipitated, resuspended in formamide loading buffer, and analyzed on a 5% polyacrylamide/7 M urea sequencing gel followed by autoradiography.

In Vitro Transcription—In all transcription experiments, plasmid DNA was preincubated with the desired PNA concentration as described for permanganate probing. Multiple rounds or single round transcription assays were then carried out as described (16), except for a reduction of the final KCl concentration from 100 to 50 mM to minimize PNA dissociation. Gel-filtration analysis of transcription products on Sepharose 2B (Amersham Pharmacia Biotech) was performed as described (20). Transcripts were quantified with the Multi-Analyst/PC software (Bio-Rad) using phosphorimaging of dried gels obtained with a Personal Imager FX (Bio-Rad).
inhibition at 200 nM. By comparison, no significant inhibition was produced by the control PNA, nor by the T10 oligonucleotide. As shown in Fig. 2A (lanes 5–7), inhibitory concentrations of the T10 PNA caused the appearance of a main shortened transcript. At maximally inhibitory PNA concentrations (lanes 6, 7), the levels of this incomplete transcript reached those of the full-length product; both transcripts were detectable in low amounts (~5% of the output of the uninhibited reaction) up to a T10 PNA concentration of 2 μM (not shown). A third, much less abundant transcript of intermediate size is barely detectable in lanes 6 and 7, but it was more evident in other experiments (see for example Fig. 4A, lane 2).

When genes with shorter terminator elements (6–8 T residues) were used in place of the tRNAile gene, the T10 PNA was less effective in inhibiting transcription. As shown in Fig. 2B, a significant inhibition could still be observed in the case of a tDNApro(TGG) with a T8 terminator (lanes 1–5), whereas only a 40% inhibition at 700 nM PNA was observed with the SUP4 tRNA gene, having a T7GT6 terminator (lanes 10–13), and no inhibition was detected in the case of the T6 terminator of the tRNAglu(TTC) gene (lanes 6–9). Along with the results of permanganate probing experiments (Fig. 1), these data indicate that the T10 PNA selectively binds to, and inhibits the transcription of, tRNA genes with a terminator element made up of at least 8 consecutive T residues.

Effect of Template Supercoiling on PNA Inhibition—The T10 PNA half-inhibitory concentration measured in the case of the tRNAile gene (100 nM) is 5–10 times lower than that determined in previous studies using templates containing an artificially positioned T10 cassette to analyze the PNA-induced arrest of phage RNA polymerases (10) and human RNA polymerase II (8). These studies, however, were all conducted with linearized DNA templates (to allow for runoff transcription), whereas the tRNA genes utilized in our experiments were all in the form of negatively supercoiled plasmids. An enhanced binding of PNAs to supercoiled DNA has been reported previously (19). The plasmid containing the tRNAile gene was thus linearized by restriction digestion and used to test whether template supercoiling is responsible for the higher sensitivity of tRNA gene transcription to PNA inhibition. As shown in Fig. 3, this appears to be the case, because switching from a supercoiled (lanes 1–4) to a linearized (lanes 5–8) template caused a ~10-fold increase (from 100 nM to 1 μM) in the half-inhibitory concentration of the T10 PNA.

Mechanism of Transcriptional Inhibition by the T10 PNA—The mechanism of PNA inhibition was investigated by limiting transcription of preformed PNA:tDNAile complexes to a single round, a condition that by preventing reinitiation should allow to monitor the effect of the PNA on the initiation and elongation steps of the first transcription cycle. Reported in Fig. 4A are the results of an experiment comparing the effect of the T10 PNA on either multiple rounds (8 cycles; lanes 1 and 2) or a single round (lanes 3–6) of tRNAile gene transcription. The PNA reduced the total output of multiple round transcription to levels comparable to those of a single round reaction (cf. lanes 2 and 3). Interestingly, however, it did not appreciably affect the total amount, but rather the size of the products of single round transcription reactions, with the appearance of two shorter transcripts at a saturating PNA concentration (lane 6). The PNA thus appears to arrest elongating pol III before it enters the terminator region, thereby strongly inhibiting subsequent recycling. Closer inspection of lanes 2 and 6 in Fig. 4A shows that, although very drastic, the PNA inhibition of recycling is not complete. In fact, although the amounts of the incomplete transcripts produced under multiple-round
part of arrested ternary complexes. As shown in Fig. 4 that can resolve unbound RNAs from RNA molecules that are molecular filtration on Sepharose-2B, a gel-permeation matrix produced in PNA-inhibited reactions was next investigated by precursor (24, 25). The physical state of the transcripts do not originate from pol III-catalyzed cleavage of a longer inhibited reactions (data not shown), thus suggesting that they was revealed by a time-course analysis of single round, PNA-shortened transcripts, with respect to the full-length product, possibility that they are synthesized by a second, colliding pol the first round of PNA-inhibited transcription rules out the some residual reinitiation took place on a few PNA-free tem-

Fig. 4. Single-round transcription analysis of T10 PNA inhibition. A, the tDNA10(TAT)-containing plasmid was preincubated with the indicated concentrations of T10 PNA, and stalled elongation complexes, containing a 7-nt long nascent RNA, were allowed to form by the omission of CTP from the reaction mixture. Transcription was then resumed by the addition of CTP, either alone to allow for multiple rounds of transcription (lanes 1–2, MR) or together with heparin (200 µg/ml) to limit transcription to a single round (lanes 3–6, SR). The output of individual reactions, relative to the output of the uninhibited multiple-round reaction of lane 1 (arbitrarily set to 100) is indicated below each lane (Txa). The migration position of the full-length pre tRNA10 is indicated on the left. The position of the most abundant shortened transcript is marked by an asterisk. B, a scaled-up transcription reaction (programmed with tDNA10(TAT) previously incubated with 400 nM T10 PNA) was blocked with 20 µM EDTA, then loaded onto a 1-ml Sepharose-2B column. Twenty fractions (50 µl each) were collected, and transcripts contained in the indicated fractions (6–17) were analyzed by gel-electrophoresis; an aliquot of the unfractionated mixture was analyzed in parallel (input). The migration positions of full-length and arrested transcripts are indicated on the left. (lane 2) and single-round (lane 6) conditions at 200 nM PNA are roughly the same, the full-length transcript is more abundant in the PNA-inhibited multiple-round reaction (lane 2), as if some residual reinitiation took place on a few PNA-free templates. The fact that the shortened transcripts are produced in the first round of PNA-inhibited transcription rules out the possibility that they are synthesized by a second, colliding pol III molecule. Moreover, no delay in the appearance of these shortened transcripts, with respect to the full-length product, was revealed by a time-course analysis of single round, PNA-inhibited reactions (data not shown), thus suggesting that they do not originate from pol III-catalyzed cleavage of a longer precursor (24, 25). The physical state of the transcripts produced in PNA-inhibited reactions was next investigated by molecular filtration on Sepharose-2B, a gel-permeation matrix that can resolve unbound RNAs from RNA molecules that are part of arrested ternary complexes. As shown in Fig. 4B, full-length RNAs produced in a PNA-inhibited, multiple-round reaction behaved as free, released transcripts (fractions 13–17), whereas incomplete transcripts (the shortest one corresponding to ~90% of the total) eluted in the void volume (fractions 7–11), as expected for unrelease RNAs that are part of large, PNA-arrested ternary complexes. A terminator-bound PNA thus acts as a roadblock for elongating pol III, in such a way that the polymerization site of the enzyme becomes arrested at a discrete distance from the upstream border of the (T10 PNA)2-

Fig. 5. PNA-induced arrest sites on different class III genes. A, sizing PNA-induced transcript shortening on the tRNA10 gene. The tRNA10 gene, either in a supercoiled form (lanes 1–2, circular) or after digestion with XhoI (cutting 15 bp upstream of the first T of the terminator) (lanes 3–4, XhoI) was transcribed for multiple rounds in the absence (−) or in the presence (+) of T10 PNA (400 nM). The migration position and length of the complete tDNA10 transcript (150 nt) and of the XhoI runoff product (130 nt) are indicated on the right. B, PNA-induced transcript shortening on other class III genes. Following preincubation with the indicated concentrations of the T10 PNA, the tDNA10(TAT) (lanes 1–4) or tDNA10(GTT) (lanes 5–7) were transcribed for multiple rounds. The migration positions of transcripts ending at the T5 or T13 termination sites of the tRNA10 gene are indicated on the right. The main shortened transcript of the SNR6 gene is marked by an asterisk in lane 4. The two shortened transcripts of the tRNA10 gene are bracketed in lane 11. C, schematic representation of a PNA-roadblock pol III ternary complex. The double-headed arrow indicates the distance between the position of transcription arrest and the upstream border of the (PNA)2-DNA adduct; the polymerase active site is arbitrarily drawn in the middle. Genes—The size of the main unreleased product of PNA-inhibited tRNA10 gene transcription was estimated by comparison with two reference transcripts: the 150-nt long, full-length pre-tRNA10 transcript (whose size was estimated on the assumption that 5 U residues are incorporated before actual termination (26)) and the 130-nt runoff transcription product of the same gene cut at an XhoI site located between the B-block and the terminator. Because of the loss of the terminator, and thus of the fast recycling pathway (3), the synthesis of this transcript was totally insensitive to PNA inhibition (Fig. 5A, lanes 3 and 4) and much less efficient than correctly terminated transcription (cf. lanes 1 and 3). From the gel reported in Fig. 5A, a length of ~125 nt could be calculated for the incomplete transcript associated to PNA-arrested complexes. Fig. 5B illustrates the PNA-induced transcription blockage observed with

PNA-induced Transcription Arrest on Different Class III
two other class III genes, both containing a terminator element make up of 10 or more T residues. In the case of the SNR6 gene, which codes for the yeast U6 small nuclear RNA and has a T10 terminator, the addition of the T10 PNA (100 nM) caused an 80% inhibition of transcription and the appearance of an ~15-nt-shortened transcript (marked by an asterisk in lane 4). At variance with the residual, single-round transcription observed with the tRNAHis gene (lane 2), the T10 PNA at a 500 nM concentration completely inhibited SNR6 transcription (lane 6). This is most likely due to weak PNA binding to the T7 sequence at position ~18 of the SNR6 gene and to the ensuing inhibition of TFIIIB assembly (27). Particularly informative was the case of a tRNAAsp gene having the more complex terminator sequence T5CT4CT13 (Fig. 5B, lanes 8–12). In this gene, the first T5 element induces termination by about half of the elongating polymerases with the production of a 95-nt-long RNA, whereas pol III molecules reading through this sequence end up at the more distal T13 site and produce a 106-nt-long transcript (lane 8). The T10 PNA can only bind to the latter element with a small (~3 nt) positional heterogeneity. As shown in Fig. 5B (lanes 10–12) a PNA bound to such an element not only causes the disappearance of the T13-terminated transcript but also determines a strong reduction in the levels of the T5-terminated transcripts, with the appearance of two early terminated products (bracketed in lane 11) 15–20 nt shorter than the full-length (106 nt) transcript. Considering that 5 U residues, generated by transcription through the first 5 bp of the terminator element, are incorporated into the full-length transcripts (26), the observed transcript-shortening values indicate that the distance between the polymerase active site and the upstream border of the (PNA)10-DNA adduct ranges from 10 to 20 bp on different templates (Fig. 5C).

**DISCUSSION**

This report provides an in-depth account of the mode of action of a DNA-binding drug specifically targeting the transcriptional terminators of eukaryotic class III genes. Our analysis utilized a highly purified RNA polymerase III transcription system from *S. cerevisiae* in which reinitiation, relying on a facilitated pol III-recycling mechanism, takes place at high efficiency. Central to this process is a T-rich terminator element, which by an as yet unidentified mechanism allows a terminating pol III to efficiently reinitiate by bypassing the slow, *de novo* enzyme recruitment step (3). Targeting the complementary poly(dA) tract of the class III gene terminator with an oligothymine PNA resulted in the formation of a stable (PNA)10-DNA adduct acting as a sequence-specific roadblock for elongating pol III. We found that a terminator-located PNA roadblock exerted two main effects on class III gene transcription. The first one is a dramatic reduction in overall transcription efficiency, due to the fact that pol III molecules get stuck during the first transcription cycle, with the consequent loss of pol III recycling. The second, somewhat unexpected effect is the blockage of transcribing pol III well ahead of the PNA roadblock, as revealed by the fact that the PNA-arrested ternary complex contains an unreleased RNA that, depending on the template, is 15–25 nt shorter than the full-length transcript and does not result from pol III-catalyzed hydrolysis of a longer precursor. This suggests that the polymerization site of a transcribing pol III lies 10–20 bp ahead of the leading edge of the enzyme that first senses the upstream border of the PNA-DNA adduct (see Fig. 5C). This distance is larger than that observed in previous studies employing either phage T3 and T7 RNA polymerases (10) or human RNA polymerase II (8). In these studies, in which artificial templates containing a dA10dT10 cassette were transcribed in the presence of a T10 PNA, elongation became arrested at the very upstream border of the PNA-binding site. Although the discrepancy between pol III and phage RNA polymerases can be explained by the much larger size of the eukaryotic enzyme, the lack of an earlier arrest in the case of pol II is more difficult to reconcile with our data. The possibility that TFIIIC, bound to the intragenic B-block, might cooperate with a terminator-bound PNA in inducing premature pol III arrest seems to be ruled out by the fact that an early blockage of transcription also occurred in the case of the SNR6 gene, in which the B-block is extragenically positioned 120 bp downstream of the terminator. Also, incomplete transcripts of the same size were produced in PNA-inhibited transcription reactions carried out in the presence or absence of TFIIIC on the tRNAAsp gene (data not shown (16)). A more likely explanation of the observed early blockage of transcription is that, in the context of the sequence surrounding its termination site, pol III has an extended conformation that is especially sensitive to the presence of a downstream roadblock. Indeed, exonuclease III protection studies conducted on RNA polymerase II arrested at different positions along the template have shown that the active site to leading edge distance can vary from 7 to 20 bp depending on the DNA sequence context of individual positions (28). The range of these distances is close to what we found with PNA-arrested pol III, and the variation in transcript shortening observed with different class III genes may similarly reflect sequence-dependent changes in the pol III active site to leading edge distance. Noticeably, a DNA occupancy of about 40 bp has been reported for yeast pol III having transcribed through the first 17 bp of the SUP4 tRNA gene (29), a space length that is fully compatible with an active site to leading edge distance of ~20 bp. The effect of a protein roadblock on RNA polymerase elongation and termination has previously been studied by placing either the bacterial lac repressor (30–33) or a cleavage-defective EcoRI endonuclease (5, 34) at various positions along the transcribed sequence. The EcoRI roadblock has been shown to efficiently arrest both elongating and terminating *E. coli* RNA polymerases, and a detailed analysis of the arrested complexes has suggested a model in which the leading edge of the transcribing polymerase precedes by 7–9 bp the site at which polymerization occurs (34). More consistent with our observations, studies of bacterial RNA polymerase blockage by a psoralen diaduct have shown that the leading edge of the enzyme can extend further downstream, being separated by as much as 18–20 bp from the growing point of the RNA chain (35).

The T10 PNA very effectively inhibited transcription of various class III genes with a half-inhibitory concentration (100 nM) 5- to 10-fold lower than that previously determined for phage (10) and human RNA polymerases (8). This difference is likely due to the negatively supercoiled state of the templates utilized for our pol III experiments, because we observed a 10-fold increase of the PNA half-inhibitory concentration upon linearization of the tDNA5- containing plasmid. By influencing the dynamics of base pair breathing, DNA supercoiling enhances PNA binding (19), a most important effect in view of the possible *in vivo* use of PNAs as negative modulators of chromatin-assembled (i.e. supercoiled) target genes. Because the pol III transcription machinery, unlike pol I and pol II, efficiently terminates and reinitiates *in vitro* on supercoiled templates, it lends itself as the most sensitive and biochemically appropriate system for studying the anti-transcriptional activity of PNAs.

The possibility of controlling eukaryotic gene transcription with small effector molecules has important pharmacological implications. Several studies have recently demonstrated the transcriptional inhibition of specific genes *in vitro* and *in vivo* by triple-helix forming oligonucleotides (36, 37), synthetic poly-
amides containing N-methylimidazole and N-methylpyrrole amino acids (38, 39), and PNAs (40–43). In all of these studies, DNA ligands were designed so as to target specific sequences either involved in transcription initiation, thereby inhibiting transcription complex assembly, or located in the middle of a transcriptional unit to block transcription elongation. Our present findings extend the above results by showing that targeting a well-conserved control element at the end of class III transcriptional units also causes an elongation arrest. Eukaryotic genes transcribed by RNA polymerase III, especially the tRNA and the 5 S rRNA genes, are novel potential targets of anti-gene strategies. In fact, because of their key roles in protein biosynthesis, the products of these genes are essential for cell proliferation, and their deregulation in neoplastic cells has been proposed to contribute to the loss of cell growth control that accompanies tumor formation (44). The present demonstration that oligothymine PNAs strongly and specifically inhibit pol III transcription thus sets the ground for future studies aimed to optimize the in vivo exploitation of these class III terminator ligands for pharmacological purposes.

Acknowledgments—We are grateful to Christophe Carles and Emmanuel Favry for the gift of purified RNA polymerase III, and to Claudio Rivetti for helpful suggestions and comments on the manuscript. Encouragement and support from Gian Luigi Rossi are also gratefully acknowledged.

REFERENCES

1. Kassavetis, G. A., Braun, B. R., Nguyen, L. H., and Geiduschek, P. E. (1990) Cell 60, 235–245
2. Kassavetis, G. A., Kumar, A., Letts, G. A., and Geiduschek, E. P. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 9196–9201
3. Dieci, G., and Sentenac, A. (1996) Cell 84, 245–252
4. Allison, D. S., and Hall, B. D. (1985) EMBO J. 4, 2657–2664
5. Nudler, E., Kashlev, M., Nikiforov, V., and Goldfarb, A. (1995) Cell 81, 351–357
6. Nielsen, P. E. (1999) Curr. Opin. Biotechnol. 10, 71–75
7. Nielsen, P. E., Egholm, M., and Buchardt, O. (1994) J. Mol. Recognit. 7, 163–170
8. Hanvey, J. C., Peffer, N. J., Bisi, J. E., Thomson, S. A., Cadilla, R., Josey, J. A., Rice, D. J., Hassman, C. F., Benham, M. A., Au, K. G., Carter, S. G., Bruckenstein, D. A., Boyd, A. L., Noble, S. A., and Babiss, L. E. (1992) Science 258, 1481–1485
9. Nielsen, P. E., Egholm, M., Berg, R. H., and Buchardt, O. (1993) Anticancer Drug Des. 8, 55–63
10. Nielsen, P. E., Egholm, M., and Buchardt, O. (1994) Gene 149, 139–145
11. Larsen, H. J., and Nielsen, P. E. (1996) Nucleic Acids Res. 24, 458–463
12. Haaiama, G., Lobse, A., Buchardt, O., and Nielsen, P. E. (1996) Angew. Chem. Int. Ed. Engl. 35, 1939–1942
13. Puscel, A., Sforza, S., Haaiama, G., Dahl, O., and Nielsen, P. E. (1998) Tetrahydrobenz. Lett. 39, 4707–4710
14. Christensen, L., Fitzpatrick, R., Gildea, B., Petersen, K. H., Hansen, H. F., Koch, T., Egholm, M., Buchardt, O., Nielsen, P. E., Coull, J., and Berg, R. H. (1995) J. Pept. Sci. 1, 175–183
15. Sforza, S., Girardi, S., Corradini, R., DOSsena, L., and Marchelli, R. (2000) Eur. J. Org. Chem. 2905–2913
16. Dieci, G., Pecurandi, R., Guiolodori, S., Bottarelle, O., and Ottonello, S. (2000) J. Mol. Biol. 299, 601–613
17. Gabrielsen, O. S., and Oyen, T. B. (1987) Nucleic Acids Res. 15, 5699–5713
18. Burnol, A. F., Margotin, F., Schultz, P., Marsolier, C. M., Oudei, P., and Sentenac, A. (1993) J. Mol. Biol. 233, 644–658
19. Bentin, T., and Nielsen, P. E. (1996) Biochemistry 35, 8863–8869
20. Dieci, G., Hermann-Le Demat, S., Lakhlanov, E., Thuriaux, P., Werner, M., and Sentenac, A. (1995) EMBO J. 14, 3766–3776
21. Nielsen, P. E., Egholm, M., Berg, R. H., and Buchardt, O. (1991) Science 254, 1497–1500
22. Sforza, S., Haaiama, G., Marchelli, R., and Nielsen, P. E. (1999) Eur. J. Org. Chem. 197–204
23. Lagriffoule, P., Wittung, P., Eriksson, M., Jensen, K. K., Nordén, B., Buchardt, O., and Nielsen, P. E. (1997) Chem. Eur. J. 3, 912–919
24. Whitehead, S. R., Bardeleben, C., and Kassavetis, G. A. (1994) J. Biol. Chem. 269, 2299–2306
25. Bokkova, E. V., and Hall, B. D. (1997) J. Biol. Chem. 272, 22832–22839
26. Matsuzaki, H., Kassavetis, G. A., and Geiduschek, E. P. (1994) J. Mol. Biol. 235, 1173–1192
27. Gerlac, V. L., Whitehall, S. K., Geiduschek, E. P., and Brown, D. A. (1995) Mol. Cell. Biol. 15, 1455–1466
28. Samkursavilii, I., and Luse, D. S. (1996) J. Biol. Chem. 271, 23495–23505
29. Egholm, M., Durkovich, D., Kassavetis, G. A., and Geiduschek, E. P. (1993) Mol. Cell. Biol. 13, 942–952
30. Deuschel, U., Genta, R., and Bujard, H. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 4134–4137
31. Deuschel, U., Hipkiss, R. A., and Bujard, H. (1996) Science 248, 480–483
32. Sellitti, M. A., Pavco, P. A., and Steege, D. A. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 3199–3203
33.Jeong, S. W., Lang, W. H., and Reeder, R. H. (1995) Mol. Cell. Biol. 15, 5929–5936
34. Pavco, P. A., and Steege, D. A. (1990) J. Biol. Chem. 265, 9960–9969
35. Shi, Y. E., Gamber, H., Van Houten, B., and Hearst, J. E. (1988) J. Mol. Biol. 199, 577–593
36. Pareseth, D., Gueysses, A. L., and Helene, C. (1999) Biochim. Biophys. Acta 1488, 181–206
37. Faria, M., Wood, C. D., Peresault, L., Nelson, J. S., Winter, A., White, M. R., Helene, C., and Giovannangeli, C. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 3862–3867
38. Gottesfeld, J. M., Neely, L., Trauger, J. W., Baird, E. E., and Dervan, P. B. (1997) Nature 387, 292–295
39. Dickinson, I. A., Gulizia, S. A., Trauger, J. W., Baird, E. E., Moser, D. E., Gottesfeld, J. M., and Dervan, P. B. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 12890–12895
40. Cutrona, G., Carpaneto, E. M., Ulivi, M., Roncella, S., Landt, O., Ferrarini, M., and Boffa, L. C. (2000) Nat. Biotechnol. 18, 300–303
41. Mologni, L., Nielsen, P. E., and Gambacorti-Passerini, C. (1999) Biochem. Biophys. Res. Commun. 264, 537–543
42. Gambacorti-Passerini, C., Mologni, L., Bertazzoli, C., le-Couteur, P., Marchesi, E., Grignani, F., and Nielsen, P. E. (1996) Blood 88, 1411–1417
43. Vickers, T. A., Griffith, M. C., Ramasamy, K., Risen, L. M., and Freier, S. M. (1995) Nucleic Acids Res. 23, 3003–3008
44. White, R. J. (1997) Trends Biochem. Sci. 22, 77–80
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*J. Biol. Chem.* 2001, 276:5720-5725.
doi: 10.1074/jbc.M009367200 originally published online November 9, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M009367200

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