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Synthetic polyamines stimulate *in vitro* transcription by T7 RNA polymerase

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ABSTRACT

The influence of nine synthetic polyamines on *in vitro* transcription with T7 RNA polymerase has been studied. The compounds used were linear or macrocyclic tetra- and hexaamine, varying in their size, shape and number of protonated groups. Their effect was tested on different types of templates, all presenting the T7 RNA promoter in a double-stranded form followed by sequences encoding short transcripts (25 to 35-mers) either on single- or double-stranded synthetic oligodeoxyribonucleotides. All polyamines used stimulate transcription of both types of templates at levels dependent on their size, shape, protonation degree, and concentration. For each compound, an optimal concentration could be defined; above this concentration, transcription inhibition occurred. Highest stimulation (up to 12-fold) was obtained by the largest cyclic compound called [38]N6C10.

INTRODUCTION

The development of *in vitro* transcription systems lead to an explosion of new research directions allowing the synthesis of almost any RNA either in its wild-type or mutated forms and has found applications in all fields of RNA research (1). Transcriptional systems are based on cloning of synthetic DNA coding for the RNA of interest downstream the phage promoter of a DNA dependent RNA polymerase into a plasmid vector. Transcription of plasmids, linearised at the extremity of the appropriate genes, is performed in the presence of the four ribonucleoside triphosphates (e.g. 2, 3). For synthesis of short RNAs (~20- to 80-mers), the matrix can be reduced to a double stranded promoter region followed by a single-stranded template sequence (4, 5). Alternatively, hybridised full-length oligodeoxyribonucleotides encompassing the phage promoter sequence and the sequence of interest can be used as template for RNA polymerases (6–8). Within the various phage systems available (SP6, T7, T5, T3) the RNA polymerase from phage T7 has found the widest applications since the gene of this enzyme has been cloned and purification of large amounts of the enzyme can easily be achieved from an overproducing strain (9, 10).

Despite the presence of a strong promoter, the T7 RNA polymerase shows some intrinsic limitations which can lead to low transcription levels. This is linked to the nature of its promoter which starts at nucleotide −17 and extends to nucleotide +6 in the sequence to be transcribed (11, 12). Thus variabilities in the region +1 to +6 due to the nature of the sequences to be transcribed may be responsible for modulation of transcription levels (4, 13). It is a well known experimental fact that DNA templates to be transcribed, starting at position +1 with one or several C residues, are the best substrates for the enzyme. However, not all molecules starting with C are efficiently transcribed and our knowledge about optimal sequence arrangements in this internal promoter region (4^6 possible sequences) to get optimal transcription, is still limited.

The purpose of this paper is to establish novel experimental conditions favourable for more efficient transcription with T7 RNA polymerase, especially for sequences poorly transcribed. The starting point of this investigation was our observation of an extremely poor transcription efficiency of a tRNA sequence that seemingly possessed a favourable starting sequence 5′-riboGGUUUC-3′ (14). Even using conditions which proved to optimise the enzyme activity, like the addition of spermidine (4), Triton X100 (15) or polyethylene glycol (15), transcription of this molecule was dramatically poor.

Polyamine derivatives appear as potential candidates which might modulate the efficiency of *in vitro* transcription systems, since it was shown that the concentration of natural polyamines increases when RNA transcription proceeds *in vivo* (16, 17). Therefore we have screened a series of synthetic polyamine derivatives (Fig. 1). Members of this family already proved to be efficient substitutes of natural polyamines in the stimulation of a biological reaction *in vivo*, namely polymerisation of actin (18). These artificial polyamines vary in shape, spread, and number of charges. Polyamines **1** to **3** (named LN6, L38N6, and L38N4) are linear (LxNy: L for linear, x for the total number of N plus C atoms, and y for that of N atoms). The five other

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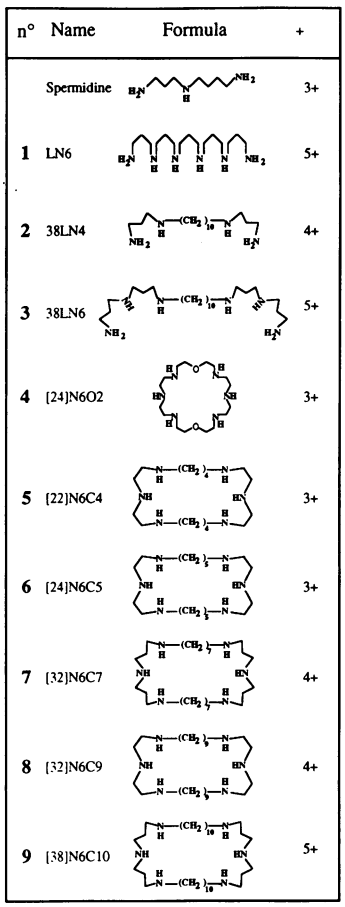

Figure 1. Structure, nomenclature, and protonation levels (+) at pH 8.1 of the various synthetic polyamines tested in this work (the degree of protonation may be higher when complexed to RNA).

compounds (**5** to **9**) are macrocycles ([x]NyCz: [ ] for cyclic, x for the total number of N and C atoms, and y and z for that of N and C atoms). L38N6 and L38N4 are derived from compound **9** ([38]N6C10). Compounds **1** and **2** are linear hexaamines containing in their framework two dipropylene triamine units connected by linear trimethylene and decamethylene chains. Compound **3** is the tetraamine analogue of compound **2**. Whereas compounds **4**, **5**, **6**, and **8** contain diethlylenetriamine units, all others contain dipropylenetriamine groups.

We report here the influence of these compounds on the *in vitro* transcription with T7 RNA polymerase of different types of single-stranded and double-stranded templates encoding short RNA sequences (19- to 35-mers) and show in particular that transcription is stimulated up to 12-fold in the presence of some of these synthetic polyamines.

MATERIALS AND METHODS

Enzymes and nucleic acids

T7 RNA polymerase (573 units/mg) was purchased from Pharmacia (St Quentin-en-Yvelines, France). Spermidine was from Sigma (France). Oligodeoxynucleotides were synthesised on an Applied Biosystem 381 A DNA synthesiser using the phosphoramidite method and purified by HPLC on a Nucleosil

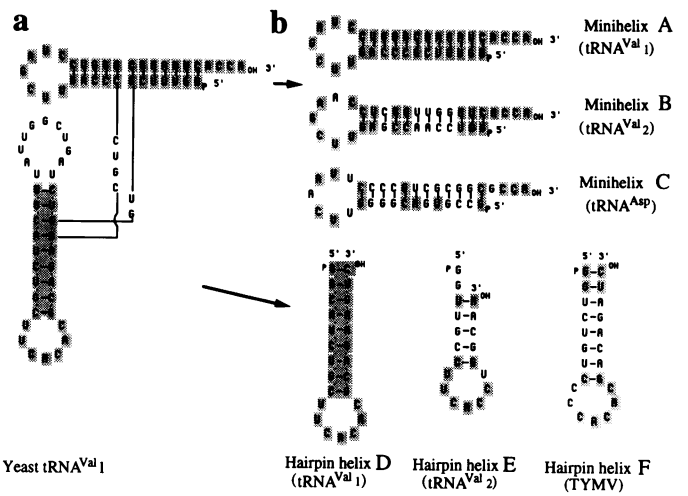

Figure 2. Sequences of minihelices derived from tRNAs. (**a**) L-shaped representation of tRNA$^{Val}_1$ derived transcript emphasising the regions corresponding to the minihelices. (**b**) Sequences of the minihelices (molecules A to F) used for *in vitro* synthesis with T7 RNA polymerase (shaded regions correspond to sequence similarities with yeast tRNA$^{Val}_1$). Shaded nucleotides highlight sequence conservation between minihelices or hairpin helices and tRNA^{Val}.

120 5c18 column. They correspond to both strands of templates used for *in vitro* transcription of several RNA minihelices. The sequences of the upstream part of the promoter is the following: 5′-TAATACGACTCACTATAG-3′. The sequences of the DNA strands to be transcribed can be deduced from those of the transcripts shown in Figure 2; these DNA strands comprised also the (−) upstream promoter sequence. A plasmid encompassing the sequence of microhelix A and the T7 promoter (2712 base pairs) was constructed according to [3]. [α-^{32}P] ATP (400Ci/mmol) was from Amersham (Les Ulis, France).

Synthesis of polyamines

Polyamines were synthesised according to the following references: LN6 (19), 38LN6 and 38LN4 (18, 20, 21), [24]N6O2 (22), [22]N6C4, [24]N6C5 and [32]N6C9 (23), [32]N6C7 (20), and [36]N6C9 (23).

In vitro transcription

Two types of templates for T7 RNA polymerase were used in this study. (i) Single-stranded synthetic templates containing the consensus upstream promoter sequence (position −17 to +1) of T7 RNA polymerase. Annealing of the (−) strand oligodeoxyribonucleotide to a 17-mer (+) strand oligodeoxyribonucleotide allowed the formation of the double-stranded promoter. (ii) Double-stranded synthetic templates were obtained by annealing complete (+) and (−) strands. Annealing of both types of templates was performed by heating for 3 min at 65°C in 250 mM Tris−HCl pH 8.1, 125 mM $MgCl_2$ and 2.5 μM of each oligodeoxyribonucleotide and cooling down slowly to room temperature.

In vitro transcriptions were performed at 37°C in mixtures containing 40 mM Tris−HCl pH 8.1, 40 mM $MgCl_2$, 5 mM dithiothreitol, 4 mM of each nucleoside triphosphate, 50 μg/ml bovine serum albumin, 45 mCi/ml [α-^{32}P ATP], 1.25 units/ml of commercial T7 RNA polymerase, and 400 nM of each DNA strand for synthetic templates (or 0.4 μg linearised plasmid per

μl). Incubations were performed during 3 hours. Different polyamines were present at final concentrations varying from 0.1 to 10 mM. Reaction were stopped by addition of one volume of RNA 'loading buffer' containing 8 M urea, 300 mg/ml saccharose and 0.1% (w/v) xylene cyanol and bromopenol blue. The transcription products were separated on 15% denaturing polyacrylamide/8 M urea gels. Results were quantified by autoradiography analysis on a Bio-Imaging Analyser (Fuji Photo Film Co. Ltd, Japan). Arbitrary units were defined so that numerical data given in this paper are directly comparable. From a practical point of view, counts from the Bio-Imaging Analyser (corrected for background) corresponding to a transcription product were normalysed to the length of the transcript and the number of incorporated labelled nucleotides.

RESULTS AND DISCUSSION

Various tRNA-derived minihelices and their differential transcription yields

To study the aminoacylation properties of the amino acid acceptor arms of tRNAs disconnected from the rest of the tRNA structures, minihelices recapitulating the acceptor and the anticodon arms of tRNAs (24) were designed for synthesis by *in vitro* transcription with T7 DNA dependent RNA polymerase (14, 25, 26). Our study was focused on three different acceptor minihelices derived from yeast $tRNA^{Val}_1$ (minihelix A), $tRNA^{Val}_2$ (minihelix B), and $tRNA^{Asp}$ (minihelix C), respectively, as well as on three different anticodon hairpin minihelices derived from yeast $tRNA^{Val}_1$ (minihelix D), yeast $tRNA^{Val}_2$ (minihelix E), and from the turnip yellow mosaic virus tRNA-like domain (minihelix F) (Fig. 2). Although all three acceptor minihelices are of the same length (35-mers) they differ markedly in sequence. Anticodon minihelices differ both in size (19-, 23-, and 25-mers) and sequence.

Since the size of the RNA fragments to be produced is rather short (19- to 35-mers), we decided to avoid cloning steps for the preparation of templates for *in vitro* transcription and instead, favoured the very quick preparation of templates by hybridisation of two synthetic oligodeoxyribonucleotides (4). Thus, one oligodeoxyribonucleotide corresponding to the (+) strand of the promoter region and six oligodeoxyribonucleotides corresponding to the (−) template strands were synthesised and annealed adequately before transcription.

The efficiency of transcription observed for the various templates studied is shown in Figure 3a. The transcription yields are weak (~ 2 to 30 pmoles of transcript per pmole of single-stranded template) as compared to those (30 to 500-fold higher) observed on average when templates are linearised double-stranded plasmids. Nevertheless, there are marked differences within the synthesis of the six minihelices and a dramatic result is observed for minihelix A where less than 2.5 pmoles of transcript are obtained per pmole of single-stranded template. This low yield could be increased by a factor of two in the presence of 0.5 mM spermidine. Scaling up of the reaction mixture did not allow to obtain sufficient amount of minihelix A to perform functional studies neither did tentatives to optimise the transcription by increasing the concentration of template oligodeoxyribonucleotides, or addition of Triton X100 (0.01%) or the presence of polyethylene glycol 8,000 (80 mg/ml) (results not shown).

The low level of transcription of molecule A may be the consequence of the presence of three consecutive U residues within the first six nucleotides to be polymerised by the T7 RNA polymerase which would weaken the ternary complex between the polymerase and the DNA−RNA duplex (Fig. 3b). Following this hypothesis, it becomes understandable that short and abortive RNA sequences are synthesised predominantly during transcription of this molecule (results not shown). The differences in transcription levels of the other minihelices may be due to structural differences between the sequences of these RNAs outside the internal promoter region which would modulate the interaction of the nascent RNA chain with the polynucleotide binding domain of the polymerase (27). We have observed similar differences when comparing the transcription levels of a series of $tRNA^{Asp}$ variants mutated far away from the 5′-terminal region.

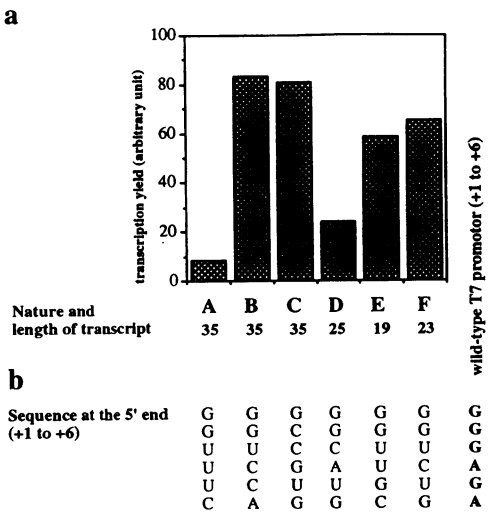

Figure 3. (**a**) Transcription levels of several templates (corresponding to RNA minihelices A to F in Fig. 2) by T7 RNA polymerase in the absence of any polyamine and (**b**) comparison of the 5′-terminal sequences (+1 to +5) of the transcripts with the optimal polymerase promoter sequence.

Stimulation of transcription of a single-stranded template by synthetic polyamines

The effect of nine synthetic polyamines on the transcription of acceptor minihelix A was tested. In a first series of experiments, polyamine **1**, as well as compounds **4**−**9** were assayed at three different concentrations (0.1, 1.0, and 10 mM) and transcription yields were compared to those obtained in the absence of synthetic polyamines or in the presence of 1 mM spermidine. As seen on the autoradiographs and the corresponding scans presented in Figure 4, spermidine and all synthetic polyamines increase transcription levels. It is worth noting that beside the full-length transcripts (n family), abortive products and especially two other RNA families differing in length by one (n+1 family) and two (n+2 family) additional nucleotides were also present. The amount of these undesired transcripts is strongly dependent upon the concentration and the nature of the polyamine added in the transcription mixtures. For instance with spermidine they represent less than 5% but can reach more than 50% with polyamines **1**, **7** or **9** (Fig. 4a). Interestingly, when the amount of full-length transcripts (n family) is high, that of the smallest abortive products is low. Such heterogeneities in transcription products were also observed by others (e.g. 4).

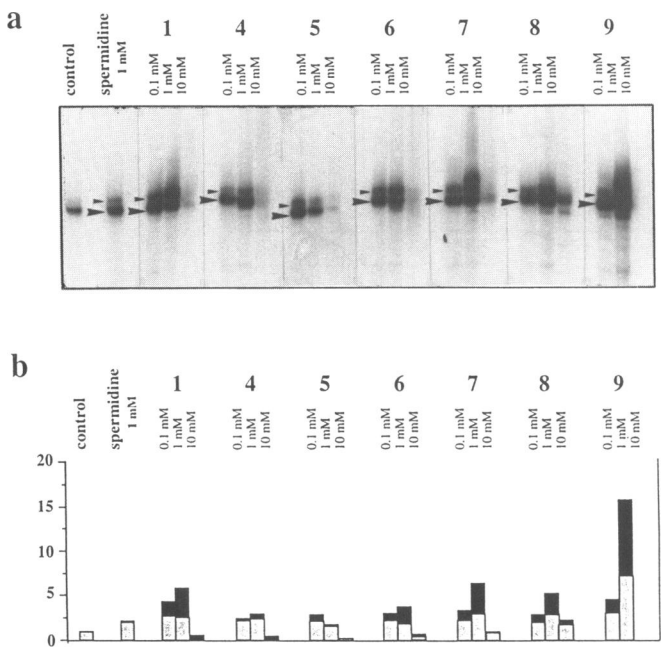

Figure 4. Effect of seven synthetic polyamines on *in vitro* transcription of minihelix A (see Fig. 2) by T7 RNA polymerase. (**a**) Autoradiography of a 12% denaturing polyacrylamide gel. Same amounts of radioactivity were introduced in the different reaction mixtures and deposited on the gels. (**b**) Quantitation of the results by scanning of the gels. The shadowed parts in the histograms correspond to the stimulations of full-length transcript synthesis, the black parts account for the stimulations due to the n+1, n+2 transcripts, and the longest abortive products.

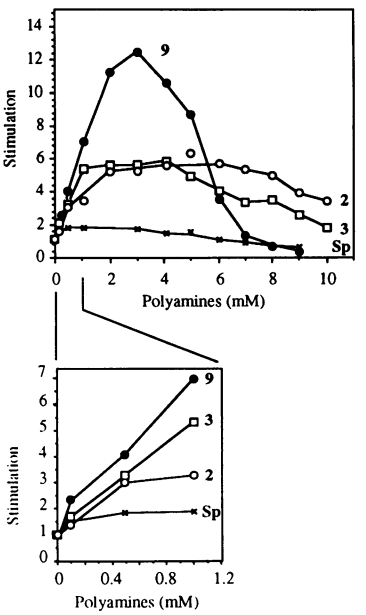

Figure 5. Dependence of the concentration of spermidine (Sp) and synthetic polyamines (compound **2**, **3**, and **9**) on the transcription efficiency of template A (corresponding to the minihelix derived from yeast $tRNA^{Val}_1$). Data correspond to synthesis levels of full-length transcripts.

Based on these experimental facts, stimulation of transcription was analysed either by comparing the total amount of transcripts (the n, n+1, and n+2 families, plus the longest abortive products, as seen on Fig. 4a) obtained per mole of template in the presence of the polyamines to the amount produced in their absence, or by comparing solely the amount of the full-length products obtained in the absence or in the presence of the polyamines (Fig. 4b). Stimulation is clearly dependent upon the concentration of the polyamines. Whereas low concentrations lead to stimulation, high concentrations generally have an opposite effect and inhibit transcription (with compound **9** at 10 mM, transcription is even suppressed). At 1 mM concentration compounds **1**, **4**, **5**, **6**, **7**, **8**, and spermidine have globally the same effect with stimulation of ~ 2- to 4-fold of the full-length transcripts as compared to the control (Fig. 4b). However, with the synthetic polyamines the overall stimulation is markedly increased, especially with compounds **1**, **7**, and **8** which stimulate production of n+1 and n+2 families up to 5- to 7-fold. Most efficient transcription is obtained with macrocyclic polyamine 9 at 1 mM, with stimulations of ~ 3-fold as compared to transcription levels in the presence of spermidine and 7-fold above the control level in the absence of **9**. If all transcription products are considered, the overall stimulation is up to 16-fold. Interestingly, this same polyamine is also the most efficient inducer for polymerisation of actin (18)

Since compound **9** showed the greatest effects in terms of transcription stimulation and inhibition, its concentration dependence on T7 RNA polymerase action was further investigated in a second series of experiments including also assays of its two other structural analogues **2** and **3**. These analogues are open chain tetra and hexaamines (Fig. 1). As seen in Figure 5, stimulation of full-length transcript production can reach a 12-fold increase in the presence of 3 mM of the macrocycle **9**. The concentration dependence is stringent since at 7 mM of polyamine **9**, the transcription level is the same as for the control experiment in the absence of **9**. The inhibitory effect, as well as all transcription inhibitions observed in the present study, is perhaps not due to a direct inhibition of the polymerase, but rather to DNA aggregation and precipitation of DNA templates, similar to tRNA which can be precipitated by spermine (28). The linear analogues **2** and **3** also enhance transcriptions of single-stranded template encoding minihelix A, even at concentrations below 1 mM, but their action is less stringent and efficient than that of the macrocycle. Here, the highest stimulation for full-length RNA synthesis is only 6-fold.

Effects of polyamines on templates of different sequences

The effects of the three most efficient synthetic polyamines (compounds **2**, **3**, and **9**) in the concentration range from 0.5 to 5 mM on *in vitro* transcription by T7 RNA polymerase was further tested on two other single-stranded templates that differed from template A only by their sequences. These templates code for the amino acid acceptor arms of isoacceptor $tRNA^{Val}_2$ (minihelix B) and of yeast $tRNA^{Asp}$ (minihelix C). Figure 6 (upper panels) summarises the results obtained for templates B and C and enables comparison with data collected for template A. As mentioned before, the level of transcription of both of these templates in the absence of the polyamine is higher than for template A. In general, transcription of template B is only weakly stimulated by polyamines. Only compound **3** maintains its effect over the concentration range tested. Spermidine as well as compound **9**, from 2 to 5 mM, inhibit transcription. Compound **2** has an inhibitory effect at all concentrations tested. As for template B, transcription of template C is only weakly or not at all stimulated in the presence of polyamines **2**, **3** or **9**. Spermidine becomes inhibitory already at 2 mM. Compound **9**

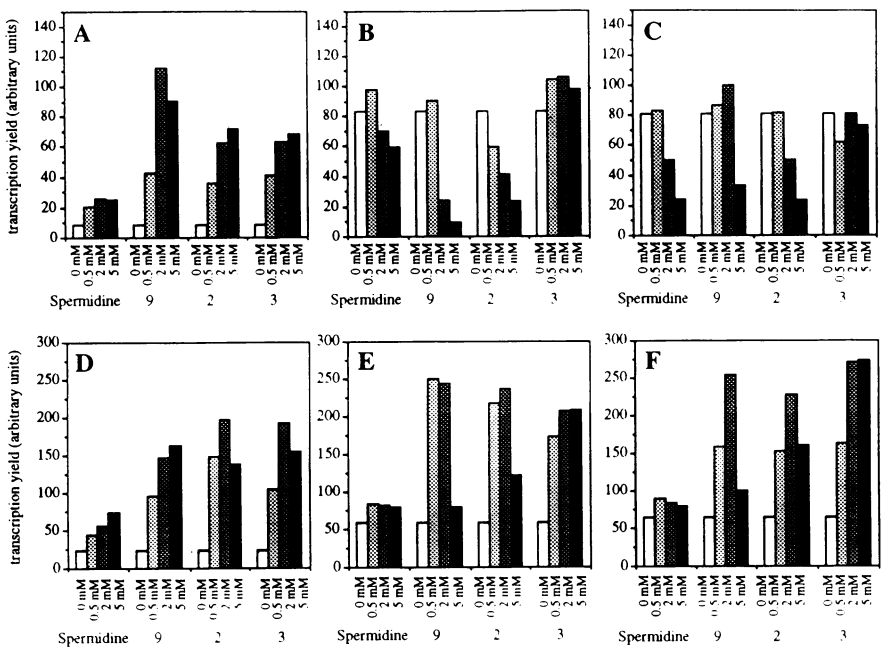

Figure 6. Effect of spermidine and polyamines **2**, **3**, and **9** on the transcription of different DNA templates (coding for RNA minihelices A to F in Fig. 2). Data correspond to transcription yields of full-length transcripts.

Table I. Ranking of templates into two families depending on their optimal transcription in the presence of synthetic polyamines

Template*	Basic transcription without polyamine (arbitrary units)	Most efficient polyamine (arbitrary unit)	Optimal transcriptions	Optimal stimulation (x-fold)
A	8.1	n° **9**	99	12.2
B	83.5	n° **3**	112.5	1.3
C	80.8	n° **2**	97	1.2
D	24.2	n° **2/3**	196	8.1
E	58.4	n° **9**	257	4.4
F	65.2	n° **3**	215	3.3

*For sequences of templates, see Figure 2.

stimulates transcription weakly at concentrations between 0.5 and 2 mM and inhibits it at 5 mM. Compound **2** has the same effect as spermidine and polyamine **3** has no effect at all.

Effects of polyamines on templates of different length

Templates D, E, and F encode anticodon hairpin helices of different length , 25-, 19-, and 23-mers, respectively (Fig. 1). Their basic levels of transcription (in the absence of polyamine) shows that templates E and F are about twice more efficiently transcribed than template D (Fig. 3). In the presence of 5 mM spermidine, their levels of transcription become however identical (Fig. 6, lower panels). The effect of polyamines **2**, **3**, and **9** were also tested at three different concentrations. Although their effects were almost the same, their optimal activity was obtained with different concentrations and reached stimulations of up to 4.3-fold for templates E and F and 8.1-fold for template D (Table I).

Differential effects of polyamines on templates of different nature

A completely double-stranded synthetic template derived from minihelix A (Fig. 2) was prepared by annealing two complementary 52-mer oligodeoxyribonucleotides and its transcription compared to that of the single-stranded version. As seen in Figure 7, under comparable experimental conditions, the basic level of transcription of this double stranded-template is ~ 6-fold higher than that of the equivalent single-stranded template. Spermidine has a weak stimulatory effect on transcription of both templates, the single-stranded being transcribed ~ twice better in the presence of 0.5 mM spermidine, whereas for double-stranded template stimulation does not exceed 1.3-fold the control level (Fig. 6). Low concentrations of polyamines **2**, **3**, and **9** stimulate strongly transcription of the single-stranded template, especially macrocycle **9** (at most 13-fold). This effect becomes moderate (1.85-fold in the best case) or even is erased for the double-stranded template. With the three polyamines **2**, **3** or **9** at high concentrations transcription of double-stranded template is inhibited, especially by the linear polyamine **3**.

For the sake of completeness, the effect of polyamines **2**, **3**, and **9** were also analysed on transcription of a linearised plasmid corresponding to minihelix A. As expected from transcription levels of synthetic templates of that molecule, those for the

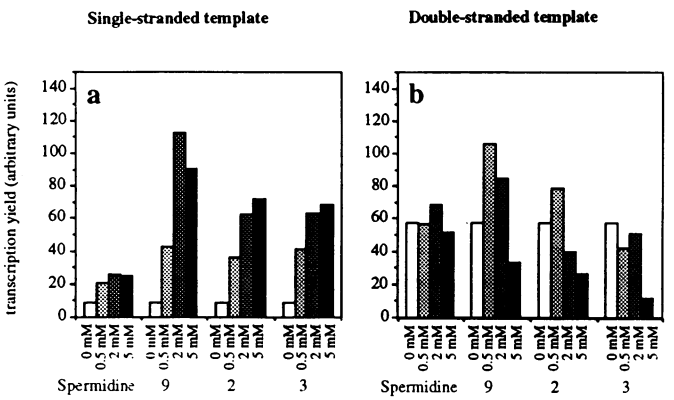

Figure 7. Comparison of the effect of polyamines **2**, **3**, and **9** on transcription of different forms of a same template (corresponding to RNA minihelix A in Fig. 2). (**a**) Transcription of a single-stranded and (**b**) of a double-stranded template. Data correspond to transcription yields of full-length transcripts.

plasmid were extremely low in the absence of polyamine. Addition of polyamines **2** and **3** (1 to 5 mM) had no effect; however polyamine **9** (at 2 mM) enhances transcription 3.6-fold. This stimulating effect was not observed with another plasmid containing a well transcribable sequence, i.e. that of yeast $tRNA^{Asp}$ [3] (results not shown).

In summary, stimulation by polyamines is less strong when the template is double-stranded and occurs at lower concentrations. Interestingly, it is worth noting that the optimal amount of transcript obtained starting from either single or double-stranded template is approximately the same. With plasmid transcription, stimulation by polyamine **9** is significant, however the overall efficiency of the trancription remains low.

Rate of nucleotide incorporation during transcription

Rates of nucleotide incorporation during transcription, under the conditions where the polyamine effects are optimal, are given in Table I. On the basis of these values, substrates of the T7 RNA polymerase can be classified into two groups. The first group contains templates A, B, and C coding for 35 nucleotide-long transcripts and the second one comprises templates D, E, and F which encode for shorter transcripts of 25, 19, and 23 nucleotides, respectively. In this second group transcription rates are higher by a factor of two than in the first one.

The difference in transcription rates for both families of transcripts may be correlated with the folding of DNA templates. Indeed, all template sequences may form hairpins since they encode minihelices. Templates from the first group may present a four nucleotide long single-stranded domain, overlapping the end of the double-stranded T7 promoter region. This is not the case in the second family of templates. Thus, a short single-stranded DNA fragment may prevent an efficient binding of the T7 RNA polymerase on its promoter.

Possible interpretation of polyamine action

Polyamines are involved in a large series of cellular processes like cell proliferation, DNA replication, protein synthesis, and RNA transcription (e.g. 16, 17, 29). Despite their ubiquity, their role and mechanism of action are not yet well understood. *In vitro*, natural polyamines such as spermine and spermidine have been widely used as counter ions of nucleic acids and also as additives for RNA crystallisation (reviewed in 30). They have also been shown to stimulate RNA transcription with DNA dependant RNA polymerases from phages (31). These natural polycationic compounds, with a distribution of charges along the molecule, differ in their binding properties with nucleic acids from other cations, such as Ca^{2+} or Mg^{2+}, where charges are punctual. So, the major interest of polyamines is their ability to interact with, or to match, several scattered ionic sites on nucleic acids. This view is supported by the ability of polyamines to promote condensation and aggregation of DNA (32).

Although synthetic polyamines assayed in this study likely present the same type of binding features than the biogenic polyamines, they may nevertheless show different binding properties due to their different geometries and protonation degrees. Indeed they differ both by the number of ammonium groups at pH 8.1, and the localisation of these groups, as well as by their cyclic or linear structures. This diversity in structure and physical properties likely accounts for the variability of their effects on transcription observed first with single-stranded template A (see Fig. 4). Interestingly, in spite of the weak basic transcription yield obtained in the controls, the synthetic polyamines can stimulate transcription of this template with higher (up to 12-fold) efficiency than the natural spermidine. Best stimulations are obtained with polyamines **1**, **7**, **8**, and **9**. On the basis of their pK_a values (18, 20–23), these substances are expected to bear 4 or 5 positive charges, in the free state, at pH 8.1. However, the effective pK_a's on binding may increase the degree of protonation, for instance up to 6 + for compound **9**. Thus, it appears that the number and the localisation of these positive charges may play a major role during transcription. With less positively charged cyclic structures such as polyamines **4**, **5**, and **6**, stimulation of transcription is lower.

At the present state it is not possible to propose an exact description of the mode of action of polyamines on transcription. This is because of the high structural flexibility of these compounds which likely adopt specific conformations when bound either to the DNA matrix, the T7 RNA polymerase or to the ternary complex formed by the matrix, the enzyme and the growing transcript. Nevertheless two main roles can be proposed for these molecules. Polyamines may have a role in stabilising the transcription initiation ternary complex formed by the matrix DNA, the T7 RNA polymerase and the growing transcript. Indeed, one possible reason why single-stranded templates are not well transcribed in the absence of the artificial polyamines could be an accumulation of short RNA fragments (until 5 nucleotides) as can be observed in the bottom of gels used to analyse transcription products. When transcriptions are done in the presence of artificial polyamines, accumulation of these short RNAs is moderate (not shown), and concomitantly longer abortive products appear (Fig. 4a). It seems that under standard conditions (without the synthetic polyamines) and at the very beginning of the transcription process, the T7 RNA polymerase is discarded systematically from the DNA template at nucleotide positions 3, 4, and 5 at the 3'-end of the transcribed sequences, all occupied by U residues (see Fig. 3). In the presence of the artificial polyamines, a better stabilisation of the DNA-polymerase complex at the initiation step of the reaction likely occurs, thus favouring the more efficient transcription. This view is in agreement with the present knowledge of T7 polymerase functioning that requires formation of an adequately stable

initiation complex (33–35) which may occur best with internal promoter sequences starting with and internally rich in C residues (G residues in the transcript).

Finally, in addition to a possible stabilising effect on the template, artificial polyamines seem to play a role of 'cofactor' for activation of T7 RNA polymerase. Indeed, comparison of the transcription efficiencies of the same sequence either from a single-stranded or a full-length double-stranded template, in the presence or absence of polyamines, suggests that polyamines may play a dual function. For instance, transcription of the double-stranded template is increased ~ 2 times by the polyamine **9** (Fig. 7b), but is already transcribed 6 times more efficiently than the single-stranded template in the absence of polyamines (compare Figs. 7a and 7b). A similar effect, although less pronounced, is observed with polyamine **2** (Fig. 7). Thus, these polyamines may directly stimulate the T7 RNA polymerase activity. The mechanism of such a stimulation is however unclear. The ~ 12-fold improvement of the corresponding single-stranded DNA transcription by compound **9** may thus represent the additive combinations of the polyamines effects, namely a 6-fold effect on stabilisation of the ternary complex (a stabilisation occurring naturally in the case of the double-stranded template) and a 2-fold direct effect on the polymerase activity.

Practical considerations

Transcription yields of DNA templates by T7 RNA polymerase are sometimes low and therefore methods aimed to increase their level may be of practical interest. The present work illustrates the potential of artificial polyamines to fulfil this role. The effects we describe are most marked with single-stranded short templates and this allowed the preparation and functional study of $tRNA^{Val}$ derived minihelices [14]. Since many workers in the field use cloned DNA templates as substrates of the T7 RNA polymerase we examined also in two instances the effect of the most active polyamine on such DNAs. Although the transcription level of a plasmid intrinsically well transcribed was not stimulated, our data point to potential stimulation for badly transcribed plasmids. This opens possibilities for enhancing transcription of DNAs with unfavourable internal promoter sequences whatever the type of template.

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