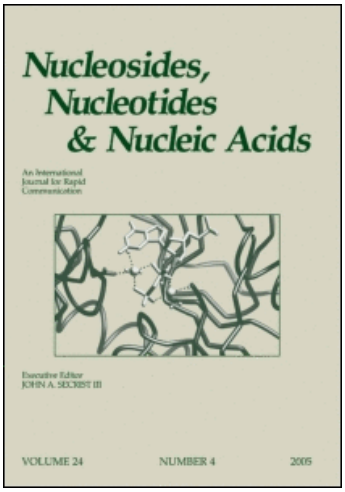

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Synthesis of Mixed Ribo/Deoxyribopolynucleotides by Mutant T7 RNA Polymerase

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SYNTHESIS OF MIXED RIBO/DEOXYRIBOPOLYNUCLEOTIDES BY MUTANT T7 RNA POLYMERASE.

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ABSTRACT: Mutant form of T7 RNA polymerase able to use both rNTP and dNTP as substrates was studied. A number of different T7 promoter-containing templates were tested for the synthesis of dNTP-containing RNA-like polynucleotides (dcRNAs). The initiation stage was shown to be the most critical in the dNTP's incorporation into the product. The dcRNAs obtained were tested as templates for HIV-1 reverse transcriptase (RT) and appear to be the effective in RT-catalyzed reaction.

Recently we have carried out the detailed study of the so-called "motif B" in T7 RNA polymerase (T7 RNAP). This motif is supposed to be responsible for NTP binding and plays an important part in catalysis [1]. The introduction of two amino acid substitutions into motif B, Y639F and S641A, results in the ability of mutant obtained to utilize both rNTPs and dNTPs as substrates [2].

Two distinct stages are characteristic of T7 RNAP transcription: the initiation, which accompanied with easy termination of growing chain and accumulation of short oligonucleotide products (8-10 bases long) and the elongation, when the reaction complex becomes stable due to the conformational rearrangements in T7 RNAP; then practically no chain termination was observed [3]. We have found that, for all types of templates used, the most critical stage for dNTP incorporation is the synthesis of short initiating fragment of polynucleotide chain (**TABLE 1**). As dGTP is obligatory for most T7 promoters in positions +1-+2(3), its incorporation into polynucleotide chain was practically negligible. The further the first dNTP position gets from transcription start, the easier the incorporation of this deoxynucleotide into the growing chain occurs. Kinetic

data have shown that the shift of dNTP position downstream of the transcription start correlated with the decrease of the K_m(dNTP) value. The increase of errors in the 5'-end sequence of the transcript was demonstrated: the "correct" dNTP sometimes appeared to be replaced by "incorrect" rNTP (mainly dGTP for rATP) [2].

Using the constructed plasmid pTZR7G (see **TABLE 1**), synthesis of dcRNAs with all possible ribo/deoxy combinations (except dG-containing) was achieved with high yields. These dcRNAs annealed with deoxyoligonucleotide primer were tested as templates in the HIV-1 reverse transcriptase (RT)-catalyzed reaction. All dcRNAs were shown to be the efficient templates for RT. The respective K_m values for dcRNAs appear to be intermediate between those for RNA and DNA templates. The correlation equation connecting apparent K_m value for template/primer and the number of dNMP substitutions in the template was proposed [4], and the good agreement between experimental and theoretical K_m values was demonstrated (**TABLE 2**).

TABLE 1. dNTP incorporation by mutant T7 RNAP

Template	Initiation sequence	efficiency of dNTP incorporation, %						
		dG	dA	dC	dT	dTdC	dAdT	dAdCdT
pGEMT	GGGAGACCGGAAGCU	3	22	48	87	37	-	-
pV18/*Hind*III	GGGAAUUCGAGCUCG	1	25	83	66	40	-	-
pPV19/*Xba*I	GGGAAGCUUGCAUGC	1	21	58	82	42	-	-
pBSKSII/*EcoR*V	GGGCGAAUUGCAGCU	1	18	62	52	40	12	-
pLys,3/*EcoR*I	GCCCGGAUAGCUCAG	1	26	1	45	-	20	-
pTZR7G/*Pvu*II	GGGGGGGAUCCACUA	0	68	80	85	68	38	29

TABLE 2.dcRNAs as templates for HIV-1 RT

	Template				
	RNA	**dC-RNA**	**dT-RNA**	**dCdT-RNA**	**DNA**
K_m app., experimental (calculated)	1200 ± 170 (1150)	370 ±20 (300)	275 ± 48 (440)	178 ±87 (120)	15 ±2 (18)

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