POSSIBLE DNA STRUCTURE IN THE REGION OF INITIATION OF THE TRANSCRIPTION

H. VENNER, H. REINERT and Ch. ZIMMER

Akademie der Wissenschaften der DDR Forschungszentrum für Molekularbiologie und Medizin Zentralinstitut für Mikrobiologie und experimentelle Therapie DDR 69-Jena, Beuthenbergstr. 11, Germany

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On the basis of the analysis of minor bases in DNA from various microorganisms a suggestion for the primary structure of the DNA region responsible for the process of initiation of the transcription is presented. In addition the recognition interaction of the oligopeptide antibiotic netropsin with dA·dT pairs in DNA is discussed in view of a possible competitor of the transcription at the specific DNA binding site.

Earlier studies on the distribution of the minor base 5-methylcytosine (m^5C) in DNA of various microorganisms led us to a model on the possible role of the minor bases m^5C and 6-methylaminopurine (m^6A) in the transcription [1]. Further studies using the in vivo incorporation of radioactive labeled methionine into DNA of *Proteus mirabilis* PG 43 met$^-$ (table 1a,b) reinforce our results previously observed on the distribution of m^5C in the DNA from *Streptomyces chrysomallus*. It has been demonstrated that nearly the total content of m^5C of the DNA is contained in the nucleotide sequence –pPu–pCpm^5CpPu– in the case of *Streptomyces chrysomallus* whereas half of the amount of m^5C was found in this sequence for *Proteus mirabilis* DNA (table 1a,b).

In accordance with our previous conclusions derived from results on the distribution of m^5C in the DNA [1,4] from the present data together with the findings of Vanyushin et al. [5] it is suggested that the minor bases are located in a start codon (m^5C) or stop codon (m^6A). The position of the methylated cytosine or adenine could induce a conformational change in the transcriptase enzyme passing the start or stop codon, which at least results in switching on of the transcription process. The preceding DNA segment representing the promotor/operator region is responsible for the specific binding of the enzyme.

Table 1a
Distribution of radioactivity in pyrimidine clusters obtained from *Proteus mirabilis* PG 43 met$^-$DNA after hydrolysis according to Burton [2]

Isostich n	counts/min	radioactivity of isostichs (%)
1	1060	7.2
2	9400	64.7
3	1580	10.8
4	840	5.7
5	740	5.0
6	500	3.4
7	300	2.0
8	180	1.2

Table 1b
Distribution of radioactivity in the pyrimidine isostich $n = 2$

Isostich $n = 2$	counts/min	radioactivity (%)
C_2P_3	1640	62.5
CTP_3	520	19.7
T_2P_3	460	17.7

45 % of the radioactivity found in the species d(pC_2p) is located in the 3′ terminal position.

For comparison in *Streptomyces chrysomallus* DNA nearly 70% or 2.5 mole-% m^5C/C respectively of the overall m^5C content has been found in the component d(pC_2p) and 4.3 mole-% m^5C/C, which means approximately all of the m^5C contained in d(pC_2p) is located in the 3′ terminal position.

The preparation of labeled DNA *Proteus mirabilis* PG 43 met$^-$ was obtained after incubation with [methyl-^{14}C]-methionine purchased from the Institute of Radioisotopes Prague (specific activity of 3.15 μCi/mM). Degradation of the DNA to pyrimidine isostichs was performed according to Burton (2) using a mixture of diphenylamine–formic acid. Fraction-

ation of pyrimidine isostichs according to their chain length and base composition was done using a column loaded with DEAE-cellulose of the chloride form or in its formate form and then eluted with a linear gradient of LiCl.

5-Methylcytosine content in pyrimidine cluster and non-isomeric components was determined by perchloric acid hydrolysis of the desalted samples and separation of the split products by thin layer or column chromatography.

Radioactivity was measured by scintillation spectrophotometry in a Packard Tricarb scintillation spectrometer.

The specificity of the polymerase binding to a discrete DNA region is involved in the transcription prior to the process of initiation. Binding of the *E. coli* RNA polymerase occurs preferentially along AT-rich sequences [6,7]. It has been shown, that the oligopeptide distamycin A acts on RNA synthesis mainly due to an interference with a reaction prior to the formation of the phosphodiester bonds [8]. In view of the binding recognition of a DNA duplex segment the specificity of the interaction of netropsin with an AT-helical region is of certain interest. Netropsin strongly inhibits the process of transcription [9] and may be a strong competitor for the enzyme binding site. The netropsin–DNA interaction may represent a possible principle of an outside binding for the recognition of an AT-rich double helical region (as discussed in a recent review, [10]). Recent CD measurements [10,11] demonstrated most efficient binding of netropsin to native AT-rich DNA and DNA polymers consisting of AT pairs. As a sensitive measure of the binding the magnitudes of the induced Cotton effects are summarized in table 2 for comparison. AT containing DNA polymers show the highest binding efficiency for netropsin, while no effect appears for poly(dG)·poly(dC). As recently demonstrated increasing GC pairs of the DNA polymer progressively decreases the netropsin affinity and introduces great sensitivity of the interaction to ionic strength [10–12]. From these binding effects and investigations of the stability conditions of the complex [12,13] as well as some structural requirements of the oligopeptide structure the concept of a recognition model which involves hydrogen bonding has been developed [10,12]. The specificity and tight binding is most probably favoured by formation of hydrogen bonds between peptide groups of the oligopeptide and keto groups of thymine facing the narrow groove together with hydrogen bonding of the guanidino and acetamidino residue to phosphate sites. The conformation of the AT containing duplex region also determines the location of reactive groups responsible for the complex formation. Thus, we suggest the oligopeptide netropsin as a competitor of an AT-rich duplex segment representing the DNA recognition-binding of the transcriptase.

Table 2

Binding of netropsin to DNA and synthetic polymers represented by $\Delta\epsilon$ of induced Cotton effects, in 0.02 M NaCl solution, at 0.1 moles netropsin per nucleotide phosphate

DNA	$\Delta\epsilon$
poly(dA)·(dT)	4.5
poly(dA-dT)·(dA-dT)	4.0
Calf thymus (58% A+T)	3.2
Streptomyces chrysomallus (28% A+T)	1.9
poly(dG)·(dC)	none

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