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DIRECTED ONE-DIMENSIONAL DIFFUSION OF ESCHERICHIA COLI RNA-POLYMERASE, A MECHANISM TO FACILITATE PROMOTER LOCATION

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INTRODUCTION

Location of a DNA signal sequence by facilitated diffusion has been proposed for a number of sequence specific binding proteins (1,2,3). Such a mechanism involves formation of unspecific protein-DNA complex and subsequent diffusion of the protein wihin or along the DNA domain until either the signal sequence is located or dissociation occurs. Sliding as a means of acceleraing the promoter search process by RNA-polymerase was proposed by P.v.Hippel (4).

It has been here designed a system which allows testing this idea by means of competition experiments in which the occupancy of promoters with flanking DNA sequences of different lengths were compared. The hypothesis was that unspecific DNA sequences serve as "antennae" along which the E.coli RNA-polymerase moves to the promoter. If this antenna effect exists, the occupancy of a promoter should be influenced by the length of the flanking sequences.

A DNA fragment was constructed with four identical promoters, A1 from phage T7 , recognized by the E.coli RNA polymerase. The upstream and downstream sequences of promoter I and IV, respectively, were varied (fig.1). The flanking sequences of the middle promoters were left unchanged.

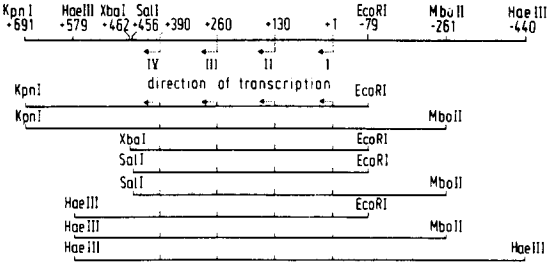

Fig.1

Map of the DNA fragments used in competition binding experiments

METHODS

The binding assay (20 µl total) of RNA polymerase (c= $3x10^{-6}$ M) with promoter-carrying DNA fragments was performed in 50mM NaCl, 6mM $MgCl_2$, 8mM Tris HCl, pH 7.9 and 10 mM mercaptoethanol. The assay was incubated 10 min at T=37°C. Heparin c=0.5 mg/ml was added. The RNA synthesis was started adding ATP, UTP, GTP, CTP, each with a concentraion of 10 mM. The enzyme to DNA ratio was<1. Run-off transcription was allowed for 10 min at 37°C. The transcripts were analyzed on a RNA sequencing gel (6). The radioactivity of the alpha ^{32}P-UMP labelled RNA was determined by scanning of autoradiograms or by cutting out the bands followed by scintillation counting.

RESULTS AND DISCUSSION

Each experiment was performed in two steps: first, A1 promoters with different lengths of flanking sequences were allowed to compete for RNA-polymerase and then nucleoside triphosphates were added to produce "run-off" transcripts in order to determine the occupancies of the competing promoters. In order to quantify the occupancy of each of the four promoters, "run-off" transcripion was allowed. The amount of radioacivity incorporated in the products indicated the number of RNA chains initiated at each of the four promoters. Reinitiaion was suppressed by addition of heparin. Therefore, the amount and size of RNA chains reflected the number of polymerase molecules associated with the different promoters.

Two sets of experiments were performed, one to study the influence of the downstream flanking sequences determining the occupancy of the promoter IV and another one to study the influence of the upstream flanking sequences using promoter I.

Downstream DNA sequences of up to 250bp increased the occupancy of the adjacent promoter (fig.2a), while upstream sequences longer than 70bp had no or only a minor effect on its occupancy (fig 2b); promoter occupancies (measured as a function of the length of the downstream flanking DNA sequences) were fit by a published theory (5) which takes into accounts an enhancement of signal sequence location by linear diffusion.

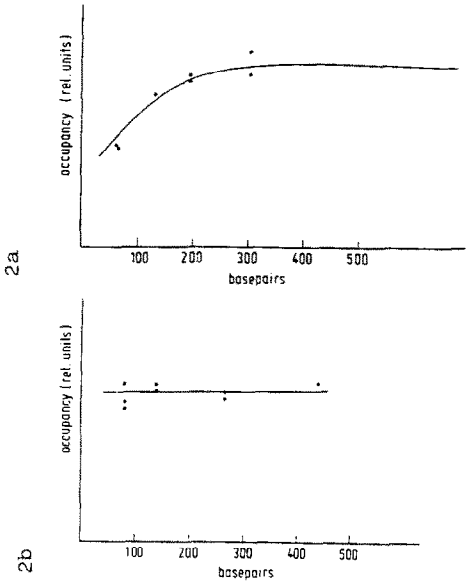

Fig.2

Compilation of the occupancies of promoters of all fragments:
a) dependence on the length of the downstream region
b) dependence on the length of the upstream region
The occupancy of promoters II and III are used for normalization

The directional effect is discussed on the basis of three models: 1) the "barrier-sink" model, 2) the "sliding-along-a-gradient" model and 3) the "sequence-induced-flagging" model. The firs two models assume a special potential along the DNA upstream and downstream of the promoter while the third model proposes a sequence induced transition state of the RNA-polymerase.

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