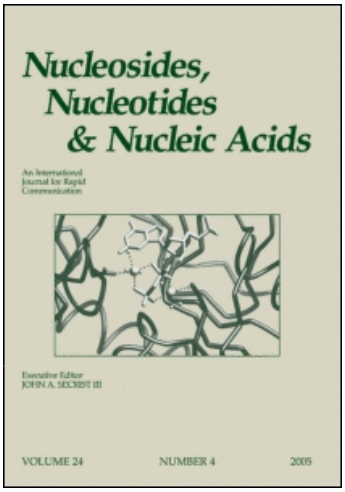

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Chemoenzymatic Synthesis of a Gene Control Region

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CHEMOENZYMATIC SYNTHESIS OF A GENE CONTROL REGION

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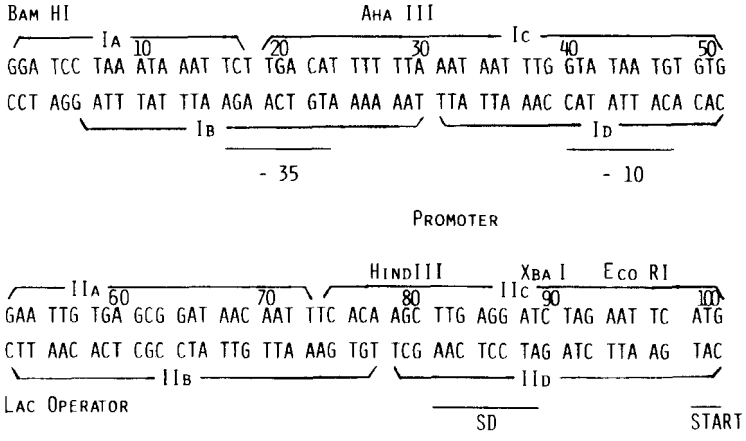
Summary: By the phosphitetriester approach we synthesized the fragments for constructing a synthetically idealized promoter (SIP). The total construction consists of the promoter, operator and Shine-Dalgarno (SD) region. This control region was fused to the gene for γ-interferon.

We chemically synthesized a control region in order to optimally express our synthetic genes considering the following criteria. For easy modifications, e.g. for structure function studies we designed each functional domain as a cassette which can conveniently be dissected by the appropriate restriction enzymes. The regulation unit consists of a promoter[1,2], an operator[3] and a Shine-Dalgarno-sequence[4] (FIG. 1).



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The first cassette consists of the promoter as a reasonably well defined structure[2] of two highly conserved DNA-sequences the -35 and the -10 region which have to be properly spaced in order to maximally interact with RNA-polymerase and thus cause high levels of transcription. The second cassette includes the lac operator which makes the system inducible and allows accumulation of the gene product in a defined time period. The third cassette is the least defined structure of all three and the essential features of a good potential ribosome binding[5] site still have to be worked out more clearly.

For this construction the above deoxyoligonucleotides Ia - d and IIa - d were synthesized according to the phosphoramidite chemistry[6,7]. As a polymer support we obtained the best results with controlled pore glass beads (CPG, LCAA, Pierce)[8]. The reaction cycle included detritylation with 3 % trichloroacetic acid in dichloromethane, followed by addition of the deoxynucleosidephosphordiisopropylamidites and tetrazole. Capping was accomplished with the aid of acetic anhydride and 4-dimethylaminopyridine followed by oxidation with iodine in water, collidine and tetrahydrofurane. The deoxyoligonucleotides Ia - d and IIa - d were purified by a 15 % polyacrylamide gel electrophoresis and after extraction from the gel desalted on Sephadex G 50. Ligation of the annealed deoxyoligonucleotides Ia - d separately and IIa - d in Tris pH 7.4 was performed as described[9]. Cloning of the resulting duplex DNA into a pUC 8 plasmid[10] and sequencing according to Maxam-Gilbert[11] established the correct sequence. The SIP-control region[12] was subsequently fused to our synthetic γ-interferon gene[13] (FIG. 2).

After expression of this construct in E. coli the cells were harvested and the amount of -interferon was monitored according to a commercial RIA (Celltech). The SIP-construction in comparison to the tac construction[14] resulted in a 1.5 times higher production of -interferon. This lac operator - wild type - in the repressed state did not fully shut off the synthesis of γ-interferon, the yield according to RIA and the cytopathic effect was 6 μg/OD·l. A base modification in the operator at position 65 rendered the promoter constitutive by generating

a down mutation. On the other hand Sadler et al.[15] have described an up mutation of the lac operator by introducing the correct symmetry axis. By cutting out the lac wild type operator with the restriction enzymes Aha III and Hind III, we could insert this idealized synthetic operator.

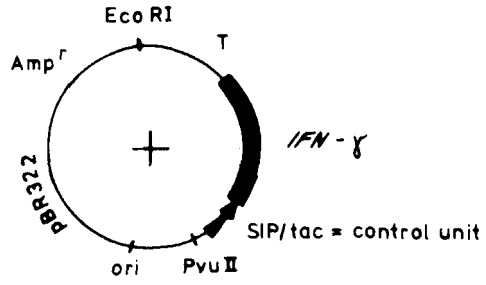

FIG. 2

The final point is the modification of the SD-sequence in order to obtain optimal translational control. Here, studies are under way to modify the SD-sequence as well as the spacer region between the SD-sequence and the start codon to gain optimal expression of e.g. γ-interferon.

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