A novel approach to design of *cis*-acting DNA structural elements for regulation of gene expression *in vivo*

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Taking advantage of the degeneracy of the genetic code we have developed a novel approach to introduce, within a gene, DNA sequences capable of adopting unusual structures and to investigate the role of such sequences in regulation of gene expression *in vivo*. We used a computer program that generates alternative codon sequences for the same amino-acid sequence to convert a stretch of nucleotides into an inverted-repeat sequence with the potential to adopt cruciform structure. This approach was used to replace a 51-base-pair *Eco*RI–*Hin*dIII segment in the N-terminal region of the β-galactosidase gene in plasmid pUC19 with a 51-bp synthetic oligonucleotide sequence with the potential to adopt a cruciform structure with 18 bp in the stem region. In selecting the 51-bp sequence, care was taken to include those codons that are preferred in *E. coli*. *E. coli* DH5α cells harbouring the plasmid containing the redesigned sequence showed drastic reduction in expression of the β-galactosidase gene compared to cells harbouring the plasmid with the native sequence. This approach demonstrates the possibility of introducing DNA secondary-structure elements to alter regulation of gene expression *in vivo*.

GENE expression is a highly regulated process, much of the regulation being exerted at the level of transcription initiation[1,2]. Recent evidences show that the regulation of gene expression can also occur at the level of transcription elongation[3,4]. Although some *cis*-acting DNA sequences[5] and RNA secondary structures[6] have been implicated in regulation of transcription elongation, no definite evidence exists to show the possibility of DNA secondary structure *per se* being involved in modulation of the transcription-elongation process *in vivo*. Several *in vitro* studies have shown that unusual DNA structures, such as the left-handed Z-form[7], cruciform DNA[4] and heteronomous DNA[8], can influence transcription. Insertion of a synthetic DNA sequence capable of adopting a cruciform structure within the promoter region was found to inhibit transcription initiation *in vivo*[9]. The sensitivity to tetracycline observed in *E. coli* cells harbouring plasmid pRM36 has been attributed to the insertion of a 'structural cassette' with short stretches of left-handed Z and right-handed B helices in the tet^R promoter region[10,11]. Our earlier studies using pBR322 form V DNA as a template for *E. coli* RNA polymerase *in vitro* showed that a supercoil-stabilized cruciform structure present within the *rep* gene could block elongation by RNA polymerase, and this block could be abrogated once the cruciform structure was destabilized[4,10]. However, because of the high superhelical density in this molecule, the physiological relevance of these results is limited.

To delineate the DNA structural elements responsible for transcriptional control *in vivo* we have developed a novel approach to introduce structural cassettes within a gene and to follow the effect of such unusual DNA structures, stabilized *in vivo*, on the transcription-elongation process. We have used expression of the β-galactosidase gene as a model system for this study. Taking advantage of the degeneracy of the genetic code we have incorporated silent mutations to redesign the 5′-end of the β-galactosidase gene in plasmid pUC19 so as to introduce an inverted-repeat sequence that has the potential to adopt a cruciform structure *in vivo*. This is, to our knowledge, the first report that demonstrates '*in vivo*' that a potential cruciform structure present within the gene could act in *cis* to down-regulate the expression of a gene.

Methods

Synthesis and annealing of complementary oligodeoxyribonucleotides

The complementary 51-nucleotide-long oligonucleotides were synthesized on a Pharmacia Gene Assembler Plus using phosphoramidite chemistry. The oligomers were purified on a 20% polyacrylamide–8 M urea gel and by passing through an NAP (Pharmacia) column[12]. To anneal the complementary strands, the oligonucleotides were mixed in equimolar ratio, heated at 95°C for 3 min, and slowly brought to room temperature. The annealed sample was stored at −20°C. Circular-dichroism (CD) spectra of the annealed product were recorded on a Jasco J-500 spectropolarimeter equipped with a data processor[12].

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