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 EcoRI–HindIII 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 exclude 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 initiation1–2. Recent evidences show that the regulation of gene expression can also occur at the level of transcription elongation3–4. Although some cis-acting DNA sequences5 and RNA secondary structures6 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-form7, cruciform DNA8 and heteronomous DNA9, 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 vivo9. 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 terR promoter region10,11. 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 destabilized4,10. 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 S'-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 oligodeoxynucleotides
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. 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.