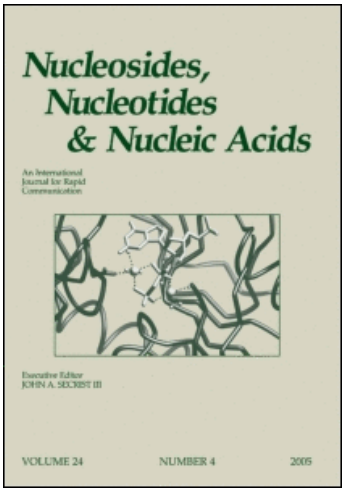

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A Large Fragment Approach to Gene Synthesis

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A LARGE FRAGMENT APPROACH TO GENE SYNTHESIS

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Summary: The total synthesis of a 232 base-pair coding sequence of the proteinase inhibitor eglin c from only six synthetic fragments is described.

All the genes synthesized so far have been constructed by assembling single-stranded fragments (10 to 20 mers) which cover both strands. This process requires the ligation of a correspondingly large number of oligonucleotides, which results in a complex assembly. An alternative method has been anticipated recently by K. Itakura et al. (1). It basically involves the chemical synthesis of large single-stranded DNA fragments which share a short stretch of complementary sequences at their 3'-termini. After annealing and in vitro repair with DNA polymerase I, a full double-stranded product is obtained.

A gene of the 70 amino acid elastase-inhibiting eglin c (from the leech Hirudo medicinalis) was synthesized by way of this approach. Six fragments (34 to 61 mers) were prepared on a polystyrene carrier using phosphotriester chemistry by condensing appropriate trinucleotides with MSNT (average coupling yields: ca. 96 %) with a self-constructed semi-automatic synthesizer. The released and deprotected fragments were purified by means of HPLC and electrophoresis. The assembled gene was shown to have the correct sequence. It was expressed in high yield in E. coli under the transcriptional control of the E. coli trp promoter. The expression product was biologically fully active, but a detailed physicochemical analysis of the purified recombinant protein indicated the presence of a post-translational modification at the amino terminus.

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1) Rossi, J.J.; Kierzek, R.; Huang, T.; Walker, P.A.; Itakura, K. The Journal of Biological Chemistry 1982, 257, 9226-9229

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