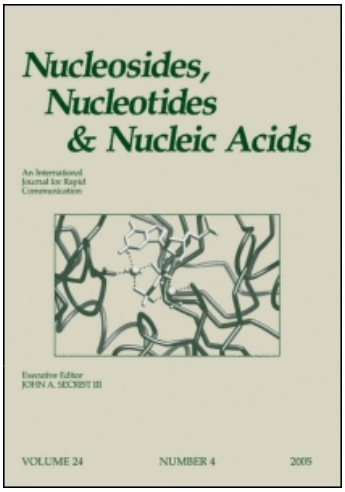

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Approach to the Synthesis of Functionally Active Polydeoxynucleotides Using DNA Amplification In Vitro

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APPROACH TO THE SYNTHESIS OF FUNCTIONALLY ACTIVE POLYDEOXYNUCLEOTIDES USING DNA AMPLIFICATION *in vitro*

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Abstract. Approach to the eukaryotic genes construction based on polymerase chain reaction (from genomic DNA by artificial splicing, or from a mixture of mRNAs via sequence-specific initiation of reverse transcription) and exemplified by the mature interleukin 1α gene is described.

Construction of native and modified genes is one of essential problems in molecular biology and biotechnology, which has been delineating successfully due to the extensive development of chemical and chemical-enzymatic polynucleotide synthesis and site-directed mutagenesis [1]. Much has been done through and still more is coming from the polymerase chain reaction (DNA amplification *in vitro*), enormously contributing into the rapid progress of various branches of biology and medicine [2]. We describe an approach to the synthesis of functionally active polydeoxynucleotides via the artificial splicing of intronized genes. The approach is exemplified by designing a gene which encodes mature human interleukin 1α (amino acid residues 113-271) and, in genomic DNA, is partitioned among three exons (5, 6, and 7) [3].

The exon fragments of the gene were prepared by means of a series of PCR with the use of synthetic primers containing recognition sites of the *Eco*31I restriction endonuclease, which cleaves DNA fragments at a definite distance from the site (GGTCTCN$^{\downarrow}$/CCAGAGNNNNN$^{\downarrow}$), thus giving tetranucleotide 5′-protruding ends of unique structure. These fragments were cloned into pUC19 and pUR vectors, the latter allowing for the expression of the fragments as C-terminal tags of β-galactosidase. Enzymatic ligation of the exon fragments of the gene (after *Eco*31I digestion) proceeded under "stringent control" of the unique protruding ends and yielded the desired polynucleotide coding for mature interleukin 1α. Its structure was proved by restriction analysis and sequencing by the dideoxy approach.

Alternative synthesis of the same gene was carried out using messenger RNA. A mixture of polyadenylated mRNAs from human monocytes was subjected to the reverse transcription specifically initiated from the mRNA encoding interleukin 1α by the polynucleotide complementary to the mRNA's coding 3'-end (i.e., the downstream primer for the 7th exon) to yield the corresponding mRNA-cDNA duplex. Under PCR conditions, with the above polynucleotide as the downstream primer and an upstream primer corresponding to the beginning of the mature interleukin 1α gene (i.e., the upstream primer for the 5th exon), the mRNA-cDNA duplex yielded the desired gene.

We have also constructed a 73-membered DNA duplex coding for the signal sequence from the *E. coli* heat-labile enterotoxin [4], to make it possible to transfer the product of the gene expression into the periplasmic space. The synthesis was carried out by ligating four chemically prepared oligonucleotides followed by the DNA polymerase extension of 3'-ends and cloning the resultant blunt-end duplex into pUC19 vector. To join together the 73-mer and the above gene, Eco31I sites were introduced into the distal end of the former and proximal end of the latter, which, after digestion with the restriction endonuclease, yield complementary protruding ends fit for the efficient ligation.

The gene, which contains translation initiation codon ATG and two adjacent termination codons TAA and TGA, and is flanked by BamHI sites, as well as its fragments and analogues can be used for producing interleukin 1α in various expression systems and studying the mechanism of its biosynthesis and structure-activity relationship.

REFERENCES

(1) Frank, R.; Meyerhans, A.; Schwellnus, K.; Blocker, H. *Meth. Enzymol.* 1987, *154*, 221-249, and subsequent papers.

(2) PCR Technology: Principles and Applications for DNA Amplification; Ehrlich, H.A., Ed.; Stockton: New York/ Macmillan: London, 1989.

(3) Furutani, Y.; Notake, M.; Fukui, T.; Ohue, M.; Nomura, H.; Yamada, M.; Nakamura, S. *Nucl. Acids Res.* 1986, *14*, 3167-3179.

(4) Yamamoto, T.; Tamura, T.; Yokota, T. *J. Biol. Chem.* 1984, *259*, 5037-5044.