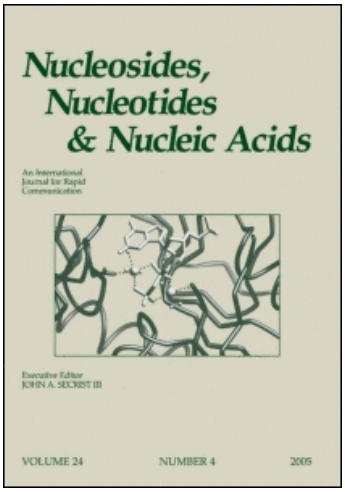

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Automated Preparation of DNA-RNA Hybrids and their Joining with RNA Ligase; An Approach to Single-Strand Gene Synthesis

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AUTOMATED PREPARATION OF DNA-RNA HYBRIDS AND THEIR JOINING WITH RNA LIGASE; AN APPROACH TO SINGLE-STRAND GENE SYNTHESIS

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ABSTRACT: 3'-riboterminated oligodeoxynucleotide hybrid strands were successively joined to a 3'-terminal deoxynucleotide using T4 RNA ligase to produce a 121 b DNA-RNA hybrid single-strand corresponding to a gene for β-endorphin (Fig. 1).

The chemical synthesis of oligonucleotide fragments and their purification and characterisation is still the most time-consuming and expensive part of a gene synthesis. This expenditure can be reduced to less than 50% by synthesizing a gene as a single-strand and subsequent filling up with DNA polymerase. The application of this strategy is demonstrated for the synthesis of a β-endorphin gene. The construction of this gene is described in Fig. 1.

The chemical synthesis of the 3'-ribonucleoside terminated deoxyoligonucleotides was done in a Biosearch SAM I synthesizer by the standard phosphor-amidite method[1] starting from silica gel supports loaded with the 2'(3')-succinate of 5'-DMTr-N-benzoyl-adenosine. The residual functions were capped by acetylation. Routine cleavage from the support, deprotection and purification by preparative gel electrophoresis gave the 3'-ribo oligonucleotides. The 3'end of the gene terminated with a deoxynucleoside (fragment I in Fig. 2) was made by the same standard technique. Sequence analysis was done by the method of Maxam and Gilbert.

The strategy of the RNA ligase reactions is given in Fig. 2. The joining between the 5'phosphorylated donor oligonucleotide and the 3'-ribonucleoside terminated acceptor fragment was performed at 17°C in 50mM Hepes (pH 7.6), 10mM $MgCl_2$, 10mM $MnCl_2$, 20mM DTT, 2mM spermine, 10% DMSO, 12% polyethylenglycol (MW 6000)[2]. Dependent on the fragment length RNA ligase reactions were incubated for 8-28h yielding 40 to 90%

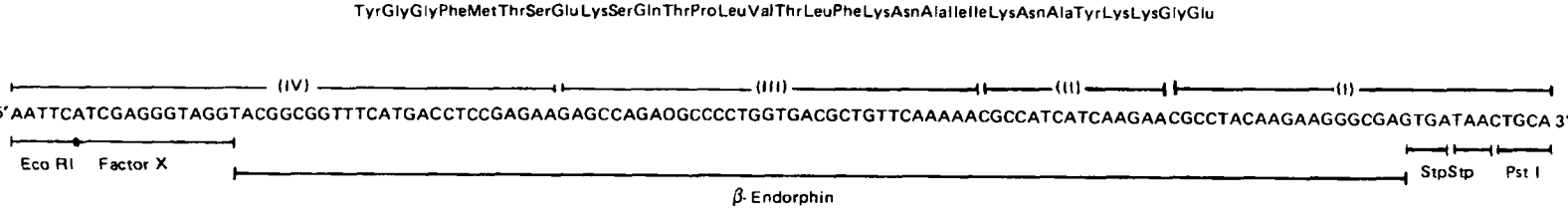

FIGURE 1

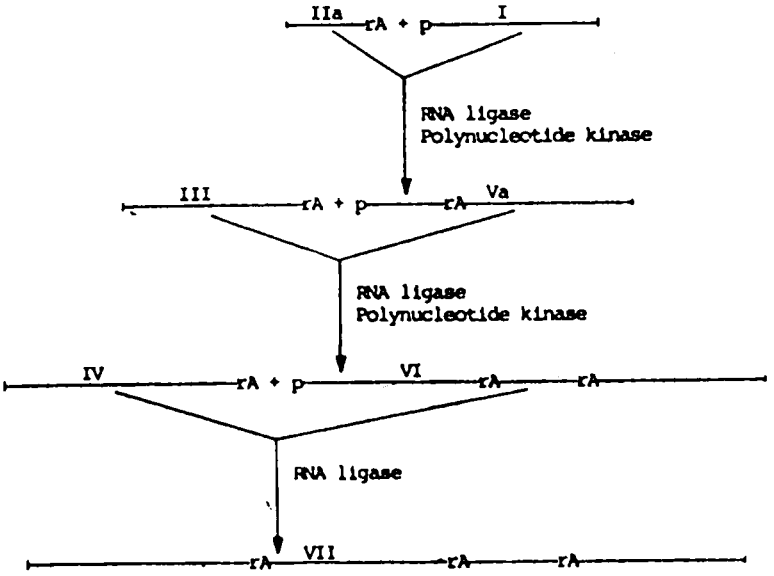

FIGURE 2

of ligation product. Two possibilities for the cloning of the single-strand oligonucleotide can be envisaged. The first route involves the ligation of the single-strand DNA directly into the linearized vector and subsequent filling up with DNA polymerase. The second route includes the preparation of the double-strand by primed DNA polymerase reaction, subsequent generation of the appropriate restriction enzyme sites and ligation into the vector. Both routes are currently under investigation.

This work demonstrates the potential of single-strand gene synthesis with fragment joining by RNA ligase. Fast and efficient methods for the preparation of DNA-RNA hybrid strands are now available[3]. Our strategy of blockwise chain extension from the 3'deoxy end largely prevents self-ligation or cyclization of donor fragments during RNA ligase reactions. Cyclization of the donor fragment was not more than 4% for short fragments and undetectable for longer ones. Careful sequence analysis of the single-strand fragments is necessary, since there is no "proof-reading" through a synthetic double-strand.

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