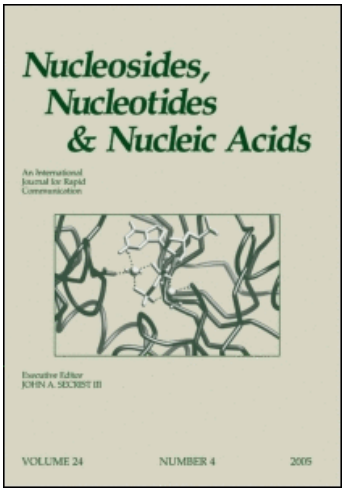

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Signal Sequence: Application of Chemical DNA Synthesis to Studies of Studies of Translocation of Expressed Proteins

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SIGNAL SEQUENCE: APPLICATION OF CHEMICAL DNA SYNTHESIS TO STUDIES OF TRANSLOCATION OF EXPRESSED PROTEINS

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In general there are three different ways in which eucaryotic genes can be expressed in E. coli.
(i) Direct expression: This mode of expression produces methionylproteins which often are unstable in E. coli especially in the case of smaller proteins. (ii) Expression as a fusion protein: The expressed protein accumulates to high yields in E. coli. The fusion has to be cleaved, however, to the mature form (in the case of cyanogen bromide cleavage, for instance, the mature polypeptide should not have an internal methionine). (iii) Transport expression: The expressed protein is excreted from the bacterial cytoplasm to the periplasmic space, the outer membrane or even into the culture medium. The advantage of this procedure is that the produced protein is not mixed with the intracellular proteins and degradation by enzymes within the cell is prevented.

The theoretical basis of the translocation process is stated in the "signal hypothesis" established by Blobel and Dobberstein [1]. As an approach to this expression mode we conceived an experimental set up in which the protein to be transported is monkey proinsulin [2]. This gene sequence is coupled on the DNA level to a signal sequence of a suitable bacterial protein. We chose the presequence of the secretory protein Alkaline Phosphatase [3], which is highly expressed in E.coli, plus five N-terminal amino acids of the mature protein which again may be part of the signal peptidase cleavage site [4]. The DNA sequence corresponding to the signal peptide required the chemoenzymatic synthe-

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sis of a 84 bp DNA fragment with cohesive ends at each side. The protruding ends of the Eco RI cleavage site allow subcloning of the signal fragment as a movable element. Unique internal restriction enzyme sites permit the alteration of specific amino acids - (the hydrophobic core sequence, the positively charged N-terminus or the signal peptidase recognition site) - as well as the coupling of any eucaryotic gene in the correct reading frame.

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