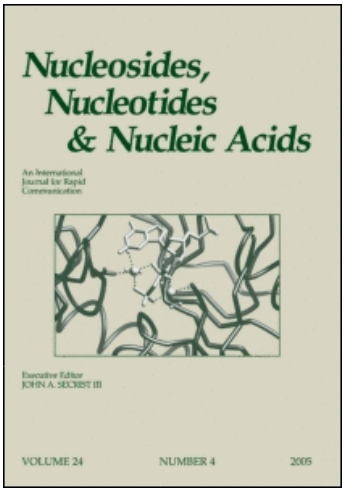

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Chemical Synthesis and Cloning of a Gene Coding for Bacillus Subjilis Hbsu Protein

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CHEMICAL SYNTHESIS AND CLONING OF A GENE CODING FOR *BACILLUS SUBTILIS* HBSU PROTEIN

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Abstract. A gene coding for *Bacillus subtilis* non-specifically DNA-binding HBsu protein has been chemically synthesized and cloned. The gene was designed to permit expression of the encoded protein in several ways.

A widely unsolved problem is how bacteria achieve a folding and organization of their DNA into a compact form. It is believed that the bacterial DNA is aggregated into a nucleosome-like structure called the nucleoprotein complex[1]. It is not known with certainty what this complex looks like and what all the constituting components are. The most extensively characterized group of prokaryotic proteins engaged in the organization of DNA has been classified DNA-binding proteins (DNABP) II[2]. From more than ten organisms, including archaebacteria, homologous proteins have been isolated. Some have also been shown to be immunologically related. None of the proteins is homologous with eukaryotic histones. *In vitro* the DNABP II cause a condensation of DNA and RNA and the formation of beaded structures[3]. However, the total amount of DNABP II in the bacterial cell is insufficient to cover more than 10% of the chromosome. Their unspecific binding to DNA affects replication and transcription.

We have decided to study the DNABP II from *Bacillus subtilis* for a number of reasons: (1) The host organism is genetically well characterized and (2) to the best of our knowledge contains only one gene for a DNABP II unlike *Escherichia coli* where two closely homologous genes exist. (3) The three-dimensional structure is known for the related protein from *Bacillus stearothermophilus* [4] and the properties of this protein and its counterpart of *Bacillus globigii* (the HB protein) have been studied over the last years[5-7]. In analogy to the latter protein and to propose a systematic nomenclature for this class of proteins we have christianed the *B. subtilis* DNABP II HBsu protein.

As a first step to studying the molecular properties and physiological role of HBsu protein as well as to the engineering of mutant protein molecules we have chemically synthesized and cloned a gene coding for HBsu protein.

RESULTS AND DISCUSSION

The DNA sequence of the gene (Fig. 1) is based on the amino acid sequence of HBsu protein (M. Kimura, unpublished). The 10.5 kD polypeptide contains 92 amino acid residues and is devoid of cysteine, histidine, tyrosine and tryptophane. A compromise between the codon preferences of *E. coli* and *B. subtilis* [8] was used for the gene of HBsu protein. With the help of the SYNGEN option of the computer program package GENMON[9] the gene was divided into 14 overlapping fragments ranging in length from 26 to 50 nucleotide units. The oligonucleotides were designed with SYNGEN so as to allow the assembly of the whole gene from the 14 fragments in a single ligation reaction. The resultant gene consists of seven cassettes, typically in the form of a 35 base pair double strand with 15 nucleotides long overhanging single strands at both ends to join with the neighbouring cassettes.

The 14 oligodeoxyribonucleotides were chemically synthesized on an Applied Biosystems automatic DNA synthesizer and initially purified by hydrophobic interaction and anion exchange FPLC. Final purification was achieved by polyacrylamide gel electrophoresis. After ligation the gene construct was cloned between the SalI and EcoRI sites of the multiple cloning region of pUC120 putting it in reverse orientation with respect to lacZ'. After superinfection with M13K07 the gene could be sequenced directly from the resulting plasmid (pHB1) by the dideoxy method[10].

In addition to the SalI and EcoRI sticky ends of the gene several other intragenic restriction sites were designed. A SacI site, for example, allows us to exchange the 5'-end of the gene by replacing synthetic fragments 1 and 2 to adapt it to several expression systems. The open reading frame is defined by an N-terminal Met codon and tandem stop codons at the end of the gene.

With respect to the expression in *E. coli* of the synthetic gene it seems desirable to yield a fusion of HBsu protein with another polypeptide since the host cell may not tolerate high levels of an unspecific DNA-binding protein. With this in mind a factor Xa cleavage site immediately upstream of the N-terminal Met of HBsu protein was designed to permit liberation of the native

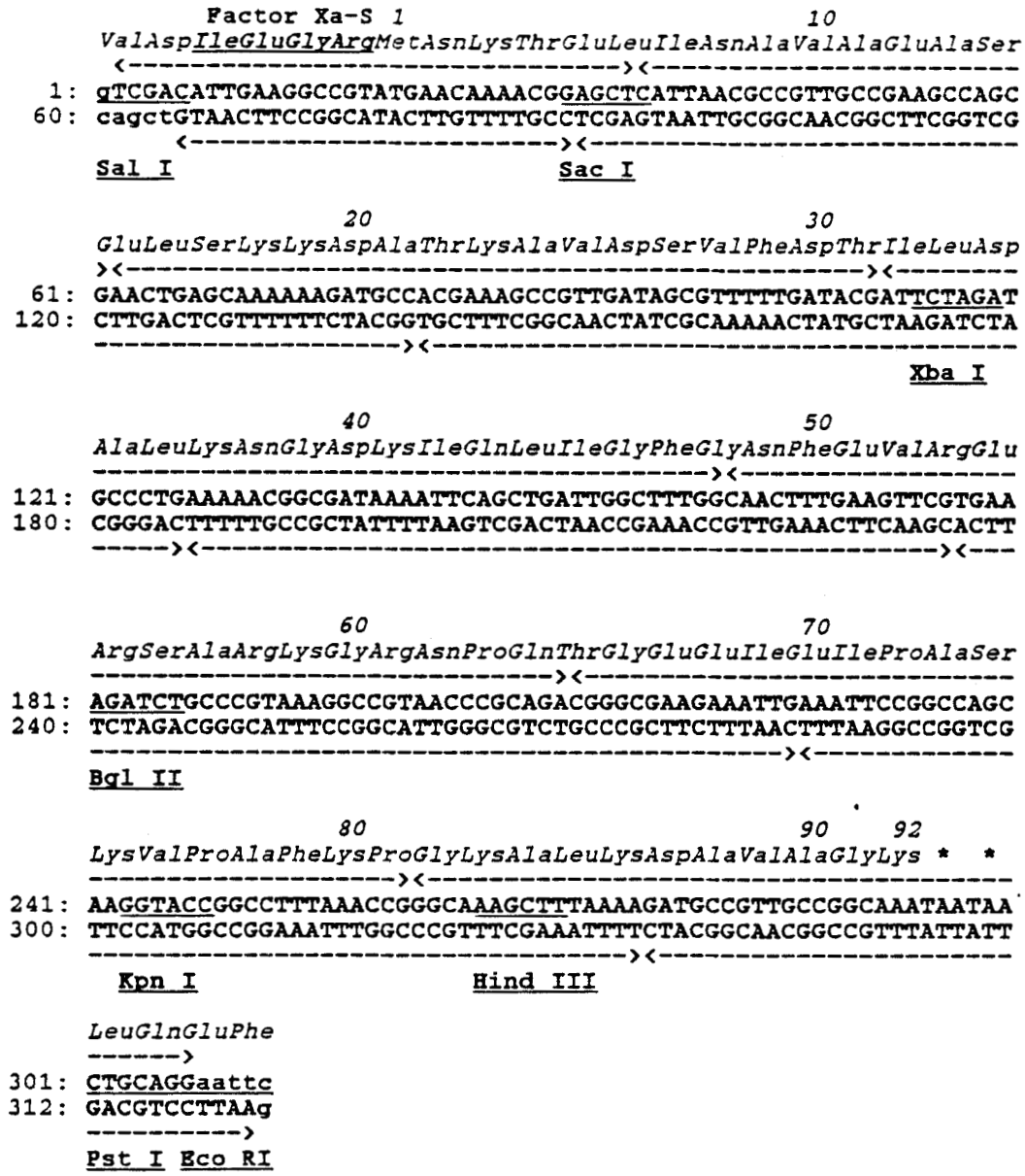

FIG. 1. Synthetic gene for B. subtilis HBsu protein. The gene sequence was designed following criteria described in the text. The amino acid sequence of HBsu protein is printed above the gene. Double-headed arrows above and below the gene mark the length of the synthetic DNA fragments. Lower case nucleotide symbols at beginning and end of the gene indicate flanking nucleotides derived from the vector. Restriction endonuclease cleavage sites are underlined in the sequence and identified below the gene.

protein. Alternatively, native HBsu protein lacking the terminal methionin may be obtained from cyanogen bromide cleavage of a fusion protein. The use of a fusion protein for overexpression of HBsu protein may also facilitate protein purification, especially from the background of homologous host proteins. At a later stage expression of native HBsu protein in *B. subtilis* and in mutants thereof is planned to study the physiological role of the protein.

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