NUCLEOTIDE SEQUENCE OF THE GENE FOR THREONINE DEAMINASE (*ILV1*) OF *SACCHAROMYCES CEREVISIAE*

by

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Keywords: Isoleucine-valine biosynthesis, allosteric enzyme, upstream open reading frame, general amino acid control, multivalent repression

The 1,728 base pair coding region of the *ILV1* gene and the adjacent 777 base pair upstream and 261 base pair downstream regions were sequenced. The size of the polypeptide deduced from the coding region is larger than previously determined values for the molecular weight of threonine deaminase, suggesting that a peptide with a molecular weight of about 15,000 is cleaved off in connection with the intracellular processing of the enzyme.

In front of the gene are found three copies of the element TGACT characteristic for genes under general amino acid control. Two of the copies are present in an orientation which is reversed to that found in front of other genes. Sites which potentially can bind strongly to the 3'-end of yeast 18 S ribosomal RNA are present at positions -5 and -24 from the start of the coding region.

An open reading frame of 31 codons is found in front of the gene. Its translation initiator codon is located at the center of a 151 base pair imperfect tandem inverted repeat which in a single-stranded nucleic acid would fold into a stem structure with a ΔG of about -33 kcal · mole⁻¹. The biological significance of this arrangement is unknown.

1. INTRODUCTION

The biosynthesis of isoleucine starts with the conversion of threonine to α -ketobutyrate and ammonia, a step catalysed by threonine deaminase (threonine dehydratase, 4.2.1.16). The enzyme in yeast has been purified (1, 14). It is a tetramer with a molecular weight of $4 \times 48,000$ (1), and complexed in mitochondria with other enzymes involved in the biosynthesis of isoleu-

cine and valine (24). It contains pyridoxal phosphate as a non-covalently bound prosthetic group. The catalytic activity is subject to (feed back) inhibition by isoleucine and is dependent in a complicated manner on the concentrations of isoleucine, threonine and valine (1). Determination of the amino acid sequence of the apoenzyme should ultimately be of help in understanding these interactions.

Abbreviations: bp = base pairs; kb = kilo bases; kD = kiloDalton

The amount of threonine deaminase in the cell is regulated. The simultaneous presence of isoleucine, valine and leucine in the growth medium causes repression (so-called multivalent repression) of the synthesis of the enzyme (5). Furthermore, its synthesis is subject to general amino acid control (cf. 29). The gene, *ILV1*, for the apoenzyme, is nuclear and located on chromosome V. It has recently been isolated on a bacterial plasmid and its approximate position on the restriction endonuclease map was determined by DNA-RNA hybridization and confirmed genetically (21). Sequencing of the gene and its adjacent regions was considered important, not only to obtain the amino acid sequence, but also as a the first step to identify DNA regions involved in multivalent repression. A minor part of the sequence determined in the present work has been previously reported (29).

2. MATERIALS AND METHODS

2.1. Preparative electrophoresis

Specific DNA fragments in restriction endonuclease digests were selected by preparative agarose gel electrophoresis using DE81 ion exchange paper (8) or dialysis membrane (30).

2.2. DNA sequencing by the dideoxy method

Standard procedures using the single-stranded bacteriophage M13 vectors (19) and dideoxynucleotide sequencing were employed (25). Thus, restriction fragments were ligated into the replicative form of M13mp8 or M13mp9 (19) and *Escherichia coli* strain JM101 (18) was transfected with the mixture and plated for development of plaques. For two *EcoRI* fragments the principles for systematic sequencing described by HONG (13) were followed.

Electrophoresis, handling of gels and sources of vectors, bacterium, chemicals and materials were as described (4, 23). Autoradiography was carried out at room temperature for 1-5 days without intensifying screens.

The nucleotide sequence data handling was carried out on a Honeywell DPS-8. The programs were kindly supplied by Dr. R. STADEN, MRC, Cambridge, England.

3. RESULTS

Plasmid pC516 contains the functional *ILV1* gene (21). Figure 1 shows the relevant part of the plasmid. It was treated with *PstI* and fragments of different sizes were prepared on agarose gels. After further treatment of the isolated fragments

Figure 1. Map of the nucleotide sequence readings from individual recombinant M13 clones used to establish the sequence of the *ILV1* gene. (A) designate clones obtained from restriction endonuclease fragments using *EcoRI* (R), *HindIII* (H) or *PstI* (P). The two sets of clones designated (B) were obtained from a 2,035 bp *EcoRI* fragment (left) and a 1.6 kb *EcoRI* fragment (right) using DNase I to generate random start-points for sequencing. The 3 clones (C) were constructed from *BglII*-*HaeIII* fragments in order to establish the sequences across the two *EcoRI* sites.

with EcoRI, Sall, HindIII or combinations thereof, subfragments were prepared. The primary PstI fragments and the subfragments were inserted in M13 vectors and sequenced. This series of experiments yielded sequences indicated by the group of arrows designated A in Figure 1.

The sequences corresponding to arrows B were obtained from two EcoRI fragments (2,035 bp and 1.6 kb) of pC516 by the DNase I method of HONG (13). To establish sequences overlapping the two EcoRI sites between the two groups of arrows (B), sequencing from a BglII site at +1,246 and an HaeIII site at +1,540 was performed after cloning of the appropriate fragments into M13mp8 (arrows C). Approximately 80% of the sequence was obtained from both strands.

Figure 2 shows the sequence spanning an EcoRI site located 777 bases upstream from the ATG of a 1,728 bp long open reading frame and a point 261 bases downstream of the last codon.

4. DISCUSSION

The open reading frame of 1,728 bases is the coding region for the *ILV1* gene since it covers

the stretch which by gene splitting has been shown to be essential for the *ILV1* function (21). In addition, an mRNA of 2,000 nucleotides is transcribed from this region (21). It is the only reading frame in this neighbourhood that can accommodate a protein of 48 kD, the size found for the monomer of threonine deaminase (1, 14). We have considered the possibility that alternative, smaller reading frames assembled with one or more introns could constitute the *ILV1* coding region, but found this highly unlikely. First, introns are rare in nuclear genes of *Saccharomyces* and they have not been found in amino acid biosynthetic genes. Secondly, an intron would expectedly contain the sequence TACTAAC (22) and this sequence does not occur here.

The *ILV1* gene can encode a polypeptide with a molecular weight of 63,719 while the monomer of threonine deaminase has been determined to be only about 48 kD. To explain this difference one could imagine that the mRNA starts within the open reading frame and that the methionine codon at +346 or +466 were used as the start of translation. However, mRNA characterization (to be published) has shown

Table 1. Codon usage in the yeast *ILV1* gene

		Second letter								
		T		C		A		G		
F i r s t l e t t e r	T	Phe	10	Ser	21	Tyr	10	Cys	5	T
		Phe	13	Ser	9	Tyr	10	Cys	0	C
		Leu	15	Ser	5	ochre	0	opal	0	A
		Leu	21	Ser	1	amber	0	Trp	1	G
	C	Leu	8	Pro	15	His	12	Arg	9	T
		Leu	0	Pro	4	His	6	Arg	1	C
		Leu	9	Pro	8	Gln	19	Arg	0	A
		Leu	2	Pro	0	Gln	4	Arg	1	G
	A	Ile	17	Thr	20	Asn	10	Ser	2	T
		Ile	14	Thr	5	Asn	16	Ser	2	C
		Ile	2	Thr	2	Lys	17	Arg	17	A
		Met	10	Thr	4	Lys	17	Arg	4	G
	G	Val	26	Ala	24	Asp	21	Gly	32	T
		Val	13	Ala	14	Asp	6	Gly	4	C
		Val	8	Ala	1	Glu	30	Gly	3	A
		Val	8	Ala	2	Glu	4	Gly	2	G

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-777
      GAATTCG TTCTCATTAC ATGCCGCCCC ACCATGCACC TTTACATTTC GTATTTCTTT
-700
CCTCTCTGCG CTATATCTTT GGGAAAGATT TGCAATCAAG GCAAACACCC GCTTGTCCCC TTTCAATTCT TGTGTTATTT
-600
CAAAAACCAT CCTTTCATAA TATATTTGTA TTTCTTTGCG CCTGGACTCT ACCAGTTCAT ATTTAGAACC TTCCACATAT
-500
CGTCCAATTC TGCAGCCAC ATCTTTTTCC ACCACGATAC GGGAAACAGA ATGGGTCTCT GGATTCTCGC TAAACAGTCT
CTCTATTCCC CTGTTCAAAA CCTCAAGAT ATTTGTTTCC CGCAACAGCT GCAATTGCAA TTGATCAATC CTATGCGAAA
-400
ATGCCGAGTT TATGTTATTC AAGACGCATT TAAAAAATT CACTAGCGGC TCCTTGAAAT TCATTATGTC TGATGAATAT
-300
GAAAACCTTT TCCTGACTAC CAAGACTCTT TAACCTCTCT CTCTTTATTG CATATTATCT CTGCTATTTT GTGACGTTCA
-200
ATTTTAATTG ACGCGAAAAA GAAAAAATAA GAAGGGCAAA AAGAAAAAGC GCAGCGGGTA GCAAATTTGG AATCGCATAA
-100
AAAGAAAAAA AAAATATCAA AGAAAAAGAG TCATCTCAAA CATATGCTCG CAGATACTTC ATTATCAGCT TTGAAAACCT
-1
TGTTGTTGCG TGCTTTGAGT TCTTCTTGT GTGAGTGCTA CAAGCCACAT TTAAACTAAG TCAATTACAC AAAGTTAGTG
      stp
+1
ATG TCA GCT ACT CTA CTA AAG CAA CCA TTA TGT ACG GTT GTT CGG CAA GGT AAA CAG TCC AAA GTG
met ser ala thr leu leu lys gln pro leu cys thr val val arg gln gly lys gln ser lys val
50
TCT GGA TTG AAC CTT TTG AGA CTA AAG GCT CAT TTG CAC AGA CAA CAC CTG TCA CCT TCC TTG ATA
ser gly leu asn leu leu arg leu lys ala his leu his arg gln his leu ser pro ser leu ile
100
AAA CTA CAC TCT GAA TTG AAA TTG GAT GAG CTG CAA ACT GAT AAC ACC CCT GAT TAC GTC CGT TTA
lys leu his ser glu leu lys leu asp glu leu gln thr asp asn thr pro asp tyr val arg leu
150
GTT TTA AGG TCC TCT GTA TAC GAT GTT ATT AAT GAA TCT CCA ATC TCT CAA GGT GTA GGT TTG TCT
val leu arg ser ser val tyr asp val ile asn glu ser pro ile ser gln gly val gly leu ser
200
TCC CGT CTA AAC ACG AAT GTC ATC TTG AAA AGA GAA GAT CTA TTG CCT GTT TTC TCT TTC AAG CTT
ser arg leu asn thr asn val ile leu lys arg glu asp leu leu pro val phe ser phe lys leu
250
CGT GGT GCC TAT AAC ATG ATT GCC AAG TTG GAC GAT TCT CAA AGA AAC CAG GGT GTT ATT GCC TGT
arg gly ala tyr asn met ile ala lys leu asp asp ser gln arg asn gln gly val ile ala cys
300
TCA GCT GGG AAT CAT GCC CAA GGT GTG GCC TTT GCT GCT AAA CAC TTG AAA ATA CCT GCT ACT ATC
ser ala gly asn his ala gln gly val ala phe ala ala lys his leu lys ile pro ala thr ile
350
GTT ATG CCT GTT TGT ACA CCA TCT ATT AAG TAT CAA AAT GTC TCG AGA TTA GGG TCT CAA GTC GTC
val met pro val cys thr pro ser ile lys tyr gln asn val ser arg leu gly ser gln val val
400
CTA TAT GGT AAC GAT TTT GAC GAG GCT AAG GCT GAA TGT GCC AAA TTG GCT GAA GAG CGT GGC TTG
leu tyr gly asn asp phe asp glu ala lys ala glu cys ala lys leu ala glu glu arg gly leu
450
ACG AAC ATT CCT CCT TTC GAT CAT CCT TAT GTC ATT GCC GGT CAA GGT ACT GTA GCT ATG GAA ATC
thr asn ile pro pro phe asp his pro tyr val ile ala gly gln gly thr val ala met glu ile
500
CTA AGA CAA GTA CGT ACC GCT AAT AAG ATC GGT GCT GTC TTT GTT CCC GTC GGC GGT GGT TTA
leu arg gln val arg thr ala asn lys ile gly ala val phe val pro val gly gly gly gly leu
550
600
650
700

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Figure 2. DNA sequence of the gene for threonine deaminase (*ILV1*) of *Saccharomyces cerevisiae*. The A of the translation initiation codon is numbered +1. The preceding G is numbered -1. The amino acid sequence of the primary translation product is given as inferred from the nucleotide sequence. Several sequence elements of possible significance can be recognized outside the coding region: The sequence TGACT and its complement AGTCA, implicated in general amino acid control, are marked with wavy lines. TATA-like sequences are designated with dotted lines. At position -117 starts a reading frame of 31 codons which is underlined. Its ATG and the preceding CAT provide the symmetry point of a large imperfect inverted repeat. The sequences TATGT and TATTTATA signify possible control elements for termination of transcription and are marked with broken lines.

750
 ATT GCT GGT ATT GGT GCT TAT TTG AAA AGG GTT GCT CCT CAT ATC AAA ACT ATT GGT GTT GAA
 ile ala gly ile gly ala tyr leu lys arg val ala pro his ile lys thr ile gly val glu
 800
 ACT TAC GAT GCG GCC ACT TTA CAT AAT TCC TTG CAA CGC AAC CAG AGA ACT CCT TTA CCT GTG
 thr tyr asp ala ala thr leu his asn ser leu gln arg asn gln arg thr pro leu pro val
 900
 GTG GGT ACT TTT GCC GAT GGT ACG TCT GTG CGT ATG ATT GGT GAA GAA ACA TTT AGA GTC GCC
 val gly thr phe ala asp gly thr ser val arg met ile gly glu glu thr phe arg val ala
 950
 CAA CAA GTG GTT GAT GAA GTT GTT CTT GTT AAC ACT GAC GAA ATC TGT GCT GCA GTA AAG GAT
 gln gln val val asp glu val val leu val asn thr asp glu ile cys ala ala val lys asp
 1000
 ATT TTT GAA GAT ACT AGA AGT ATT GTA GAA CCA TCT GGT GCC CTT TCA GTA GCC GGT ATG AAG
 ile phe glu asp thr arg ser ile val glu pro ser gly ala leu ser val ala gly met lys
 1050
 AAA TAC ATC TCT ACC GTA CAT CCA GAA ATT GAC CAC ACT AAA AAC ACC TAT GTT CCC ATC CTT
 lys tyr ile ser thr val his pro glu ile asp his thr lys asn thr tyr val pro ile leu
 1100
 TCT GGT GCT AAC ATG AAC TTT GAT AGA TTA AGA TTT GTT TCC GAA CGT GCT GTT CTT GGT GAA
 ser gly ala asn met asn phe asp arg leu arg phe val ser glu arg ala val leu gly glu
 1200
 GGA AAG GAA GTC TTC ATG TTA GTT ACT TTA CCC GAC GTC CCT GGT GCG TTC AAG AAA ATG CAA
 gly lys glu val phe met leu val thr leu pro asp val pro gly ala phe lys lys met gln
 1250
 AAG ATC ATC CAC CCA AGA TCT GTC ACT GAA TTC TCT TAC CGT TAC AAT GAA CAT CGT CAT GAG
 lys ile ile his pro arg ser val thr glu phe ser tyr arg tyr asn glu his arg his glu
 1300
 TCC TCT AGT GAA GTG CCC AAG GCT TAC ATT TAC ACT TCT TTC AGC GTC GTT GAC AGA GAA AAG
 ser ser ser glu val pro lys ala tyr ile tyr thr ser phe ser val val asp arg glu lys
 1400
 GAA ATC AAG CAA GTT ATG CAA CAG TTG AAT GCT TTA GGT TTT GAA GCT GTG GAT ATC TCC GAT
 glu ile lys gln val met gln gln leu asn ala leu gly phe glu ala val asp ile ser asp
 1450
 AAC GAA TTG GCT AAA TCT CAT GGT AGA TAC TTG GTT GGT GGT GCT TCT AAG GTT CCT AAT GAA
 asn glu leu ala lys ser his gly arg tyr leu val gly gly ala ser lys val pro asn glu
 1500
 AGA ATT ATT TCA TTT GAA TTC CCT GAA AGA CCA GGT GCC TTG ACT AGG TTC CTT GGA GGC CTA
 arg ile ile ser phe glu phe pro glu arg pro gly ala leu thr arg phe leu gly gly leu
 1550
 AGC GAT TCT TGG AAT CTT ACT TTA TTC CAT TAT AGA AAC CAT GGT GCC GAT ATC GGT AAG GTT
 ser asp ser trp asn leu thr leu phe his tyr arg asn his gly ala asp ile gly lys val
 1600
 TTA GCT GGT ATT TCC GTT CCT CCA AGG GAA AAC TTA ACC TTC CAA AAA TTC TTG GAA GAT TTA
 leu ala gly ile ser val pro pro arg glu asn leu thr phe gln lys phe leu glu asp leu
 1700
 GGC TAC ACT TAT CAT GAT GAA ACT GAT AAC ACT GTT TAT CAA AAA TTC TTG AAA TAT TAA AAC
 gly tyr thr tyr his asp glu thr asp asn thr val tyr gln lys phe leu lys tyr stp
 1800
 CGA CAA TTT ACT TTA TAA ATTTACGC AACAACTTGT TAGGTAAAAT GAACGTCGTC ATAATTTAAA TACTAGACAG
 stp
 CGCGTGTATG TTACTCATAT TTATACTCAT ATCATGTGAA CCTATAAACC AATTCAATAC AAGATTTTACA GTTCTCTTGT
 1900
 TTTCTTCTTC CAGTTTTCCT CTTTATTTTC CTGTAGAATA AACTTAAACA GTGATCATTA ATTTATAATT TTCTGCTTTG
 1989
 TAAATACGGC GGTAATGGA

that all transcription in this region starts upstream of the ATG at position +1. It is therefore suggested that the primary translation product has a size of 64 kD and is processed by proteolytic cleavage into the 48 kD monomer of threonine deaminase. In fact, such processing is known to occur for many proteins that are synthesized in the cytoplasm and transported into the mitochondria (cf. 20). Determination of the amino acid sequence of the N-terminal region of the mature protein will allow positioning of the cleavage point.

Regarding the codons of the *ILV1* gene, we have noted that the tryptophan codon is only found once. Another feature is that the first part of the gene is very rich in leucine codons, 14 out of the first 55 codons, i.e. 25% are leucine codons. This is not associated with any particular hydrophobicity of this region as judged by an analysis of the hydrophobicity along the polypeptide chain according to the method of KYTE and DOOLITTLE (15). This leucine rich part of the translation product is suggested (see discussion above) not to be present in the mature threonine deaminase. Codon usage (Table I) has a significant, but relaxed bias towards the codons commonly used in yeast (3), which might be expected for a protein present in moderate amounts in the cell (3). According to the enzyme activity of crude cell extracts as compared to purified enzyme (1, 14), threonine deaminase makes up about 0.1% of total cell protein.

Upstream to the coding region several sequence elements of interest can be recognized. DONAHUE et al. (6) and HINNEBUSCH and FINK (12) have suggested that the element TGACT plays an important role for general amino acid control. This element is found three times in front of the *ILV1* coding region, once in the orientation in which it has been identified in the 5' non-coding regions of *HIS4*, *HIS1*, *HIS3* and *TRP5* (cf. 12) and twice in reverse orientation, i.e. AGTCA in the strand presented in Figure 2. The element TGACT is the most conserved core of the larger consensus sequence A(A/T)GTGACTC (12). The sequence element in front of *ILV1* that comes closest to this consensus is AGATGACTC which is the complement to the 9 base pair sequence starting at -133 in Figure 2. Determination of threonine

deaminase activity after the addition of 5-methyltryptophan to the growth medium (cf. 29) has shown that *ILV1* is under general control. In vitro mutagenesis will permit us to determine the significance of the TGACT containing elements in front of *ILV1*.

The element TATAAA or similar sequences are common in front of yeast genes. They are believed to have promoter potential (cf. 9) and at least in some cases (10, 27), in vitro mutations give phenotypes that confirm this notion. In front of *ILV1* there are two similar elements, TATGAAA at -323 and TATCAAA at -146, the latter being at a distance from the coding region comparable to that of other yeast genes (cf. 26).

We have noticed two more features in the front region of *ILV1*. When the upstream region is searched for heptanucleotides complementary to the 3'-end of yeast 18 S ribosomal RNA, three segments at positions -177, -24 and -5 are found. With the presumed TATA-box at position -146, only the latter two possible ribosome binding sites should be considered. Using energy calculations for intramolecular base pairing according to TINOCO et al. (28) to compare bimolecular binding energies, the sequence TAAGTC (at position -24) yields a relative ΔG of $-3.6 \text{ kcal} \cdot \text{mole}^{-1}$ when binding to the 3'-end of 18S RNA and the relative ΔG is $-8 \text{ kcal} \cdot \text{mole}^{-1}$ for the sequence TAGTGAT (at position -5). Sequences complementary to 18 S ribosomal RNA of five mRNA species are discussed by ZALKIN and YANOFSKY (31). The sequence of the *ILV1* mRNA at -24 can bind to the 18 S rRNA with a strength comparable to that of these five mRNA's while the sequence at position -5 binds more strongly. In comparison to other yeast mRNA's the two putative ribosome binding sites at *ILV1* are positioned very close to the ATG. These facts make it attractive to investigate the level of gene expression in strains with site-directed mutagenesis in this region.

The other feature of the *ILV1* front region that we have noted is the following. At position -117 is the ATG of an open reading frame that could potentially code for a polypeptide of 31 amino acids. Upstream open reading frames are not uncommon to amino acid biosynthetic genes in yeast (2, 7, 12, 31). In the case of *LEU2*, the upstream reading frame is rich in leucine codons

and is associated with potential foldback structures, facts that have been proposed to be of significance for the repression of the gene by leucine (2). Subsequent deletion analysis has shown that a small palindromic G+C-rich region, but not the reading frame, is important for regulation of *LEU2* (17). Similarly, in the case of *HIS4* a deletion analysis has shown that the two open reading frames present in the 5' non-coding region are not required in either cis or trans for the regulation of *HIS4* by the general control system (16). The ATG of the open reading frame

in front of of *ILVI* and the preceding CAT provide the symmetry point of a large imperfect inverted repeat. In a computer search in the region from -777 to +330, i.e. well into the coding region, for inverted repetitions of segments of 15 bases with an allowance of up to two imperfections, this palindrome was the most pronounced and regular one. A single-stranded nucleic acid of this sequence is expected to fold into the structure represented in Figure 3. It must be emphasized that this foldback structure is hypothetical but illustrates the extent of the inverted repetition.

At the 3' end of the gene the TAA stop codon at position +1,729 is followed by a second TAA in frame at position +1,750. Two sequences have been suggested to be important for termination of transcription in yeast and both are present downstream from the *ILV1* coding region. The sequence TATGT seems to play a role in transcription termination of the *CYC1* gene (32). This sequence is found at position +1,817 of *ILV1*. At position +1,828 there is the sequence TATTTATA, differing at only one position from the sequence element TTTTTATA, suggested by HENIKOFF et al. (11) to be important for termination of the *Drosophila ADE8* message in yeast.

Figure 3. Hypothetical folding of single-stranded nucleic acid with the sequence present from -185 to -35 of the yeast *ILV1* gene. Calculation according to TINOCO et al. (28) for the corresponding RNA molecule gives a ΔG of folding of $-33 \text{ kcal} \cdot \text{mole}^{-1}$. At the top of the hairpin loop starts a reading frame potentially coding for a polypeptide of 31 amino acids.

ACKNOWLEDGEMENTS

Of particular importance for this work has been the introduction at Department of Physiology of the M13 sequencing technique by cand. polyt. SØREN K. RASMUSSEN. We are very grateful for his maintenance of equipment and software, and his readiness to always help solve problems. The skilful technical assistance by Ms. GITTE BANK and the stimulating discussions with Professor DITER VON WETTSTEIN, Dr. JULIO POLAINA and cand. scient. CLAES GJERMANSSEN are gratefully acknowledged. This work was supported by grant 16-3344.H656 from the Danish National Research Council for Technical Sciences and grant 133/001-84.154 from Teknologirådet of the Ministry of Industry to Professor DITER VON WETTSTEIN.

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Accepted by: H. KLENOW, E. LUND and S.O. ANDERSEN