OCH1 encodes a novel membrane bound mannosyltransferase: outer chain elongation of asparagine-linked oligosaccharides

Ken-ichi Nakayama, Takeshi Nagasu¹, Yoh-ichi Shimma, Jun-ro Kuromitsu¹ and Yoshifumi Jigami²

National Chemical Laboratory for Industry, Tsukuba, Ibaraki, and ¹Eizai Pharmaceutical Co. Ltd, Tsukuba, Ibaraki, Japan

²Corresponding author

Communicated by W.Tanner

The Saccharomyces cerevisiae och1 mutant shows a deficiency in the mannose outer chain elongation at the non-permissive temperature. We have cloned the OCH1 gene by complementation of temperature sensitive (ts) phenotype for growth. The integrant of OCH1 gene in the yeast chromosome can complement the ts phenotype and shows the same mapping position as that of the och1 mutation, indicating that the cloned gene is the true gene for mutation. The OCH1 gene disruptant is not lethal but ts for cell growth, and lacks mannose outer chains. The OCH1 gene sequence predicts a 55 kDa protein consisting of 480 amino acids. It contains four potential asparaginelinked (N-linked) glycosylation sites and a single transmembrane region near the N-terminus. In vitro translation/translocation analysis revealed that the large C-terminal region of the OCH1 protein is located at the lumenal side of microsomal membranes with some sugar modification, indicating a type II membrane topology. The OCH1 protein was detected in yeast membrane fractions as four forms of 58-66 kDa, which correspond to the size of a glycoprotein containing four N-linked sugar chains the length of which is almost the same or slightly larger than the inner core (Man₈GlcNAc₂) formed in the endoplasmic reticulum (ER). Finally, the OCH1 gene was found to encode a novel mannosyltransferase which specifically transfers [¹⁴C]mannose to the unique acceptor, the core-like oligosaccharide of cell wall mannan accumulated in the och1 disruptant.

Key words: mannosyltransferase/membrane spanning protein/outer chain elongation/protein glycosylation/yeast

Introduction

The asparagine-linked (N-linked) glycosylation pathways have been well studied in both mammalian and yeast cells. These pathways are similar in yeast and mammalian cells up to the oligosaccharide assembly on dolichol pyrophosphate (Dol-PP) in the endoplasmic reticulum (ER); they are different, however, in the later stage of oligosaccharide chain processing (Kornfeld and Kornfeld, 1985). In yeast the oligosaccharide chain can be larger than in higher eukaryotes, consisting of both inner core (Man₈GlcNAc₂) formed in the ER and mannose outer chain (Man₁₅₋₁₀₀) formed in the Golgi (Kukuruzinska *et al.*, 1987). In mammalian cells, several genes encoding glycosyltransferases, such as *N*-acetylglucosaminyltransferase (Kumar *et al.*, 1990), galactosyltransferase (Nakazawa *et al.*, 1988; Shaper *et al.*, 1988; D'Agostaro *et al.*, 1989) and sialyltransferase (Weinstein *et al.*, 1987), catalyzing part of the sequential reactions to form a complex type oligosaccharide from Man₃GlcNAc₂, were cloned and their common topological features were pointed out according to their amino acid sequences (Paulson and Colley, 1989).

In contrast, studies on glycosyltransferases in yeast are few. Although several genes concerned with part of the glycosylation pathway have been isolated, only two genes are known to encode a glycosyltransferase. The ALG7 gene encodes the first enzyme in the lipid-linked oligosaccharide synthesis, UDP-N-acetyl-D-glucosamine:dolichyl-phosphate N-acetyl-D-glucosamine phosphotransferase (Rine et al., 1983; Hartog and Bishop, 1987). Albright and Robbins (1990) cloned the ALG1 gene which complements the temperature sensitive (ts) phenotype of the alg1 mutant; this shows a deficiency in core oligosaccharide synthesis in the ER at the non-permissive temperature. They found that the ALG1 gene encodes a mannosyltransferase which catalyzes the formation of Dol-PP-GlcNAc2Man from GDP-Man and Dol-PP-GlcNAc₂. Both gene products are responsible for the formation of inner core oligosaccharide in the ER. However, there are no reports on yeast glycosyltransferase genes whose product is functional in outer chain elongation.

In a previous paper we reported the isolation of an *och1* mutant which shows a deficiency in mannose outer chain elongation in yeast (Nagasu *et al.*, 1992). Here we report the *OCH1* gene cloning, DNA sequence, and the topological feature of OCH1 protein, together with its function in yeast. The OCH1 protein is an integral type II membrane protein whose C-terminal major region residues in the lumenal side, and contains four N-linked oligosaccharide chains. The over-production of OCH1 protein shows an increase of an unknown mannosyltransferase activity which specifically transfers mannose to the core-like oligosaccharide acceptor accumulated in the *och1* disruptant. This suggests that the OCH1 protein is a novel mannosyltransferase which is functional in mannose outer chain elongation in yeast Golgi membranes.

Results

Cloning of OCH1 gene

The wild type *OCH1* gene was isolated by complementation of *ts* growth phenotype in the *och1* mutant. EHF-2C (*leu2 och1*) was transformed with a yeast genomic DNA library on the multi-copy plasmid pTN3200, which carries the *LEU2* gene as a selectable marker. Among a population of 2×10^4 transformants, four were able to grow at the nonpermissive temperature (36°C). This phenotype was plasmid dependent and all the transformants contained the same DNA fragment. One of the plasmids designated as pM1 was analyzed in more detail. The restriction map of pM1 revealed



Fig. 1. Restriction map of isolated DNA and DNA sequencing strategy. Cloned *OCH1* DNA was derived from plasmid pM1. Open areas and straight lines indicate cloned yeast DNA and vector DNA, respectively. In order to cause the frame shift, *Sal*I digested pM1 and *Bam*HI digested pM1 were blunt-ended by Klenow fragment and ligated. Complementation O and X show that DNA fragments can and cannot complement the *ts* phenotype of the *och1* mutant, respectively. Thin arrows below the map represent the direction and extent of sequences obtained from a given M13 clone. The thick arrow denotes the long open reading frame (ORF) proposed to encode the *OCH1* gene product.

the presence of a 5.4 kbp DNA insert (Figure 1). The Sall-HindIII 2.6 kbp fragment within this insert was found to complement the ts phenotype. To confirm that the 2.6 kbp fragment encodes the OCH1 gene itself, the plasmid DNA was integrated on the yeast chromosome with the LEU2 gene as a marker. The strain EHF-2C (MATa leu2 och1) was transformed with a linearized plasmid DNA (pTN-OCH1) containing the LEU2 and OCH1 genes, and leucine prototrophic transformants which can grow at 36°C were isolated. This transformant was crossed to YS37-4C (MAT α *leu2 cyh2*) to determine the integrated gene locus. All spores derived from 31 asci examined showed ts^+ phenotype and no progeny showed ts phenotype. Segregation of parental och1 OCH1-LEU2 and OCH1 leu2 alleles through meiosis indicates that the LEU2 gene is integrated at the och1 gene locus. The genetic linkages between integrated gene locus and neighbouring markers were also examined. The ratios of parental ditype (PD):tetra type (T):non-parental ditype (NPD) were 31:0:0 for ts^+ – *LEU2* and 11:20:0 for LEU2-cyh2. It was clearly indicated that the integration occurred at the *och1* locus because the distance between ts^+ and LEU2 was calculated as 0 cM. The mapping position of the integrated OCH1 gene as calculated from the distance between LEU2 and cyh2 (32.3 cM proximal from the cyh2 gene on the left arm of Chr VII) was almost the same as that reported previously for the och1 mutation (32.4 cM proximal from cyh2) (Nagasu et al., 1992). The results indicated that the cloned gene is the true gene responsible for the *och1* mutation, and not a multi-copy suppressor.

Sequence analysis of the OCH1 gene

The nucleotide sequence of the OCH1 gene was determined by the dideoxy method of Sanger et al. (1977). As shown in Figure 2, the unique open reading frame (ORF) of 1443 bp was found within the SalI-HindIII fragment of the OCH1 gene. This ORF encodes a protein of 480 amino acids with a molecular weight of 55 155 Daltons. A hydropathy profile of the predicted OCH1 protein indicated a membrane

2512

spanning protein with a single hydrophobic region near the N-terminus and a hydrophilic reigon at the C-terminal side. The hydrophobic region will serve as a membrane anchor but not as a signal peptide, because this region contains several positively charged amino acids immediately outside of both the N- and C-terminal ends but no typical cleavage site for signal peptidase (von Heijne, 1986). Four potential N-linked glycosylation sites (Asn-X-Ser/Thr) were found in the large C-terminal region. The amino acid sequence of OCH1 was compared with the sequence in the EMBL and GenBank databases, however, no significant homology was found with other sequences reported so far, including those of any glycosyltransferase.

Disruption of OCH1 gene

The DNA fragment having a disrupted och1 gene, in which more than half of the ORF was deleted and replaced with the *LEU2* gene instead, was introduced into wild-type diploid cells (Figure 3A). After confirming by genomic Southern blot that one copy of *OCH1* gene is replaced with the disrupted gene (Figure 3B, lane 3), the diploid cells were tetrad dissected. Surprisingly, all four spores in the asci could germinate and make colonies (Figure 3C), indicating that the *och1* gene disruption is not lethal for cell growth.

All *och1* disrupted colonies (*LEU2* colonies) were *ts* for cell growth (Figure 4A). Thus, the *OCH1* gene is not essential for cell growth but necessary for tolerance at higher temperature. Further, all the *och1* disruptants examined showed the outer chain deficiency at the permissive temperature (Figure 4B).

In vitro translation and translocation of OCH1 protein

To analyze the properties of the *OCH1* gene product, *OCH1* mRNA prepared by SP6 transcription system was translated *in vitro* in the rabbit reticulocyte lysate in the presence of [³⁵S]methionine. Further, to examine the membrane topology of the OCH1 protein, the above *in vitro* translation was performed in the presence or absence of canine

| ATAMAMA CRANTANTACTOTIC TO ALC TARGET TAGA CAN TATA ALC TATA CAN THE TATATACTATION TACK TO COME OF A CONTROL | 00 0 | ACC | GAA | NAGCI ICCTX | ATCA GCTG | TACA GATT | GACG. | AGGT | TTAC. | ATTG TCAG | CGG | GATC | CCGA | TGGA | STAT' | TGTG | ICAN GGTT/ | AGCCI | ACCG | CAT STTG | NAGG | CCCA | CAGA | ACTAG | TGT | ATAAG CGTGG | - 60 |
|--|-----------------|------------|--------------|----------------|--------------|--------------|--------------|------------|------------|--------------|------------|--------------|------------|--------------|--------------|------------|---------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|----------------|-------|
| UNTITIONAL ALLANDAL ALLANDAL ALLANDAL ALLANDAL ALLANDAL THE ALLANDAL THE THE THE ALLANDAL THE THE ALLANDAL ALLA | 00 1 | ATA | ATA | AGAGO | TGA | ATAA | PACT | TCTT | | TGA | IGAA | TAG | GIG | SCIT | SCAA | ATGCI | | ATCT/ | ATAT/ | ACA. | ATAT | TAT | ATAT | ATGT/ | ATGT | ACACC | -40. |
| TOGRACTION MACLETIAGTIC TRUCTURATION ACCOUNT MATTIC DOCUME COLLITER INTERCOMMENDATION ACCOUNT MATATION TO DOCUME CANCET AND ADDRESS AND AD | 00 0 | GAA | | JACA: | LA IG | TTCC | TOCO | | | | COCO | COM | Dir P | CACT | PPPC I | LICI | PIAP | CON | | ACC | MAA P | IGCT | TAAA | ATT I | 11GGG | DACTT | - 30. |
| TTATTAGGGATAGGTTGTTTTAGTTGTGTTGGATTGCGTTTGCATTGCAGGGCATATAGGCAGTTGGAAAAGGAAGG | 00 - | TGGT | CAGC | TTGG | TAGC | CACT | TAG | TATT | TCTG | CTT | CTTC | GAAT | ACCG | ACAT | FATT | TCTC(| GCCA | ATCC | ACAT | ICTC' | TCTC | CCA | TCTG | ATC | TTT | TATAT | -10 |
| And TCT AGG MG TTG TCC CAC CTG ATC GCT AGA AGG AM TCA TAM ACA ATA GTG GTA ACG TAT CTT ATT TAT Het Ser Arg Lys Lew Ser His Lew IIe Als Thr Arg Lys Ser Lys Thr TIE VAL VAL Thr VAL Lew Lew IIP TAT TCT TG TG TG ACA TT CAC TG TCA AAC AM AGG CTG CTT TCT CAG GTT TAT CCT AGG AM GAT GAT TT AGA GA ITT FRE LEW LEW TTT FAR THIS LEW SER ASIN Lys Arg Lew Lew Ser Gin Phe Tyr FTO SEr Lys Asp Asp Phe Lys Gin ACT CTT CTC CCT AGC ACT TCT CAT TCA CAA GAT ATA ATA TTG AGG AM ACT AATT ACA GTT AAC AGG AM AM AM AAT I TT LEW LEW FFO THT THE SER HIS SER GIN ASP IIE ASIN LEW Lys GIN IIE THT VAL AMA AMA GAG AATT ACT CTT CTC CCT AGC ACT TCT CAT TCA CAA GAT ATA ATA TTG AGG AMA CAA ATT ACA GTT AAC AGG AMA AMA AMA IAT TT LEW LEW FFO THT THE SER HIS SER GIN ASP IIE ASIN LEW LYS GIN IIE THT VAL AMA ANA AGG AGG TCI GAA TGG CAT MGT AMA GTG GG GG AGT GAT AMA GAT TTT CCT TCT GC TCA CAA CAC TAT CAA AAA ACA TGG TCT I TTG GIN TH TCH CIG AGT GAT TAT TCT CG TTT GG ATG CAT TCC AGA ACC TAT CAA AMA ACA TGG TCT I TTG GIN TT CAC GG GAT GAT AMA TTA TCT CTA TTT CCT TCT GTG AGA ACT TTT CAA AMA ACA TGG TCT I TTG GIN TT CAC GG GAT GAT CAA TAT TCT CT GTT TCG GAT GAT TCT ATT ATA CCT TTT GG GAA GAT TTT TAF GGT TGG TTA CCAA GAT ACT CAA GGG TTT AAA TGG ATG GT CT ATT ATA CCT TTT GG GAA GAT TTT TAA GI GGT GG TAC GTA GTA CTA CTC AAG GGT TT AAA TGG ATG GAT ATT ATC CTA AGG GCA ATT TCT TAA GIN SIN LEW TY ICTA TTA TTA TT CCA AGG GT GGT ATT TAC TCA GAT ATG GAT ATT ATC CTA TAT GAT TTA CTA AGG CCA ATT GAT TTA AGG GT GAT ATT TAT AGG GAA GAT GAT | 00 | TTN | TAG | GAT | GGT | TGTT | TAG | TTCT | PTGA | TTCC | STTT | TCAT | PTC A | AGAG | CAAT | ATA | GCAA | TTTG | GAAA | AGA | AAGC | AGT | | GAAA | GAAG | GATC | - |
| Het Ser Arg Lys Leu Ser His Leu IIe Ala Thr Arg Lys Ser Lys <u>Thr IIE Val Val Thr Val Val Thr Val Car</u> GAT TC AG AC AA AG CG CTG TC CC AG TT TAC CCT AGC AA GAT GAT TC AG CAA I <u>Ser Leu Leu Thr Phe</u> His Leu Ser Asin Lys Arg Leu Leu Ser Gin Phe Tyr Pro Ser Lys Aap Asp Phe Lys Gin ACT CTT CC CCT AG ACT TCT CA TCA CAA GAT ATA AAT TTG AG AAA CAA ATT ACA GTT AAC AAG AAA AAA AT 2 Thr Leu Leu Pro Thr Thr Ser His Ser Gin Aap IIe Asin Leu Lys Lys Gin IIe Thr Val Aan Lys Lys Lys Asin CAA TTG CAT AAT TTA CGT GAT CAA ATTA TCG TTT GCG TTT CCC TAG GAC TCT CAG GCC CCC ATTC CCG GAA AGG GTG 3 Gin Leu His Asin Leu Arg Asp Gin Leu Ser Phe AIa Phe Pro Tyr Asp Ser Gin Aia Pro IIe Pro Gin Arg Val TCG CAG ACC TGG AAA GTG GGC GCA GAT GAT AGA AGA TTT LCC TCT TCG TCC AGA ACC TAT CAA AAA ACA TGG TCT 3 Trp Gin Thr Ttp Lys Val Gly AIa Asp Asp Lys Asin Phe Pro Ser Ser Phe Arg Thr Tyr Gin Lys Thr Trp Ser 1 GGT TGG TAT TCA CCG GAT TAC CAA TAT TCT CTG ATT TCG GAT CAT TCT ATT ATC CCT TTT TGA GGA ACC TGG ATA CCAA TAT CC AA GGC TT AAA TGG AT ATT CCT AGA GGC GA ATT TCT CA AG CCC GTT CCG ATA GC AAA TAT TCT CTG GAT TGG GAT CAT TCT TAT ACC CT TTT TTA AGG TAC S AIa Pro Val Pro 1 Br Val 1 He Gin Aia Phe Lys Leu Het Pro Gly Asin 1 He Leu Lys Aia Asp Phe Leu Arg Tyr 1 CTA TTA TTA TTT GCC AGA GGT GGT ATT TAC TCA GAT ATT GG AT ACT ATT GC ATT TCC ATA GGC AGAT TTT TA ATT GCC TA AGG CCC TAC 6 Ser Gin Asin Lys Ser Trp Leu Asin Asin 1 He IIe Asp Leu Asin Thr MAC CT ATT CT TAT AGA GAC GAA CTA AGG CCC TCA 6 Ser Gin Asin Lys Ser Trp Leu Asin Asin 1 He IIe Asp Leu Asin Tro GAA CCA GCC GCC AGA CGA GAT CTT 7 Fro Ser Lys Asin Ser Lys Pro Ser Asp Asp Lys Asin He He Val I He Gin Ala Sep Pro Asp Asp Asp Asp I's Sin Pro Ser 2 CTT CCC AAA GTG ATG GCT AMC ACA ATA ATA CAT TGT GAT TAC CAA GCC GAA GAT GAT TC Fro Ser Gin Agn GTG TGT GAC AAC ATA ATA CAT TGT GAT TAC CAA GCC GAA CCA GAT CAT AGG CCC AAAT GC Ser Gin Asin Lys Ser Trp Leu Asin Asin 1 He IIe Asp Leu Asin Lys Pro IIe Pro Yr Lys Asin Ser Lys Pro Ser 2 CTT CCC AAA GTG AAT GG CTA ACC ACA ATA ATA CAT TGT GAT TAC GAC GAA GAC G | +1 | ATG | тст | AGG | AAG | TTG | тсc | CAC | стG | ATC | GCT | ACA | AGG | *** | TCA | *** | ACA | АТА | GTC | GTA | ACC | GTA | стт | стт | атт | TAT | 7 |
| THE TIG TIG ACA TIT CAC THE TAC ANA AGE CIG CIT CIC AG TIT TAC COT AGE AAA GAG TAT TIC ANG CAA 1 Ser Leu Leu TIT Phe His Leu Ser Asn Lys Arg Leu Leu Ser Gin Phe Tyr Pro Ser Lys Asp Asp Phe Lys Gin ACT CIT CIC CCT ACG ACT TIC CAT TCA CAG AT ATA ATT TIG AG AAA CAA ATT ACA GIT AAC AGG AM AMA ATA Thr Leu Leu Pro Thr Thr Ser His Ser Gin Asp IIe Asn Leu Lys Lys Gin IIe Thr Val Asn Lys Lys Lys Asn CAA TIG CAT AAT TTA COT GAT CAA TTA TIG ATT AG ATT TIG AG AAA CAA ATT ACC AG GIT ACC GG AA AGG GIT I Gin Leu His Asn Leu Arg Asp Gin Leu Ser Phe Ala Phe Pro Tyr Asp Ser Gin Ala Pro Tile Pro Gin Arg Val I TOG CAG ACC TIG AM CAA TTA CG TIT CIC TIT CIC TAC CAC TIT CAA AAA ACA TIG ATA AGA TAG VAI I TIG GIN TH TTA COT GAT CAA TAT TIC CAT TI GG ATT TIC CAC TIT TO TO CAA ACC TAT CAA AAA ACA TOG TIT I TIT GIN TH TIP Lys Val Giy Ala Asp Asp Lys Asn Phe Pro Ser Ser Phe Arg Thr Tyr Gin Lys Thr Tip Ser I GIT TIG TIT TCA GIG AT CAA TAT TIC CAG AT TITG GAT CAT CIT AT ATA CCT TIT TAA GG CAA ATT TITA CG Giy Ser Tyr Ser Pro Asp Tyr Gin Tyr Ser Leu IIe Ser Asp Asp Ser IIe IIE Pro Phe Leu Giu Asn Leu Tyr I GCA CCC GIT CCG ATA GC AAA TAT TIC TCA GAT ATG GAT ACT CAT TAT ATC CCT TITT AG GCA ATT TITA AGT TIC AAG GGA GIT ATT TAA TCA CAA GAT ATT TITA AGG CAA GAT TIT TAA GO TAC TS Ala Pro Val Pro IIE Val IIE GIN ALA PHE LYS LEU HET PRO GIY ASN IIE LEU LYS ALA ASP PHE LEU Arg Tyr I CTA TAT ATT ATT GCG CAA AGG GIT ATT TAC TCA GAT ATG GAT CAT ATC CAT TICA TAT GAT CAA TAG CCT A ATT A GAT CGA ATAG CAT AAC CAA ATA ATA ATA GAT TIG ATA AGA CCT ATT CAT TA GAT CAA TAG CCT ATT GAT TCA TOG CCT A Ser GIN ASN LYS SER TEP LEU ASN ASN IIE IIE ASP PHE THE ACC CAA ATT ATT GAT TCA TOG CCT A Ser GIN ASN LYS SER TEP LEU ASN ASN IIE IIE ASP THE HEC LEU LEU LYS FIO SIT 2 CTC CTC TCA AGT ATG CAT AAC ATA ATA ATA ATA ATA GAT TIG ATA CAC ATA CCT ATT CAA GAC ATA AGG CTA CAT A Ser GIN ASN LYS SER TEP LEU ASN ASN IIE IIE ASP LEU ASN LYS FOO IIE PHO TYR LSS ASN SER LYS FO SER 2 CTT CTC TCA AGT ATG GAT ATA CAA CAC ATA ATA ATA GAT TIG CAT TAC CAA GCA GAT ATC ATA C | 1 1 | Met | Ser | Arg | Lys | Leu | Ser | His | Leu | Ile | Ala | Thr | Arg | Lys | Ser | Lys | Thr | Ile | Val | Val | Thr | Val | Leu | Leu | Ile | Tyr | 2 |
| Ser Leu Leu The Phe His Leu Ser Asn Lys Arg Leu Leu Ser Gin Phe Tyr Pro Ser Lys Asp Asp Phe Lys Gin ACT CTT CTC CCT ACG ACT TCT CAT TCA CAA GAT ATA AAT TTG AAG AAA CAA ATT ACA GTT ACA GAG AAA AAA AAT 2 Thr Leu Leu Pro Thr Thr Ser His Ser Gin Asp Tie Asn Lieu Lys Lys Gin Tie Thr Val Asn Lys Lys Lys Asn CAA TTG CAT AAT TTA COT CAT CAA TTA TCG TTT GCG TTT CCT TC CAG GCC CCC AT CCG CAA AGG GTG 1 Gin Leu His Asn Leu Arg Asp Gin Leu Ser Phe Ala Phe Pro Tyr Asp Ser Gin Ala Pro Tie Pro Gin Arg Val 1 TGG CAG ACC TCG AAA GTC GC CGC GAT GAT AMG AAT TTT CCC TCT TCG TTC AGA ACC TAT CAA AAA ACA TGG TCT 1 TCP Gin Thr TCP Lys Val Giy Ala Asp Asp Lys Asn Phe Pro Ser Ser Phe Arg Thr Tyr Gin Lys Thr Trp Ser 1 GGT TCG TAT TCA CCG GAT TAC CAA TAT TCT CTG ATT TCG GAT GAT TCT ATT ATC CT TTT TTA GAA ACA TAT CC GIy Ser Tyr Ser Pro Asp Tyr Gin Tyr Ser Leu TIe Ser Asp Asp Ser TIE TIE Pro Phe Leu GIU Asn Leu Tyr 1 GCA CCC GTT CCG ATA GTC AAC CAA GGT TTA AAT GG ATT ACT ATT ATC TAT GAT ACC ATT TTT TTA AGG TAC TS Ala Pro Val Pro 11e Val Tie Gin Ala Phe Lys Leu Het Pro Giy Asn Tie CaA GCA GAT TTT TA TA AGG TAC TS Ala Pro Val Pro 11e Val Tie Gin Ala Phe Lys Leu Het Pro Giy Asn TTC GAA GCA GAT TCT TA AGG TG TY CTC TCA GAA TAG GCA GG GGT ATT AC CAG CAG GT TGG GCT ACT ACT GCT TAT CAG AGC CC TA AGG CTC AG Ser Gin Ann Lys Ser TTP Leu Asn Asn T1e TIE ASP LEU Asn TYS Lys Asn Ser Lys Pro Ser 22 CTC TC TCA AGT GAG CTA AAC AAC ATA ATA GAT TTG GCA TTG CGC ATT GCA GAA ATC CAA AGG CC TAA Fue Leu Ser Ser Asp Gu TIE Ser His GIN Pro GIY LEU Val TE OCH TT GAG CCA ATT CCC AGT GAG GTT GT TTG GAA TGG TAT GCT GTA GAG ATT CCA GC GCG CGT GT GTG TC ACT GGC AAT CCCA AGT CCA ATA CTA AG CCC GAA CGG ATA GCA TAT CAA CAAC ATA ATA GAT TTG GCA TAT CCAA GCC AAA CCAA GCT CAA CAG GAA TTC TA TT PS SER GU TTP TYr ALA ARG ATA TCA CAG CGC GCG GCT ATA CCAA GCC AAA CCCAA GGT ATT CTA TTG AGT GTA AGG ATA TCA CAAC CAG CATA ATA GAT TTG GCAA TAC CAA ACC CAA GCT AAT CTA AGG GAA TTG ATT AAT ATA ACT CCAA GCG ACT TTG GCC ACA AGC CTA AAA CCA TT ATT TTT GAA AAA ATA ATA CAA TT GT CAT AGG ACT ACA | 76 [·] | тст | TTG | TTG | ACA | TTT | CAC | TTG | TCA | AAC | *** | AGG | CTG | CTT | TCT | CAG | TTT | TAC | сст | AGC | *** | GAT | GAT | TTC | AAG | CAA | 15 |
| ACT CTT CTC CT AGG ACT TCT CAT TCA CA GAT ATA AAT TTG AAG AAC AA ATT ACA GTT ACA AG AAA AAA AAT 1 Thr Leu Leu Pro Thr Thr Ser His Ser Gin Asp Tie Asn Leu Lys Lys Gin Tie Thr Val Asn Lys Lys Lys Asn CAA TTG CAT AAT TTA CGT GAT CAA TTA TCG TTT GCG TTT GCC TC CAG GCC CCC ATC CCG CAA AGG GTG 1 Gin Leu His Asn Leu Arg Asp Gin Leu Ser Phe Ala Fhe Fro Tyr Asp Ser Gin Ala Pro Tile Pro Gin Arg Val 1 TGG CAG ACC TGG AAA GTC GGC GCA GAT GAT AAG AAT TTT CCC TCT TCG TTC AGA ACC TAT CAA AAA ACA TGG TCT 1 Trp Gin Thr Trp Lys Val Giy Ala Asp Asp Lys Asn Phe Pro Ser Ser Phe Arg Thr Tyr Gin Lys Thr Trp Ser 1 TGG CAG ACC TGG AAA GTC GGC GCA GAT GAT AAG AAT TTT CCC TCT TCG GTC AGA ACC TAT CAA AAA ACA TGG TCT 1 GT TCG TAT TCA CCG GAT TAC CAA TAT TCT CTG ATT TCG GAT GAT TCT ATT ATC CCT TTT TG GAG AAT CTT TAC GY Ser Tyr Ser Pro Asp Tyr Gin Tyr Ser Leu His Ser Asp Bay Ser Tie Tie Pro Phe Leu Glu Asn Leu Tyr 1 GCA CCC GTT CCG ATA GTC AAA GGG TTT TAA TTG ATG GAT GAT ATT ATC CTA AAG GCA ATT GTT TA AGG TAC CAA Ala Pro Val Pro Tie Val Tie Gin Ala Phe Lys Leu Het Pro Giy Asn Tie Leu Lys Ala Asp Phe Leu Arg Tyr 1 TCT ATTA TTA TTT GCA AGA GGT GAT TTA TCA GAT ATG GAT ACT ATA GTT TTA AGA CTA AG GCC TCA 6 Ser Gin Ann Lys Ser Trp Leu Asn Asn T1 E TIE ASP TAP Het Asp Thr Het Leu Luy Sr Ala Asp Phe Leu Arg Tyr 7 Leu Leu Leu Phe Ala Arg Giy Giy Tie Tyr Ser Asp Het Asp Thr GAC ATT GAT GGA AT CCT ATT GAG AAT CAT AG GCT CAA Ser Gin Ann Lys Ser Trp Leu Asn Asn T1E TIE ASP LEU Asn Lys Pro TIE Pro Tyr Lys Asn Ser Lys Pro Ser 2 CTT CTC ACA GT GAT GAG ATA TCA CAC CAG GCA GTT TG GTC ATC GGC ATT GAG GCA ATC CGA GAC GAT GTT TA Leu Leu Ser Ser Asp Glu TIE Ser His Gin Pro Giy Leu Val TIE Gin Ala Sps Pro Giy Asp | 26 | Ser | Leu | Leu | Thr | Phe | His | Leu | Ser | Asn | Lys | Arg | Leu | Leu | Ser | Gln | Phe | Tyr | Pro | Ser | Lys | Asp | Asp | Phe | Lys | Gln | 5 |
| The Leu Leu Pro The The See Hins See Clin Asp Tile Ash Leu Lys Lys din Tile The Vail Ash Lys Lys Lys Ash CAA TTG CAT AAT TTA COT GAT CAA TTA TCG TTT GCG TTT CCC TAC GAC TCT CAG GCC CCC CAC CCG CAA GGG GTG 1 Gin Leu His Aan Leu Arg Asp Gin Leu See Pre Hall Phe Per Dy Asp See Clin Ala Pre Tile Pro Clin Arg Vail TGG CAG ACC TGG AAA GTC GGC GCA GAT GAT AAG AAT TTT CCC TCT TCG TTC AGA ACC TAT CAA AAA ACA TGG TCT 1 Trp Gin The Trp Lys Val Gly Ala Asp Asp Lys Asn Phe Pro See See Phe Arg The Tyr Gin Lys The Trp See T GGT TCG TAT TCA CCG GAT TAC CAA TAT TCT CTG ATT TCG GAT GAT TCT ATT ATC CCT TTT TG GA GAT ATT TTA G Gly See Tyr See Pro Asp Tyr Gin Tyr See Leu Tile See Asp Asp See Tile Tile Tile Pro Phe Leu Glu Asn Leu Tyr 1 GCA CCC GGT CCG ATA GTC AAC GG TTT AAA TG ATG GCT GGAA AT ATC CTA AMG GAC GAT TTT TA AGG TAC 5 Ala Pro Val Pro Ile Val Tile Gin Ala Phe Lys Leu Het Pro Gly Asn Tile Leu Lys Ala Asp Phe Leu Arg Tyr 1 CTA TTA TTA TTT GCA AGA GGT GGT ATT TAC TCA GAT ATG GAT ACT ATG CTT TGG AMG CAA TT GAT TCA TGA TAGG CCT 6 Leu Leu Leu Phe Ala Arg Gly Gly Tile Tyr See Asp Het Asp Thr Het Leu Lu Lys Pro Tile Asp See Trp Pro 2 TCT CCA GAT AGG CCA GGA GTA ATT AC TCA GAT ATG GAT ACT ATG CTT TTG AMG CAC TAT GAT TCA TAG ACC CCA 6 See Gin Asn Lys See Trp Leu Asn Asn Tile Tile Asp Leu Asn Lys Pro Tile For Tyr Lys Asn See Lys Pro See 2 CTT CTC TCA AGT GAT GAG ATA TCA CAC CCA GGC AGT TG GGC AAT CGA GGC AAC CCA AGA GAT GAT 7 Leu Leu See See Asp Glu Tile See His Gin Pro Gly Leu Val Tile Glu Ala Asp Pro Asp Arg Asp Asp 2 CGT GTA AA TGG ATA GGA ATA TCA CAC CCA GGC ATT TGG GGC ATA TCC AGG CAAC CCA CCA ATT CTA GAA TGG ATT GCT GTA AAG AAC AGC ATA AGT ATT GG TTA GGC ATA TCC AAG GCC AAA CAC GGA CAG CAT CTA AFG Glu Leu Jrp Tyr Ala Arg Arg Tile Gin Phe Cys Gin Trp Thr Tile Gin Ala Lys Fro Gly His Pro Tal See Glu The Tyr Lys His 3 ATG GAA TGG ATA CTA TAA AGA ATA TTA GGA TTA GG CTA AACA CCA AGA CTA AAC ACC 9 File Asp Pro Arg Phe Glu Glu Asp Tyr Asn Val Asn Tyr Arg His Lys Arg Arg His Asp Glu The Tyr Lys His 3 ACC GAA TGG AAA AAA ATA TTA GA GGG | 51 | ACT | СТТ | стс | сст | ACG | ACT | TCT | CAT | TCA | CAA | GAT | ATA | AAT | TTG | AAG | *** | CAA | ATT | ACA | GTT | AAC | AAG | *** | *** | AAT | 22 |
| CAA THG CAT AAT THA CET GAT CAA THA TEG THT GEG THT GEG THT CEC TAE GAC TET CAG GEC CEC AFE GEG AA AGG GTG 1 Gin Leu His Asin Leu Arg Asp Gin Leu Ser Phe Ala Phe Pro Tyr Asp Ser Gin Ala Pro IIe Pro Gin Arg Val 1 TGG CAG ACC TGG AA GTG GGE GGA GAT GAT AG ANT THT CEC TT TG GTG CAG ACC TAT CAA AAA ACA TGG TET 1 TJP GIN THIT TIP LYS Val GIY ALA ASP ASP LYS ASIN PHE PRO SER SER PHE Arg THIT TYF GIN LYS THIT THE SER 1 GGT TCG TAT TCA CCG GAT TAC CAA TAT TCT CTG ATT TCG GAT GAT AT AT CC CT THT TTG GAG AAT CTT TAC GIY Ser TYF SER PRO ASP TYF GIN TYF SER LEU IIE SER ASP ASP SER IIE IIE PRO PHE LEU GIU ASIN LEU TYF 1 GCA CCC GTT CCG ATA GTC ATC CAA GGT THT TAAA TG ATG GCT GGA AAT ATC CTA ATG GCG GAG AT TTT TTA AGG TAC GAN CCC GAT GCC ATA GTC ATC CAA GGT TAT TAC TGA AT GG CT ATA ATA CTA ATG GCG GAG AT TTT TTA AGG TAC GAN CCC GTT CCG ATA GTC ATC CAA CGA TAT TAC TCA GAT ATG GAT ATA CTA TAG GCG AAT TTAT TT | 51 . | rnr | Leu | Leu | Pro | Thr | Thr | ser | HIS | Ser | Gin | Asp | TTe | Asn | Leu | L.YS | Lys | GIN | He | Thr | vai | Asn | Lys | Lys | Lys | ASN | |
| Gin Leu NIS KAIL LEU KUY KAY OLIT LEU SEL THE ALE THE FIG FIT TUT CUT TUT TUT THE THE HE HE HE THE THE FIG TUT TUT GIN THE | 26 | CAA | TTG | CAT | AAT | TTA | CGT | GAT | CAA | TTA | TCG | TTT | GCG | TTT | CCC | TAC | GAC | TCT | CAG | GCC | CCC | ATC | CCG | CAA | AGG | GTG | 30 |
| TGG CAG ACC TGG AAA GTC GGC GCA GAT GAT AAG AAT TTT UCC TGT TGG TTC CAG ACC TAT CAA AAA ACA TGG TCT Trp Gin Thr Trp Lys Val Giy Ala Asp Asp Lys Asn Phe Pro Ser Ser Phe Arg Thr Tyr Gin Lys Thr Trp Ser GGT TGG TAT TCA CAG GAT TAC CAA ATA TCT CTG ATT TGG GAT GAT TCT ATT ATC CCT TTT TGG GA AAT CTT TAC GGT CGG TAT CCG ATT AC CAA GCG TTT AAA TTG ATG GCT GGA AAT ATC CTA AAG GCA GAT TTT TTA AGG TAC Ser Ser Tyr Ser Fro Asp Tyr Gin Tyr Ser Leu Ile Ser Asp Asp Ser Ile Ile Pro Phe Leu Glu Asn Leu Tyr GCA CCC GTT CCG ATA GTC ATC CAA GCG TTT AAA TTG ATG CCT GGA AAT ATC CTA AAG GCA GAT TTT TTA AGG TAC Ala Pro Val Pro Ile Val Ile Gin Ala Phe Lys Leu Met Pro Giy Asn Ile Leu Lys Ala Asp Phe Leu Arg Tyr CTA TTA TTA TTT GCA AGA GGT GGT ATT TAC TCA GAT ATG GAT ACT ATG CTT TTG AAG CCA ATT GAT TCA TGG CCT C Leu Leu Leu Phe Ala Arg Giy Giy Ile Tyr Ser Asp Met Asp Thr Met Leu Leu Lys Pro Ile Asp Ser Trp Pro 2 CTC CAG AAT AAG TCA TGG CTA ACA AAC ATA ATA GAT TTG GAT ATA GAT CCT TAT AAG AAC TCA AAG CCC CTCA Ser Gin Asn Lys Ser Trp Leu Asn Asn Ile Ile Asp Leu Asn Lys Pro Ile Pro Tyr Lys Asn Ser Lys Pro Ser 2 CTT CTC AA GT GAT GAG ATA TCA CAC CAG CCA GGT TTG GTC ATC GGC ATT GAG GCA GAT CCG GAC AGA GAT GAT 1 Leu Leu Ser Ser Asp Glu Ile Ser His Gin Pro Giy Leu Val Ile Giy Ile Glu Ala Asp Pro Asp Arg Asp Asp 2 CTG CAA TG GAT GAT GAT GCT AGG ATC CAG CTT TGG GC GAT ACC AAG CCA GGT ACC CCA ATT CTA 8 AFG Glu GAT TGA TCT TTA ATT ACT GCA ACG ATA TGG GCA GAT ACC AAG CCA GGT ACC CCA ATT CTA 8 ATT GAT CCA AGG TAT GAT CCT CGA AGG ACT CCAG GTT TGG GG GAT ACC AAG CCA GGT ACC AAT CTA C 8 ATT GAT CCA AGA TTO ATC TTA ATT ACT GCA CGA CTA GA AAC ATT GG GAC CTA AAG ACC GG GAT CTG GT AGT AAA AGA 4 7 7 7 7 7 7 7 7 7 7 7 7 7 | /0 1 | GIN | Leu | HIS | Ash | Leu | Arg | ASD | GIN | Leu | Ser | Phe | Ald | Phe | PIO | TÀI | Asp | ser | GIN | Ald | PIO | ne | PIO | GIN | AIG | vai | 10 |
| GGT TCG TAT TCA CCG GAT TAC CAA TAT TCT CTG ATT TCG GAT GAT TCT ATT ATC CTT TT TG GAG GAT CTT TAT GIJ SET TY SEF PTO AND TYP SET LEW LIE SEF ASP ASP SET LIE LIE PTO PHE LEW GU AND LEW TYP GCA CCC GTT CCG ATA GTC ATC CAA GCG TTT AAA TTG ATG CCT GGA AAT ATC CTA AAG GCA GAT TTT TTA AGG TAC 5 Alla PTO VAL PTO LIE VAL LIE GIN ALLA PHE LYS LEW MET PTO GJY ASN LIE LEW LYS ALLA SAP PHE LEW ATG TYP 1 CTA TTA TTA TTT GCA AGA GGT GGT ATT TAC TCA GAT ATG GAT ACT ATG CTT TTG AAG CCA ATT GAT TCA TGG CTC LEW LEW LEW PHE ALLA ATG GLY GGT ATT TAC TCA GAT ATG GAT ACT ATG CTT TTG AAG CCA ATT GAT TCA TGG CCT CTA GTA AAG TCA TGG CTA AAC AAC ATA ATA GAT TTG AAT AAA CCT ATG CTT TTG AAG AAC TCA AAG CCC CTC CCT CCA GAT AAG TCA TGG CTA AAC AAC ATA ATA GAT TTG AAT AAA CCT ATT CCT TAT AGA AC TCA AAG CCC TCA SET GIN ARE LYS SET TUP LEW ASN ASN LIE LIE ASP LEW ASN LYS PTO LIE PTO TYL LYS ASN SET LYS PTO SET CTT CTC AAG TAT GAT GAG ATA TCA CAC CAG CCA GGT TTG GTC ATC GAG ATT GAG GCA GAT CCG GAC AGA GAT GAT LEW LEW SET SET ASP GLU LIE SET HIS GIN PTO GJY LEW VAL LIE GJY LIE GU ALLA SP PTO ASP ATG ASP ASP CTT CTC AA GT GAT GAC TAT GCC CAG CAG CTA TTG CAA TGG ACT ATC CAA GGC CAAA CCA GGT CAC CCA ATT CTA TEP SET GLU TEP TYP ALLA ATG CAA CGA ACT TTG GCG GAC GTA ACCAA GGC AAA CCA GGT CAC CCA ATT CTA TTG AGT GAA TGG TAT GCT CGT AGG ATC CAG TTT GGC GAC GTA TCC AAG CAA GGA GAT CCT GTC AGT AGA AGC TTG GAG GAT TTG ATT AAT ATT ACT GCA ACG ACT TTG GCG GCC GTA AAA ACA CCA GGT CTC GT AGT AGA ATT GAT CCA AGA ATT ATT ACT GCA ACG ACT TTG GCG GCA CTA AAA ACA CCA GGT CTC AGT AGA ACC AFG GLU LEU LIE LEW ASN LIE THA ALL THA CTA GCA ACC TTA GGC ACA AAA CAC CT AGG GGT CTC AGT AGA ACC AC ACG AAT TCC AAA AAT AAC AAA AAT GTT GAT GAG CTC AGT ATA ATG GAT TCG TAT ATG AGG ACT TAC TAAC AAC ACA IIE ASP PTO ATG PHE GLU GLU ASP TYP ASN VAL ASN TYP ATG HIS LYS ATG HIS ASP GLU THT TYP LYS HIS 3 TCC GAA TTC AAAA AAT AAC AAA AAT GTT GAT GAT CCA GAT ACT AAC GAA TGC AGG AATT TTC TCT ATT AAC CCA AGA CTA CTA ACT AAG GAA GTA GT GGA GAG CAC CCA GAT ACT AAC GAAT ACT ATG GG | 01 . | TGG | CAG | ACC | TGG | AAA | GTC | GGC | GCA | GAT | GAT | AAG | AAT | TTT | CCC | TCT | TCG | TTC | AGA | ACC | TAT | CAA | AAA | ACA | TGG | TCT | 37 |
| GGT TCG TAT TCA CCG GAT TAC CAA TAT TCT CTG ATT TCG GAT GAT TCT ATT ATC CCT TTT TTG GAG AAT CTT TAC 4 Gly Ser Tyr Ser Pro Asp Tyr Gln Tyr Ser Leu Ile Ser Asp Asp Ser Ile Ile Pro Phe Leu Glu Asn Leu Tyr 1 GCA CCC GTT CCG ATA GTC ATC CAA GCG TTT AAA TTG ATG GCT GGA ATA TAC CTA AAG GCA GAT TTT TA AGG TAC 5 Ala Pro Val Pro Ile Val Ile Gln Ala Phe Lys Leu Het Pro Gly Asn Ile Leu Lys Ala Asp Phe Leu Arg Tyr 1 CTA TTA TTA TTT GCA AGA GGT GGT ATT TAC TCA GAT ATG GAT ACT ATG CTT TTG AAG CCA ATT GAT TCA TGG GCT Leu Leu Phe Ala Arg Gly Gly Ile Tyr Ser Asp Met Asp Thr Het Leu Leu Lys Pro Ile Asp Ser Trp Pro 2 TCT CAG AAT AAG TCA TGG CTA AAC AAC ATA ATA GAT TTG AAT AAA CCT ATT CCT TAT AAG AAC TCA AAG CCC TCA 6 Ser Gln Asn Lys Ser Trp Leu Asn Asn Ile Ile Asp Leu Asn Lys Pro Ile Pro Tyr Lys Asn Ser Lys Pro Ser 2 CTT CTC ACA GAT GAG ATA TCA CAC CA CCA GCT GTG GTC ATC GGC ATT GAG GCA GAT CCG GAC AGA GAT GAT 7 Leu Leu Ser Ser Asp Glu Ile Ser His Gln Pro Gly Leu Val Ile Gly Ile Glu Ala Asp Pro Asp Arg Asp Asp 2 TGG AGT GAA TGG ATA GCT GGT AGG ATC CAG TT TG GCG ACT CAG GCC AAT CCA AGC CAAT CCA AGT CAT 7 Trp Ser Glu Trp Tyr Ala Arg Arg Ile Gln Phe Cys Gln Trp Thr Ile Gln Ala Lys Pro Gly His Pro Ile Leu 2 AGA GAA TTG ATC TTA AAT ATT ACT GCA ACG TAC TAT GGC GAC AAA ACC CCA GGG TTCC GTC AGT GAA ATG 9 Arg Glu Leu Ile Leu Asn Ile Thr Ala Thr Thr Leu Ala Ser Val Gln Asn Pro Gly Val Pro Val Ser Glu Met 3 ATT GAT CCA AGA TTT GAA GAA CAC TAC GAC TA AGT TAG GCA CAA AAC CCA GGA GTT CCT GTC AGT GAA ATG 9 Ile Asp Pro Arg Phe Glu Glu Asp Tyr Asn Val Asn Tyr Arg His Lys Arg Arg His Asp Glu Thr Tyr Lys His 3 TCC GAA TTG ANA AAT AAC AAA ATT GTG CCC CCA CAA AAT TAA TG AAT GG ACG CAC GAC ATT TCT TCC CG AL 10 Luy Asn Asn Lya Asn Cya Asp Gly Ser Asp Ile Met Asn Trp Thr Gly Pro Gly Ile Phe Ser Asp 3 ATT ATT TTC GAA TAC ATG GAA CAT GGT CCC CCA CAC ACA ATA ATG AAT TGA TGC GCC AGGT ATT TCT TCT TTT TTT TA AAG AAAT ACA AAA ATT GTA GCC TAC CAA ATG ATT GTA GCC CCA GAT ATT TCT TCT CT TTT TTG TTT TAT AGA GAA ATG GT GGA CCC ACA CAC CAA AAT GTG CCC CCA CCA CAC AAA | | | 0111 | | | Lys | • 4 1 | 017 | ~10 | ~3¥ | | 2,3 | | | | | 561 | | | •••• | .,. | 0111 | .,,, | •••• | | 501 | |
| GCA CC GTT CCG ATA GTC ATC CAA GCG TTT AAA TTG ATG CCT GGA AAT ATC CTA AAG GCA GAT TTT TTA AGG TAC AIA PTO VAI PTO IL VAI ILE GIN ALA PHE LAYS LEU MET PTO GIY ASN ILE LEU LYS AIA ASD PHE LEU ATG TYT CTA TTA TTA TTT GCA AGA GGT GGT ATT TAC TCA GAT ATG GAT ATG GAT ATG CTT TTG AAG CCA ATT GAT TCA TGG GT ATT TAC TCA GAT ATG GAT ATG ATT GAT GCT ATT ATA TTA TTG GA GAG GGT ACT AAC AAC ATA ATA GAT TTG AAT AAG CCA ATT GAT TGA TAG ACT CA AAG CCT CA LEU LEU LEU PHE ALA ATG GIY GIT ATA TA CAA ATA ATA GAT TTG AAT AAA CCT ATT CCT TAT AAG AAC TCA AAG CCC TCA SET GIN ASN LYS SET TFP LEU ASN ASN ILE ILE ASP LEU ASN LYS PTO ILE PTO TYT LYS ASN SET LYS PTO SET CTT CTC TC AAGT GAT GAG ATA TCA CAC CAG CCA GGT TTG GTC ATC GGC ATT GAG GCA GAT CCG GAC AGA GAT GAT LEU LEU SET SET ASP GIU ILE SET HIS GIN PTO GIY LEU VAI ILE GIY ILE GIU ALA ASP PTO ASP ATG ASP ASP 2 TGG AGT GAA TGG TAT GCT CGT AGG ATC CAG TTT GTC CA TCG ACC ATT CAA GCC AAA CCA GGT CAC CCA ATT CTA 8 TTG SET GIU TCP TYT ALA ATG ATG ILE GIN PHE CYS GIN TTP TTT ILE GIN ALA LYS FTO GIY HIS PTO ILE LEU 2 AGA GAA TTG ATC TTA AAT ATT ACT GCA ACG ATA TTA GG CAC GAT CAA GCC GAG GTT CCT GCA GTG GAA ATG 9 ATG GIU LEU ILE LEU ASN TIT GTA ACG ACT TTG GCG ACC GTA CAA AAC CCA GGA GTT CCT GCA GAT GAA AGG 4 ATT GAT CCA AGA TTT GAA GAA GCC TAC AAC GTA AAC TAT AGG CAC AAA AAC CCA GGA GTT CTT GCA AGC TAC AAAA CCA GA ATT TTT TTC CCA AGA GAT TTA GTA GAA ACC TAA AAA CCA AAA CCA GA GAT TTG GAA GAA GAT GTA TTG ATG GAA GAC TAC AAC GTA AAC TTA AGG CAC TAC AAAA CCA AAA CCA GA GAT TTT GAA GAA GAT TTG ATT GAA GAA | 76 26 | GGT Glv | TCG Ser | TAT TVr | TCA Ser | CCG Pro | GAT | TAC Tvr | CAA Gln | TAT Tvr | TCT Ser | CTG Leu | ATT | TCG Ser | GAT | GAT | TCT Ser | ATT | ATC Ile | CCT Pro | TTT Phe | TTG Leu | GAG Glu | AAT Asn | CTT Leu | TAC TVI | 45 |
| GCA CCC GTT CCG ATA GTC ATC CAA GCG TTT AAA TTG ATG CTG GGA AAT ATC CTA AAG GCA GAT TTT TTA AGG TAC 5 Ala Pro Val Pro Ile Val IIE GIn Ala Phe Lys Leu Met Pro Gly Asn Tie Leu Lys Ala Asp Phe Leu Arg Tyr 1 CTA TTA TTA TTT GCA AGA GGT GGT ATT TAC TCA GAT ATG GAT ACT ATG GTT TTG AAG CCA ATT GAT TCA TOG CTT 6 Leu Leu Leu Phe Ala Arg Gly Gly Ile Tyr Ser Asp Met Asp Thr Met Leu Leu Lys Pro Ile Asp Ser Trp Pro 2 TCT CAG AAT AAG TCA TGG CTA AAC AAC ATA ATA GAT TTG AAT AAA CCT ATT CCT TAT AAG AAC TCA AAG CCC TCA 6 Ser Gln Asn Lys Ser Trp Leu Asn Asn Ile Ile Asp Leu Asn Lys Pro Ile Pro Tyr Lys Asn Ser Lys Pro Ser 2 CTT CTC TCA AGT GAG ATA TCA CAC CAG CCA GGT TTG GTC ATC GGC ATT GAG GCA GAT CCG GAC AGA GAT GAT 7 Leu Leu Ser Ser Asp Glu Ile Ser His Gln Pro Gly Leu Val Ile Gly Ile Glu Ala Asp Pro Asp Arg Asp Asp 2 TGG AGT GAA TGG TAT GCT CGT AGG ATC CAG TTT GT CA AGG ACT ATC CAA GGC CAA CCA GGT CAC CCA ATT CTA 8 Trp Ser Glu Trp Tyr Ala Arg Arg Ile Gln Phe Cys Gln Trp Thr Ile Gln Ala Lys Fro Gly His Pro Ile Leu 2 AGA GAA TTG ATC TTA AAT ATT ACT GCA AGG ACT TTG GCG AGC GAA AGA GAT CCT GCT GA GT GAA TGG TAT GAT CAA GAC TAC AAC GTA AAC TAT AGG CAC AAA AGC CGT CAT GAT GAG ACC TAC AAA CAC 9 Arg Glu Leu Ile Leu Asn Ile Thr Ala Thr Thr Leu Ala Ser Val Gln Asn Pro Gly Val Pro Val Ser Glu Thr 7 Jr Is Asn ATT ACT GCA AGA CTA CAC GTA AAT ATA AGG CAC AAA AGC CGT CAT GAT GAG ACC TA AAA CAC 9 Ile Asp Pro Arg Phe Glu Glu Asp Tyr Asn Val Asn Tyr Arg His Lys Arg Arg His Asp Glu Thr Tyr Lys His 3 TCC GAA TTG AAA AAT ATT ACT GCT GCT CGA TAC GCT CA GAT ATT ATA AAT TAT TC GCA AGA CTA AAA CAC 7 Ser Glu Leu Lys Asn Asn Lys Asn Val Asp Gly Ser Asp Ile Met Asn Trp Thr Gly Pro Gly Ile Phe Ser Asp 3 ATT ATT TTC GAA TAC ATG AAC AAT GTG GTC CGA TAC AAT AGC GAT ATC TTG TTA ATC AAC CCA AAC CTA AAC AAG 11 Ile Ile Phe Glu Tyr Met Asn AXT GTG GTC CCA ACA CAC ACA ACA ACA CTA AAC AAG GT GT GAA GAG GAT AGT GAG AGT GGT G | | | | .,. | | | | | | | | | | | | | | | | | | | | | | • | |
| CTA TTA TTA TTT GCA AGA GGT GGT ATT TAC TCA GAT ATG GAT ACT ATG CTA TG CA AGA CCA ATT GAT TCA TGG CCT 6 Leu Leu Phe Ala Arg Gly Gly Ile Tyr Ser Asp Met Asp Thr Het Leu Leu Lys Pro Ile Asp Ser Trp Pro 2 TCT CAG AAT AAG TCA TGG CTA AAC AAC ATA ATA GAT TTG AAT AAA CCT ATT CCT TAT AAG AAC TCA AAG CCC TCA 6 Ser Gln Asn Lys Ser Trp Leu Asn Asn Ile Ile Asp Leu Asn Lys Pro Ile Pro Tyr Lys Asn Ser Lys Pro Ser 2 CTT CTC TCA AGT GAG ATA TCA CAC CAG CCA GGT TTG GTC ATC GGC ATT GAG CA ATT CAG AGA GAT GAT T Leu Leu Ser Ser Asp Glu Ile Ser His Gln Pro Gly Leu Val Ile Gly Ile Glu Ala Asp Pro Asp Arg Asp Asp 2 TGG AGT GAA TGG TAT GCT CGT AGG ATC CAG TTT GTC CATC GGC ATT CCA AGC CAA ACCA GGT CAC CCA ATT CTA 8 Trp Ser Glu Trp Tyr Ala Arg Arg Ile Gln Phe Cys Gln Trp Thr Ile Gln Ala Lys Pro Gly His Pro Ile Leu 2 AGA GAA TTG ATC TTA AAT ATT ACT GCA AGG ACT TTG GCG AGC GAC CAA AAC CCA GGT CAC GCA ATT CTA 8 Arg Glu Leu Ile Leu Asn Ile Thr Ala Thr Thr Leu Ala Ser Val Gln Asn Pro Gly Val Pro Val Ser Glu Met 3 ATT GAT CCA AGA TTT GAA GAA GAA TAA CG TA AAC TAT AGG CAC AAA AGA CGT CAT GAT GAG ACC TAC AAA CAC 9 Ile Asp Pro Arg Phe Glu Glu Asp Tyr Asn Val Asn Tyr Arg His Lys Arg Arg His Asp Glu Thr Tyr Lys Asn Asn Lys Asn Val Asn Tyr Arg His Lys Arg Arg His Asp Glu Thr Tyr Lys Asn Asn Cro GAA TTG CCA AGC TAA AAC ATT GAT GAA CAA ATT GT GA TGA GAA CAA ATT ATA TAT ATT ATT TTC GAA TAG AAT GTG GC CCA GAT ATT ATT ATT TTC GAA TAC AAT GTG GTC CGA TAC AAT AAC ATT TTG TAC AAC CCA AAA CAC CA ASP Phe Glu Glu Asp Tyr Asn Val Asp Gly Ser Asp Ile Met Asn Trp Thr Gly Pro Gly Ile Phe Ser Asp 3 ATT ATT TTC GAA TAC ATG AACT AAT GTG CTC CGA TAC AAT AGC GAT ATT ATC AAC CCA AAC CTA AAC AAG 11 Ile Ile Phe Glu Tyr Met Asn Asn Val Leu Arg Tyr Asn Ser Asp Ile Leu Leu Ile Asn Pro Asn Leu Asn Lys 3 AAC GAC GAA GAA GAT GAT GAG GAC GCC ACC ACC ACC ACA CAC ACA ATT CA ATG ACG CTG GAA TTC TTC TCT TTT TTG 12 Asn Asp Glu Glu Gly Ser Glu Ser Ala Thr Thr Pro Ala Lys Asp Val Asp Asp Thr Leu Ser Lys Ser Thr 4 AGA AAA TTT TAT AMG AAA ATA TCA GAG GTC TTT GCA ATG CTA CAAT TCA ATG AC | 51 · 51 · | GCA Ala | CCC Pro | GTT Val | CCG Pro | ATA Ile | GTC Val | ATC Ile | CAA Gln | GCG Ala | TTT Phe | AAA Lys | TTG Leu | ATG Met | CCT Pro | GGA Gly | AAT Asn | ATC Ile | CTA Leu | AAG Lys | GCA Ala | GAT Asp | TTT Phe | TTA Leu | AGG Arg | TAC Tyr | 52 |
| CTA TTA TTA TTA GCA AGA GGT GGT ATT TAC TCA GAT ANG GAT ANG GAT ANG GAT ANG GAT TIG ANG GCA ATT GAT TCA TUG CCT Leu Leu Phe Ala Arg Gly Gly Ile Tyr Ser Asp Met Asp Thr Met Leu Leu Lys Pro Ile Asp Ser Trp Pro 2 TCT CAG AAT AMG TCA TGG CTA AAC AAC ATA ATA GAT TTG AAT AMA CCT ATT CCT TAT AMG AAC TCA AMG GCC TCA 6 Ser Gln Asn Lys Ser Trp Leu Asn Asn Ile Ile Asp Leu Asn Lys Pro Ile Pro Tyr Lys Asn Ser Lys Pro Ser 2 CTT CTC TCA AGT GAT GAG ATA TCA CAC CAG CCA GGT TTG GTC ATC GGC ATT GAG GCA GAT CCG GAC AGA GAT GAT T Leu Leu Ser Ser Asp Glu Ile Ser His Gln Pro Gly Leu Val Ile Gly Ile Glu Ala Asp Pro Asp Arg Asp Asp 2 TGG AGT GAA TGG TAT GCT CGT AGG ATC CAG TTT TGT CAA TGG ACT ATC CAA GCC AAA CCA GGT CAC CCA ATT CTA 8 Trp Ser Glu Trp Tyr Ala Arg Arg Ile Gln Phe Cys Gln Trp Thr Ile Gln Ala Lys Pro Gly His Pro Ile Leu 2 AGA GAA TTG ATC TTA AAT ATT ACT GCA AGG ACT TTG GCG AGC GTA CAA AAC CCA GGA CAT CCT GCT AGT GAA TAG 9 Arg Glu Leu Ile Leu Asn Ile Thr Ala Thr Thr Leu Ala Ser Val Gln Asn Pro Jy Val Pro Val Ser Glu Met 3 ATT GAT CCA AGA TTT GAA GAA GAC TAC AAC GTA AAC TAT AGG CAC AAA AGA CGT CAT GAT GAG ACC TAC AAA CAC 9 Ile Asp Pro Arg Phe Glu Glu Asp Tyr Asn Val Asn Tyr Arg His Lys Arg Arg His Asp Glu Thr Tyr Lys His 3 TCC GAA TTG AATC ATG AGA ACA TGT GAT GGC TCA GAT ATA ATG AAT TGG ACG GGT CCA GGT ATT TTC TCC GCA 10 Ser Glu Leu Lys Asn Asn Lys Asn Val Asp Gly Ser Asp Ile Met Asn Trp Thr Gly Pro Gly Ile Phe Ser Asp 3 ATT ATT TTC GAA TAC ATG AGA GAT GCC CAC ACC ACA CAA ACA CAC CAA ACC CAA ACC CAAC CTA AAC AAT GTG CTC GAA TGA TT TAC GCC AGA AGA GAT GCT GTG TA ATC ACA 12 Asn Asp Glu Glu Gly Ser Ala Thr Thr Pro Ala Lys Asp Val Asp Asp Asp Tr Asn Ser Asp Thr Leu Ser Lys Ser Thr 4 AGA AAA TTT TAT AMG AAA ATA TCA GAG GTC CCA CAC ACC ACA CAA AGA GAT GTT GAT AACG GAT ACG CTG GAA TCC ACA CAA 12 Asn Asp Glu Glu Gly Ser Glu Ser Ala Thr Thr Pro Ala Lys Asp Val Asp Asp Asp Thr Leu Ser Lys Ser Thr 4 AGA AAA TTT TAT AMG AAA ATA TCA GAG GTC TTT GCA ACT ACT AAT CAA AGA GTT GTG GGG CAG ATTC TTC TCT TTT TTT G1 Lys Glu Fro Val Ile Val Asp As | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TCT CAG AAT AAG TCA TOG CTA AAC AAC ATA ATA GAT TTG AAT AAA CCT ATT CCT TAT AAG AAC TCA AAG CCC TCA Ser Gln Asn Lys Ser Trp Leu Asn asn lle Ile Asp Leu Asn Lys Pro Ile Pro Tyr Lys Asn Ser Lys Pro Ser 2 2 CTT CTC TCA AGT GAG ATA TCA CAC CAG CCA GG CAG GTT TG GTC ATC GGC ATT GAG CAG AGT CCG GAC AGA GAT GAT T Leu Leu Ser Ser Asp Glu Ile Ser His Gln Pro Gly Leu Val Ile Gly Ile Glu Ala Asp Pro Asp Arg Asp Asp 2 TGG AGT GAA TGG TAT GCT CGT AGG ATC CAG TTT GTC CAA TGG ACT ATC CAA GCC AAA CCA GGT CAC CCA ATT CTA TTP Ser Glu Trp Tyr Ala Arg Arg Ile Gln Phe Cys Gln Trp Thr Ile Gln Ala Lys Pro Gly His Pro Ile Leu 2 AGA GAT TG ATC GAA AGT CTA TAT CTG GAA GAG GAT TG ATC GAG AGT TG ATT GAT CAA AGA GAT TTA TT ACT GCA AGG ACT TG GCG AGC GAA CAA AGA CCA GGT CAC CCA ATT CTA TA GAT GAT TT AAT ATT ACT GCA AGG ACT TTG GCG AGC GAA AGA CCA GGT CAT GAT GAG GAA TGG ATT GAT GAA GAA GAT TTA ATT ACT GCA AGG ACT TAT ATT AGG CAC AAA AGA CGT CAT GAT GAG ACC TAC AAA CAA AGA GT CTA AAA TT A ATT ACT GCA AGG GA CTTA CAG GCA CAA AGA CGT CAT GAT GAG ACC TAA AAA CAC 9 ILe Asp Pro Arg Phe Glu Glu Asp Tyr Asn Val Asn Tyr Arg His Lys Arg Arg His Asp Glu Thr Tyr Lys His 3 TCC GAA TTG AAG AGA TGT GAT GTG GAT CTG GAT ATG GAT GG CAA AAA GAT GGT CAT GAT GAG ACC TA AAC CAA AGA GAT GTT TTC TCC GAA TG ATG ATT GGA CG GAT ATT TTC TCC GAC 10 Ser Glu Leu Lys Asn Asn Lys Asn Val Asn TGA GGC TCA GAT ATA ATG AAT TGG ACG GGT CCA GGT ATT TTC CC GAC 11 Ile Phe Glu Tyr Het Asn Asn Val Leu Arg Tyr Asn Ser Asp Ile Het Asn Tro Anc CCA AAA CCA AAG CTA ACC AAC CTA AAC AAT GTG GC CAA CAA CAAA GAT GTT GAT AACG GAT AGG AGA GAT GGT AGT GGA GAT GCC ACC ACA CAA CAA CAA AGA GAT GTT GAT AACG GAT ACG CTG GAA ATCA ACA 12 Asn Asp Glu Glu Gly Ser Ala TTr Thr Pro Ala Lys Asp Val Asp Asn Asp Thr Leu Ser Lys Ser Thr 4 AGA AAA TTT TAT AGG AAA ATT CA ACG ACT ACA CAA CCA AAA GAT GTT GAT AAC GAT ACG CTG GAA ATCA ACA 12 Asn Asp Glu Glu Gly Ser Ala Thr Thr Pro Ala Lys Asp Val Asp Asn Asp Thr Leu Ser Lys Ser Thr 4 AGA AAA TTT TAT AGG AAA ATT CA CGA CTA CAA CAA CCA ATT CAT CTT CTT TTT TT | 26 76 | CTA Leu | TTA Leu | TTA Leu | Phe | GCA Ala | AGA Arg | GGT Gly | GGT Gly | Ile | TAC Tyr | TCA Ser | GAT Asp | ATG Met | GAT Asp | ACT Thr | ATG Met | Leu | TTG Leu | AAG Lys | Pro | Ile | Asp | Ser | TGG Trp | Pro | 20 |
| Ser Gin Ani Awa Tex hoc Lik Awa Awa Ani Alk Awa Hi Alk Awa Ti Tib Ahi Awa Cer Lik Lik Awa Cer Lik Lik Awa Cer Lik | | . | ~ • • | | | ~ `` | moo | | | | | | | - | | | | | | | | | ** * | | | ** ** | 67 |
| CTT CC AGT GAT GAG ATA TCA CAC CAG CCA GGT TTG GTC ATC GGC ATT GAG GCA GAT CCG GAC AGA CAT GAT T Leu Leu Ser Ser Asp Glu II e Ser His Gln Pro Gly Leu Val II e Gly II e Glu Ala Asp Pro Asp Arg Asp Asp TGG AGT GAA TGG TAT GCT CGT AGG ATC CAG TTT TGT CAA TGG ACT ATC CAA GCC AAA CCA GGT CAC CCA ATT CTA Trp Ser Glu Trp Tyr Ala Arg Arg II e Gln Phe Cys Gln Trp Thr II e Gln Ala Lys Pro Gly His Pro II e Leu AGA GAA TTG ATC TTA AAT ATT ACT GCA ACG ACT TTG GCG ACC GTA CAA AAC CCA GGT CAC CCA ATT CTA Trp Ser Glu Leu II e Leu Asn II e Thr Ala Thr Thr Leu Ala Ser Val Gln Asn Pro Gly Val Pro Val Ser Glu Met 3 ATT GAT CCA AGA TTT GAA GAA GAC TAC AC GTA AAC TAT AGG CAC AAA AGC CTA GAT GAG ACC TAC AAA CAC 11 e Asp Pro Arg Phe Glu Glu Asp Tyr Asn Val Asn Tyr Arg His Lys Arg Arg His Asp Glu Thr Tyr Lys His 3 TCC GAA TTG AAA AAT AAC AAA ATT GT GCA GC CCA GAT ATA ATG AAT TGG ACG GGT CCA GGT ATT TTC TCC GCC 10 Leu Lys Asn Asn Axc AAA AAT GTT GAT GGC TC GAT ATA ATG AAT TGG ACG GGT CCA GGT ATT TTC TCC GCC 11 e Phe Glu Tyr Het Asn Ash N'G I Asg GIY Ser Asp II e Het Asn Trp Thr GIY Pro GIY II e Phe Ser Asp 1 Het Asn Asn Tyr Asg N's Ser Asp II e Leu Leu II e Asn Pro Asn Leu Asn Lys 3 ATT ATT TTC GAA TGA GAG AGT GCC ACC ACC CAC CCA CCA ACA GT ATA ATG AAT CGG ATA CGC AGG TAT CTA ACC AAC CTA AAC AAB 11 e 11 e Phe Glu Tyr Het Asn Asn Val Leu Arg Tyr Asn Ser Asp II e Leu Leu II e Asn Pro Asn Leu Asn Lys 3 ACC GAC GAA GAA GAT GAT GAG AGT GCC ACC ACA CAC ACA CAA AAG GAT ACG CTG GAA TTC ATC TTC TTT TTC TTT TTT TTT TAT AAG AAA ATA TCA GAG TCT TTG CCA AAT CCA AAT CA ACA 12 Asn Asp Glu Glu Gly Ser Glu Ser Ala Thr Thr Pro Ala Lys Asp Val Asp Asn Asp Thr Leu Ser Lys Ser Thr 4 AGA AAA TTT TAT AAG AAA ATA TCA GAG GTT CTT GCA ATG GTC AAAT TCA ATG ACG GCA GAA GAT GTG GGG CAG ATG GCC GA ATG GCG GAA GTA ATC ATG ATG GAT GAT GAT GTT GTA TA CAA GAT TTT TCC CAC GAA TGG GGG CAG ATG GGC GAA TG GGG CAG ATG GGG GAA GTT CTTC TCT TTT TTT 4 AGA AAA TTT TAT AAG AAA ATA TCA GAG GTT CTT CCA ATA ACG AAT TCA A | 01 | Ser | Gln | Asn | Lys | Ser | Trp | Leu | Asn | Asn | Ile | Ile | Asp | Leu | Asn | Lys | Pro | Ile | Pro | Tyr | Lys | Asn | Ser | Lys | Pro | Ser | 22 |
| CIT CLC LCA LGA WALL LGA CAC CAG CCA GC CAG CA AT CA ATC GAG CAT CAA GCC ATC CCA AAC CTA AAC AAC II Leu Leu Ser Ser Asg Glu II ESEr Glu Ser Leu GLI Ser Val CLC GUI II Glu Alla Asp Pro Asg Para Asp Asg Asg Para TGG AGT GAA TGG TAT GCT CGT AGG ATC CAG TTT TGT CAA TGG ACT ATC CAA GCC AAA CCA GGT CAC CCA ATT CTA E Trp Ser Glu Trp Tyr Alla Arg Arg II E GIN Phe Cys GIN Trp Thr II E GIN Alla Lys Pro Gly His Pro II E Leu 2 AGA GAA TTG ATC TTA AAT ATT ACT GCA ACG ACT TTG GCG AGC GTA CAA AAC CCA GGA GTT CCT GTC AGT GAA ATG S Arg Glu Leu II E Leu Asg II E Thr Alla Thr Thr Leu Alla Ser Val GIN Asg Pro Val Ser Glu Het 1 ATT GAT CCA AGA TTT GAA GAA GAC TAC AAC GTA AAC TAT AGG CAC AAA AAC CCA GGA GTT CCT GTC AGT GAA ATG S II E Asg Pro Arg Phe Glu Glu Asg Tyr Asg Val Asg Tyr Arg His Lys Arg Arg His Asg Glu Thr Tyr Lys His 3 TCC GAA TTG AAA AAT AAC AAA AAT GTT GAT GGC TCA GAT ATA ATG AAT TGG ACG GGT CCA GGT ATT TTC TCC GAC 10 Ser Glu Leu Lys Asg Asg Val Asg Gly Ser Asg II E Het Asg Trp Thr Gly Pro Gly II E Phe Ser Asg 3 ATT ATT TTC GAA TAC AGG AAC AAT GTG CTC CGA TATA ATG AAT TGG ATA CAC GCG GT CCA AAC CTA AAC CAS 4 II Ie ILEU Lys Asg Asg AGT GCC ACC ACA CAA CAA AGC GAT ATA TC AAC GCA TACC CAAAA CCA AAA CAA CAA AGA TTT TTC TCC GAA TGA GTG CCC GAAT ATG AAT GTG ATT GAT ACA CAA CAA CAA AGA ATT TT TAC GAA TAC AGG AGT AGC GAC CAAC CA | 76 | ~~~~ | ~ | • | | ~~~ | ~ • • | | ~ | | ~ ~ | | . | - | . | | | | ~* ~ | | ~ • • | | C1 C | | | | 76 |
| TGG AGT GAA TGG TAT GCT CGT AGG ATC CAG TTT TGT CAA TGG ACT ATC CAA GCC AAA CCA GGT CAC CCA ATT CTA Trp Ser Glu Trp Tyr Ala Arg Arg Ile Gln Phe Cys Gln Trp Thr Ile Gln Ala Lys Pro Gly His Pro Ile Leu AGA GAA TTG ATC TTA AAT ATT ACT GCA ACG ACT TTG GCG AGC GTA CAA AAC CCA GGA GTT CCT GTC AGT GAA ATG Arg Glu Leu Ile Leu Asn Ile Thr Ala Thr Thr Leu Ala Ser Val Gln Asn Pro Gly Val Pro Val Ser Glu Het ATT GAT CCA AGA TTT GAA GAA GAC TAC AAC GTA AAC TAT AGG CAC AAA AGA CGT CAT GAT GAG ACC TAC AAA CAC Ile Asp Pro Arg Phe Glu Glu Asp Tyr Asn Val Asn Tyr Arg His Lys Arg Arg His Asp Glu Thr Tyr Lys His TCC GAA TTG AAA AAT AAC AAA AAT GTT GAT GGC TCA GAT ATA ATG AAT TGG ACG GGT CCA GGT ATT TTC TCC GAC Ser Glu Leu Lys Asn Asn Lys Asn Val Asp Gly Ser Asp Ile Het Asn Trp Thr Gly Pro Gly Ile Phe Ser Asp ATT ATT TTC GAA TAC ATG AAC ATT GTG CTC CGA TAC AAT AGC GAT ATC TTG TTA ATC AAC CCA AAA CAC AAC GAC GAA GAA GGT ACT GAG ACT GCC ACC ACA CAC GCA AAA GAT GTT GAT AAC GAT AGC GCG TCC AAAT TCA AAC AAG Ile Ile Phe Glu Tyr Het Asn Asn Val Leu Arg Tyr Asn Ser Asp Ile Leu Leu Ile Asn Fro Asn Leu Asn Tys ACG GAC GAA GAA GGT ACT GAG ACT GCC ACC ACA CAC GCA AAA TCA ACG AT AGC GTG TCC AAAT TCA ACC ACG ACG GAA GAA GGT ACT GAG ACT GCC ACC ACA CAC GCA AAA GAT GTT GAT AAC GAT ACG CTG TCC AAAT TCA ACC ASn Asp Glu Glu Gly Ser Glu Ser Ala Thr Thr Pro Ala Lys Asp Val Asp Asn Asp Thr Leu Ser Lys Ser Thr ACG AAAA TTT TAT AAG AAA ATA TCA GAG TCT TTG CCA ATT CA ATT CA ATG ACG CCTG GGA ATTC TTC TCT TTT TTT Arg Lys Phe Tyr Lys Lys Ile Ser Glu Ser Leu Gln Ser Ser Asn Ser Het Pro Trp Glu Phe Phe Ser Phe Leu AAA GAA CCA GTT ATC GTT GAT GAT GTG GTG GTT GA ACG ATG GCG GAA AGG ATG GCG CAG ATG GCG CAG ATG AGC GAC AAA ATG ACG GTT GTT GA AAC AAG TTT TCA CCA GAT GTG GGG CAG ATG GGC CGG CAG TTG CTT AGT GAC GAC GAA AAAT TT CA CGA GAT GTT GTA CAA ATCA ACG ATT TTT TCA CCA GAT GTG GGG CAG ATG GGC CGG CAG GTT ACT GAT GAT GAT GTG GTG GTT CTA CCA ATA ACC AGT TTT TCA CCA GAT GAA GAT GTT GAT GAT GAT GAT GTT GAT GA | 26 | Leu | Leu | Ser | Ser | Asp | Glu | Ile | Ser | His | Gln | Pro | Gly | Leu | Val | Ile | Gly | Ile | Glu | Ala | Asp | Pro | Asp | Arg | Asp | Asp | 25 |
| TTP SET GIU TEP TYF ALA AFF AFG ILE GIN PHE CYS GIN TEP THF ILE GIN ALA LYS FRO GLY HIS PRO ILE LEU 2 AGA GAA TTG ATC TTA AAT ATT ACT GCA ACG ACT TTG GCG AGC GTA CAA AAC CCA GGA GTT CCT GTC AGT GAA ATG 5 Arg Glu Leu ILE LEU ASN ILE THF ALA THF THF LEU ALA SET VAL GIN ASN PRO GLY VAL PRO VAL SET GLU HET 3 ATT GAT CCA AGA TTT GAA GAA GAC TAC AAC GTA AAC TAT AGG CAC AAA AGA CGT CAT GAT GAG ACC TAC AAA CAC 5 ILE ASP PRO Arg PHE GLU GLU ASP TYF ASN VAL ASN TYF AFG HIS LYS AFG ATG HIS ASP GLU THF TYF LYS HIS 3 TCC GAA TTG AAA AAT AAC AAA AAT GTT GAT GGC TCA GAT ATA ATG AAT TGG ACG GGT CCA GGT ATT TTC TCC GAC 10 SET GLU LEU LYS ASN ASN VAL ASP GLY SET ASP ILE HET ASN TTF THT GLY PRO GLY ILE PHE SET ASP ATT ATT TTC GAA TAC ATG AAC AAT GTG GTC CGA TAC ATA AGG GAT ATC TTG TTA ATC AAC CCA AAC CTA AAC AAG ILE PHE GLU TYF HET ASN ASN VAL ASP GLY SET ASP ILE HET ASN TFP THF GLY PRO GLY ILE PHE SET ASP ATT ATT TTC GAA TAC ATG AAC AAT GTG CTC CGA TAC AAT AGC GAT ATC TTG TTA ATC AAC CCA AAC CTA AAC AAG ILE ILE PHE GLU TYF HET ASN ASN VAL LEU AFG TYF ASN SET ASP ILE LEU LEU ILE ASN FFO ASN LEU ASN LYS 3 AAC GAC GAA GAA GGT AGT GAG AGT GCC ACC ACA CCA CCA CAA CAA AGT GTT GAT AAC GAT AGC GTG TCC AAA TCA ACC 12 ASN ASP GLU GLU GLY SET GLU SET ALA THT THF PRO ALA LYS ASP VAL ASP ASN ASP THF LEU SET LYS SET THF AGA AAA TTT TAT AAG AAA ATA TCA GAG TCT TTG CAA TCG ATA AAC AAG TTT TCA CCA GAT GTG GGG CAG TG GCA ATG AGT GTG GAT GTG GAT ATT ATCA ACC AGT ATT CTT TCT TTT TTG LYS LYS LYS LLS LLE SET GLU SET LEU GLN SET EANS SET ASN SET HET PRO TYP GLU FHE PHE SET PHE LEU 4 AAA GAA CCA GTT ATC GTT GAT GAT GTG GTG GTA GTA CCA ATA ACCA AGT TTT TCA CCA GAT GTG GGG CAG ATG GGC CL LYS GLU PRO VAL ILE VAL ASP ASP VAL HET VAL LEU PRO ILE THT SET PHE SET PRO ASP VAL GLY GLN HET GLY AGC CCAG GTT AGT GAC GAC AAA ATG GCT TTT GTA AAG CAC GTT TAT ACCA GGG GGA AGG GCG GAAA GAA GAT GCT GAT AAA GAA GTA GTT GAC AAA GAT GTT GAT AAG GAT GTT GAT AAC AGT TTT ACC CAG GAA GAAG ATG GCT GAAT AAA GAA GAT GCT AGT GAC CAC AAA ATG ACT TTT GTA AAG CAC ATT ACCA AGT TTT ACC | 51 | TGG | AGT | GAA | TGG | тат | CCT | CGT | AGG | ATC | CAG | TTT | тот | CAA | TGG | ACT | ATC | C & & | 000 | | CC.A | GGT | CAC | CC.A | атт | CTA | 82 |
| AGA GAA TTG ATC TTA AAT ATT ACT GCA ACG ACT TTG GCG AGC GTA CAA AAC CCA GGA GTT CCT GTC AGT GAA ATG 5 Arg Glu Leu Ile Leu Asn Ile Thr Ala Thr Thr Leu Ala Ser Val Gln Asn Pro Gly Val Pro Val Ser Glu Het ATT GAT CCA AGA TTT GAA GAA GAC TAC AAC GTA AAC TAT AGG CAC AAA AGA CGT CAT GAT GAG ACC TAC AAA CAC Ile Asp Pro Arg Phe Glu Glu Asp Tyr Asn Val Asn Tyr Arg His Lys Arg Arg His Asp Glu Thr Tyr Lys His TCC GAA TTG AAA AAT AAC AAA AAT GTT GAT GGC TCA GAT ATA ATG AAT TGG ACG GGT CCA GGT ATT TTC TCC GAC Ser Glu Leu Lys Asn Asn Lys Asn Val Asp Gly Ser Asp Ile Het Asn Trp Thr Gly Pro Gly Ile Phe Ser Asp ATT ATT TTC GAA TAC ATG AAC AAT GTG CTC CGA TAC AAT AGC GAT ATC TTG TTA ATC AAC CCA AAC CTA AAC AAG Ile Phe Glu Tyr Het Asn Asn Val Leu Arg Tyr Asn Ser Asp Ile Leu Leu Ile Asn Pro Asn Leu Asn Lys ACC GAC GAA GAA GGT ACT GAG ACT GCC ACCA CAC ACA GCT AAT AGC GAT ATC TTG TTA ATC AAC CCG AAAC CTA AAC AAG Ile Ile Phe Glu Gly Ser Glu Ser Ala Thr Thr Pro Ala Lys Asp Val Asp Asn Asp Thr Leu Ser Lys Ser Thr AGA AAA TTT TAT AAG AAA ATT TCA GAG TCT TTG CAA TCG ATA TCA ATG CAG GGA TCC TTC TCT TTT TTG Arg Lys Phe Tyr Lys Lys Ile Ser Glu Ser Leu Gln Ser Ser Asn Ser He Pro Trp Glu Phe Phe Ser Phe Leu AAA GAA CCA GTT ATC GTT GAT GAT GTG GTG GTG TCA CAA ATCA AAT GAA ATG ACA GAT GTG GGG CAG ATG GGC CAG Lys Asp Asp Asp Nal Ile Ser Fhe Leu AAA GAA CCA GTT ATC GTT GAT GAT GTG GTG GTT CA CAC ATA ACA AGT TTT TCA CCA GAT GTG GGG CAG ATG GGC CI Lys Glu Pro Val Ile Val Asp Asp Val Het Val Leu Pro Ile Thr Ser Phe Ser Pro Asp Val Gly Gln Het Gly GCC CAG TCT AGT GAC GAC AAA TG GCT TTT GTA AAG CAT GTT GAA GGG GGA GAG GGC GAA ALG GAG GAT GAC ACA AAA GAT GTT GAT GAA GGA GGA GGT GGA GAA GA | 51 | Trp | Ser | Glu | Trp | Tyr | Ala | Arg | Arg | Ile | Gln | Phe | Cys | Gln | Trp | Thr | Ile | Gln | Ala | Lys | Pro | Gly | His | Pro | Ile | Leu | 27 |
| Arg Glu Leu Ile Leu Asn Ile Thr Ala Thr Thr Leu Ala Ser Val Gln Asn Pro Gly Val Pro Val Ser Glu Met 3 ATT GAT CCA AGA TTT GAA GAA GAC TAC AAC GTA AAC TAT AGG CAC AAA AGA CGT CAT GAT GAG ACC TAC AAA CAC 5 Ile Asp Pro Arg Phe Glu Glu Asp Tyr Asn Val Asn Tyr Arg His Lys Arg Arg His Asp Glu Thr Tyr Lys His 3 TCC GAA TTG AAA AAT AAC AAA AAT GTT GAT GGC TCA GAT ATA ATG AAT TGG ACG GGT CCA GGT ATT TTC TCC GAC 10 Ser Glu Leu Lys Asn Aan AAC AAT AGT GTG CTC CGA TAT ATA ATG AAT TGG ACG GGT CCA GGT ATT TTC TCC GAC 11 ATT ATT TTC GAA TAC ATG AAC AAT GTG CTC CGA TAC AAT AGC GAT ATC TTG TTA ATC AAC CCA AAC CTA AAC AAG 11 Ile Ile Phe Glu Tyr Met Asn Asn Val Leu Arg Tyr Asn Ser Asp Ile Leu Leu Ile Asn Pro Asn Leu Asn Tys 3 AC GAC GAA GAA GGT ACT GAG ACT GCC ACC ACA CCA CAC ACA GAT ATC TTG TATA ATA CAT ACG CTG TCC AAA TCA ACA 12 Asn Asp Glu Glu Gly Ser Glu Ser Ala Thr Thr Pro Ala Lys Asp Val Asp Asn Asp Thr Leu Ser Lys Ser Thr 4 AG AAA TTT TAT AAG AAA ATA TCA GAG TCT TTG CAA TCG CAC AAT TCA ATC AAC AGT TTT TCA CCA GAT ATC TTC TCT TTT TTT TTG 12 Arg Lys Phe Tyr Lys Lys Ile Ser Glu Ser Leu Gln Ser Ser Asn Ser Her Pro Trp Glu Fhe Phe Ser Phe Leu 4 AGA AAA TTT TAT AG AAA ATA TCA GAT GTG GTT CTA CCA ATA ACA AGT TTT TCA CCA GAT ATC TTC TCT TTT TTT TTT 12 Arg Lys Phe Tyr Lys Lys Ile Ser Glu Ser Leu Gln Ser Ser Asn Ser Met Pro Trp Glu Fhe Phe Ser Phe Leu 4 AGA CCA CTT ATT GAT GAT | 26 | AGA | GAA | TTG | ATC | тта | ААТ | ATT | ACT | GCA | ACG | ACT | TTG | GCG | AGC | GTA | саа | AAC | CCA | GGA | GTT | сст | GTC | AGT | GAA | ATG | 90 |
| ATT GAT CCA AGA THT GAA GAA GAC TAC AAC GTA AAC TAT AGG CAC AAA AGA CGT CAT GAT GAG ACC TAC AAA CAC S Ile ASP Pro Arg Phe Glu Glu Asp Tyr Asn Val Asn Tyr Arg His Lys Arg Arg His Asp Glu Thr Tyr Lys His TCC GAA THT AAA AAT AAC AAA AAT GTT GAT GGC TCA GAT ATA ATG AAT TGG ACG GGT CCA GGT ATT TTC TCC GAC Ser Glu Leu Lys Asn Aan AAT ATC AAA AAT GTT GAT GGC TCA GAT ATA ATG AAT TGG ACG GGT CCA GGT ATT TTC TCC GAC ATT ATT TTC GAA TAC ATG AAC AAT GTG CTC CGA TAC ATA AGC GAT ATC TTG TTA ATC AAC CCA AAC CTA AAC AAG Ile Ile Phe Glu Tyr Met Asn Asn Val Leu Arg Tyr Asn Ser Asp Ile Leu Leu Ile Asn Pro Asn Leu Asn Lys AC GAC GAA GAA GGT ACT GAG ACT GCC ACC ACA CCA GCA AAA GAT GTT GAT AAC GAT AGC GTG TCC AAA TCA ACA A Asn Asp Glu Glu Gly Ser Glu Ser Ala Thr Thr Pro Ala Lys Asp Val Asp Asn Asp Thr Leu Ser Lys Ser Thr AG AAA TTT TAT AAG AAA ATA TCA GAG TCT TTG CAA TCG ATA CA AT CA AAC GAT AGC CTG GCC ATC TTT TTT AGA AAA TTT TAT AAG AAA ATA TCA GAG TCT TTG CAA TCG ATA CA AT TCA ATG CTG GGG GA GC GG CAG ATG GGC AAG AAA TTT TAT AAG AAA ATA TCA GAG TCT TTG CAA TCG ATA CA ATT CA ATG ASP Ser Met Pro Trp Glu Phe Phe Ser Phe Leu AAG GAA CCA GTT ATC GTT GAT GAT GTG GTG TTA CCA ATA ACA AGT TTT TCA CCA GAT GTG GGG CAG ATG GGC Lys Glu Pro Val Ile Val Asp Asp Val Met Val Leu Pro Ile Thr Ser Phe Ser Pro Asp Val Gly Gln Met Gly GCC CAG TCT AGT GAC GAC AAA TCG GTT TTT GTA AAG CAT GTT AGC GGG AGG GGG AAG GCT GAT ANA GAT GTT GAA GAT GTT GAA GAT GTT GAA GAT GTT GAA AGA GTT GTT | 76 | Arg | Glu | Leu | Ile | Leu | Asn | Ile | Thr | Ala | Thr | Thr | Leu | Ala | Ser | Val | Gln | Asn | Pro | Gly | Val | Pro | Val | Ser | Glu | Met | 30 |
| Ile Asp Pro Arg Phe Glu Glu Asp Tyr Asn Val Asn Tyr Arg His Lys Arg Arg His Asp Glu Thr Tyr Lys His 3 TCC GAA TTG AAA AAT AAC AAA AAT GTT GAT GGC TCA GAT ATA ATG AAT TGG ACG GGT CCA GGT ATT TTC TCC GAC 10 Ser Glu Leu Lys Asn Asn Lys Asn Val Asp Gly Ser Asp Ile Het Asn Trp Thr Gly Pro Gly Ile Phe Ser Asp 3 ATT ATT TTC GAA TAC ATG AAC AAT GTG CTC CGA TAC AAT AGC GAT ATC TTG TTA ATC AAC CCA AAC CTA AAC AAG 11 Ile Ile Phe Glu Tyr Het Asn Asn Val Leu Arg Tyr Asn Ser Asp Ile Leu Leu Ile Asn Pro Asn Leu Asn Lys 3 ACC GAC GAA GAA GGT AGT GAG AGT GCC ACC ACA CCA GCA AAA GAT GTT GAT AAC GAT AGC GTG TCC AAA TCA ACA 12 Asn Asp Glu Glu Gly Ser Glu Ser Ala Thr Thr Pro Ala Lys Asp Val Asp Asn Asp Thr Leu Ser Lys Ser Thr 4 AGA AAA TTT TAT AAG AAA ATA TCA GAG TCT TTG CAA TCG ATA CTA ATC AAT GG CTG GCG GATC GTC TTT TTT 12 Arg Lys Phe Tyr Lys Lys Ile Ser Glu Ser Leu Gln Ser Ser Asn Ser Her Pro Trp Glu Phe Phe Ser Phe Leu 4 AGA GAA CCA GTT ATC GTT GAT GAT GTG GTT CTA CCA ATA ACA AGT TTT TCA CCA GAT GTG GGG CAG ATG GGC 14 Lys Glu Pro Val Ile Val Asp Asp Val Het Val Leu Pro Ile Thr Ser Phe Ser Pro Asp Val Gly Gln Het Gly 4 GCC CAG TCT AGT GAC GAC AAA ATG GCT TTT GTA AAG CAG TTT ASP AGG AGA GAA GAT GTT GAA GAA GAT GTT ASP ASP ASH VAL ASP ASP ASA PVA GLY CAGA AAA SP ASP ASP ASA ATA SP ASP ASP ASP ASP ASA ATA TCA GAT GAT GAT GAT GAT GAT GAT GAT GAT GA | 01 . | атт | GAT | CCA | AGA | ттт | GAA | GAA | GAC | TAC | AAC | GTA | AAC | TAT | AGG | CAC | *** | AGA | CGT | CAT | GAT | GAG | ACC | TAC | *** | CAC | 97 |
| TCC GAA TTG AAA AAT AAC AAA AAT GTT GAT GGT GGT CA GAT ATA ATG AAT TGG ACG GGT CCA GGT ATT TTC TCC GAC 10 Ser Glu Leu Lys Asn Asn Lys Asn Val Asp Gly Ser Asp Ile Met Asn Trp Thr Gly Pro Gly Ile Phe Ser Asp 3 ATT ATT TTC GAA TAC ATG AAC AAT GTG CTC CGA TAC AAT AGC GAT ATC TTG TTA ATC AAC CCA AAC CTA AAC AAG 11 Ile Ile Phe Glu Tyr Met Asn Asn Val Leu Arg Tyr Asn Ser Asp Ile Leu Leu Ile Asn Pro Asn Leu Asn Lys 3 AAC GAC GAA GAA GGT ACT GAG ATG GCC ACC ACA CCA GCA AAA GAT GTT GAT AAC GAT AGC GTG TCC AAA TCA ACA 12 Asn Asp Glu Glu Gly Ser Glu Ser Ala Thr Thr Pro Ala Lys Asp Val Asp Asn Asp Thr Leu Ser Lys Ser Thr 4 AGA AAA TTT TAT AAG AAA ATA TCA GAG TCT TTG CAA TCG ATA AAT TCA ATG CCT GG GAA TTC TTC TCT TTT TTG Arg Lys Phe Tyr Lys Lys Ile Ser Glu Ser Leu Gln Ser Ser Asn Ser Met Pro Trp Glu Phe Phe Ser Phe Leu Lys Glu Pro Val Ile Val Asp Asp Val Met Val Leu Pro Ile Thr Ser Phe Ser Pro Asp Val Gly Gln Met Gly 4 GCC CAG TCT AGT GAC GAC AAA TCG GCT TTT GTA AAG CAT GTT AGC GGG AGA GAG GCT GAAA AAFG GCT TTT GTA GAA GAT GTT GAC GAC AAA GCA GTT TTA GCA GAG GAG GCG GAAA GAA GAT GTT GAA GAA GCA GTA TAT ATG ATG GG GCA TTT TTG TTA CAA ATG ASP VAI ILE VAL LEU PRO ILE TH SER PHE SER PRO ASP VAI GIJ GIN MET GJY 4 AGA GAA CCA GTT ATC GTT GAA GAT GTT TTT GTA AAG CAG TTT TCG CGG AAG GCA GAG GCG CAG ATG GGC Lys Glu Pro Val Ile Val Asp Asp Val Met Val Leu Pro Ile Thr Ser Phe Ser Pro Asp Val Gly Gln Met Gly GCC CAG GTT AGT GAC GAC AAA ATG GCT TTT GTA AAG CA GTT TTA GC GGG AGC GGA AGA GAT GCT GAT AAA GAA GAT GTA GAC GAC AAA ATG GCT TTT GTA AAG CAG GTT AGC GGG AAG GAA GAA GAT GCT GAT AAA ATG ACT TTT GTA AAA ATG ACA TTT AAG AAA ATG GCT TTT GTA AAA CA AGT TTT AGC AGA GAA GAA GAT GCT GAT AAA ATG GCT TAT GAA GAA GAA GAA GAA GAA GAA GAT GCT GAA AAA GAA GAT GCT GAA AAA GAA GAA GAA GAA GAA GAA GAA GA | 01 | Ile | Asp | Pro | Arg | Phe | Glu | Glu | Asp | туr | Asn | Val | Asn | Τyr | Arg | H15 • | Ly s | Arg | Arg | His • | Asp | Glu | Thr | Τyr | Lys | His | 32 |
| Ser Glu Leu Lys Asn Asn Lys Asn Val Asp Gly Ser Asp Ile Het Asn Trp Thr Gly Pro Gly Ile Phe Ser Asp 3 ATT ATT TTC GAA TAC ATG AAC AAT GTG CTC CGA TAC AAT AGC GAT ATC TTG TTA ATC AAC CCA AAC CTA AAC AAG 11 Ile Ile Phe Glu Tyr Het Asn Asn Val Leu Arg Tyr Asn Ser Asp Ile Leu Leu Ile Asn Pro Asn Leu Asn Lys 3 AAC GAC GAA GAA GGT AGT GAG ATT GCC ACC ACCA CCA GCA AAA GAT GTT GAT AAC GAT AGC GTG TCC AAA TCA ACC 12 Asn Asp Glu Glu Gly Ser Glu Ser Ala Thr Thr Pro Ala Lys Asp Val Asp Asn Asp Thr Leu Ser Lys Ser Thr 4 AG AAA TTT TAT AAG AAA ATA TCA GAG TCT TTG CAA TCG ATC AAT TCA AATG CCTG GCA ATTC TTC TCT TTT TTG Arg Lys Phe Tyr Lys Lys Ile Ser Glu Ser Leu Gln Ser Ser Asn Ser Het Pro Trp Glu Phe Phe Ser Phe Leu AAA GAA CCA GTT ATC GTT GAT GAT GTG GTG TTA CCA ATA ACC AGT TTT TCA CCA GAT GTG GGG CAG ATG GGC 13 Lys Glu Pro Val Ile Val Asp Asp Val Het Val Leu Pro Ile Thr Ser Phe Ser Pro Asp Val Gly Gln Het Gly GCC CAG TCT AGT GAC GAC AAA ATG GCT TTT GTA AAG CAC ATT ACC GGG AGG CGG AAA GAC GGT TAG GAC AAAA ATA TTA TAG GAC TTT TTG TTA GAC GGG AGG CTG AAAA GAT GTT GAC GAG AAA GAT GTT GAC GAC GAAA AAA GAA CCA GTT ATC GTT GAT GAT GTG GTT TTA CAA CTA ATA ACA AGT TTT TCA CCA GAT GTG GGG CAG ATG GGC 14 Lys Glu Pro Val Ile Val Asp Asp Val Het Val Leu Pro Ile Thr Ser Phe Ser Pro Asp Val Gly Gln Het Gly 4 GCC CAG TCT AGT GAC GAC AAA ATG GCT TTT GTA AAG CAC ATT TTCA CGG GG AGA GGT GAAA GAA GAT GCT GAT AAA ATA ASA ASP VAL ASP AN HET VAL HEU PRO ILE TTA GCC GGG AAA GAA GAT GCT GAAA AAA ASP ASP VAL ASP AN HET VAL HEU PRO ILE TTA GCC GGG AAA GAA GAT GCT GAAA AAA ASP ASP ASP ASP AN HET VAL ASP ASP AN HET AAA ASP ASP ASP ASP ASP AN ASP | 76 | rcc | GAA | TTG | *** | AAT | лас | *** | AAT | GTT | GAT | GGC | TCA | GAT | АТА | ATG | AAT | TGG | ACG | GGT | CCA | GGΤ | ATT | TTC | тсс | GAC | 105 |
| ATT ATT TTC GAA TAC ATG AAC AAT GTG CTC CGA TAC AAT AGC GAT ATC TTG TTA ATC AAC CCA AAC CTA AAC AAG 11 Ile Ile Phe Glu Tyr Met Aan Aan Val Leu Arg Tyr Aan Ser Aap Ile Leu Leu Ile Aan Pro Aan Leu Aan Lys 3 AAC GAC GAA GAA GGT AGT GAG AGT GCC ACC ACCA CCA GCA AAA GAT GTT GAT AAC GAT ACG CTG TCC AAA TCA ACC A [2 Aan Asp Glu Glu Gly Ser Glu Ser Ala Thr Thr Pro Ala Lys Asp Val Asp Aan Asp Thr Leu Ser Lys Ser Thr 4 AG AAA TTT TAT AAG AAA ATA TCA GAG TCT TTG CAA TCG ATA ATC AAT CCA ATG CTG GG GAA TCC TTC TTT TTG Arg Lys Phe Tyr Lys Lys Ile Ser Glu Ser Leu Gln Ser Ser Aan Ser Met Pro Trp Glu Phe Phe Ser Phe Leu AAA GAA CCA GTT ATC GTT GAT GAT GTG GTG GTT CTA CCA ATA ACCA AGT TTT TCA CCA GAT GTG GGG CAG ATG GGC Lys Glu Pro Val Ile Val Asp Aap Val Met Val Leu Pro Ile Thr Ser Phe Ser Pro Aap Val Gly Gln Met Gly GCC CAG TCT AGT GAC GAC AAA ATG GCT TTT GTA AAG CAC ATT ACG GGG AGC GGG AAG GGT GAT ATG GTT GAA GAA GTT TTA GAA GA | 26 | Ser | Glu | Leu | Lys | Asn | Asn | Lys | Asn | Val | Asp | Gly | Ser | Asp | Ile | Met | Asn ∳ | Trp | Thr | Gly | Pro | Gly | Ile | Phe | Ser | Asp | 35 |
| Ile Ile Phe Glu Tyr Met Asn Asn Val Leu Arg Tyr Asn Ser Asp Ile Leu Leu Ile Asn Pro Asn Leu Asn Lys 3 AAC GAC GAA GAA GGT AGT GAG AGT GGC ACC ACA CCA GCA GAA GAT GTT GAT AAC GAT AGG CTG TCC AAA TCA ACA 11 Asn Asp Glu Glu Gly Ser Glu Ser Ala Thr Thr Pro Ala Lys Asp Val Asp Asn Asp Thr Leu Ser Lys Ser Thr 4 AG AAA TTT TAT AAG AAA ATA TCA GAG TCT TTG CAA TCG TCA AAT TCA ATG GCC TGG GAA TTC TTC TCT TTT TTG Arg Lys Phe Tyr Lys Lys Ile Ser Glu Ser Leu Gln Ser Ser Asn Ser Met Pro Trp Glu Phe Phe Ser Phe Leu AAA GAA CCA GTT ATC GTT GAT GAT GTG ATG GTT CTA CCA ATA ACA AGT TTT TCA CCA GAT GTG GGG CAG ATG GGC 4 Lys Glu Pro Val Ile Val Asp Asp Val Met Val Leu Pro Ile Thr Ser Phe Ser Pro Asp Val Gly Gln Met Gly GCC CAG TCT AGT GAC GAC AAA ATG GCT TTT GTA AAG CAC ATG TTT AGC GGG AGA GAG AGT GCT GAT AAA 14 Ala Gin Ser Ser Asp Asp Lys Met Ala Phe Val Lys His Met Phe Ser Gly Ser Trp Lys Glu Asp Ala Asp Lys 4 | 51 | ATT | ATT | TTC | GAA | TAC | ATG | AAC | AAT | GTG | стс | CGA | TAC | AAT | AGC | GAT | ATC | TTG | TTA | ATC | AAC | CCA | AAC | CTA | AAC | AAG | 112 |
| AC GAC GAA GGA GGA AGG GCC AGC AGC ACA CCA GCA AAA GAT GTT GAT AAC GAT AGC GTG TCC AAA TCA ACA 11 Asn Asp Glu Glu Gly Ser Glu Ser Ala Thr Thr Pro Ala Lys Asp Val Asp Asn Asp Thr Leu Ser Lys Ser Thr 4 AG AAA TTT TAT AAG AAA ATA TCA GAG TCT TTG CAA TCG TCA AAT TCA ATG GCC TGG GAA TTC TTC TCT TTT TTG Arg Lys Phe Tyr Lys Lys lie Ser Glu Ser Leu Gln Ser Ser Asn Ser Het Pro Trp Glu Phe Phe Ser Phe Leu AAA GAA CCA GTT ATC GTT GAT GAT GTG GTG GTT CTA CCA ATA ACA AGT TTT TCA CCA GAT GTG GGG CAG ATG GGC Lys Glu Pro Val 11e Val Asp Asp Val Het Val Leu Pro 11e Thr Ser Phe Ser Pro Asp Val Gly Gln Het Gly GCC CAG GTC TAGT GAC GAC AAA ATG GCT TTT GTA AAG CAC ATG TTT AGC GGG AGA GGA GGT GAT GAT GAT GAT ATG ATG | 51 | Ile | Ile | Phe | Glu | Tyr | Met | Asn | Asn | Val | Leu | Arg | Tyr | Asn | Ser | Asp | Ile | Leu | Leu | Ile | Asn | Pro | Asn | Leu | Asn | Lys | 31 |
| AST ASP GIU GIU GIY SET GIU SET AIA ITT THE PTO AIA LYS ASP VAI ASP AST ASP INT LEU SET LYS SET INT 4 AGA ANA THT TAT ANG ANA ATA TCA GAG TCT THG CAA TUG TCA AAT CCA ATG CCC TGG GAA TTC THC TTT TTG II Arg LYS Phe TYT LYS LYS IIS SET GIU SET LEU GIN SET SET AST SET MET PTO TTP GIU Phe Phe Set Phe Leu ANA GAA CCA GTT ATC GTT GAT GAT GTG GTG GTT CTA CCA ATA ACA AGT TTT TCA CCA GAT GTG GGG CAG ATG GGC LYS GIU PTO VAI IIE VAI ASP ASP VAI MET VAI LEU PTO IIE THT SET PHE SET PTO ASP VAI GIY GIN MET GIY GCC CAG TCT AGT GAC GAC ANA ATG GCT TTT GTA ANG CAC ATG TTT AGC GGG AGG TGG ANA GAA GAT GCT GAT ANA 14 AIA GIN SET SET ASP ASP LYS MET AIA PHE VAI LYS HIS MET PHE SET GIY SET GLY SET UA SP AIA SP LYS AIB GIN SET SET ASP ASP LYS MET AIA PHE VAI LYS HIS MET PHE SET GIY SET UA SP AIA SP ASP ASP VAI | 26 | AAC | GAC | GAA | GAA | GGT | AGT | GAG | AGT | GCC | ACC | ACA | CCA | GCA | *** | GAT | GTT | GAT | AAC | GAT | ACG | CTG | тсс | *** | TCA | ACA | 120 |
| AGA ANA THT TAR ANG ANA ATA TCA GAG TCT TTG CAA TCG TCA ANT CCA ATG CCC TGG GAA TTC TTC TCT TTT TTG 11 Arg Lys Phe Tyr Lys Lys II ser Glu Ser Leu Gin Ser Ser Asn Ser Het Pro Trp Glu Phe Phe Ser Phe Leu AMA GAA CCA GTT ATC GTT GAT GAT GTG GTG GTT CTA CCA ATA ACA AGT TTT TCA CCA GAT GTG GGG CAG ATG GGC ILys Glu Pro Val IIe Val Asp Asp Val Het Val Leu Pro IIe Thr Ser Phe Ser Pro Asp Val Gly Cin Het Gly GCC CAG TCT AGC GAC ANA ATG GCT TTT GTA AGA CATG TTT AGC GGG AGC TGG AMA GAA GAT GCT GAT ANA 14 Ala Gin Ser Ser Asp Asp Lys Het Ala Phe Val Lys His Het Phe Ser Gly Ser Trp Lys Glu Asp Ala Asp Lys | /6 | ASN | Asp | GIU | GIU | GIY | ser | GIU | ser | Ala | Thr | Thr | Pro | AIA | Lys | Asp | vai | Asp | Asn ♦ | Asp | Thr | Leu | Ser | Lys | ser | int | 40 |
| AND LYS FILE IYE LYS LYS THE SET GIU SET LEU GIN SET SET AST SET RET FO THE GIU FILE DEU S ANA GAA CCA GTT ATC GTT GAT GAT GTG TG GTG GTG TTA CCA ATA ACA AGT TTT TCA CCA GAT GTG GGG CAG ATG GGC 13 Lys Glu Pro Val 11e Val ASP ASP Val Het Val Leu Pro 11e Thr Ser Phe Ser Pro Asp Val Gly Gin Het Gly 4 GCC CAG TCT AGT GAC GAC ANA ATG GCT TTT GTA ANG CAC ATG TTT AGC GGG AGC TGG ANA GAA GAT GCT GAT ANA 14 Ala Gin Ser Ser Asp Asp Lys Het Ala Phe Val Lys His Het Phe Ser Gly Ser Trp Lys Glu Asp Ala Asp Lys 4 | 01 | AGA | *** | TTT | TAT | AAG | *** | ATA | TCA | GAG | тст | TTG | CAA | τcg | TCA | AAT | TCA | ATG | ccc | TGG | GAA | TTC | TTC | тст | TTT | TTG | 127 |
| ANA GAA CCA GTT ATC GTT GAT GAT GTG ATG GTT CTA CCA ATA ACA AGT TTT TCA CCA GAT GTG GGG CAG ATG GGC 11 Lys Glu Pro Val 11e Val Asp Asp Val Met Val Leu Pro 11e Thr Ser Phe Ser Pro Asp Val Gly Gln Met Gly 4 GCC CAG TCT AGT GAC GAC ANA ATG GCT TTT GTA ANG CAC ATG TTT AGC GGG AGC TGG ANA GAA GAT GCT GAT ANA Ala Gln Ser Ser Asp Asp Lys Met Ala Phe Val Lys His Met Phe Ser Gly Ser Trp Lys Glu Asp Ala Asp Lys 4 | 01 | Arg | Lys | Phe | 191 | Lys | Lys | 11e | Set | GIU | ser | Leu | GIN | Ser | Ser | ASN | ser | Met | PIO | πp | 010 | Phe | Phe | Ser | rite | Leu | • 4 |
| Lys Glu Pro Val 11e Val Asp Asp Val Met Val Leu Pro 11e Thr Ser Phe Ser Pro Asp Val Gly Gin Met Gly 4 GCC CAG TCT AGT GAC GAC AAA ATG GCT TTT GTA AAG CAC ATG TTT AGC GGG AGC TGG AAA GAA GAT GCT GAT AAA 14 Ala Gln Ser Ser Asp Asp Lys Met Ala Phe Val Lys His Met Phe Ser Gly Ser Trp Lys Glu Asp Ala Asp Lys 4 | 76 | *** | GAA | CCA | GTT | ATC | GTT | GAT | GAT | GTG | ATG | GTT | CTA | CCA | ATA | ACA | AGT | TTT | TCA | CCA | GAT | GTG | GGG | CAG | ATG | GGC | 135 |
| GCC CAG TCT AGT GAC GAC AAA ATG GCT TTT GTA AAG CAC ATG TTT AGC GGG AGC TGG AAA GAA GAT GCT GAT AAA 14 Ala Gin Ser Ser Asp Asp Lys Met ala Phe Val Lys His Met Phe Ser Giy Ser Trp Lys Giu Asp ala Asp Lys 4 | 26 | Lys | Glu | Pro | Val | Ile | Val | Asp | Asp | Val | Met | Vai | Leu | Pro | Ile | Thr | Ser | Phe | Ser | Pro | Asp | vai | GIY | GIN | Met | GIY | 43 |
| Ala Gin Ser Ser Asp Asp Lys Met Ala Phe Val Lys His Met Phe Ser Giy Ser Trp Lys Giu Asp Ala Asp Lys | 51 | GCC | CAG | TCT | AGT | GAC | GAC | *** | ATG | GCT | TTT | GTA | AAG | CAC | ATG | TTT | AGC | GGG | AGC | TGG | *** | GAA | GAT | GCT | GAT | AAA | 142 |
| | 51 | ~1 d | 01N | ser | ser | АБр | ASD | LYS | met | AIG | Phe | vai | Lys | HIS | Met | Phe | ser | 61Y | Ser | пр | LYS | 010 | ASP | A18 | ASP | LYS | |
| AAT GCA GGT CAT AAA TAACGAGGCTCTCCTTTCATCATACCCTCTCTAAATAAAATTTATTCCTAGTTATTCCTTCTTCTTCATATCCTTAATC 15 Asn Ala Gly His Lys *** | 26 | AAT Asn | GCA Ala | GGT Gly | CAT His | AAA Lys | таа ••• | CGAG | GCTC | тсст | TTCA | TCAT | ACCC | TC TC | TAAA | TAAA | ATTT. | ATTC | CTAG | TAT | тсст | TTCT | TCTT | CATA | TCCT | TAATC | 152 |
| | | | - | | - | | | | | | | | | | | | | | | | | | | | | | |
| ATGCATTCACTGCCATGTGCAAATAAAGGCTCTTGTGGTAGAAACTTTTAGCTCAAACGTGGTAAAACTATCTCAACGTCCTTCCGTAGACAACTGGTGA GCAGTGCTTCTGTAACACTGCAGATCTCCAATGATAAATCGATACCGTACTAACTGCCCGCACTATAATGAACTCTTTGTATCCGTAACTATTTAAAATA 1° | 521 521 | ATG | CATT GTGC | CACT TTCT | GCCA GTAA | TGTG CACT | GCAG | ATCT | GGCT | CTTG TGAT | TGGT | АСАА ССАТ | ACTT | ТТАС ТАСТ | CTCA AACT | AACG | TGGT GCAC | АААА ТАТА | СТАТ АТСА | CTCA ACTC | ACGT TTTG | UCTT TATC | CCGT CGTA | AGAC ACTA | ааст ттта | UGTGA AAATA | 16 |

Fig. 2. Nucleotide and deduced amino acid sequences of the OCH1 gene. Amino acids are abbreviated with the three letter code. The putative transmembrane domain is underlined. Rhombuses are located below the four potential N-glycosylation sites. Asterisks show the basic amino acids cluster. The sequence data is available from EMBL/GenBank/DDBJ under accession number D11095.

pancreatic microsomal membranes. While the translation product in the absence of membranes showed the 55 kDa band (Figure 5, lane 1), which is identical to the predicted molecular weight of the OCH1 protein, four discrete bands >55 kDa were observed in the presence of membranes. The molecular weight of each band corresponds to 57, 60, 63 and 66 kDa (Figure 5, lane 2). These bands were protected from proteinase K digestion, indicating that the large C-terminal region of the OCH1 protein was located in the lumenal side, with some modification (Figure 5, lane 3). When the OCH1 protein which was translated and modified *in vitro*, was treated with endoglycosidase H (endo H), a band corresponding to 55 kDa was observed (Figure 5, lane 5), confirming that the OCH1 protein is a glycoprotein containing four N-linked sugar chains.

Expression of OCH1 gene in yeast

We analyzed the localization and properties of the OCH1 protein in yeast cells using the anti-peptide antibody. The

yeast strains, KK4 (OCH1 wild type), YS52-1-1B (och1 disruptant derived from KK4) and a KK4 transformant harboring the plasmid YEp51-OCH1 (OCH1 protein overproducer), were grown in the SG + 0.5% glucose medium at the permissive temperature (25°C). The whole cell extracts were subjected to Western blot analysis. The KK4 transformant showed a 6-fold higher amount of OCH1 protein ranging from 58 to 66 kDa than that of the KK4 wild type cell, while the corresponding bands were not observed in the och1 disruptant (Figure 6A). Further, the cell extract of the KK4 transformant was separated into cytosolic and membrane fractions. It is reported that the ER membrane is mainly recovered in low speed pellet (LSP) (65-76%) and the Golgi membrane is separated into LSP (45%) and high speed pellet (HSP) (40%) (Nakano et al., 1988). The 58-66 kDa OCH1 protein bands were observed in the LSP and HSP fractions (Figure 6B, lanes 2 and 3), while no specific bands were observed in the high speed supernatant (HSS) fraction (Figure 6B, lane 4). The above data may





Fig. 4. Growth and glycosylation phenotype of the *och1* disruptant. (A) A set of meiotic progeny, (1) YS57-5A, (2) YS57-5B, (3) YS57-5C and (4) YS57-5D, were grown on (a) YPD at 25° C, (b) YPD at 37° C, and (c) SD without leucine. (B) Active staining of invertase. Mobility of invertase from meiotic progeny was analyzed by 8% SDS-PAGE, lanes 1-5 are YS57-5A, YS57-5B, YS57-5C, YS57-5D and wild type cell YS54-6B, respectively. Invertase active staining was carried out as described in Materials and methods.



Fig. 5. In vitro translation and translocation of the OCH1 gene product. The OCH1 mRNA which was transcribed under the SP6 promoter and RNA polymerase system was translated in rabbit reticulocyte lysate in the presence (lanes 2 and 3) or absence (lane 1) of canine pancreatic membranes. After 90 min of translation in the presence of canine pancreatic membranes, the samples were either incubated with proteinase K in the presence (lane 4) or absence (lane 3) of 1% Triton X-100 or treated with endoglycosidase H (lane 5). The positions shown on the left side indicate the ¹⁴C-labeled molecular weight standards (Amersham), bovine serum albumin (69 kDa), ovalbumin (46 kDa) and carbonic anhydrase (30 kDa). The arrows show the OCH1 protein with (66 kDa) and without (55 kDa) oligosaccharide chains. The reaction products were resolved on 7.5% SDS – PAGE and [³⁵S]methionine labeled OCH1 protein was visualized by autoradiography.

Fig. 3. Disruption of the och1 gene. The wild type diploid strain YS57 was transformed with the ochl disrupted DNA fragment in which the central half of the ORF was deleted and replaced with the LEU2 gene instead, and tetrad dissected after sporulation. (A) Construction of the och1::LEU2 disrupted DNA fragment. The 742 bp of the HpaI-AatII fragment in the ORF of och1 was deleted and the LEU2 fragment was inserted instead. The resulting 3.5 kb och1::LEU2 fragment was introduced into the wild type diploid strain by one-step replacement. (B) Southern blot analysis. The Sall-HindIII fragment of the wild type strain gives a 2.6 kb band, and that of the och1 disrupted strain gives a 3.5 kb band. Lane 1, size marker; lane 2, the wild type diploid; lane 3, the och1::LEU2 fragment introduced diploid strain; lanes 4-7, meiotic progenies of the disrupted diploid. In lanes 5 and 7, the 2.6 kbp band was slightly shifted to the slower migration position, because salt concentrations in these samples were higher than other samples. (C) Tetrad dissection of och1 disrupted diploid. Most of the segregants showed two large and two small colonies.

suggest that the OCH1 protein is a type II membrane protein which resides in the ER and/or Golgi membranes *in vivo*.

The HSP fraction solubilized with 2% Triton X-100 was treated with endo H and analyzed by Western blotting. Most of the OCH1 protein bands were shifted from 58-66 kDa to 55 kDa but two minor bands were also observed at 58 and 60 kDa, indicating incomplete endo H digestion (Figure 6C, lane 2). The results indicate that the OCH1 protein probably has four oligosaccharide chains and the length of each oligosaccharide is relatively short and equivalent to 2-3 kDa. This is in accordance with the results of in vitro translation/translocation analysis that demonstrated the presence of four larger endo H sensitive bands (57, 60, 63 and 66 kDa) and the unglycosylated OCH1 protein band (55 kDa). The length of the oligosaccharide chain produced in yeast was almost the same or slightly larger than the inner core size formed in the ER because the sizes of the OCH1 protein estimated by in vitro translation/translocation and Western blotting were almost identical. This result also



Fig. 6. Western blot analysis of the OCH1 protein. Polyclonal anti-OCH1 antibody (anti-OCH1-C) was prepared using the synthetic peptide corresponding to the amino acid sequence of the C-terminal region (465-480 amino acids) of the OCH1 protein. (A) Whole cell lysate. The *och1* disruptant cells (lane 1), wild type haploid cells (KK4) (lane 2) and KK4 transformant cells harboring a multi-copy plasmid YEp51-OCH1 (KK4/YEp51-OCH1) (lane 3), were disrupted by glass beads and the cell lysates were electrophoresed on 4-20% gradient SDS-PAGE and transferred to a polyvinylidene difluoride membrane filter. OCH1 proteins were detected with anti-OCH1-C antiserum and alkaline phosphatase conjugated second antibody. (B) Subcellular fractionation and OCH1 protein localization. The whole cell lysates from KK4/YEp51-OCH1 (lane 1) were fractionated into high speed pellet (lane 2) and high speed supernatant (lane 4) by centrifugation (10 000 %, 60 min). Electrophoresis and detection method are the same as above. (C) Endoglycosidase H treatment. HSP fraction was solubilized by 2% Triton X-100 and treated with endo H. Lane 1, solubilized HSP; lane 2, endo H treated sample.

| Table I. Assay of mannosyltransferase activ | . Assay of mannosyltransferase acti | vit |
|---|-------------------------------------|-----|
|---|-------------------------------------|-----|

| Acceptor | Mannosyltransferase activity [pmol mannose/h/mg (ratio)] | | | | | | |
|--|---|-----------|-----------------|--|--|--|--|
| | och1 disruptant | KK4 | KK4(YEp51-OCH1) | | | | |
| α -methyl-mannoside (2 mM) | 241(0.72) | 334(1.00) | 315(0.94) | | | | |
| α -1.6-mannobiose (2 mM) | 233(0.91) | 255(1.00) | 277(1.09) | | | | |
| mannotetraose(α -1,3- α -1,2- α -1,2) (2 mM) | 5(0.32) | 15(1.00) | 10(0.68) | | | | |
| Man _o GlcNAc (0.06 mM) | 8(0.44) | 18(1.00) | 17(0.93) | | | | |
| $\Delta och l$ mannan (0.03 mM) ^a | 4(0.36) | 12(1.00) | 58(4.79) | | | | |

^aThe molar concentration was calculated on the basis of the average molecular weight (70 kDa) of och1 disruptant mannan.

suggests that the OCH1 protein may exist in the ER and/or Golgi membranes.

derived from the endo H treated inner core oligosaccharide accumulated in the ER.

Assay of mannosyltransferase activity

Since it is most likely that the OCH1 protein may function in the Golgi as a mannosyltransferase itself or its modifier protein to control enzyme activity, several mannosyltransferase activities were measured and compared among the cells of och1 disruptant, KK4 transformant and KK4 wild type. The small oligosaccharides, methyl- α -mannoside and α -1,6-mannobiose, are known as good acceptors for two different α -1,2-mannosyltransferases (Lewis and Ballou, 1991), and α -1,3- α -1,2- α -1,2-mannotetraose is an acceptor for the conventional α -1,6 mannosyltransferase (Nakajima and Ballou, 1975). However, no significant differences were observed in these enzyme activities among the above cells. In contrast, when the core-like oligosaccharide of cell wall mannoprotein which accumulated in the ochl disruptant cells, was used as an acceptor, a 4.8-fold higher amount of ¹⁴C]mannose transfer from GDP-Man to the acceptor molecule was observed in the KK4 transformant than in the control cells (Table I). It is noteworthy that no differences are observed between the cells when examined for mannose transfer activity from GDP-Man to Man₈GlcNAc, which is The addition of EDTA (Figure 7A) and the immunoprecipitation of OCH1 protein by the antibody against OCH1 peptide (Figure 7B) caused a reduction of the above mannose transfer reaction in a dose dependent manner. Since the Mn^{2+} requirement is a common feature of glycosyltransferase (Paulson and Colley, 1989) but not of protein kinase which can modify the glycosyltransferase activity (Bunnell *et al.*, 1990), the above data indicate that the OCH1 protein functions as a novel mannosyltransferase which specifically recognizes a unique core-like oligosaccharide structure as an acceptor molecule.

Discussion

We have succeeded in cloning the OCH1 gene which complements ts growth phenotype of och1 mutant, and have shown that this DNA encodes a membrane spanning protein with 480 amino acid residues (Figure 2). The *in vitro* and *in vivo* analyses suggest that the OCH1 protein is a type II membrane protein containing four N-linked sugar chains. The OCH1 protein shows common properties with a group of mammalian glycosyltransferases existing in the Golgi



Fig. 7. Inhibition by EDTA and decrease by immunoprecipitation of mannosyltransferase activity. The mannosyltransferase activity of high speed pellet fraction from KK4 transformant was measured as described in Materials and methods using the och1 disruptant mannan as an acceptor. (A) Inhibition of mannosyltransferase activity by the addition of EDTA. Assay of mannosyltransferase activity was performed in 0, 1, 3, 5, 7, 9 and 10 mM EDTA, respectively. (B) Decrease of mannosyltransferase activity by immunoprecipitation of OCH1 protein. The OCH1 protein was precipitated by antibody against OCH1-C peptide before the measurement of mannosyltransferase activity. The antibody concentrations were 1/10, 1/50, 1/500, 1/5000 and 1/50000.

membranes. They all have a short N-terminal cytoplasmic tail, a 16-20 amino acid membrane anchor domain, and an extended stem region which is followed by the large Cterminal catalytic domain (Paulson and Colley, 1989). The length of oligosaccharides (Man₉₋₁₃GlcNAc₂) of invertase accumulated in the och1 mutant (Nagasu et al., 1992), is very close to that of the mnn9 mutant, which is reported to accumulate the Man₁₀₋₁₃GlcNac₂ oligosaccharide that contains one additional α -1,6 mannose residue, together with a few other α -1,2 and/or α -1,3 mannose residues, into the inner core (Man₈GlcNAc₂) (Hernandez et al., 1989). Although the mnn9 mutation affects the expression of mannosyltransferase activities, all the evidence suggests that the mutation is not in the structural genes for such enzymes (Gopal and Ballou, 1987; Ballou et al., 1989; Devlin and Ballou, 1990).

Clear evidence for the OCH1 protein function was

obtained by the measurement of several mannosyltransferase activities. By using the core-like oligosaccharide formed in the ochl disruptant as an acceptor, the OCH1 protein overproducing cells showed a 4.8-fold higher activity of mannose transfer than the control wild type cells, whereas the ochl disruptant showed a reduced activity (36%) of control cells. This residual activity in the *och1* disruptant may be contributed by the other α -1.2 or α -1.3 mannosyltransferase which can transfer mannose from GDP-Man to the above core-like acceptor. The OCH1 protein dependence for the reaction and inhibition of the reaction by EDTA are also supporting evidence for the demonstration of mannosyltransferase function of the OCH1 gene product. It is noteworthy that neither the endo H treated inner core oligosaccharide (Man₈GlcNAc) nor the smaller oligosaccharides which are commonly used to measure α -1,2 or α -1,6 mannosyltransferase serve as an acceptor. We did not measure the α -1,3 mannosyltransferase activity, because this reaction takes place within the side chain of the α -1,6 mannose backbone (Nakajima and Ballou, 1975) and is not concerned with the outer chain elongation which is deficient in the och1 disruptant.

In yeast cells the α -1.6 mannose should be added to the inner core (Man₈GlcNAc₂) to form Man₉GlcNAc₂ by the initiation specific α -1,6 mannosyltransferase (Romero and Herscovics, 1988; Reason et al., 1991). Ballou et al. (1990) proposed that the mannose outer chain elongation will be started by the different elongation specific α -1,6 mannosyltransferase. In this step, if the α -1,2 mannose instead of α -1.6 mannose is added to Man₉GlcNAc₂ by the termination specific α -1,2 mannosyltransferase, mannose outer chain elongation will be stopped (Ballou et al., 1990). It is noteworthy that Man₈GlcNAc does not serve as an acceptor of mannose transfer by OCH1 protein, whereas the mannose oligosaccharide prepared from the och1 disruptant does (Table I). It is confirmed that the oligosaccharide of invertase prepared from the och1 disruptant consists of $Man_{9-13}GlcNAc_2$ (data not shown), which is the same as that preparaed from the och1 mutant grown at the nonpermissive temperature (Nagasu et al., 1992). It is most likely that Man_{10-13} GlcNAc₂ is formed by the addition of mannose to the side chain of Man₉GlcNAc₂ by the α -1,2 and/or α -1,3 specific mannosyltransferase. Therefore Man₉GlcNAc₂ will be the true substrate accumulated in the och1 mutant and disruptant. The OCH1 protein may be either the elongation specific α -1,6 mannosyltransferase or the termination specific α -1,2 mannosyltransferase, both of which use Man₉GlcNAc₂ as an acceptor. However, the latter possibility looks unlikely, because the lack of α -1,2 mannosyltransferase does not show the deficiency in mannose outer chain elongation observed in the och1 mutant and disruptant. Therefore, it is most likely that the OCH1 protein is the novel α -1,6 mannosyltransferase which initiates the elongation of Man₉GlcNAc₂. The detailed structure of the acceptor molecule for OCH1 protein dependent mannose transfer should be elucidated in future.

Materials and methods

Strains and media

Escherichia coli strain XL1-Blue [endA1 hsdR17 (r_k^-, m_k^-) supE44 thi-1 recAl gyrA96 relAl $\Delta(lac)$ (F' proAB lacl^AZ Δ M15 Tn10 (tet^R)] (Frischauf et al., 1983) was used for preparation of plasmids and single strand DNA. Saccharomyces cerevisiae strain EHF-2C (MATa och1 leu2-3

leu2-112 pep4-3) (Nagasu et al., 1992) was used for cloning the complementary gene and integration of the OCH1 gene. KK4 (MATa ura3 his1 or his3 trp1 leu2 gal80) (Nogi et al., 1984) was used for preparation of microsomal membranes, overproduction of OCH1 protein and OCH1 gene disruption. EHA-1C (MATa leu2-3 leu2-112 pep4-3) was used for OCHI gene disruption. YS37-4C (MATa leu2cyh2) was used for tetrad analysis. EG1-103 (MATa ura3 leu2 trp1) obtained from Dr B.Hall, University of Washington, was used for the construction of the yeast genomic DNA library. Escherichia coli cells were grown in LB-broth (1% Bacto-tryptone, 0.5% yeast extract, 0.5% NaCl and 0.2% glucose). Saccharomyces cerevisiae cells were grown in YPD (2% Bacto peptone, 1% yeast extract and 2% glucose), SD -leu (0.67% Bacto-yeast nitrogen base w/o amino acids, 2% glucose and $20-400 \mu g/ml$ amino acids mixture lacking leucine) (Sherman et al., 1986a) and SG + 0.5% glucose (0.67% Bacto-yeast nitrogen base w/o amino acids, 2% galactose, 0.5% glucose and 20-400 µg/ml amino acids mixture).

Materials

Plasmids pUC19, M13mp18 and 19 were purchased from Toyobo Co. pSP65 was obtained from Amersham. Bluescript SK+, Phagescript SK and Exo/Mung Deletion kit were from Stratagene. The dye primers for DNA sequencing, reagents for DNA and peptide synthesis were obtained from Applied Biosystems Inc. pUC12 and dATP, dCTP, dTTP were purchased from Pharmacia Co. 7-deaza-dGTP, dideoxy-NTP, DIG-RNA labeling and detection kits and 1-deoxy-mannojirimycin were purchased from Boehringer-Mannheim. Sequenase Ver.2 (Tabor and Richardson, 1989) was from United States Biochemical Co. Restriction endonucleases were from Toyobo Co. Mutagene in vitro mutagenesis kit (Kunkel, 1985), Dowex 1-X8 and Bio-Gel P2 were obtained from Bio-Rad Laboratories. SP6 System (in vitro transcription kit) (Melton et al., 1984), rabbit reticulocyte lysate and canine pancreatic microsomal membranes were purchased from Amersham. L- $[^{35}S]$ methionine (specific activity, 37 TBq/mmol) and GDP-[14C]mannose (specific activity, 8.8 GBq/mmol) were from Du-Pont-New England Nuclear. m-Maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), proteinase K and phenylmethylsulfonyl fluoride (PMSF) were from Sigma. Endoglycosidase H and zymolyase 100T were from Seikagaku Kogyo Co. Polyvinylidene difluoride (PVDF) membrane filter was purchased from Millipore. Keyhole limpet hemocyanin (KLH) was from Calbiochem Co. Goat anti-rabbit IgG alkaline phosphatase conjugate was from Cappel. Antipain, chymostatin, leupeptin and pepstatin A were purchased from Peptide Institute Inc. BCA protein assay reagent was from Pierce chemical company. Clear-sol II and a-methyl-mannoside were obtained from Nakarai tesque. Man₈GlcNAc was purchased from Genzyme corporation. α -1,2-mannobiose was kindly provided by Dr T.Nakajima (Tohoku University, Japan).

Plasmid construction

The LEU2 gene was excised from pCV13 by digestion with AccI and HpaI. The fragment overhang was made blunt-ended by Klenow fragment of DNA polymerase I. pTN0103 was constructed by inserting this LEU2 fragment into the Smal site of pUC12. Yeast 2 µ DNA was digested from YEp51 (Broach et al., 1983) with PstI and XbaI, and the 1.3 kbp fragment containing REP3 and replication origin was inserted into pTN0103 PstI-XbaI sites. This plasmid (pTN3200) was used for construction of the yeast genomic DNA library. The yeast genomic library was constructed by inserting the Sau3A partially digested genomic DNA isolated from EG1-103 strain into the BamHI site of pTN3200 (Nakayama et al., 1985). Plasmid pM1 was isolated from the yeast genomic DNA library by complementation of the och1 ts mutant. It carried the OCH1 5.4 kbp fragment and was digested with Sall and HindIII. This 2.6 kbp Sall-HindIII OCH1 fragment was inserted into SalI/HindIII sites of pTN0103, Bluescript SK+ and M13mp19, respectively. The OCH1 inserted pTN0103 (pTN-OCH1) was used for chromosomal integration of the OCH1 gene to the och1 strain, and the OCH1 inserted M13mp19 was used for site directed mutagenesis.

The OCH1 gene was mutagenized to make a SphI site at ATG by Mutagene in vitro mutagenesis (Kunkel, 1985). The mutagenized OCH1 gene was digested with SphI and HindIII, and the 1.8 kbp fragment was inserted into pUC19 SphI/HindIII sites. This plasmid was cut by SaII and HindIII and the 1.8 kbp fragment was inserted into SaII/HindIII sites of pSP65 (Melton et al., 1984) and of YEp51. These plasmids (pSP-OCH1 and YEp51-OCH1) were used for in vitro transcription and overproduction of OCH1 protein in yeast, respectively.

The OCH1 gene disruption was carried out by replacing a central part of OCH1 gene with LEU2 gene. The LEU2 gene was excised from plasmid pTN0103 by digestion with SacI and BamHI. The fragment overhangs were blunt-ended by digestion with Mung Bean nuclease. The blunt-ended LEU2 fragment was inserted into the Hpal-AatII site of the OCH1 gene that was located in Bluescript SK+. This plasmid (pBL- Δ och1) in which the 0.7 kbp *Hpa*I-*Aat*II *OCH1* fragment was replaced with the 1.6 kbp *LEU2* gene was used for disruption of the *OCH1* gene.

Cloning of the OCH1 gene

EHF-2C (*och1*) was transformed with a yeast genomic DNA library in pTN3200 by the lithium acetate procedure (Ito *et al.*, 1983). The *LEU2* transformants were grown on a SD – leu plate and *OCH1* transformants were isolated by complementation of *ts* phenotype at 36°C. The plasmids were isolated from these transformants by the yeast minipreparation method (Sherman *et al.*, 1986b) and used to transform competent *E. coli* XL1-Blue cells from which plasmids were recovered by the alkali method (Maniatis *et al.*, 1982).

DNA sequencing

Restriction fragments containing a portion of the OCH1 gene were subcloned into M13mp18, 19 or Phagescript. Deletions were made of the DNA inserts in Phagescript using the Exo/Mung deletion system. The clones were sequenced by the dideoxy method (Sanger *et al.*, 1977) with dye primers using ABI DNA sequencer (model 370A).

Integration and disruption of the OCH1 gene

The targeted OCH1 gene integration was according to Rothstein (1991). EHF-2C (MATa och1) cells were transformed with HpaI digested pTN-OCH1 by lithium acetate method. One of the transformants (LEU2 OCH1) was crossed to YS37-4C (MATa leu2 cyh2) and 31 tetrads of integrant were analyzed by standard genetic methods (Sherman and Hicks, 1991) to determine the integration site.

One step gene disruption was performed according to Rothstein (1991). Plasmid pBL- Δ och1 was digested by *Sal*1 and *Hin*dIII and *och1::LEU2* DNA was excised. The 3.5 kbp linear DNA fragment was used to transform the EHA-1C/KK4 diploid cells, which require leucine for growth. *LEU* transformants were selected on SD –leu medium and these transformants were allowed to sporulate to make disruptants. Gene disruption was confirmed by Southern blot analysis and one of the meiotic disruptants designated as YS52-1-1B was used for mannosyltransferase assay.

Southern blot analysis

Total chromosomal DNA was isolated from the EHA-1C/KK4 diploid, from one of the $LEU2^+$ transformants derived from the above diploid, and from a set of segregants after tetrad dissection. These chromosomal DNAs were digested with *SaII* and *HindIII*, and separated by 0.8% agarose gel electrophoresis. After electrophoresis, digested DNAs were transferred to Nylon membranes using electro-blotter (Nippon eido, Japan). The *OCH1* probe was made by the DIG-RNA labeling method and detection was carried out by the DIG-detection system (Dooley *et al.*, 1988) according to the supplier's protocol.

In vitro translation/translocation

The plasmid pSP-OCH1 (5 μ g) was linearized by digestion with *Hin*dIII and transcribed *in vitro* by SP6 RNA polymerase system containing m⁷GpppG in 50 μ l reaction mixture (Melton *et al.*, 1984). The reaction mixture was ethanol precipitated to remove nucleotides and was dissolved in 10 μ l of deionized water. *In vitro* translation was performed at 30°C for 60 min in solution containing 40 μ l of rabbit reticulocyte lysate, 7 μ l of [³⁵S]methionine and 1 μ l of the above reaction mixture as a source of transcribed mRNA according to Pelham and Jackson (1976). *In vitro* translation/translocation was carried out at 30°C for 90 min as described by Walter and Blobel (1983) in solution containing 20 μ l of rabbit reticulocyte lysate, 8 μ l of [³⁵S]methionine, 20 μ l of canine pancreatic microsomal membranes, 1 μ l of transcribed mRNA and 11 μ l of H₂O. Proteinase K and endo H digestion were carried out by the method of Hansen *et al.* (1986).

Preparation of polyclonal antibodies against chemically synthesized peptides

Three peptides ranging from 16 to 17 amino acids, which were chosen as a representative of N-terminal, central and C-terminal domains of the OCH1 protein, were synthesized by peptide synthesizer ABI model 470A. These synthetic peptides (Met1-Thr16, Val295-Val311 and Phe465-Lys480), were coupled to keyhole limpet hemocyanin (KLH) through the cysteine residue of the peptides by the method of Lerner *et al.* (1981). These conjugates were injected into rabbit to obtain anti-peptide antibodies. Among three synthetic peptides only the C-terminal peptide (OCH1-C, Phe465-Lys480) was successful in preparing the polyclonal antibody which was able to cross react with the OCH1 protein. This anti-OCH1-C antibody was purified on a C-terminal peptide column (Hunt *et al.*, 1985).

Western blot analysis

The yeast KK4 was transformed with YEp51-OCH1 by the lithium acetate method. YS52-1-1B (och1 disruptant), KK4 (wild type), KK4 harboring the YEp-OCH1 (KK4 transformant) were grown in 250 ml of SG + 0.5%glucose, in the presence or absence of leucine to mid-logarithmic phase at 25°C. After confirming that the cells had exhausted glucose and OCH1 gene expression under the GAL10 promoter was induced by galactose, the cells were collected by centrifugation at 3000 g for 5 min, washed with 1% KCl and resuspended in 5 ml of 50 mM Tris-HCl (pH 7.5), 10 mM MnCl₂, 1 mM PMSF, 5% glycerol and 2 µg/ml each of proteinase inhibitor (antipain, chymostatin, leupeptin and pepstatin A). Glass beads (0.45-0.50 mm) were added to half of the cell suspension volume and homogenized with B. Brown homogenizer for $1 \min \times 3$ times at 4°C. Homogenates (WCL; whole cell lysate) were filtered by G1 glass filter and centrifuged at 10 000 g for 20 min. Pellet was collected as LSP (low speed pellet), and supernatant was further centrifuged at 100 000 g for 1 h. Pellet (HSP. high speed pellet), and supernatant (HSS, high speed supernatant) were collected. LSP and HSP were resuspended in a buffer containing 50 mM Tris-HCl (pH 7.5), 10 mM MnCl₂, 1 mM PMSF, 5% glycerol and proteinase inhibitor mixture (2 µg/ml each). Protein concentration was determined by BCA protein assay reagent.

HSP fraction from KK4 transformants was solubilized with 2% Triton X-100 by sitting the HSP on ice for 2 h. Endo H treatment of HSP solubilized with 2% Triton X-100 was carried out by the method of Hansen *et al.* (1986).

A 170 µg protein of each cell fraction was separated by SDS-PAGE and proteins were transferred to PVDF membrane filter by electro-blotter (Atto, Japan) at room temperature under the constant current (0.4 A) for 1 h. After 1 h incubation in a blocking buffer containing 3% gelatin, 20 mM Tris-HCl (pH 7.5) and 500 mM NaCl, this filter was transferred to 10 ml of antibody buffer containing 1% gelatin, 20 mM Tris-HCl (pH 7.5), 500 mM NaCl, 0.05% Tween 20 and affinity purified anti-OCH1-C antibody at a dilution of 1:5000. The membrane filter was incubated overnight at room temperature, washed three times with washing buffer containing 20 mM Tris-HCl (pH 7.5), 500 mM NaCl, 0.5% Tween 20 for 10 min each, and then incubated for 1 h with anti-rabbit IgG alkaline phosphatase conjugate at a dilution of 1:1000 in 10 ml of antibody buffer. The membrane filter was washed three times with washing buffer for 10 min each, and incubated with 10 ml of 100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 50 mM MgCl₂ containing nitroblue tetrazolium salt (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (X-phosphate). Coloring reaction was stopped by washing the membrane filter for 5 min with 50 ml of 10 mM Tris-HCl (pH 8.0), 1 mM EDTA.

Active staining of invertase

Active staining of invertase was carried out by the method of Gabriel and Wang (1969). The yeast strains YS57-5A, YS57-5B, YS57-5C, YS57-5D and YS54-6B were grown to mid-logarithmic phase in YPD at 25°C. A 5 ml aliquot of culture was centrifuged, washed with water and resuspended in 5 ml of YP containing 0.05% glucose. The cells were cultured for 2 h at 25°C, centrifuged and resuspended in 50 mM Tris-HCl (pH 6.8), 1 mM PMSF, 1 mM DTT, 2% SDS and 10% glycerol. Glass beads (0.45-0.50 mm) were added up to the surface of cell suspension, and vortexed for 1 min with cooling down on ice. After adding an equal volume of 50 mM Tris-HCl (pH 6.8), 1 mM PMSF, 1 mM DTT, 2% SDS and 10% glycerol and spinning in centrifuge for 5 min, supernatant was loaded on an 8% SDS-polyacrylamide gel and electrophoresis was performed. After the SDS-PAGE, the gel was bathed in 0.1 M sucrose, 0.1 M sodium acetate (pH 5.1) at 37°C for 20 min. The gel was washed with water, placed in 0.1% 2,3,5-triphenyltetrazolium chloride, 0.5 M NaOH and boiled to detect red bands.

Mannosyltransferase assay

Mannosyltransferase activity was measured by a modification of Nakajima and Ballou (1975). A 200 μ g protein of HSP was incubated in 50 μ l of 50 mM Tris-HCl (pH 7.5), 10 mM MnCl₂, 0-2 mM acceptor, 0.6% Triton X-100, GDP-[¹⁴C]mannose (740 Bq), 0.5 mM 1-deoxymannojirimycin at 25°C for 30-120 min. 1-Deoxy-mannojirimycin was used as an inhibitor of α -mannosidase in yeast (Jelinek-Kelly *et al.*, 1985). Excess GDP-[¹⁴C]mannose was removed by passing the solution through a Dowex 1-X8 column (1 ml). The neutral products were eluted with 1 ml of water and radioactivity was counted in 10 ml of clear-sol II. In this assay, α -methyl-mannoside, α -1,6-mannobiose, mannotetraose (α -1,3- α -1,2- α -1,2), Man₈GlcNAc, OCH1 disruption mannan were used as acceptors.

The EDTA was added to the assay mixture containing HSP from the KK4 transformant and mannan from the *och1* disruptant, until the final concentration of 0-10 mM. Immunoprecipitation of OCH1 protein using

antibody against OCH1-C peptide was performed as follows. A 200 μ g protein of HSP (from the KK4 transformant) was incubated in 15 μ l of 2% Triton X-100 and anti-OCH1-C antibody added to final concentrations of 1/10, 1/50, 1/500, 1/50000. After incubation for 1 h on ice, 15 μ l of 20% protein A – sepharose (Pharmacia) was added and incubated for 1 h on ice. The sample was centrifuged and the supernatant used for the mannosyltransferase assay.

Yeast KK4 (*OCH1*) and YS52-1-1B ($\Delta och1$) mannans were isolated by Peat *et al.* (1965). KK4 mannan was hydrolyzed by the acetolysis method (Kocourek and Ballou, 1969) and mannotetraose (α -1,3- α -1,2- α -1,1) was isolated by Bio-Gel P2 column (2.6 × 200 cm) according to the method of Raschke *et al.* (1973).

References

- Albright, C.F. and Robbins, P.W. (1990) J. Biol. Chem., 265, 7042-7049.Ballou, L., Alvarado, E., Tsai, P.-K., Dell, A. and Ballou, C.E. (1989) J. Biol. Chem., 264, 11857-11864.
- Ballou, L., Hernandez, L.M., Alvarado, E. and Ballou, C.E. (1990) Proc. Natl. Acad. Sci. USA, 87, 3368-3372.
- Broach, J.R., Li, Y.-Y., Wu, L.-C.C. and Jayaram, M. (1983) In Inouye, M. (ed.), *Experimental Manipulation of Gene Expression*. Academic Press, New York, pp. 82-117.
- Bunnell, B.A., Adams, D.E. and Kidd, V.J. (1990) Biochem. Biophys. Res. Commun., 171, 196-203.
- D'Agostaro, G., Bendiak, B. and Tropak, M. (1989) Eur. J. Biochem., 183, 211-217.
- Devlin, C. and Ballou, C.E. (1990) Mol. Microbiol., 4, 1993-2001.
- Dooley, S., Radtke, J., Blinand, N. and Unteregger, G. (1988) Nucleic Acids Res., 16, 11839.
- Frischauf, A.-M., Lehrach, H., Poustka, A. and Murray, N. (1983) J. Mol. Biol., 170, 827-842.
- Gabriel, O. and Wang, S.-F. (1969) Anal. Biochem., 27, 545-554.
- Gopal, P.K. and Ballou, C.E. (1987) Proc. Natl. Acad. Sci. USA, 84, 8824-8828.
- Hansen, W., Garcia, P.D. and Walter, P. (1986) Cell, 45, 397-406.
- Hartog, K.O. and Bishop, B. (1987) Nucleic Acids Res., 15, 3627.
- Hernandez, L. M., Ballou, L., Alvarada, E., Gillece-Castro, B. L., Burlingame, A.L. and Ballou, C.E. (1989) J. Biol. Chem., 264, 11849-11856.
- Hunt, J.S., McGiven, A.R., Groufsky, A., Lynn, K.L. and Taylor, M.C. (1985) *Biochem. J.*, 227, 957-963.
- Ito,H., Fukuda,Y., Murata,K. and Kimura,A. (1983) J. Bacteriol., 153, 163-168.
- Jelinek-Kelly, S., Akiyama, T., Saunier, B., Tkacz, J.S. and Herscovics, A. (1985) J. Biol. Chem., 260, 2253-2257.
- Kocourek, J. and Ballou, C.E. (1969) J. Bacteriol., 100, 1175-1181.

Kornfeld, R. and Kornfeld, S. (1985) Annu. Rev. Biochem., 54, 631-664.

- Kukuruzinska, M.A., Bergh, M.L.E. and Jackson, B.J. (1987) Annu. Rev. Biochem., 56, 915-944.
- Kumar, R., Yang, J., Larsen, R.D. and Stanley, P. (1990) Proc. Natl. Acad. Sci. USA, 87, 9948–9952.
- Kunkel, T.A. (1985) Proc. Natl. Acad. Sci. USA, 82, 488-492.
- Lerner, R.A., Green, N., Alexander, H., Liu, F.-T., Sutcliffe, G. and Shinnick, T.M. (1981) Proc. Natl. Acad. Sci. USA, 78, 3403-3407.
- Lewis, M.S. and Ballou, C.E. (1991) J. Biol. Chem., 266, 8255-8261.
- Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 90–91.
- Melton, D.A., Krieg, P.A., Rebagliati, M.R., Maniatis, T., Zinn, K. and Green, M.R. (1984) Nucleic Acids Res., 12, 7035-7056.
- Nagasu, T., Shimma, Y., Nakanishi, Y., Kuromitsu, J., Iwama, K., Nakayama, K., Suzuki, K. and Jigami, Y. (1992) *Yeast*, 8, in press.
- Anakanishi, Nakajima, T. and Ballou, C.E. (1975) Proc. Natl. Acad. Sci. USA, 72, 3912-3916.
- Nakano, A., Brada, D. and Schekman, R. (1988) *J. Cell Biol.*, **107**, 851–863. Nakayama, N., Miyajima, A. and Arai, K. (1985) *EMBO J.*, **4**, 2643–2648. Nakazawa, K., Ando, T., Kimura, T. and Narimatsu, H. (1988) *J. Biochem.*, **104**, 165–168.
- Nogi, Y., Shimada, H., Matsuzaki, Y., Hashimoto, H. and Fukasawa, T. (1984) Mol. Gen. Genet., 195, 29-34.
- Paulson, J.C. and Colley, K.J. (1989) J. Biol. Chem., 264, 17615-17618.

Peat, S., Whelan, W.J. and Edwards, T.E. (1961) J. Chem. Soc., 29-34. Pelham, H.R.B. and Jackson, R.J. (1976) Eur. J. Biochem., 67, 247-256.

Raschke, W.C., Kern, K.A., Antalis, C. and Ballou, C.E. (1973) J. Biol. Chem., 248, 4660-4666.

- Reason, A.J., Dell, A., Romero, P.A. and Herscovics, A. (1991) *Glycobiology*, **1**, 387-391.
- Rine, J., Hansen, W., Hardeman, E. and Davis, R.W. (1983) Proc. Natl. Acad. Sci. USA, 80, 6750-6754.
- Romero, P.A. and Herscovics, A. (1989) J. Biol. Chem., 264, 1946-1950.
- Rothstein, R. (1991) Methods Enzymol., **194**, 281-301.
- Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA, 74, 5463-5467.
- Shaper, N.L., Hollis, G.F., Douglas, J.G., Kirsch, I.R. and Shaper, J.H. (1988) *J. Biol. Chem.*, **263**, 10420-10428.
- Sherman, F. and Hicks, J.B. (1991) Methods Enzymol., 194, 21-37.
- Sherman, F., Fink, G.R. and Hicks, J.B. (1986a) Methods in Yeast Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 163-167.
- Sherman, F., Fink, G.R. and Hicks, J.B. (1986b) Methods in Yeast Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 127-128.
- Tabor, S. and Richardson, C.C. (1989) J. Biol. Chem., 264, 6447-6458.
- von Heijne, G. (1986) Nucleic Acids Res., 14, 4683-4690.
- Walter, P. and Blobel, G. (1983) Methods Enzymol., 96, 84-93.
- Weinstein, J., Lee, E.U., McEntee, K., Lai, P.-H. and Paulson, J.C. (1987) *J. Biol. Chem.*, **262**, 17735-17743.

Received on March 12, 1992