Sequence Analysis of the Translational Elongation Factor 3 from *Saccharomyces cerevisiae*  

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The gene YEF-3 encoding the elongation factor for protein synthesis in *Saccharomyces cerevisiae* is an essential gene as shown by one-step gene disruption and is located on chromosome XII as determined by orthogonal field alternation gel electrophoresis. The nucleotide sequence of the gene was determined from a sequential series of subclones generated from the YEF-3 gene cloned into bacteriophage M13. The HOMOL1 sequence and the RPG box, which are considered to be enhancer elements involved in coordinate regulation of transcription of the genes coding for yeast ribosomal proteins and protein synthesis factors, are found in the 5'-flanking region of the gene. A dyad symmetry that enables hairpin loop formation in the DNA molecule is found in the 3'-terminal at the termination site of transcription. An open reading frame of 3132 nucleotides codes for a deduced protein of 115,860 Da. A striking feature of the elongation factor 3 deduced polypeptide is the internal repeat of a region with approximately 200 amino acids which includes an ATP-binding site and shares similarity with some transport and drug-resistant proteins. Another characteristic is the presence of a highly charged C-terminal region composed of three basic polylysine blocks, suggesting interaction with RNA. The sequence supports the hypothesis that YEF-3 encodes a protein synthesis factor and suggests that its main role may be to transduce nucleoside triphosphate energy into mechanical energy for translocation during translation.

The elongation factor 3 (EF-3) was discovered by Skogerson and collaborators (1976, 1977) to be required for *in vitro* protein synthesis by yeast ribosomes in addition to elongation factors 1 and 2 which are interchangeable with the equivalent factors from other eukaryotes. EF-3 has been purified from several fungi species and found to consist of a single polypeptide chain with a molecular weight of about 125,000 (Dasmahapatra and Chakraburtty, 1981; Uritani and Miyazaki, 1988a). The evidence that EF-3 is a protein synthesis factor is compelling. *In vitro* protein synthesis systems directed by poly(U) and natural mRNA exhibits a 30-fold dependence on elongation factor 3 (Skogerson and Wakatama, 1976; Skogerson and Engelhardt, 1977; Herrera et al., 1984). Immuno-inactivation experiments with monoclonal antibodies specific for EF-3 demonstrated that EF-3 was required for protein synthesis (Hutchison et al., 1984). A temperature-sensitive yeast mutant blocked in the elongation phase of protein synthesis was shown to have a thermolabile EF-3 (Herrera et al., 1984). The gene encoding EF-3 has been isolated and shown to complement the temperature-sensitive defect in the yeast mutant with the thermolabile EF-3 (Qin et al., 1987).

In some respects EF-3 is the best characterized elongation factor. However, its mechanism of action remains unclear despite a number of studies (Skogerson and Wakatama, 1976; Skogerson and Engelhardt, 1977; Dasmahapatra and Chakraburtty, 1981; Hutchison et al., 1984; Herrera et al., 1984; Miyazaki et al., 1988a, 1988b). The basic problem is that although the overall elongation reaction with both natural and synthetic mRNAs strongly depends on EF-3, it has not been possible to assign EF-3 to a specific step in the elongation process. We have undertaken to sequence the gene encoding EF-3 to determine if the sequence can illuminate the mechanism of action of EF-3.

Our results indicate that the YEF-3 is an essential gene located at chromosome XII. The presence of the consensus sequences HOMOL1 and RPG box as well as thymidine-rich sequences 3' of RPG box included the gene in the group of coordinate regulated genes of the translational machinery.

The unique open reading frame of this gene codes for a predicted peptide of 1044 amino acids with a molecular mass of 115.86 kDa. The polypeptide seems to be a soluble protein both by amino acid composition and hydropathy profile. The amino acid sequence indicates the presence of internally repeats which share a common evolutionary origin, bind ATP, and probably serve to couple ATP hydrolysis to a different biological process (Higgins et al., 1986).

**EXPERIMENTAL PROCEDURES**

**Gene Disruption**—A one-step gene disruption experiment was carried out as described by Rothstein (1983). A 3.5-kb XbaI fragment containing almost the complete YEF-3 gene was cloned into pUC19. A 3.0-kb fragment containing the selectable marker LEU2 was then inserted into the *Bgl*II site within the YEF-3. The disrupted YEF-3 by LEU2 was linearized with XhoI and used to transfer a diploid strain SSU10 leu2 (Fig. 1). leu" transformants were allowed to sporulate for tetrad analysis.
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FIG. 1. Restriction map of the disrupted YEF-3 by LEU2.
Symbols used for restriction enzymes are: Ba, BamHI; B, BglII; PI, PstI; PII, PvuII; Xh, XhoI; Xb, XbaI; A, EcoRI. The hollow arrow symbolizes the YEF-3 transcript. A 3.0-kb BglII fragment containing the LEU2 gene was inserted in the BglII site within the YEF-3.

FIG. 2. Nucleotide sequence strategy. The vertical line is the insert from pEF-3. The hollow arrow is the transcript unit of YEF-3 (Qin et al., 1987). The bottom line is the fragment that was used to construct M13 subclones for dideoxy nucleotide sequence analysis. The arrows indicate the direction and extent of nucleotide sequences obtained from subclones. The restriction enzyme sites are shown as follows: B, BglII; X, XbaI; Xh, XhoI; A, EcoRI.

Orthogonal Field Alternation Gel Electrophoresis (OFAGE) and Southern Blot Hybridization—Sample preparations from yeast strain DC04 and OFAGE were performed as published (Schwartz and Cantor, 1984; Carle and Olsen, 1984, 1985). After staining with ethidium bromide and destaining, the gel was soaked in 0.25 N HCl for 20 min and then transferred to Zetabind membrane with 0.4 N NaOH for 3 h. The blot was cut into three strips and hybridized with chromosome probes and the YEF-3 DNA, respectively.

Cloning of Fragments—3.5-kb XbaI fragment containing the major part of the transcription unit of YEF-3 and 1.5-kb EcoRI-XhoI fragment containing the upstream sequence were prepared from plasmid pEF-3 (Qin et al., 1987; Fig. 2). The 3.5-kb fragment was cloned into M13mp18, and two clones with inserts in opposite orientation were used to derive a series of overlapping subclones. The 1.5-kb fragment was cloned into M13mp18 as well as M13mp19. A 2.5-kb XhoI-BglII fragment was also cloned into M13mp18 to be used for determining the junction sequence of the 1.5- and 3.5-kb fragments (Fig. 2).

Nucleotide Sequencing—The sequencing strategy is shown schematically in Fig. 2. Cyclone Biosystem (International Biotechnologies, Inc.) was used to produce a sequential series of overlapping subclones on single-stranded M13 DNA. Clones covering the complete sequence in both orientations were obtained. DNA sequence was determined by the dideoxy chain termination procedure (Sanger and Coulson, 1975) using α-32P-dATP and the modified T7 DNA polymerase (United States Biochemical Corp.).

Computer Analysis—Computer searches in the NBRF-PIR database, local similarities, dot plots, statistical significance of similarities, and hydropathy values were carried out using the FASTA, LFASTA, TLFASTA, RDP2, GREASE, and TGREASE routines of the FASTA program (Pearson and Lipman, 1988). Open reading frames, amino acid composition of the predicted polypeptide, and the search for short recognition sequences in the DNA were carried out using the SNAP-Sequence Analysis Program (Schwidering and Warner, 1984). Inverted repeats, best alignments, the alignments of the different sequences in a group, and the consensus sequence were performed using the programs STEMLOOP, BESTFIT, LINEUP, PROFILEGAPS, and PRETTY of the University of Wisconsin Genetics Computer Group (Devereux et al., 1984) software.

RESULTS AND DISCUSSION

YEF-3 Is an Essential Gene—To determine whether YEF-3 performs an essential function in vivo, a gene disruption experiment was undertaken (Rothstein, 1983). Linear DNA containing the YEF-3 gene disrupted by LEU2 gene was used

FIG. 3. Demonstration that YEF-3 is an essential gene. Part A, after sporulation of a transformed leu+ diploid, 12 ascii were dissected. Spores from a single ascus are aligned vertically. After this photograph was taken, the phenotypes of viable spores were determined. All of them were leu-.

FIG. 4. Identification of YEF-3 on chromosome XII. Part A, 1.2% OFAGE agarose gel stained with ethidium bromide of yeast strain DC04. Part B, southern blot of the OFAGE gel, hybridized by the probes LEU1, chromosome VII (lane 1); rDNA, chromosome XII (lane 3) and 3.5-kb XbaI YEF-3 fragment (lane 2).
FIG. 5. Nucleotide sequence of YEF-3. The nucleotide and the deduced amino acid sequences of the YEF-3 gene are shown. In the 5'-flanking region, box 1 is HOMOL1; box 2 is RPG box, and TATA boxes are underlined. In the coding region, the sequence motifs of regions A and B are underlined and double underlined, respectively. In the 3'-flanking region, the polyadenylation signal sequence is double underlined. The signal for transcriptional termination proposed by Henikoff et al. (1983) is underlined, and the transcription terminal signal proposed by Zaret and Sherman (1982) is overlined.

to transform a leu- diploid yeast strain Ssu10. Three stable leu+ transformants were obtained and one of them was allowed to sporulate for tetrad analysis. Fig. 3A shows 12 tetrads. Only two of the four spores of each tetrad were viable for 10 out of the 12 tetrads. All viable spores are leu-. This segregation pattern shows that the disruption of the gene encoding EF-3 is lethal in haploids.

To confirm the site of integration of the transforming DNA in the genome of Ssu10, the DNA from the parental diploid, the leu+ transformants, and a pair of viable spores was prepared and digested with PvuII or EcoRI, followed by blotting and hybridization with YEF-3 and LEU2 DNA fragments, respectively. Since YEF-3 is a single copy gene and there are no PvuII sites within YEF-3 and LEU2, only one band can be seen in the parental diploid (Ssu10) and the pair of viable spores after hybridization with the YEF-3 probe (Fig. 3B, lanes 1, 3, and 4), while from the leu+ transformants two bands are found (Fig. 3B, lane 2), which was expected if the linear YEF-3::LEU2 fragment had been integrated into one of the two haploid genomes. In Fig. 3C, the EcoRI-digested DNAs were hybridized with a LEU2 probe. Because there is an EcoRI site within LEU2, two bands can be seen in the parental diploid and the pair of viable spores (lanes 1, 3, and 4), while four bands are seen from the leu+ transformant (lane 2).
TABLE I
Amino acid composition of the deduced sequence of EF-3

| Nonpolar | No. % | Polar | No. % | Acidic | No. % |
|----------|-------|-------|-------|--------|-------|
| Ala      | 91    | 8.7   | Gly   | 63     | 6.0   |
| Val      | 70    | 6.7   | Ser   | 60     | 5.8   |
| Leu      | 88    | 8.4   | Thr   | 63     | 6.0   |
| Ile      | 77    | 7.4   | Cys   | 15     | 1.4   |
| Pro      | 43    | 4.1   | Tyr   | 20     | 1.9   |
| Met      | 25    | 2.4   | Asn   | 49     | 4.7   |
| Phe      | 25    | 2.4   | Asp   | 58     | 5.6   |
| Arg      | 45    | 4.3   | Glu   | 92     | 8.8   |
| His      | 24    | 2.3   | Glu   | 29     | 2.8   |
| Total    | 442   | 42.3  | Total | 299    | 28.6  |
| Total    | 153   | 14.7  | Total | 299    | 28.6  |

Fig. 6. Hydrophy profile of the EF-3 protein. The amino acid sequence was deduced from the open reading frame shown in Fig. 5. The hydrophaty plot was obtained by computer assisted analysis (FASTA program) using the algorithm and hydrophaty values of Kyte and Doolittle (1982) for a window of 11. Hydrophobic regions are above the line, and hydrophylic regions are below.

2). The results described above indicate that the lethal mutation is genetically linked to the disrupted YEF-3 gene. Therefore, YEF-3 is an essential gene.

YEF-3 Gene Is Localized in Chromosome XII—In order to determine in which chromosome the YEF-3 gene is localized, a number of OFAGE studies were carried out using the yeast strain DCO4 (Carle and Olsen, 1985) at different gel concentrations followed by blotting and probing with the YEF-3 fragment and different chromosomal probes. We ruled out that the YEF-3 was in chromosome IV, VII, or XV (data not shown) and found that the band probed with YEF-3 always corresponded to the band probed with rDNA, the probe for chromosome XII. This chromosome behaves anomalously and irreproducibly under the OFAGE conditions. However, occasionally it migrates as a well defined band. One such gel is shown in Fig. 4 and demonstrates that YEF-3 is on chromosome XII.

YEF-3 Gene Has Only One Open Reading Frame—The complete nucleotide sequence of the 5014-base pair fragment of the YEF-3 gene and the deduced amino acid sequences are shown in Fig. 5. Only one open reading frame with 3132 amino acids was found with no splicing sequences in the cloned fragment, which is consistent with the results of the S1 mapping previously reported (Qin et al., 1987). The molecular mass of the predicted EF-3 protein is 115,860 daltons, which is somewhat less than the 125,000 Da determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Dasmahapatra and Chakraburtty, 1981). Both amino acid composition (42.3% nonpolar amino acids) (Table I) and the hydropathy profile (Fig. 6) based on hydropathy values of Kyte and Doolittle (1982) indicate that EF-3 should be a soluble protein which is consistent with the purification procedures previously published (Skogerson and Wakatama, 1976; Dasmahapatra and Chakraburtty, 1981; Qin et al., 1987; Uritani and Mivasaki, 1988a). The amino acid composition indicates that elongation factor 3 is an acidic protein with an isoelectric point of 5.7. The codon usage in the YEF-3 coding region is presented in Table II. A codon bias of 0.87 obtained for this gene reflects the preferential codon usage of yeast genes expressed at high levels such as glucose-3-phosphate dehydrogenase and ADH1 (Bennetzen and Hall, 1982).

The Flanking Sequences Have Potential Enhancer-like Elements of Transcription in the 5'-Terminal Region and Potential Hairpin Loop Structures in the 5'- and 3'-Terminal Re-
Fig. 7. Dot matrix. A, internal repeats in the predicted EF-3 polypeptide, compared with itself using the PAM 250 scoring matrix (Dayhoff, 1978) in the LFASTA program. B-E, diagonal plots of the regions of similarity shared by EF-3 and Nod1, PetB, HisP, MbpX, MalK, ChlD, HlyB and Mdr1, respectively.

Fig. 8. Alignment of regions A and B of each repeated domain in the EF-3 protein. Regions A and B of each repeat are shown. Amino acids are represented by one-letter notation. Identical residues are indicated by a colon and conservative replacements by a period. Gaps are indicated by a hyphen. X denotes the ends of the initial region found by LFASTA.

Regions of the Gene—The initiation codon AUG is preceded by 1552 nucleotides that presents stop codons in all reading frames, indicating that this region is untranslated. Observations in Saccharomyces cerevisiae show that the genes encoding the components of translation and transcription apparatus, such as r-proteins, elongation factors, and RNA polymerase subunits, contain a number of conserved elements in their 5'-flanking regions (Teem et al., 1984), as well as in the elongation factor 1 LY genes TEF1 and TEF2 (Huet et al., 1985), suggests that the transcription of YEF-3 gene may be coregulated with other genes encoding for the components of translational apparatus in yeast. Four potential TATA boxes were found. Two of them are located around positions −124 and −165, respectively, and the other two are far from the translation initiation site (−584 and −640). According to the transcription unit map of YEF-3, the transcription initiation may start in a purine-rich site between −49 and −42 nucleotides, which resembles the transcription initiation regions of other yeast genes, viz. the sequence PyAAPu (Burke et al., 1983). The environment of the initiation codon AUG in the YEF-3 gene with an A at position −3 is associated with the most efficient translation in eukaryotes (Kozak, 1981; Bairn and Sherman, 1988 and fits well with the consensus sequence of the initiator region 5′-A/YAA/UAAUGUCU-3′ found in most of the yeast genes (Cigan and Donahue, 1987).

The coding region ends with a TAA terminator codon followed by a segment of about 350 nucleotides of the 3′-untranslated region. Several sequence elements have been proposed to be involved in transcription termination and polyadenylation in yeast. A putative polyadenylation signal AATAAA was found 50 nucleotides downstream of the stop

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The amino acid sequence motifs of regions A and B in the EF-3 polypeptide and in other nucleotide-binding proteins

The sequence motifs are in bold. The consensus KTGT following the aspartate residue in P-ATPases is underlined.

| Protein       | Region A   | Region B   |
|---------------|------------|------------|
| EF-3a         | RYGCIGPNCGRSTL  | DIILLDEPNTNLD  |
|               | 469         | 968         |
|               | 707         | 701         |
|               | 51          | 168         |
| NodIb         | CFGL1GPNCGRSTL  | QLLL1DEATSALD  |
|               | 45          | 176         |
| HISPc         | VISSIGGPNCGRSTF  | DWLL1DEPTSALD  |
|               | 453         | 557         |
| MDR(5′)d      | TLAVGSGCGRSTV  | HILL1DEATSALD  |
|               | 647         | 872         |
| U1 RNAe       | AT1GSGCGRSTL  | 258         |
| ATPase Bovinef | KILFGGAGVGRTVF  | VLLF1DNIFRFTQ  |
|               | 21          | 210         |
| Adenylate kinaseg | I1FVVGGPGSGBKGTQ | LLLYV1DAGPETMT |
| II′-ATPaseh   |            | 378         |
| Na-K-ATPasei  |            | 375         |
| EF-1b         | NVVV1HGVDGSRSTL  | STICSDKTGLTLQ  |
|               | 20          | 375         |
| EF-Tu′        | NVGT1HGVDGRTTL  |            |
|               | 24          | 375         |
| IF-2j         | VVT1MGHDGRTSL  |            |
|               | 404         | 375         |
| RAS 1k        | K1VVVGGGGVGRSL  |            |
|               | 23          | 375         |

This work.

* Evans and Downie, 1986.

† Higgins et al., 1982.

‡ Gros et al., 1986.

§ Dolittle et al., 1986.

‖ Walker et al., 1982.

¶ Serrano, 1988.

‖ Cotrelle et al., 1985.

¶ Laursen et al., 1981.

‰ Sacerdot et al., 1984.

‡ Powers et al., 1984.

‡ This work.

The amino acid sequence motifs of regions A and B in the EF-3 polypeptide and in other nucleotide-binding proteins were found in the YEF-3 gene, as well as the sequence TTTTTATA 37 base pairs downstream of the stop codon (Fig. 5), which is proposed by Zaret and Sherman (1982) on the basis of Henikoff et al.’s (1983) model for transcription termination. Based on the transcription unit map published previously (Qin et al., 1987), the termination site of transcription is located about 110 bp downstream of this consensus sequence. Interestingly, at this site we found a dyad symmetry that is exactly the same as we found in the upstream region, but in the opposite orientation (AAGAGAAAGG/TTTTTTTTTTTC) at positions 13263 and +3323. Moreover, at the end of the coding region of the gene, in sites that correspond to three clusters of lysine in the C-terminal region of the protein, other dyad symmetries were found. Because of the rapidity of tandem in the AAG codons in these clusters and the presence of pyrimidine residues (T/C) between them, hairpin loops could be formed in the mRNA molecule.

Internal Repeats in the Amino Acid Sequence Share an ATP-binding Domain, and the C-terminal of the Polypeptide Presents a Highly Charged Region Composed of Three Basic Polysine Blocks—Dot matrix analysis shows the presence of internal repeats in the EF-3 predicted polypeptide (Fig. 7A). The alignment of EF-3 with itself demonstrates the existence of two large repeated sequences encompassing residues 435 to 608 and 673 to 961 (Fig. 8). When the codon preference of these two repeats was examined the same codon bias was found, suggesting that a gene duplication probably occurred during the evolution of EF-3. Each repeat consists of two regions of high similarity (Fig. 8). Regions A of both repeats exhibit 26.9% identity in amino acid sequence and regions B 33% identity. If conservative replacements are considered, similarities of 76 and 78% are obtained. These regions present two short amino acid sequence motifs, GXXGXGKS/T and (np)4 D, which seem to occur in many different proteins that bind nucleotides (Walker et al., 1982; Brands et al., 1986), suggesting that these repeats have a nucleotide-binding domain.
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| MalK | Npt60-98 | 8-49 |
|------|-----------|------|
| PstB | Npt60-98 | 8-49 |
| HisP | Npt60-98 | 8-49 |
| Nodl | Npt60-98 | 8-49 |
| MbpX | Npt60-98 | 8-49 |
| HlYb | Npt60-98 | 8-49 |
| CidH | Npt60-98 | 8-49 |
| Mdp1 | Npt60-98 | 8-49 |
| Efasa | Npt60-98 | 8-49 |
| Efg | Efg60-98 | 8-49 |

Fig. 9. Alignment of EF-3 repeated domains with Nodl, PstB, HisP, MbpX, MalK, CidH, HlYb, and Mdp1. The alignment was obtained by LINEUP and PROFILEGAP. The consensus was obtained with a minimum plurality of 2.0 running PRETTY. In each column positions that shares consensus are in upper case. Gaps are indicated by periods. The * marks the amino acid identity shared by the EF-3 domains and the consensus.

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**et al., 1986,** is not found in the EF-3 polypeptide. This structural feature together with the biochemical studies (Skogerson and Wakatake, 1976; Skogerson and Engelhardt, 1977; Uritani and Miyazaki, 1982, 1988) suggest that the nucleotide code of the repeats is an ATP-binding domain. In the archetype adenylyl kinase mode of ATP binding, the lysine in region A binds to the -P of ATP and the aspartate at sequence B binds to the - and -P of ATP through the chelated magnesium. However, other modes of ATP binding exist in nature like the ATP-binding site of phosphofructokinase, phosphoglycerate kinase, hexokinase, pyruvate kinase, and particularly rich in basic amino acids with three clusters of Elongation Factor 3 phosphorylation. The ATPases the aspartate residue is always followed by the same cation pumps suggests that one must seek direct evidence of ATP-Phosphorylation.

The EF-3 polypeptide also contains a consensus target sequence for potential modification by cAMP-dependent protein kinases (Cohen, 1985) at position 982-986 in the N-terminal region of the protein. Five potential sites for N-linked glycosylation (NXT/S) were found throughout the protein. The C-terminal region of the protein is highly hydrophilic and particularly rich in basic amino acids with three clusters of lysine residues, which may interact directly with nucleic acid residues.
acid (Clarck and Felsenfeld, 1971). Similar domains were found in the yeast and human eukaryotic initiation factor 2 in the N-terminal of the polypeptide (Donahue et al., 1988; Pathak et al., 1988).

**EF-3 Is a Member of a Family of Closely Related ATP-binding Proteins**—Searches of the NBRF-PIR data bank using the FASTA program show that although EF-3 is unique it is similar to some proteins that belong to a family of ATP-binding proteins with 14 members already identified (Higgins et al., 1988). This similarity extends over an entire internal repeat of about 200 amino acids. The proteins with the highest scores of similarity to EF-3 include Escherichia coli phosphate transport subunit, pstB (Amemura et al., 1985); E. coli ribose transport subunit, rbsA (Bell et al., 1986); E. coli maltose transport subunit, malK (Gilsone et al., 1982); Salmonella typhimurium periplasmic permease subunits, oppD (Higgins et al., 1985) and oppF (Hiles et al., 1987); S. typhimurium histidine transport subunit, hisP (Higgins et al., 1982); Rhizobium leguminosarum nodulation protein, nodI (Evans and Downie, 1986); *E. coli* hemolysin secretion protein, hlyB (Femlee et al., 1985); liverwort chloroplast predicted protein mbpX (Oyama et al., 1986); E. coli chloride-resistant protein, chlD (Johann and Hinton, 1987) and human and mouse drug-resistant F-glycoproteins, mdr (Chen et al., 1986; Gros et al., 1986; Gerlach et al., 1986), pstB, oppD, oppF, malK, hisP, and rbsA are known components of bacterial active transport transmembrane complexes (Ames, 1986). The other proteins are associated with nodulation in *Rhizobium*, export of hemolysin in *E. coli*, drug resistance in *E. coli* and tumor cells, and one with an unknown function encoded by the mbpX gene of liverwort chloroplast.

Fig. 9 shows the alignment of domain I (435 to 608 amino acid residues) and domain II (673 to 961 amino acid residues) of the predicted EF-3 polypeptide with some of the proteins with the best scores of similarity and that are involved in different cellular processes. The statistical significance of similarities was examined to 100 shuffled comparisons with the window for local shuffling set to 10 amino acids. The initial score obtained by the alignment of the EF-3 polypeptide domain with each other was 17.35 S.D. above the mean, while the initial scores obtained by the alignment of domain I with pstB, mbpX, hisP, malK, nodI, mdr1, chlD, and hlyB were 21.5, 22.0, 19.2, 18.55, 17.9, 19.53, 11.97, and 7.9 S.D. above the mean, respectively. The initial scores obtained by the alignment of those proteins with domain II were 15.52, 16.53, 10.07, 14.43, 14.47, 6.93, 5.82, and 5.93 S.D. above the mean, respectively. The similarities, considering identities and conservative replacements (BESTFIT), using the algorithm of Smith and Waterman (1981), of domain I with each one of the above proteins is 54.3, 50.9, 51.1, 50.0, 51.2, 55.2, 50.9, and 54.4%, respectively, and of domain II is 50.2, 42.8, 53.6, 43.3, 45.8, 51.6, 48.6, and 43.6%. When these proteins were analyzed for local similarity with EF-3, the highest level of similarity was observed in the regions that bear the nucleotide-binding site. Nevertheless, the similarity is not restricted to these sites. The dot plots in Fig. 7 showing the internal repeats of EF-3 (Fig. 7A) and the alignment of each one of the related proteins with the two domains of EF-3 (Fig. 7, B-F) clearly demonstrate the extensive similarity among them. Moreover, it is important to emphasize that no similarity could be detected between EF-3 and any other nucleotide-binding proteins, in spite of the preservation or the consensus sequences (Table III), indicating that additional functional similarities arise from potential nucleotide-binding properties could be detected between EF-3 and this group of proteins. Fig. 9 shows that the 174 amino acid residues of EF-3 domain I match very well with this group of proteins, being almost restricted to the regions of highest similarity, as can be seen by the consensus sequence. On the other hand, the 289 amino acid residues of the domain II bear a region of about 45 amino acid residues after the G-G-GKS/T motif with very few matches. Nevertheless, domain II extends through all the length of the alignment and shares identities with the different sequences of the group as though it was the sum of these sequences.

The proteins of this family are associated with a variety of different biological processes including membrane transport, cell division, protein export, DNA repair, and multidrug resistance. The inclusion of the eukaryotic elongation factor 3 in this family brings a new function to these ATPases and expands the discussion about the mechanisms of action of these proteins.

Binding and hydrolysis of ATP have been demonstrated until now for only one single member of this family, the UvrA protein (Seeberg and Steinum, 1985). ATP hydrolysis has not yet been demonstrated by any of the transport components, although the binding of ATP has unambiguously been shown to oppD, hisP, and malK (Higgins et al., 1985; Hobson et al., 1984). This fact has raised the question whether ATP-binding sites play a role other than coupling ATP hydrolysis to this biological process, for instance a regulatory (Chen et al., 1986; Ames et al., 1986) or a structural role (Cross and Nalin, 1982). The fact that ATP hydrolysis is clearly associated with the function of EF-3 and that EF-3 shares an extensive similarity with this family of proteins is consistent with the suggestion that ATP hydrolysis supplies the energy necessary for the specific biological processes with which the other components of this family are associated (Hiles et al., 1987; Higgins et al., 1988). Most of the ATP-binding proteins are associated with membrane events. The members of the bacterial transport protein family interact with periplasmatic substrate-binding proteins as well as with hydrophobic integral membrane proteins, are relatively hydrophilic, and seem likely that they are peripheral membrane proteins (Ames, 1986). The other proteins HlyB, P-glycoproteins, and NodI are also thought to be membrane-associated. However, the UvrA protein which is a member of this family is not a membrane-associated protein since it binds to both single- and double-stranded DNA and, when complexed with UvrB and UvrC, cuts damaged DNA at two sites separated by 12 or 13 nucleotides (Sancar and Rupp, 1983). Likewise, the EF-3 protein is not a membrane protein because it purifies as a soluble protein and must interact with other elongation factors and the ribosome itself to accomplish its function.

The internal repeats in the EF-3 polypeptide expand the suggestion of Hiles et al. (1987) that the proteins of the bacterial transport family function as dimers, since the *E. coli* oligopeptide transport system requires two ATP-binding subunits, oppD and oppF. Moreover, the RbsA subunit of the *E. coli* ribose transport contains two ATP-binding domains in tandem (Bell et al., 1986), as well as the UvrA protein. In eukaryotes, the mammalian multidrug-resistant membrane glycoproteins (Chen et al., 1986; Gros et al., 1986; Gerlach et al., 1986) and the white protein in the fruit fly *Drosophila melanogaster* (Dreessen et al., 1988), members of this family of ATP-binding proteins, also provide a corresponding pair of ATP-binding domains required for the decrease of drug accumulation in multidrug-resistant cells and for the deposition of pteridine pigments in the compound eyes and other tissues, respectively. Moreover, all the prokaryote members of this family belong to a multi-component system of proteins that...
could be energized by ATP hydrolysis. In eukaryotes, the organization of the single protein is similar to the multi-component system of prokaryotes (Higgins et al., 1988).

These extensive sequence similarities suggest that the basic role of EF-3 is to transduce chemical energy stored in nucleoside triphosphate into mechanical energy involved in translocating the various components of the protein synthesis system during the elongation process. The fact that EF-3 interacts with the ribosome is demonstrated by the ribosome dependence of its ATPase activity. The C-terminal region of the protein with its polylysine blocks could interact with tRNA. EF-3 is required in every cycle of chain elongation (Hutchison et al., 1984) but merely stimulates the partial reactions carried out by EF-1, EF-2, and peptidyltransferase (Skogerson and Engelhardt, 1977; Dasmahapatra and Chakraburtty, 1981; Uritani and Miyazaki, 1988a). It may be that EF-3 carries out an as yet undefined step in the elongation process, but an alternative interpretation is that it acts to speed up the whole process and removes a rate-limiting step, probably involving the dissociation of the factors or tRNA molecules from the ribosomes, not assayed by the partial reactions. Although yeast has 80 S ribosomes with a molecular mass around 4.5 x 10^6 Da like other eukaryotes they achieve a rate of protein synthesis only a little slower than bacteria with their 70 S, 2.5 x 10^6 Da ribosomes. E. coli with 70 S ribosomes can achieve a rate of amino acid polymerization of 17 amino acids/s/ribosome (Dennis and Bremer, 1974), three times the rate of protein synthesis in yeast which is 5.5 amino acids/s/ribosome (Waldron and Lacroute, 1975). The rate of protein synthesis of Chinese hamster ovary cells in culture is 0.60 amino acid/s/ribosome, calculated from data presented by Fischer and Moldave (1981) and Harlow and Lane (1988). Mammalian ribosomes are eight times slower than yeast in spite of the similarities of the ribosome particles in both eukaryotes.

The energy for EF-3 role in protein synthesis almost certainly comes from ATP. EF-3 affinity for GTP (Uritani and Miyazaki, 1988a). The concentration of ATP in yeast is five times higher than the concentration of GTP (Swedes et al., 1979). This 10–15-fold preference for ATP over GTP is somewhat surprising. GTP is the favored energy source for protein synthesis in all organisms examined. If the role of EF-3 is to accelerate the elongation process on a eukaryote 80 S ribosome to approach the speed of a 70 S ribosome, the use of ATP as an energy source may allow fungi to directly regulate the rate of protein elongation in response to energy limitation. Studies on the regulation of protein synthesis in yeast during energy limitation have demonstrated that yeast inhibits both initiation and elongation in response to energy limitations. The effect on initiation is most likely due to the impact of changes in the GTP:GDP ratio on eukaryotic initiation factor 2 which is inhibited by GDP (Wallou and Gill 1975a, 1975b, 1976). The effect on elongation is not explained by the known affinities of EF-1 and EF-2 for GTP or GDP and may well involve the regulation of EF-3 activity.

We and others (Skogerson and Wakatama, 1976; Dasmahapatra and Chakraburtty, 1981; Herrera et al., 1984; Hutchison et al., 1984; Qin et al., 1987; Uritani and Miyazaki, 1988a) have been able to find elongation factor 3 in several fungal species. Bacterial, plant, and mammalian sources have been examined without success for enzymatic activity that would complement a yeast extract depleted in EF-3 and supernatant proteins that would cross-react with monoclonal and polyclonal antibodies against yeast EF-3. Uritani and Miyazaki (1988a) have noticed that polyclonal antibodies raised against yeast EF-3 cross-react with ribosomal proteins from *Tetrahymena* brucei, shrimp, and rat liver. We have extended their observation to include cross-reaction with ribosomal proteins from mouse ascites cells, soybean, and corn. The meaning of the cross-reaction with ribosomal proteins awaits further study, but it is clear that there is no evidence for a supernatant factor such as EF-3 in mammalian or plant ribosomes. Negative results are intrinsically unsatisfying, but sufficient effort has been expended to suggest that EF-3 is characteristic of fungal protein synthesis and is not present in higher plants and animal. It may mark a major step in the evolutionary history of protein synthesis. The question whether EF-3 activity is only required for fungal ribosomes or whether it assumes a different form, presumably as a ribosomal protein(s), in mammalian and plant ribosomes awaits further study as does the broader evolutionary question of whether EF-3 appears outside the fungal kingdom.

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**REFERENCES**

- Amemura, M., Makino, K., Shimagawa, H., Kobayashi, A., and Nakata, A. (1985) *J. Mol. Biol.* 184, 241–250
- Ames, G. F. L. (1986) *Annu. Rev. Biochem.* 55, 397–425
- Baint, S. H., and Sherman, V. (1988) *Mol. Cell. Biol.* 8, 1591–1601
- Bell, A. W., Buckel, S. D., Groarke, J. M., Hope, J. N., Kingstone, D. H., and Hermodson, M. A. (1986) *J. Biol. Chem.* 261, 7652–7658
- B Eaton, J. L., and Hall, B. D. (1982) *J. Biol. Chem.* 257, 3026–3031
- Brandis, H. G. M., Maassen, J. A., van Hemert, F. J., Amos, R., and Moller, W. (1986) *J. Biochem. (Tokyo)* 155, 167–171
- Burke, R. L., Tekamp-Olson, P., and Najarin, R. (1983) *J. Biol. Chem.* 258, 2985–2991
- Carle, G. F., and Olsen, M. V. (1984) *Nucleic Acids Res.* 12, 5647–5664
- Carle, G. F., and Olsen, M. V. (1985) *Proc. Natl. Acad. Sci. U. S. A.* 82, 3766–3769
- Chen, C-M., Misra, T. K., Silvers, S., and Rosen, B. P. (1986) *J. Biol. Chem.* 261, 15000–15006
- Cigan, A. M., and Donahue, T. (1987) *Gene* (Amst.) 59, 1–18
- Clark, R. J., and Felsenfeld, G. (1971) *Nature New Biol.* 229, 101–106
- Cohen, P. (1985) *Eur. J. Biochem.* 151, 439–448
- Coutelle, P., Thiele, D., Price, V. L., Memet, S., Micouin, J-Y., March, L., Borger, J-M., Sentenac, A., and Fromageot, P. (1985) *J. Biol. Chem.* 260, 3067–3074
- Cross, R. L., and Nalin, C. M. (1982) *J. Biol. Chem.* 257, 2874–2881
- Dasmahapatra, B., and Chakraburtty, K. (1981) *J. Biol. Chem.* 256, 9999–10004
- Davhoff, M. (1978) *Atlas of Protein Sequence and Structure*, Vol. 5, Suppl. 3, National Biomedical Research Foundation, Silver Spring, MD
- Dennis, P. P., and Bremer, H. (1974) *J. Mol. Biol.* 84, 407–422
- Devereux, J., Haebler, P., and Smithies, O. (1984) *Nucleic Acids Res.* 12, 387–395
- Dobson, M. J., Tuite, M. F., Roberts, N. A., Kingsman, A. J., and Kingsman, S. M. (1982) *Nucleic Acids Res.* 10, 2625–2637
- Donahue, T. F., Cigan, M., Pabich, E. K., and Valavicius, B. C. (1988) *Cell* 54, 621–632
- Dolittle, R. F., Johnson, M. S., Husain, I., Van Houten, R., Thomas, D. C., and Sancar, A. (1986) *Nature* 323, 451–453
- Dreesen, T. D., Johnson, D. H., and Henikoff, S. (1986) *Mol. Cell. Biol.* 6, 5206–5215
- Evans, J. A., and Downie, J. A. (1986) *Gene* (Amst.) 43, 95–101
- Feinle, T., Pellel, S., and Welch, R. A. (1985) *J. Bacteriol.* 163, 94–105
- Fischer, I., and Moldave, K. (1981) *Anai. Biochem.* 113, 13–26
- Gerlach, J. H., Endicott, J. A., Juranka, P. F., Henderson, G., Sarangi, P., Deuchars, K. L., and Ling, V. (1986) *Nature* 324, 485–489

2 S. Qin, unpublished results.
Sequence analysis of the translational elongation factor 3 from Saccharomyces cerevisiae.

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