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Research Article

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Molecular Cloning of the Sec61p γ Subunit Homologue Gene from the Mole Cricket, *Gryllotalpa orientalis*

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The Sec61 trimeric complex (α, β, and γ subunits) is one of the Sec-complex responsible for post-translational protein translocation across the endoplasmic reticulum membrane in diverse organisms. In this study, a cDNA encoding the Sec61p γ subunit homologue was isolated from the cDNA library of the mole cricket, *Gryllotalpa orientalis*. Sequence analysis of a 442-bp cDNA clone showed it to contain an open reading frame of 68 amino acid residues consisted of 204-bp. The homologues of the gene were found in the GenBank database in a diverse organism including insect, mammals, fungi, and plants. The deduced amino acid sequence of Sec61p γ subunit homologue of the mole cricket showed the highest homology to the gene of the singly known insect, *Drosophila melanogester* (93% identity), and the least homology to that of the baker's yeast, *Saccharomyces cerevisiae* (37.2%). Phylogenetic analysis also confirmed a close relationship between the insect Sec61p γ subunit homologues of *G. orientalis* and *D. melanogester*. Hydropathy analysis of the cricket mole and published other data suggested that the hydrophobic segment close to C-terminus is predicted to be the putative membrane anchor. Multiple alignment of the Sec61p γ subunit homologue among several organisms showed the presence of several conserved domains including the conserved proline at position 28.

Key words: Sec61p γ subunit, Post-translational protein translocation, Mole cricket, *Gryllotalpa orientalis*

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Introduction

The Sec61 trimeric complex (α, β, and γ subunits) is one of the Sec-complex responsible for post-translational protein translocation across the endoplasmic reticulum (ER) membrane in diverse organisms (Rapoport *et al.*, 1999). The Sec61 complex forms the hydrophilic pore in the membrane through which the nascent polypeptide is translocated, and it is responsible for the tight binding of the ribosome to the ER membrane during the co-translational transport process (Kalies *et al.*, 1994; Greenfield and High, 1999). Moreover, the Sec61p complex is involved in the recognition of the signal sequence regulating the insertion of the nascent polypeptide chain into the translocation channel (Kathrin *et al.*, 1998; Herskovits and Bibi, 2000). Also, Valcárcel *et al.* (1999) found that *Drosophila* Sec61p β subunit is required during development of organisms. That is, although neither the *Saccharomyces cerevisiae* Sect61p β nor its functional *Escherichia coli* homologues are essential for viability or for protein translocation, the *Drosophila* Sect61p β is essential for embryonic development.

It has been shown that the common theme, protein transport across the ER membrane in eukaryotes and the cytoplasmic membrane in bacteria has been well preserved in the course of evolution. The core component of Sec61p complex is one of the well-conserved systems (Jungnickel *et al.*, 1994). Moreover, β and γ-subunits of Sec61 complex are highly conserved in the protein translocation apparatus of all classes of organisms (Hartmann *et al.*, 1994).

The mole cricket, *Gryllotalpa orientalis* (Burmeister), is a singly known species of the Family Gryllotalpidae in Korea, and distributed in Asia and many European and African countries. Information on this species, particu-

larly on the genetic aspect, is extremely rare, not enough to cite scientifically (Nevo *et al.*, 2000).

In order to obtain genetic information of the mole cricket, we have constructed cDNA library from the whole bodies (adult and juvenile) of *G. orientalis*. In this study, we have cloned, sequenced, and characterized a cDNA encoding the Sec61p γ subunit homologue from the cDNA library of *G. orientalis*.

Materials and Methods

Animal

Several hibernating adult and juvenile *G. orientalis* were collected in Kimhe City, Kyungsannam-do Province in Korea on December 19, 2001 by digging ground.

cDNA library screening

A cDNA library wa constructued from poly (A^+) mRNA isolated from the whole body of adults and juveniles by Uni-ZAP XR vector and Gigapack III Gold Packing Extract (Stratagene, La Jolla, USA). *E. coli* XL2-Blue MRF strain was infected by the Uni-ZAP XR library harboring *G. orientalis* cDNA and the bacteria were cultured on the NZY agar medium. Each plaque was suspended in SM buffer (5.8 g/l NaCl, 2 g/l $MgSO_4 \cdot 7H_2O$, 0.05 M Tris-Cl, pH 7.5 and 0.01% gelatin solution) containing 0.02% (v/v) chloroform and stored at 4°C for one day. The pages were eluted into SM buffer. The pBluescript phagemids were *in vivo* excised from the Uni-ZAP XR vector using ExAssist helper phage. *E. coli* strain, SOLR cell (Stratagene, La Jolla, USA), was infected by the excised phagemids and plated on LB-Amp medium (50 μg/ml medium ampicillin). Plasmid DNA from the overnight culture was isolated by Wizard mini-preparation kit (Promega, Medison, WI). For the construction of the expressed sequence tags (ESTs) profile, cDNA clones randomly selected from the cDNA library were sequenced.

Nucleotide sequencing and data analysis

Sequence of each cDNA clone was determined using an automatic sequencer (model 310 Genetic Analyzer; Perkin-Elmer Applied Biosystems, CA). The sequences were compared using the BLAST programs (Altschul *et al.*, 1990) provided by the NCBI using the option "Nucleotide query-Protein db [blastx]". Hydropathy analysis was performed using MacVector (ver. 6.2) as described in Kyte and Doolittle (1982).

Phylogenetic analysis

Phylogenetic analysis was performed to infer the relationships among the amino acid sequences of *G. orientalis* Sec61p γ subunit homologue and other GenBank-registered Sec61p γ subunit genes using PAUP (Phylogenetic Analysis using Parsimony) version 3.0 (Swofford, 1993). The accession numbers of the sequences in the GenBank are as follows: *G. orientalis* (AF540908, from this study), *Drosophila melanogaster* (AY070594), *Branchiostoma belcheri* (AF395915), *Canis familiaris* (L25086), *Homo sapiens* (AF054184), *Mus musculus* (U11027), *Caenorhabditis elegans* (U53785), *Oryza sativa* (P38385), *Arabidopsis thaliana* (AL035523), *Neurospora crassa* (AL513463), *Schizosaccharomyces pombe* (Z56276), *Saccharomyces cerevisiae* (X74499), *Encephalitozoon cuniculi* (AL590445), and *Plasmodium falciparum* (AE001395).

Results and Discussion

A cDNA library was constructed from the whole body RNA of *G. orientalis*. The sequencing of randomly selected clones harboring cDNA inserts was performed to generate the ESTs of *G. orientalis*. Among these ESTs, one exhibited similarity to the reported Sec61p γ subunit. Thus, this clone was selected for further characterization.

The nucleotide and deduced amino acid sequences of the cDNA encoding the Sec61p γ subunit of *G. orientalis* is presented in Fig. 1. The complete subunit cDNA sequence comprised of 442 bp with 68 amino acid residues consisted of 204 bp (GenBank accession number AF540908). In the Sec61p γ subunit gene sequence, a polyadenylation signal sequence (AATAAA) was found at nucleotides 301, twenty-seven bp upstream of the poly(A) tail. In the pairwise comparisons among amino acid sequences of the Sec61p γ subunit, the least divergence was found in a comparison between the two unique insect species, *G. orientalis* and. *D. melanogaster* (6%). On the other hand, the highest divergence was found in a comparison between *A. thaliana* vs. *S. cerevisiae* (70.9%), but overall substan-

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-96                                    GGGCACGAGGTAATCGAACGTGGACGCGGAGATCAG
-60  CTGCGGAGAAACCAGCGAGTGTTGATTGCTCCTGAAAAACCAACAAATCCCCAGGCCACA
  1  ATGGATCAGGTAACAAAGTTTATCGAGCCAGGAAGGCAGTTTGCAAAGGATTCTATTCGT
  1   M  D  Q  V  T  K  F  I  E  P  G  R  Q  F  A  K  D  S  I  R

 61  CTTGTCAAGCGTTGCACAAAGCCTGACAGGAAAGAATTTCAGAAAATTGCAGTTGCCACT
 21   L  V  K  R  C  T  K  P  D  R  K  E  F  Q  K  I  A  V  A  T

121  GCTATTGGATTTTGCATTATGGGTTTTATTGGATTCTTTGTTAAGTTGATCCATATTCCC
 41   A  I  G  F  C  I  M  G  F  I  G  F  F  V  K  L  I  H  I  P

181  ATTAATAACATCATTGTGGGCTCGTAATCTATCATGGCGTGAGCTTCCGTTTTCTGGACA
 61   I  N  N  I  I  V  G  S  *

241  TTTTTACACCAAGTTGTTAACAGATGGATTTTCCTTGGTTTATATATGTTATTTTTGAGT
301  AATAAATTATACTTTATACACATATTTAAAAAAAAAAAAAAAAAAA
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Fig. 1. The nucleotide and deduced amino acid sequences of *G. orientalis* Sec61p γ subunit homologue gene. The start codon ATG is boxed and the termination codon is asterisked. The polyadenylation signal AATAAA is underlined. The GenBank accession number is AF540908.

Table 1. Pairwise comparisons among amino acid sequences of the *G. orientalis* Sec61p γ subunit gene and the known homologous genes

Species	GenBank No.	1	2	3	4	5	6	7	8	9	10	11	12
1. *G. orientalis*	AF540908	–	0.070	0.093	0.093	0.093	0.093	0.128	0.221	0.221	0.419	0.442	0.628
2. *D. melanogaster*	AY070594	6	–	0.116	0.128	0.128	0.128	0.174	0.233	0.244	0.430	0.442	0.651
3. *B. belcheri*	AF395915	8	10	–	0.070	0.070	0.070	0.093	0.221	0.233	0.430	0.442	0.640
4. *C. familiaris*	L25086	8	11	6	–	0.000	0.000	0.116	0.233	0.233	0.407	0.430	0.616
5. *H. sapiens*	AF054184	8	11	6	0	–	0.000	0.116	0.233	0.233	0.407	0.430	0.616
6. *M. musculus*	U11027	8	11	6	0	0	–	0.116	0.233	0.233	0.407	0.430	0.616
7. *C. elegans*	U53785	11	15	8	10	10	10	–	0.267	0.256	0.430	0.442	0.628
8. *O. sativa*	P38385	19	20	19	20	20	20	23	–	0.070	0.453	0.465	0.686
9. *A. thaliana*	AL035523	19	21	20	20	20	20	22	6	–	0.442	0.465	0.709
10. *N. crassa*	AL513463	36	37	37	35	35	35	37	39	38	–	0.337	0.616
11. *S. pombe*	Z56276	38	38	38	37	37	37	38	40	40	29	–	0.605
12. *S. cerevisiae*	X74499	54	56	55	53	53	53	54	59	61	53	52	–
13. *E. cuniculi*	AL590445	47	49	46	47	47	47	48	46	48	52	50	59
14. *P. falciparum*	AE001395	48	48	48	47	47	47	49	49	52	56	55	54

Numbers above the diagonal are mean distance values; numbers below the diagonal are absolute distance values.

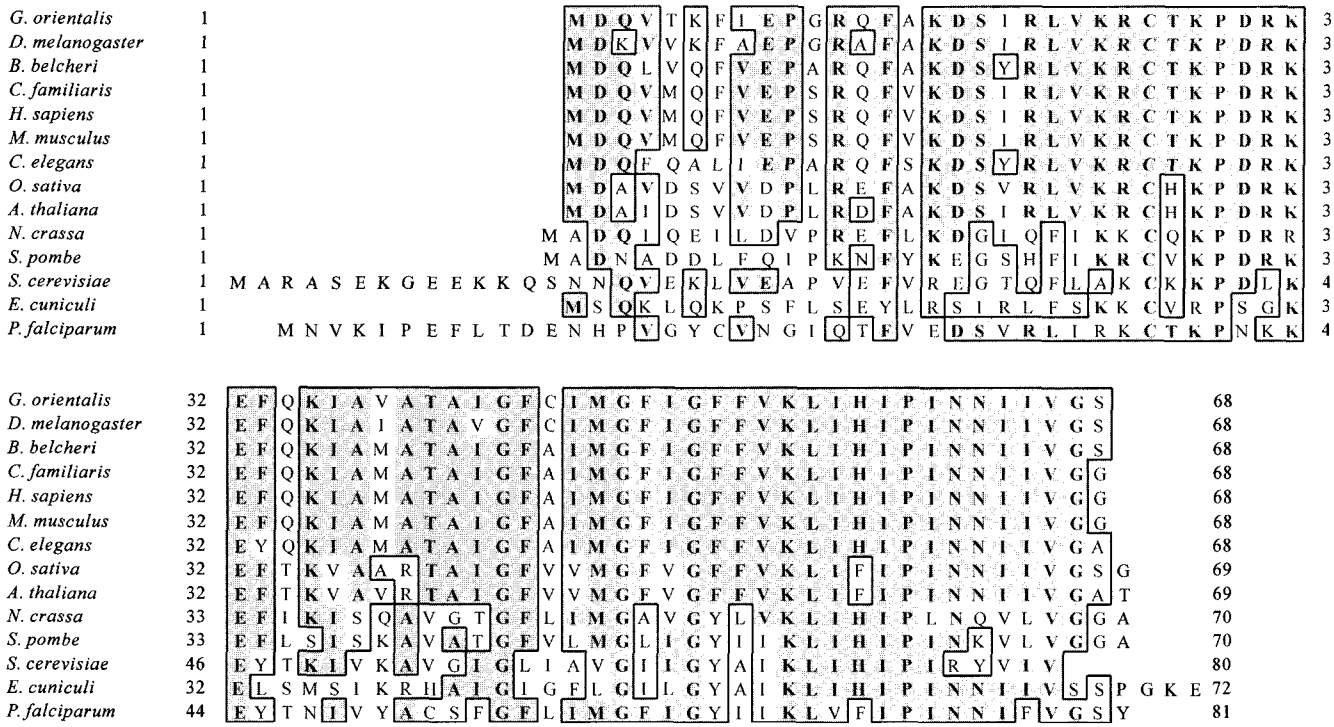

Fig. 2. Multiple sequence alignment of the deduced amino acid sequences of the *G. orientalis* Sec61p γ subunit and of other organisms. The solid boxes are the residues that are identical to those of *G. orientalis*. Note that the putative ER membrane anchors are lined above the *G. orientalis* sequence. The conserved proline (marked with *) in the Sec61p γ subunit is well conserved in all organisms surveyed.

tially high homology was present in a diverse organism (Table 1). From the perspective of Sec61p γ subunit of the mole cricket, the highest divergence was found in the bakers yeast, *S. cerevisiae* (37.2%).

A multiple sequence alignment of the deduced protein sequence of Sec61p γ subunit gene with those of other organisms is shown in Fig. 2. The multiple alignment showed an extensive identity among those of animals,

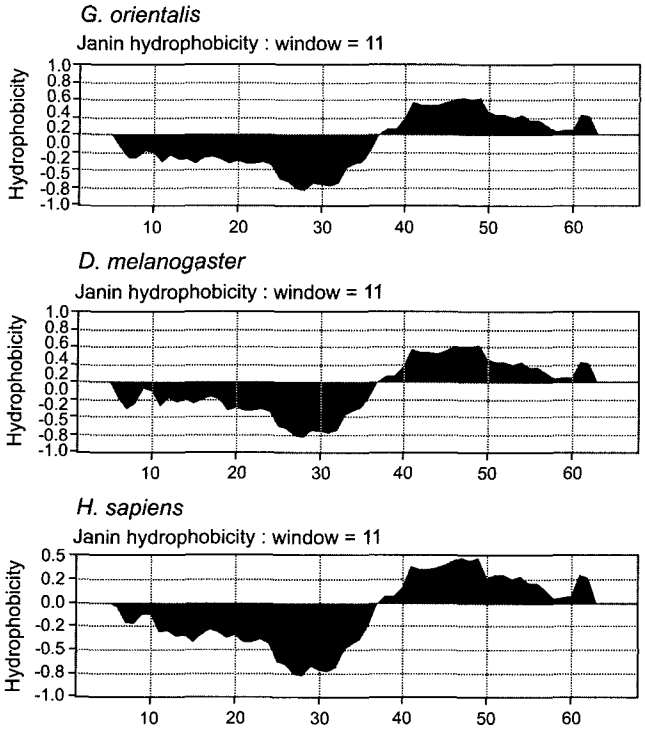

Fig. 3. The hydropathy profiles of *G. orientalis*, *D. melanogaster*, and *H. sapiens* Sec61p γ subunit. Hydropathic analysis was performed as described by Kyte and Doolittle (1982).

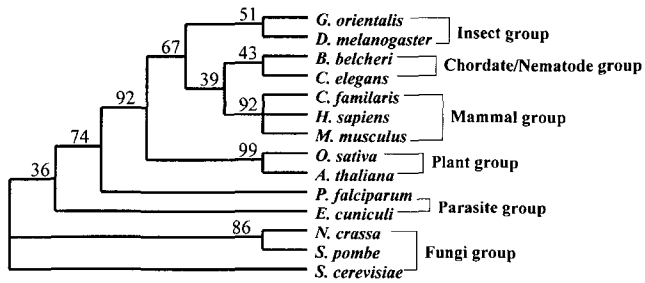

Fig. 4. A phylogenetic tree for aligned amino acid sequences of the *G. orientalis* Sec61p γ subunit protein and the known Sec61p γ subunit proteins. The GenBank accession number of the already known sequences is listed in the text and Table1. The tree was obtained by bootstrap analysis with the option of heuristic search for 100 replicates using PAUP (Swofford, 1993).

plant, parasite and fungi groups. Excluding parasites and fungi groups, the length of the amino acid was highly conserved (68–69 aa). The putative ER membrane anchors (amino acid position 41 ~ 61 in canine Sec61p γ subunit; Hartmann *et al.*, 1994) are very well conserved among organisms compared, particularly in the animal species (Fig. 2).

The hydropathy plot of Sec61p γ subunit was analyzed by the Kyte and Doolittle method (1982), and compared with that of *D. melanogaster* (unpublished, GenBank accession number AY070594) and *H. sapiens* (Zhang *et al.*, 2000). The comparison resulted in a pattern showing high similarity among *G. orientalis*, *D. melanogaster*, and *H. sapiens* in that the Sec61p γ subunit od these organisms possesses a putative membrane anchor at hydrophobic segment close to C-terminus. Hartmann *et al.* (1994) predicted that the protein spans the membrane once, with a hydrophobic segment close to the C-terminus and have its N-terminus located in the cytoplasm. The conserved proline (amino acid position at 28) is well conserved at the same position in all organisms surveyed.

Pairwise comparison among Sec61p γ subunit of the diverse organisms showed the highest sequence homology between the two unique Sec61p γ subunit of insect species, *G. orientalis* (from this study) and *D. melanogaster* (Table 1). Sequence divergence between them only was 7% (6 amino acids among 68), although they are belonged to different taxonomic order. Phylogenetic analysis of the Sec61p γ subunit of the diverse organisms showed that taxonomically closer organisms formed inclusive groups by Sec61p γ subunit protein without exception (Fig. 4). Particularly, the two insect species, *G. orientalis* and *D. melanogester* also formed a subgroup exclusively, but bootstrap analysis did not support the branch strongly (51%) as much as other groups such as mammals (92%), plants (99%), and fungi (86%) (Fig. 4). This may suggest that insect Sec61p γ subunit is an ancient and divergent through its evolution.

In summary, we illustrated gene nucleotide sequence of the cDNA encoding a Sec61p γ subunit homologue from the mole cricket, *G. orientalis* in this study.

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