Asian-Aust. J. Anim. Sci.
Vol. 19, No. 7 : 953 - 957
July 2006
www.ajas.info

Molecular Characterization and Expression Patterns of Porcine Eukaryotic Elongation Factor 1 A

H. L. Wang[1, 2], H. Wang[1, 2], Z. M. Zhu[1], S. L. Yang[1], S. T Feng[1] and K. Li[1, *]
[1]Department of Gene and Cell Engineering, Institute of Animal Science
Chinese Academy of Agricultural Sciences, Beijing 100094, P. R. China

ABSTRACT : The eukaryotic elongation factor 1 A (EEF1A) participates in protein synthesis by forming the eEF1A·GTP·tRNA complex to deliver aminoacyl-tRNA to the A site of ribosomes. This study described cDNA sequences and partial genomic structure of porcine *EEF1A1*. The porcine *EEF1A1* gene encoded a protein with 462 amino acids, which shared complete homology with human, chimpanzee and dog. The temporal expression pattern showed the diversity of *EEF1A1* level in mRNA was relatively minor in prenatal embryo skeletal muscle, however, the expression decreased during aging after birth in skeletal muscle of the Chinese Tongcheng pig. The spatial expression patterns indicated that the gene expressed in skeletal muscle, heart, lung, liver, kidney, fat and spleen. In addition, we assigned the gene to porcine chromosome 1 using a radiation hybrid panel. (**Key Words :** *EEF1A1*, Expression, Chromosome, Genome, Porcine)

INTRODUCTION

A variety of molecules have been identified in the complicated network system of protein synthesis, such as Guanine triphosphate (GTP), ribosome, initial factors, elongation factors and termination factors. In protein translational elongation process, elongation factor 1 A (EF1A) contributes to the binding of aminoacyl-tRNA to the A site of ribosome at the presence of GTP and maintaining EF1A-GTP concentration in tRNA channeling which EF1βγ subunits were for exchange of GDP/GTP (Sanders et al., 1993). The elongation factor 2 promotes the translocation of tRNA with the nascent polypeptide from the A-site to the P-site of the ribosome (Joseph and Noller, 1998).

Eukaryotic EF1A has structurally been identified as a GTP-binding protein characterized consensus sequences including G-X4-G-K, D-X2-G and N-K-X-D sequences (Dever et al., 1987), which the three highly conserved motifs showed the important roles in the regulatory process of translational elongation by some previous mutation studies (Carr-Schmid et al., 1999; Knudsen et al., 2001; Chiron et al., 2005). Functions of EEF1A were also reviewed in cell process (Lamberti et al., 2004).

In this paper, we isolated the full-length cDNA of porcine *EEF1A1* gene from a skeletal muscle cDNA library of porcine embryos, where the porcine eukaryotic translation termination factor 1 (*ETF1*) gene was also analyzed (Shao et al., 2005), characterized the genomic structure and patterns for temporal and spatial expression, identified the localization on porcine chromosome. All these will be the bases for a better understanding of *EEF1A1* gene.

MATERIAL AND METHODS

Isolation of porcine *EEF1A1* gene

The cDNA sequence of *EEF1A1* gene was obtained from a porcine cDNA library of 55d-fetus skeletal muscle as methods described previously (Zhu et al., 2005), and shared higher homology with human *EEF1A1* gene.

In order to analyze the genomic DNA sequence of *EEF1A1* gene, the primers (Table 1) were designed in exons to amplify potential introns according to the comparing results of porcine cDNA and human DNA sequences of *EEF1A1* gene in NCBI BLAST database (http://www.ncbi.nlm.nih.gov/blast/bl2seq/). Following optimization of all PCR conditions in PTC-100 (MJ research), every PCR fragment was purified and sequenced as described by Yang

* Corresponding Author: Kui Li. Tel: +86-10-6283-3312, Fax: +86-10-6289-9982, E-mail: kuili@iascaas.net.cn
[2] Department of Animal Genetics and Breeding, School of Animal Science and Technology, Huazhong Agricultural University, Wuhan 430070, P. R. China.
Received November 18, 2005; Accepted February 21, 2006

Table 1. Amplification primers for porcine *EEF1A1*

Primers	Sequence (5'-3')	Annealing temperature (°C)	Product size (bp)
P1L[1]	CTCACATCAACATCGTCGTC	59	693
P1R[2]	CCGTTCTTGGAAATACCTG		
P2L	TGGCTACAACCCTGATACA	58	638
P2R	CTTCCATAGGTGGGTCATT		
P3L	GGTGGTATTGGTACAGTCCC	58	900
P3R	GCAAACAGTTCTGAGACCGT		

[1, 2] Primers of chromosome mapping.

et al. (2005). Genomic DNA sequence of *EEF1A1* was obtained through sequences assembling using DNAstar software. The genomic organization was identified and determined for the exon and intron size and junction phase of porcine *EEF1A1*. The information about genomic structure for porcine *EEF1A1* gene was shown in Figure 1.

Chromosome localization

Using the primers (Table 1), PCR was performed using the INRA-Minnesota Porcine Radiation Hybrid (IMpRH) panel in 10 μl reaction containing 1×PCR buffer, 20 ng panel DNA, 0.3 μM each primer, 75 μM each dNTP, 1.5 mM $MgCl_2$ and 1.0 U Taq DNA polymerase. In addition, the controls were designed containing pig, hamster genomic DNA and a blank control without DNA. The PCR protocol was 5 min at 94°C; 35 cycles of 20 s at 94°C, 20 s at 59°C, and 30 s at 72°C, and a final extension for 5 min at 72°C. The genotypes were electronically submitted and automatically analyzed by the IMpRH mapping program (Milan et al., 2000) available on the IMpRH web site (http://imprh.toulouse.inra.fr/).

Analysis of expression patterns

Total RNA was extracted using TRIzoL reagent kit (Promega) from seven tissues samples (skeletal muscle, heart, liver, lung, spleen, kidney and fat of Landrace), and skeletal muscle of six time points (prenatal 33 d-, 65 d-, 90 d-, postnatal 2 d-, 28 d- and adult Tongcheng pigs) and reverse transcript PCR (RT-PCR) was performed (Pan et al., 2003). PCR products were amplified as templates for cDNA in a volume of 20 μl containing 1×PCR buffer, 0.3 μM each primer, 75 μM each dNTP, 1.5 mM $MgCl_2$ and 2.0 U Taq DNA polymerase, which the program was 3 min at 94°C, 28 cycles of 20 s at 94°C, 20 s at 58°C, and 30 s at 72°C and a final 5-min extension at 72°C. Glyseraldehyde-3-phosphate dehydrogenase *(GAPDH)* was used as an internal reference to standardize the targeted samples and the conditions were same above. The relative expression levels were analyzed using KODAK1D 3.6 software.

RESULTS AND DISCUSSION

Analysis of full-length cDNA of porcine *EEF1A1*

A 1,783 bp sequence was obtained by double-direction sequencing and sequences assembling, and had 92% identity to both human and mouse *EEF1A1* (GenBank Acc. No. NM_001402 and NM_010106). Open reading frame was predicted using ORF finder tool (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). As a result, the cDNA sequence contained 90 nucleotides of 5' untranslation region, 1386 nucleotides of open reading frame and 307 nucleotides of 3' untranslation region including polyA signal (ATTAAA) at nucleotide 1,744-1,750 upstream of a 19 bp polyA tail.

Protein characterization was analyzed in the website (http://kr.expasy.org/tools/). The protein predicted encoded 462 amino acids with molecular weight 50.14 kDa and theoretical isoelectric point 9.10. The predicted results in the PROSITE database showed the protein contained the typical structure of GTP-binding protein for the three consensus sequences, GHVDSGK was localized at 13-19 amino acids and DAPG localized at 100-104 amino acids for 58 amino acids upstream of NKMD (Figure 1).

Alignment of EEF1A1 amino acids sequences among different species was performed in NCBI BLAST database (http://www.ncbi.nlm.nih.gov/blast/bl2seq/). The results indicated the degree of homology is very high (up to 100%) between pig and human, Chimpanzee, Macaque and dog, but relatively low (99%, 99%, 96% and 91% respectively) between pig and mouse, rat, chicken and zebrafish. Therefore, we suggested the gene might derive from a common gene and be very conserved in evolution according to high homology among species and its central roles in various cell processes. Simultaneously, the results supported the closer relationship is between pig and human, which provides further foundation for organ xenotransplantation (pig to human).

Analysis of expression profiles

In order to observe expression alternations of *EEF1A1* gene during skeletal muscle development, six important periods for muscle development of pigs were chosen and the gene was considered to be up-regulated when the intensity ratio between *EEF1A1* and *GAPDH* was ≥1.8. Analysis of semi-quantity reverse transcriptase PCR (RT-PCR) assay showed the *EEF1A1* expression trend was basically invariable during skeletal muscle development

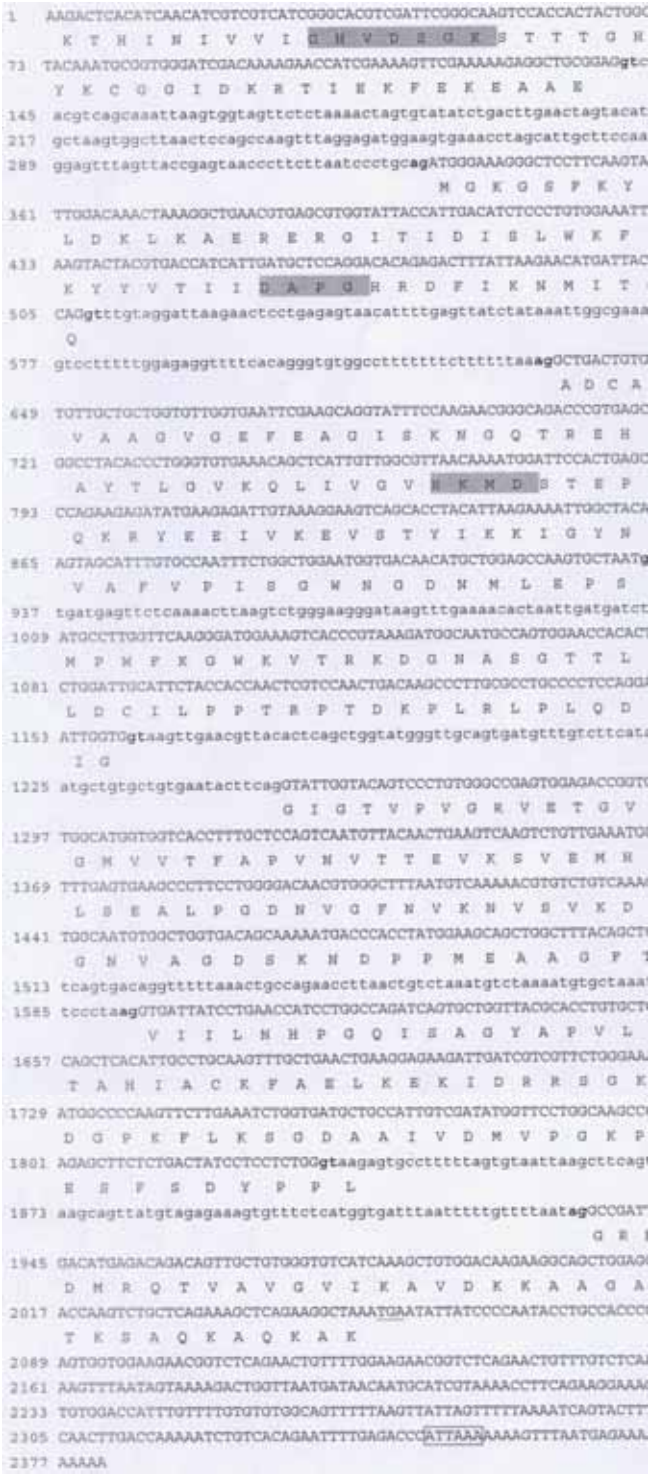

Figure 1. Nucleotide and the predicted partial protein sequences of porcine *EEF1A1*. The exons are showed in uppercase and the introns are showed in lowercase. 5' splice donor and 3' splice acceptor (gt and ag) are in boldface. The stop codon (TGA) is indicated in underlined type. The polyadenylation signal (ATTAAA) is boxed. The three consensus sequences (GHVDSGK, DAPG and NKMD) are in shadow.

before birth, but the expression was decreased with age after birth in Tongcheng pigs. It was demonstrated that the different expression of *EEF1A1* was existed between prenatal (33 day- and 65 day-) and postnatal muscle in Tongcheng pigs (Figure 2). The previous study showed the *EEF1A* gene was detected down-regulation expression from 75 day- fetal to 1 week- postnatal muscle in pig using cDNA macroarray method (Zhao et al., 2003). Another study showed *EEF1A1* expression level was decreased with aging in human fibroblasts (Cavallius et al., 1986).

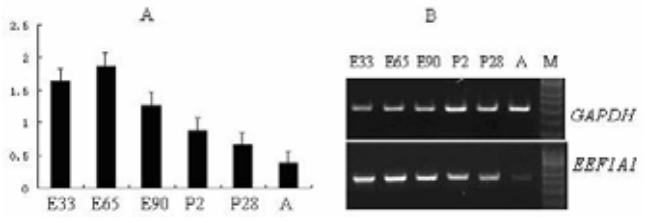

Figure 2. Temporal expression profile of porcine *EEF1A1*. E33, E65, E90, P2, P28, A represent the samples for skeletal muscle from prenatal 33 d-, 65 d-, 90 d-, postnatal 2 d-, 28 d- and adult Tongcheng pigs, respectively. M is for 100 bp Ladder. A is for the histogram representing expression level in different muscle developmental times, and repeat number of the sample in every stage is for three (n = 3). B is for the electrophoretic picture in 2.0% gel.

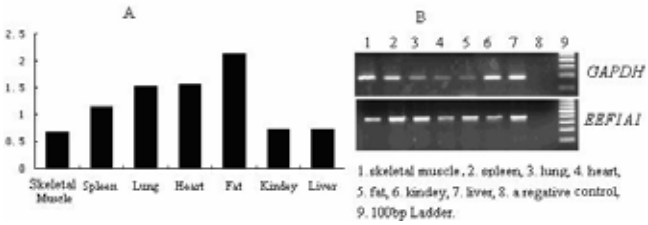

Figure 3. Spatial expression profile of porcine *EEF1A1*. A is for the histogram representing the expression level in different tissues. B is for the electrophoretic picture in 2.0% gel.

Therefore, our results were consistent with the previous studies. As we know, the increase of muscle mass was mainly due to the increase of muscle fibre number before birth and that of muscle fibre size after birth. So it was suggested that *EEF1A1* may play an important role in formation of muscle fibres.

The result of spatial expression profile showed *EEF1A1* expressed in every tissue detected, and higher in fat than in skeletal muscle, liver, heart, spleen, lung and kidney (Figure 3). It was thought that the wide expression was related to the important functions of *EEF1A1* in protein elongation.

Chromosome localization

The result of chromosome physical mapping showed *EEF1A1* was assigned to porcine chromosome 1 using 90 of 118 clones of IMpRH panel. Two-point analysis revealed the gene was closely linked with microsatellite marker *SW1514* at a distance of 85 cR7000 with LOD score 3.07. Based on comparative genome information, our mapping result was agreed with that of human *EEF1A1* gene on chromosome 6q14.1 using fluorescence *in situ* hybridization (FISH) and somatic cell hybrid panel (SCHP) methods (Lund et al., 1996).

Analysis of genomic structure

The genomic organization of porcine *EEF1A1* was described (Figure 1). We obtained all intron sequences except for the first intron. The sequence assembled spanned 2,362 bp length containing seven exons (132, 180, 297, 151, 257, 235, 435 bp, respectively) and six introns (195, 121, 83, 88, 88, 100 bp, respectively) and conformed to the GT-AG rule for the splice donor and acceptor sites. The structure is similar with that of human and mouse *EEF1A1*. Information of the genomic sequence will provide a good way to polymorphism detection and functional analysis.

CONCLUSION

In summary, we obtained the full-length cDNA sequence and analyzed the characterization of porcine *EEF1A1* gene. The temporal and spatial expression profiles, chromosome localization and partial genomic organization were described in this study. All will give a good pathway to study the function of *EEF1A1* gene.

ACKNOWLEDGEMENT

The authors would like to thank Dr. Martine Yerle for providing the RH panel (INRA, Castanet-Tolosan, France). This research was supported by the National 10th Five Year Scientific Project of China for Tackling the Key Problems (2004BA717B), National Natural Science Foundation of China (30571330), Key Project of National Natural Science Foundation of China (30330440), Scientific Research Foundation of the State Human Resource Ministry for Returned Chinese Scholars and State Platform of Technology Infrastructure (2004DKA30450) and National 973 Project of China.

REFERENCES

Carr-Schmid, A., N. Durko, J. Cavallius, W. C. Merrick and T. G. Kinzy. 1999. Mutations in a GTP-binding Motif of Eukaryotic Elongation Factor 1A Reduce Both Translational Fidelity and the Requirement for Nucleotide Exchange. J. Bio. Chem. 274:30297-30302.

Cavallius, J., S. I. Rattan and B. F. Clark. 1986. Changes in activity and amount of active elongation factor 1 alpha in aging and immortal human fibroblast cultures. Exp. Gerontol. 21:149-157.

Chiron, S., A. Suleau and N. Bonnefoy. 2005. Mitochondrial Translation Elongation Factor Tu Is Essential in Fission Yeast and Depends on an Exchange Factor Conserved in Humans but Not in Budding Yeast. Genet. 169:1891-1901.

Dever, U. E., M. J. Glynias and W. C. Merrick. 1987. Biochemistry GTP-binding domain: Three consensus sequence elements with distinct spacing. Proc. Natl. Acad. Sci. 84:1814-1818.

Joseph, S. and H. F. Noller. 1998. EF-G-catalyzed translocation of anticodon stem–loop analogs of transfer RNA in the ribosome. EMBO. 17:3478-3483.

Knudsen, C., H. J. Wieden and M. V. Rodnina. 2001. The Importance of Structural Transitions of the Switch II Region for the Functions of Elongation Factor Tu on the Ribosome. J. Biol. Chem. 276:22183-22190.

Lamberti, A., M. Caraglia, O. Longo, M. Marra, A. Abbruzzese and P. Arcari. 2004. The translation elongation factor 1A in tumorigenesis, signal transduction and apoptosis: Review article. Amino Acids. 26:443-448.

Lund, A., S. M. Knudsen, H. Vissing, B. Clark and N. Tommerup. 1996. Assignment of Human Elongation Factor 1a Genes: EEF1A Maps to Chromosome 6q14 and EEF1A2 to 20q13.3. Genomics. 36:359-361.

Milan, D., R. Hawken, C. Cabau, S. Leroux, C. Genet, Y. Lahbib, G. Tosser, A. Robic, F. Hatey, L. Alexander, C. Beattie, L. Schook, M. Yerle and J. Gellin. 2000. IMpRH Server: an RH mapping server available on the Web. Bioinformatics. 16:558-559.

Pan, P. W., S. H. Zhao, M. Yu, B. Liu, T. A. Xiong and K. Li. 2003. Identification of differentially expressed genes in the longissimus dorsi muscle tissue between duroc and erhualian pigs by mRNA differential display. Asian-Aust. J. Anim. Sci. 16:1066-1070.

Sanders, J., R. Raggiaschi, J. Morales and W. Moller. 1993. The human leucine zipper-containing guanine-nucleotide exchange protein elongation factor-1 delta. Biochim. Biophys. Acta. 1174:87-90.

Shao, M. Y., H. Wang, H. Y. Ren, Z. M. Zhu, S. L. Yang and K. Li. 2006. Assignment of CRSP9, ETF1 and TMEM59 genes to porcine chromosomes. Cytogenet Genome Res. 112:3421 (DOI.10.1159/000089899).

Yang, J., M. Yu, B. Liu, B. Fan, M. Zhu, T. Xiong and K. Li. 2005. Cloning and Initial Analysis of Porcine MPDU1 Gene. Asian-Aust. J. Anim. Sci. 18:1237-1241.

Zhao, S. H., D. Nettleton, W. Liu, C. Fitzsimmons, C. W. Ernst, N. E. Raney and C. K. Tuggle. 2003. Complementary DNA macroarray analyses of differential gene expression in porcine fetal and postnatal muscle. J. Anim. Sci. 81:2179-2188.

Zhu, Z. M., J. B. Zhang, K. Li and S. H. Zhao. 2005. Cloning, mapping and association study with carcass traits of the porcine SDHD gene. Anim. Genet. 36:191-195.