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Polymorphic Diversity of Actin-Like Protein from cDNA Isolated from Korean Native Chicken

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Abstract: We report the isolation and characterization of a chicken cDNA which putatively encodes a chicken actin-like protein (cALP). A clone, KNC-NDS-7 (Genbank, access No, AY466164.1) will be a other form of actin-like protein with these low about 10% homology. DNA sequence (34 - 748 nt) of KNC-NDS-7 was translated into amino acids (237aa) which was compared with actin-like protein of 99% homology with chicken. We found that three important sites have been deleted or substituted. Thereafter, the amino acid profile has been changed. The deleted site (729-731 nt) was not translated to glutamate (E), and the substituted site (711-714 nt) was expressed to leucine (L) instead of proline (P) in the all other species. The third position (465-459 nt) was changed to lysine and threonine (KT) instead of glutamine and lysine (QK). These changes will have important affect to actin structure in KNC. In these results, KNC-NDS-7 is may another form of acin-like protein.

Key words: Actin-like protein, Korean native chicken, cDNA, Muscle growth

Introduction

Korean native chicken (KNC) is a conserved breed for pedigree, family line, and improving economic value. The production cost is high due to low growth rate and long period to reach marketing weight. However, the commercial broilers have low preference and palatability although they have good economic value with high growth rate. Therefore, the KNC is needs to be conserved for unique taste and meat quality to their people (Sang *et al*., 2002). Also, KNC may have strong resistance against some pathogenic microorganisms (personal communication). Genetic resources of native animals will be important in world market for future bio-industry. Meat quality is closely related especially with the DNA expression of muscle protein, actin, and myosin. Actin is generally expressed muscle protein and utilized as an indicator for the research of cellular and molecular biology study. If the actin expression is different among breed depending on developmental stage, the muscle structure and meat quality will be affected by their DNA expression

Actins are a family of highly conserved proteins that are ubiquitously found among eukaryotic organisms. The isoactins are encoded by a set of structurally related genes that probably evolved from a common ancestor (Hightower and Meagher, 1986; Mounier *et al*., 1992). Actin represents approximately 22% of total myofibrillar protein in skeletal muscle (Yates and Greaser, 1983). The skeletal muscle a-actin isoform (sk-a-actin) represents >95% of all actin present in adult skeletal muscle (Caravatti *et al*., 1982; Garner *et al*., 1989). b-actin utilize three important pathway for DNA transcription; first, a component of ATP-dependent chromatin remodeling complex (Olave *et al*., 2002), second, the RNP component (Percipalle *et al*., 2001), third, utilize three RNA polymerization enzyme in the nucleolus of eukaryote (Percipalle *et al*., 2006). The objectives of this study are to identify specific functional genes which related with growth in Korean native chicken. We recently isolated a chicken cDNA bearing a short ORF with sequence homology to chicken actin-like protein (cALP).

Materials and Methods

Animals: The Korean native chickens (KNC, red brown, 12 months old, 2.41kg±0.24) and Cornish chickens (16 month old, 2.76±3.04kg) were obtained from the Daejeon branch of the National Livestock Research Institute, Korea. Pectoralis muscles were dissected and frozen immediately in liquid nitrogen. Total RNA was isolated by RNA isolation kit (Clonetech, Korea). mRNA was analyzed by the methods of Poly (A)+ purification using PolyATtract mRNA isolation System (Promega, USA).

mRNA Subtraction and Cloning: Two breed mRNAs were subtracted by the methods of subtractive suppression hybridization. mRNA was subtracted by suppression hybridization method using PCR-select cDNA Subtraction Kit. Cloned cDNA were inserted into PCR4 Blunt-TOPO vector. The cloned vector was transformed in TOP 10 electroporation cells. The cDNA was then used as a probe to screen 0.5x106 lysate plates. A 1.2kb cDNA, chicken actin-like protein (cALP), KNC-NDS-7 was thus isolated. The sequence (847nt) of this cDNA is given in Fig. 2. Specific clones were constructed by the subtraction from cDNA of Korean native chicken to cDNA of Cornish chicken.

cDNA Library Preparation: Isolated RNA was used Sub-

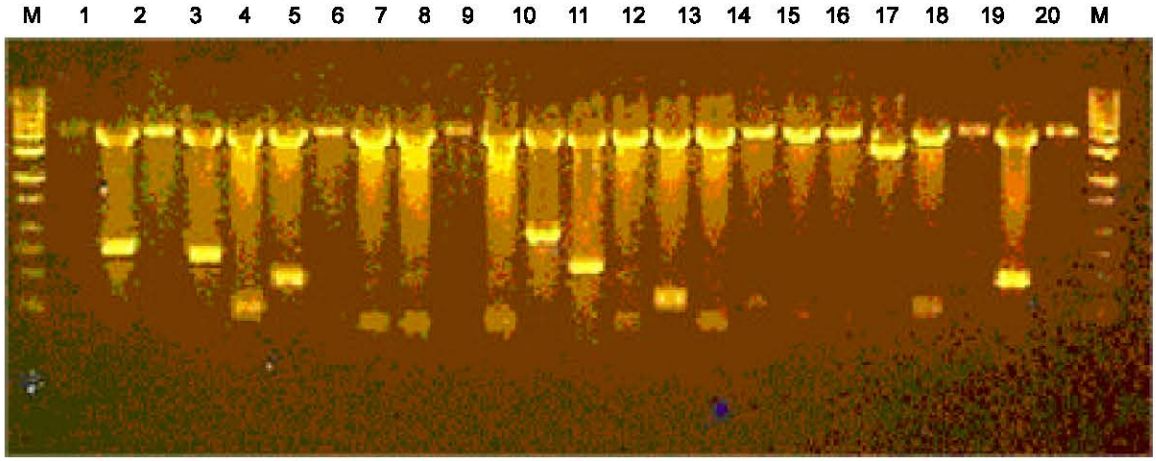

Fig. 1: Insert size determination. twenty colonies are randomly picked and extracted plasmid DNA. plasmid DNAs are digested with EcoR 1. Digested insert DNA were electrophoresed on a 1% TAE agarose gel. Lane M: 1kb DNA ladder (Stratagen), lane 1-20: Digested insert DNA.

Lib DNA (Eugentech, Korea). To select *E. coli* contained incorporated plasmid, *E.coli* (0.1 ml) was spread on LB/AMP/KAN/X-Gal plate was incubated for overnight at 37°C. The cultured colony was adjusted to 1 x 104 cfu per 10 ng of vector DNA. From cultured colony, the single white colony was inoculated in LB/AMP/KAN, and was incubated for 16-18 hrs in shaking incubator until 0.6-0.8 O.D. value at 37°C, 170rpm. The cultured liquid (817 ul) was mixed with 80% glycerol (183 ul), and then these culture stock was stored in -70°C. These culture stocks (1.0ml) in 1.5ml tube were centrifuged at 3000rpm for 15min, and the E.coli cell pellet was collected. Vector ligation was used 80ng of cDNA and 10ng of PCR4 Blunt-TOPO vector. cDNA inserted vector was transformed into TOP 10 electroporation cells (Invitrogen). The cell pellet was dissolved completely in 250 ul resuspension buffer (P1) in 1.5ml tube, and then the plasmid was isolated using QIAprep Miniprep kit (Invitrogen). The mixture was gently mixed with 250 ul lyses buffer (P2), and incubated for 4 min at RT. 250 ul Neutralization buffer (N3) was added, incubated for 5 min, and then centrifuged for 10 min at 10,000rpm. Supernatant was transferred into binding column tube, centrifuged 60 sec, and discarded filtered liquid. 750 ul PE buffer was added into binding column tube, centrifuged for 1 min at 12000 rpm. The binding column tube was completely dried without ethanol. Binding column tube was connected into 1.5ml tube, add 50ul Elution buffer in the middle, rested for 1 min, and centrifuged for 1 min at 13000rpm. The isolated plasmid was digested with restriction enzymes, and identified the DNA size with electrophoresis. The insert was sequenced (Macrogen, Korea), and the homology was compared with other breeds and species in GenBank.

Results and Discussion

The inserted DNA size into vector was 0.5-1.0kb from agarose gel electrophoresis (Fig. 1). DNAs were sequenced and they were identified mostly as triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, 11-beta-hydroxysteroid dehydrogenase, carbamoyl phosphate synthase, endo-1,4-beta-D-glucanase, and makorin etc. Most genes were not specific in KNC. However, several candidate genes were identified as the KNC specific DNA. The function was not confirmed yet, but they are related with pectoral muscle development.

DNA sequence was compared homology in GenBank of NCBI. Clone, KNC-NDS-7, has relatively high 47% homology with in human of actin-related protein, about 12% homology with cattle, pig, and chicken, 12% homology with pet animals such as dog, cat, rabbit, and horse, and about 13% homology with chimpanzee, rat, and frog. This clone, KNC-NDS-7, will be a other form of actin-like protein with these low about 10% homology. Similar results were reported by Michaille *et al.*, (1995) of actin-like protein in chicken. These three actin-like proteins are Drosophila melanogaster (ARP14D), Caenorhabditis elegans (ACTL), and Saccharomyces cerevisiae (ACT2), and have 81, 70, and 67% of amino acid homology, respectively. We registered our result of DNA sequence (Fig. 2) to Genbank (access number, AY466164.1).

DNA sequence (34-748 nt) of KNC-NDS-7 was translated into amino acids. The untranslated region was 1-33 nt and 749-847 nt. Amino acid sequence was compared with actin-like protein of 99% homology with chicken (*Gallus gallus*), 97% homology with human (*Homo sapiens*) and dog (*Canis familiaris*) and, 95% homology with rodents (*Rattus norvegicus*) and cattle (*Bos taurus*). In these results, KNC-NDS-7 is another form of acin-like protein. Actin is expressed similarly in muscle protein of most species. In these result, the amino acid sequence of KNC was similarly expressed

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  26  TGTTGCCTTCCTCTGCCTGACATGAGTTTCTTCATGAGTTAGAAATCACAGACCTTGTTT
   1        G I L P E Q S L E T A K A I K E K

  86  CCATTAGACAAGCATTATGACATGACTCCAAACACAGGATTATGACGACAAATGCTAGGG
  17  Y C Y I C P D I V K E F A K Y D G D P R

 146  CCATATTCTTCATAGTCttt...ttttGTGTGACATACTTGGAAGAACTCCGGTGTTGAAGCC
  37  K W I K Q Y T G I N A I N K T K F V I D

 206  AGCATGGAACCGCCAAACCAAACCGCATAGCGCTGCATATGATGTGTTATCACTTGAACT
  57  V G Y E R F L G P E I F F H P E F A N P

 266  TCAACTGGCTTGGGTTTTATCCGACCACCACTGAGCTCCTCACTAAGCCT CAATCTTGCA
  77  D F M E S I S D V V D E V I Q N C P I D

 326  TCCACTACTCTTTTCAAATCCCTTTGCAGTCGTCGTCCAAAGTCCCTGAACATTGTGGAT
  97  V R R P L Y K N V V L S G G S T M F R D

 386  CCTCCCGAGAGCACCACATTCTTATATAATGGACGCCGGACATCAATGGGACAGTTCTGT
 117  F G R R L Q R D L K R V V D A R L R L S E

 446  ATAACTTCATCAACTACATCCG4A4ATGGATTCCATAAAATCAGGATTAGCAAACTCGGGA
 137  E L S G G R I K P K P V E V Q V I T H H

 506  TGAAAGAAAATTTCAGGTCCAAGGAACCTTTCATAACCAACATCTATAACAAATTTGGTT
 157  M Q R Y A V W F G G S M L A S T P E F F

 566  TTGTTGATTGCATTGATGCCAGTATACTGTTTGATCCATTTTCGGGGATCTCCATCATAC
 177  Q V C H T K K D Y E E Y G P S I C C L S

 626  TTAGCAAATTCTTTCACAATGTCGGGGCAAATATAACAATATTTCTCCTTTATGGCTTTT
 197  N G N K V C D F S R H N P V F G V M S S

 686  GCTGTCTCCAGAGATTGTTCAGGGAGAATTCCCACTTCCCTCTNCCTCAGNAGTTGTTGA
 217  L M K K L M S G R G R Q Q V P A R A A A

 746  ATAAAGTAAGTAATATCTCTACCTGCATAGGAATATGTTTGATGCAACTTCCATTACATA
(237)  R

 806  GCCTTCTGCCACAGGAATACATGGGTCACTNCATCACACT AT
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Fig. 2: Partial nucleotide sequence of KNC-NDS-7 cDNA (GenBank/NCBI accession No. AY466164.1) of Korean native chicken (847 bases). Nucleotide residues are numbered on the left. Translated sequence (34-748 nt) is underlined. The 1-33 nt is untranslated region. The actin-like protein cDNA includes 749-847 nt open reading frame in Korean native chicken. The deduced 237aa (single letter code) sequence of the chicken ALP protein in given under the nucleotide sequence. The KNC-NDS-7 cDNA was isolated by screening $0.5x10^6$ lysate plates of an adult chicken pectoral muscle cDNA library constructed in Eugentech (Daejon, Korea) in Blunt-TOPO vector.

in other breeds at same DNA position. Also, actin-binding protein (ABP) may affect to skeletal muscle structure which constructed by actin protein (Peitsch *et al.*, 2006). Also, He reported that serum response factor (SRF) will affect to actin for cellular growth rate, cell migration, and myogenesis. DNA sequence of black carp b-actin has 98.1% homology with chicken (Feng *et al.*, 2006).

```
NDS-7    AGRDITYFIQQLLR X REVGI L PEQSLETAKAIKEKYCYICPDIVKEFAKYD G DPRKWIKQ
Chicken  ---------- E ----- P ---------------------------------- - ---------
Dog      ---------- E ----- P ------------------------------ V ---------
Human    ---------- E ----- P ------------------------------ V ---------
Rat      ---------- E ----- P ------------------------------------------
Cattle   ---------- E ----- P ------------------------------ V ---------

NDS-7    YTGINAIN  KT  KF V IDVGYERFLGPEIFFHPEFANPDFMESISDVVDEVIQNCPIDVRRPL
Chicken  --------- -- -- - ------------------------------------------------
Dog      --------  QK -- I ------------------------------------------------
Human    --------  QK ------------------------------------------------------
Rat      --------  QK -- I ------------------------------------------------
Cattle   ---V---  QK ----------------------------------E-------------------

NDS-7    YKNVVLSGGS TMFRDFGRRL QRDLKRVVDA RLRLSEELSG GRIKPKPVEV QVITHHMQRY
Chicken  ------------------------------------------------------------------------
Dog      ------------------------------------------------------------------------
Human    ----------------------------------------------------V-------------------
Rat      ---I--------------------------.K--Q-----------------------------------
Cattle   --------------------------------K---------------------------------------

NDS-7    AVW FGGSMLASTP EFFQVCHTKK DYEEYGPSIC RHNPVFGVMS                          *
Chicken  ------------------------------------------------------------------------ *
Dog      ------------------------------------------------------------------------ *
Human    ------------------------------------------------------------------------ *
Rat      ------------------------------------------------------------------------ *
Cattle   ------------------------L-----------C------------------------------------ *
```

Fig. 3: Comparison of 237 amino scid sequence of KNC-NDS-7 with other species. Highly conserved region was changed (729-731 nt, 711-714 nt, and 465-459 nt) by substitution.

We found that three important positions have been mutated, deleted or substituted. Thereafter, the amino acid profile has been changed (Fig. 3). The deleted site (729-731 nt) was not translated to glutamate (E), and the substituted site (711-714 nt) was expressed to leucine (L) instead of proline (P) in the all other species. The third position (465-459 nt) was changed to lysine and threonine (KT) instead of glutamine and lysine (QK). These changes will have important affect to actin structure in KNC. The deletion (730 nt) contained highly conserved amino acid of the 3' end of the coding region, the in-frame stop codon, a less frequently used poly(A) signal that is normally found 126 nt downstream of the stop codon, and a large portion of the 3' UTR. Because of this deletion, glutamic acid was substituted and the open reading frame was extended for an additional 15 amino acids before reaching the transcriptional termination site. The predicted amino acid sequence of the novel carboxyl-terminus cALP of KNC is largely hydrophobic with a poly-lysine tail, whereas the carboxyl-terminus of ALP of other species is composed of hydrophilic amino acids. Actin gene can be used for a primer for identifying different breed and species due to highly conserved sequence (Lockley and Bardsley, 2002; Hopwood *et al*., 1999; Reecy *et al*., 1996). Most vertebrate genomes contain numerous actin genes with high sequence homology in comparable exons, but considerable variation in intron number and size (Weber and Kabsch, 1994).

Actin amino acid is interacting with ATP, Ca^{2+} and myosin, and involved in actin-actin interactions, as determined by X-ray crystallography and other methods (Holmes *et al*., 1990; Holmes and Kabsch, 1991). The changes sites (729-732 nt, 711-714 nt) in this result will be a binding site for ATP and Ca^{2+} Chicken actin probably bind ATP or Ca^{2+} because most of the 15aa that contact ATP and the three aa that contact Ca^{2+} are conserved (Michaille *et al*., 1995). Some regions required for actin polymerization are also conserved, but others have highly diverged. Furthermore, most of the sited interacting with myosin has considerably diverged too. Actin-related protein of the actin-like protein group may thus be involved in some unusual interactions with other actin, and probably do not interact with myosin.

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