

Molecular Characterization of a Polyubiquitin Gene from Duck, *Anas Platyrhynchos* (Aves: Anatidae)

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Abstract. Molecular amplification and sequencing of genomic DNA encoding duck (*Anas platyrhynchos*) polyubiquitin was performed by polymerase chain reaction (PCR). One of the several DNA fragments obtained on amplification conformed to 345 bp, which on sequencing yielded a polyubiquitin fragment (PUBD1). The PUBD1 sequence was found to be 89 and 93% similar to the sequences of human UBC and chickens UB1 respectively. This fragment translated into 115 amino acids corresponding to three fused units of ubiquitin (one complete and two partial units), indicating polyubiquitin coding sequences. Furthermore, genomic DNA was digested with *EcoR* I, *Bam*H I and *Hind* III and subsequently single-strand amplification was performed with ubiquitin specific primers. It was concluded that ducks genome contains at least four loci for polyubiquitin genes. This is the first report of a newly characterized polyubiquitin gene from ducks.

Key words: *Anas platyrhynchos*, Duck, Polyubiquitin.

Introduction

Polyubiquitin genes encode one of the highly conserved proteins and are characterized by the presence of tandem repeats of 228 bp, ubiquitin monomer. Ubiquitin consists of 76 amino acids. It is found in all eukaryotes from unicellular organism to higher plants and animals. Ubiquitin is both a cytoplasmic and nuclear protein and plays an important role in the proteolysis process in the cell where it becomes covalently attached to the target proteins as a recognition signal for protein degradation (Hershko & Ciechanover, 1986; Hershko, 1991; Rechsteiner, 1991; Varshavsky, 1997; Hochstrasser, 1996; Vierstra, 1996). It is also involved in the cellular heat shock response (Bond & Schlesinger, 1985), DNA repair, gene transcription regulation, cell cycle regulation, signal transduction and cell recognition (Ciechanover, & Schwartz, 1994;

Wilkinson, 1994). It has also been proposed that ubiquitin is highly expressed during spermatogenesis and plays an essential role in the differentiation of germinal cells, particularly in the structural changes of chromatin taking place at the end of the process (Mezquita and Mezquita, 1991). Ubiquitin is also found in neurofibrillary tangles typical of Alzheimer disease (Perry, *et al.*, 1987; Mori, *et al.*, 1987). It may also play a role in morphogenesis (Pfeifer, *et al.*, 1993).

Although ubiquitin protein sequence is highly conserved, nucleotide sequences are quite divergent in different organisms. In contrast to the coding regions, the 5' and 3' non-coding sequences of some members of ubiquitin gene family are entirely different from one another (Xia and Mahon, 1998). The conservation of ubiquitin throughout the phylogenetic tree is remarkable. Its amino acid sequence is identical from arthropod to mammals. Moreover, multiple polyubiquitin

genes are present in various organisms and distinguished by different number of ubiquitin repeats (Wiborg, *et al.*, 1985; Arribas, *et al.*, 1986; Baker and Board, 1987; Neno, *et al.*, 1994; Giorda, and Ennis, 1987; Mezquita, *et al.*, 1997; Al-Khedhairy, 2004).

In this study, molecular characterization of a polyubiquitin gene from duck (*Anas platyrhynchos*) was achieved by polymerase chain reaction (PCR) using various sets of primers.

Materials and Methods

Genomic DNA preparation

DNA was extracted from duck blood with GenomicPrep™ Cells and Tissue DNA Isolation Kit (Amersham Bioscience, USA). Primers were designed on the basis of the DNA sequence data for human polyubiquitin C (Wiborg *et al.*, 1985). Polymerase chain reaction was performed using PuRe Taq Ready-To-Go PCR Beads (Amersham Bioscience, USA) with different sets of primers (Table 1).

Polymerase chain reaction

A 200-300 ng of duck genomic DNA was used as a template in 25µl reaction. Genomic DNA was amplified for 40 cycles. Each cycle consisted of 94°C for 30 sec, 52°C for 30 sec, 72°C for 1 min.

PCR products obtained were separated by electrophoresis on 1.5% agarose gel in TAE buffer, visualized by ethidium bromide fluorescence. Fragments with the expected size were cut from the gel and purified using GFX PCR DNA Gel Band Purification Kit (Amersham Bioscience, USA).

DNA sequencing and analysis

Purified DNA segments were sequenced using both forward and backward primers (Al-Khedhairy, 2004). Subsequently, the results were analyzed and compared with the sequences of polyubiquitin genes from human and chickens using blast-2 software (NCBI, Bethesda, USA). The DNA sequence was also translated into amino acid sequence and compared with that of polyubiquitin from human and chickens and homology was determined.

Determination of gene copy

Genomic DNA was digested with a number of restriction enzymes (*EcoR* I, *Bam*H I, *Hind* III). All enzymes have no sites within PUBD1. The digests were amplified using single primers specific for ubiquitin. DNA fragments obtained were separated on agarose gel and compared to calculate their sizes.

Results

Duck genomic DNA was amplified by PCR to yield polyubiquitin coding sequence. The amplification generated various DNA fragments that were all sequenced. One of these fragments conformed to 345 bp in size and yielded PUBD1 DNA sequence. The sequence data for the fragment showed that it was a polyubiquitin DNA coding sequence as it contains one complete (228 bp) and two partial units of ubiquitin. Comparison of PUBD1 nucleotide sequence with its counterparts of polyubiquitin from human and chickens indicated a high sequence homology. The PUBD1 nucleotide sequence was found to be 89

Table 1. Set of primers used.

Set	Forward primer (F)	Backward primer (R)
1	5'-ctg acc agc aga ggy tga tct t-3'	5'-gtc ttg cca gtg agt gtc ttc a-3'
2	5'-tga aga ccc tgt ctg gta aga c-3'	5'-tgg act ctt tct gga tgt tgt ag-3'
3	5'-aag atg gac gca ccc tgt ctg act aca aca-3'	5'-ctt cct tat ctt gga tct ttg cct tga cat t-3'
4	5'-cct gtc tga cta caa cat cca gaa gtc gac -3'	5'-atc ttc cag ctg ttt ccc agc aaa gat caa cct-3'

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PUBD1 : cagaggtgatctttgcaggcaagcagcttgaagatgggcgaccctgtctgactacaac 60
        |||
UBC   : cagaggtgatctttgctgggaaacagctggaagatggacgcaccctgtctgactacaac
        |||
UB1   : cagaggtgatctttgctggcaagcagctggaagatgggcgaccctgtctgactacaac

PUBD1 : atccagaaggaatccaccctccacctgtcctgcgctgagaggtggcatgcagatcttt 120
        |||
UBC   : atccagaaagagtcaccctgcacctggtgctccgtcttagaggtggatgcagatcttc
        |||
UB1   : atccagaaagagtcaccctgcatctggtgctgcgctgaggggagcatgcagatcttt

PUBD1 : gtgaagaccctgactggcaagaccatcaccttgaggttgagccagtgacaccattgag 180
        |||
UBC   : gtgaagaccctgactggttaagaccatcactctcgaagtgagccagtgacaccattgag
        |||
UB1   : gtgaagaccctgactggcaagaccatcaccttgaggttgagccagtgacacaattgag

PUBD1 : aatgtgaaggccaagatccaggacaaagagggcattccccctgaccagcagaggttgatc 240
        |||
UBC   : aatgtcaaggcaaagatccaagacaaggaaggcatccctcctgaccagcagaggttgatc
        |||
UB1   : aatgtgaaggccaagatccaggataaagaaggcattcctcctgatcagcagaggttgatc

PUBD1 : tttgctggcaagcagctggaagatggtcgaccctgtctgactacaacatccagaaagag 300
        |||
UBC   : tttgctgggaaacagctggaagatggacgcaccctgtctgactacaacatccagaaagag
        |||
UB1   : tttgctggttaagcagctggaagacgggcgaccctgtctgactacaacatccagaaagag

PUBD1 : tccaccctgcatcttgtgctgcgctgagaggtggcatgcagatc 345
        |||
UBC   : tccaccctgcacctggtgctccgtcttagaggtggatgcagatc
        |||
UB1   : tccaccctgcatctggtgtgcgctgagaggtggatgcagatc

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Fig 1. Comparison of nucleotide sequences of ducks PUBD1, human UBC and chickens UB1

and 93% similar to the sequences of human UBC and chickens UB1, respectively. Figure 1 shows synonymous nucleotide differences between ducks PUBD1, human UBC and chickens UB1.

PUBD1 translated into 115 amino acids (Fig. 2) corresponding to one complete and two incomplete units of ubiquitin protein indicating that PUBD1 contained at least 3 units of ubiquitin. These three repeats are not separated by any intervening sequences and immediately

adjacent to each other, similar to the sequences of polyubiquitin genes from different species.

Heterogeneous amplification of genomic DNA with single primers (forward or backward) specific for ubiquitin was obtained, which indicated the presence of multiple polyubiquitin loci (Fig. 3). Determination of polyubiquitin gene copy number revealed the presence of at least four loci for polyubiquitin in the ducks genome (Fig. 4).

PUBD : QRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRGGMQIFVKTLTGKTITLEVEPSDTIE 60
 UB1 : QRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRGGMQIFVKTLTGKTITLEVEPSDTIE
 PUBD1 : NVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRGGMQI 115
 UB1 : NVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRGGMQI

Fig. 2. Comparison of amino acid sequences of ducks PUBD1 and chickens UB1

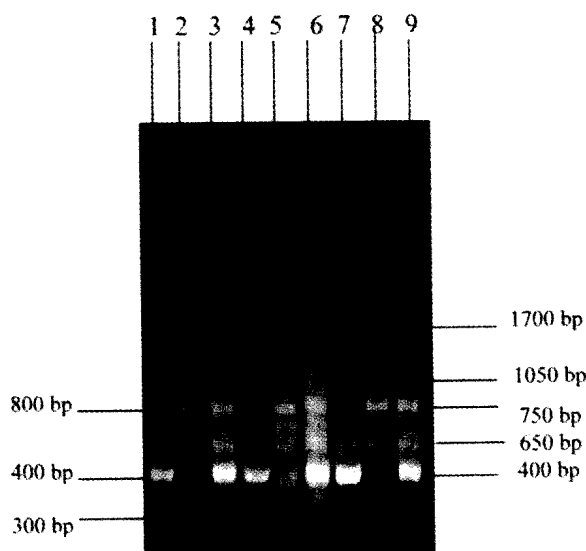


Fig 3. Heterogenous amplification of genomic DNA with ubiquitin single primer after digestion with different restriction enzymes.

Lane 1 *EcoR* I digest amplified using F1 primer
 Lane 2 *EcoR* I digest amplified using R1 primer
 Lane 3 *EcoR* I digest amplified using both F1 and R1 primers
 Lane 4 *BamH* I digest amplified using F1 primer
 Lane 5 *BamH* I digest amplified using R1 primer
 Lane 6 *BamH* I digest amplified using both F1 and R1 primers
 Lane 7 *Hind* III digest amplified using F1 primer
 Lane 8 *Hind* III digest amplified using R1 primer
 Lane 9 *Hind* III digest amplified using both F1 and R1 primers

Discussion

Although, PUBD1, human UBC and chickens UB1 nucleotide sequences were found to have several base differences. These differences among the nucleotide sequence of the PUBD1, chicken UB1 and human UBC are silent nucleotide substitutions. Notably, none of the several base changes resulted in amino acid

substitution and the amino acid sequence encoded by PUBD1 was similar to ubiquitin from other species. Comparison of amino acid sequence of the PUBD1 with those of other species showed that it was well conserved in various species. This conservation is in accordance with the previous reports on different organisms where the ubiquitin sequence is highly conserved while nucleotide sequences are quite divergent with homologies ranging from 77 to 95%. Okubo *et al.* (2002) compared DNA sequences of rainbow trout and eleven other species and found several variations in the nucleotide sequences but the encoded amino acid sequences were fully conserved.

Moreover, the repeat number of ubiquitin coding units in polyubiquitin genes varies between species ranging from 3 units in human and chickens to about 30 units in *Trypanosoma brucei* (Baker and Board, 1987; Giorda and Ennis, 1987; Wong and Campbell, 1989). Mammalian genomes including that of human have been reported to contain a ubiquitin multigene family with varied DNA sequence organization. Some of the genes in this family encode for polyubiquitin containing three, four or nine repeats of ubiquitin while some encodes for only one ubiquitin sequence (Wiborg, *et al.*, 1985; Baker and Board, 1987; Einspanier, R, 1987). In the present study, one complete and two incomplete units of ubiquitin coding sequences were amplified from DNA fragment using specific primers. Further, these three repeats are immediately adjacent to each other, not separated by any sequence as expected in polyubiquitin gene sequence. Ducks ubiquitin

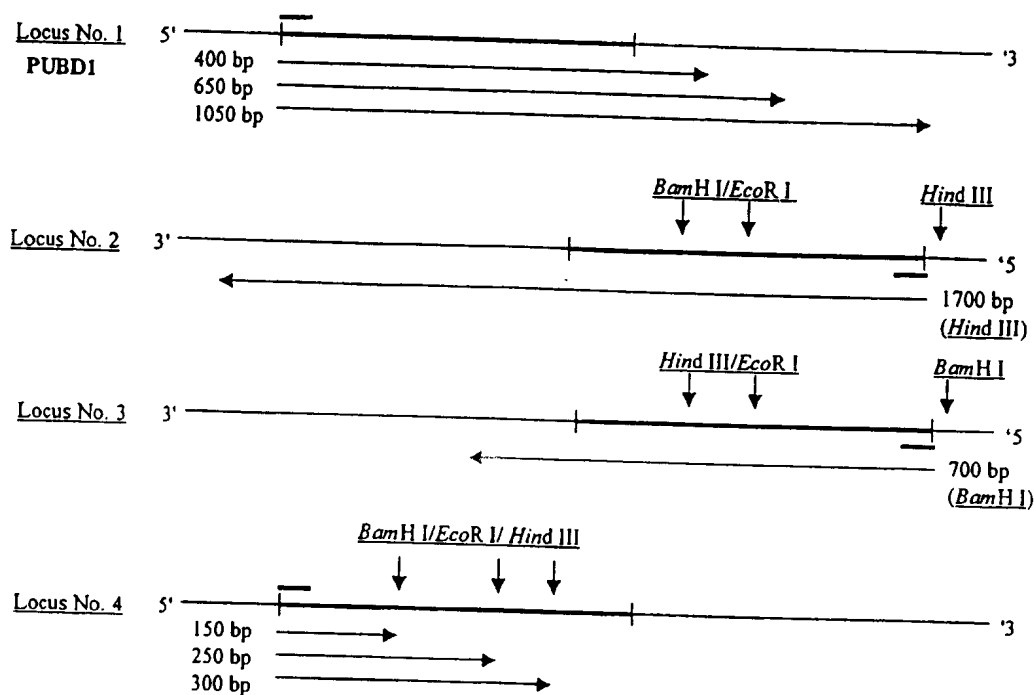


Fig 4. Hypothetical illustration of polyubiquitin gene copy number in the ducks Genome.

genetic organization seems to be similar to those of human and other mammals having multiple loci, encoding repeats of ubiquitin, of which DNA sequences are not conserved while enclosed amino acid sequences are fully conserved.

In order to analyze gene copy number, three restriction enzymes were utilized, where all these enzymes have no sites within PUBD1. It was concluded that heterogenous amplification of genomic DNA with ubiquitin single primers indicated the presence of polyubiquitin sequences at multiple loci (Fig. 3).

Several single-strand fragments were amplified using forward primer. These were calculated to be of 400 bp, 650 bp and 1050 bp in size. Similarly, fragments of 400 bp, 650 bp and 750 bp in size were amplified with backward primer. Since the amplification was noticed from genomic DNA preparations digested separately with *EcoR* I, *BamH* I and *Hind* III that have no sites within PUBD1, it was

concluded that these fragment might have originated from a minimum of one locus for polyubiquitin gene (PUBD1) without sites for such enzymes.

Further more, a single fragment of 1.7 kb was amplified only with backward primer using DNA preparations digested with *Hind* III but not with *EcoR* I and *BamH* I. Therefore, it was concluded that a second locus may exist for polyubiquitin in the ducks genome with restriction sites for *EcoR* I and *BamH* I.

Also, a single fragment of 700 was amplified only with backward primer using DNA preparations digested with *BamH* I but not with *EcoR* I and *Hind* III. Therefore, it was concluded that a third locus must exist for polyubiquitin in the ducks genome with restriction sites for *EcoR* I and *Hind* III.

Finally, amplifications with either forward or reverse polyubiquitin primers generated smaller fragments ranging from 150 to 300 bp using DNA preparations digested separately with each restriction enzyme. This indicated the

existence of a fourth locus for polyubiquitin in the ducks genome with restriction sites for all three enzymes, *Bam*H I, *Eco*R I and *Hind* III. Therefore, ducks genome contains at least four copies of polyubiquitin genes. Figure 4 illustrates a hypothetical illustration for the presence of at least four polyubiquitin loci in the ducks genome.

This is the first report of a polyubiquitin gene from ducks. The polyprotein contains at least 3 direct repeats of the ubiquitin amino acid sequence with no spacer sequence separating the ubiquitin repeats and coding regions that are not interrupted by any intervening sequences. Also, the ducks genome contains at least four loci for polyubiquitin genes. The complete conservation of amino acid sequence of ubiquitin observed in the functional genes sequenced so far is in accordance with the fact that ubiquitin is completely conserved throughout the animal kingdom, which indicates that a strong selection pressure must be operating in order to maintain the conservation of amino acid sequences as suggested by Wiborg *et al.* (1985).

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توصيف جزيئي لمورث عديد اليوبكوتين من البط المحلي (*Anas Platyrhncchos* (Aves: Anatidae)

عبدالعزیز بن علی الخضیری

قسم علم الحيوان - كلية العلوم - جامعة الملك سعود

ص.ب. ٢٤٥٥ - الرياض ١١٤٥١ - المملكة العربية السعودية

ملخص: في هذا البحث تم اكنثار وقراءة تتابع النيوكليوتيدات لدنا جينومي حامل للشفرات الوراثية لبروتين اليوبيكوتين العديد من البط (طائر البركة *Anas platyrhncchos*) باستخدام تفاعل البلمرة المتسلسل Polymerase Chain Reaction. وكانت واحدة من عدة قطع دنا تم الحصول عليها بحجم ٣٤٥ نيكليوتيدة وذات تتابعات مطابقة لبروتين اليوبيكوتين العديد وأعطى الرمز (PUBD1). وقد لوحظ أن التتابعات كانت ٨٩% ، ٩٣% مشابهة لتتابعات الدنا لبروتين اليوبيكوتين العديد من الإنسان (UBC) والدجاج (UB1) على التوالي. وقد تم ترجمة هذه القطعة إلى ١١٥ حمض أميني تمثل ثلاث وحدات مندمجة لبروتين اليوبيكوتين (وحدة كاملة ووحدين غير مكتملتين)، وهو ما يشير إلى تتابعات مشفرة لبروتين اليوبيكوتين العديد. إضافة إلى ذلك فقد تم تقطيع الدنا الجينومي بثلاث إنزيمات حصر هي *EcoR I* ، *BamH I* ، *Hind III* ومن ثم إكنثار خيط مفرد للدنا باستخدام بادئات (Primers) خاصة بيوبكوتين. وقد وجد أن جينوم البط يحتوي على الأقل على أربع مواقع وراثية لبروتين اليوبيكوتين العديد. ويعتبر ذلك أول بحث يصف مورث لبروتين اليوبيكوتين العديد من البط.