

Characterization and Expression of Chicken Selenoprotein U

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Abstract Selenoprotein U (SelU) may regulate a myriad of biological processes through its redox function. In chicks, neither the nucleotide sequence nor the amino acid sequence is known. The main objectives of this study were to clone and characterize the chicken *Selu* gene and investigate *Selu* messenger RNA (mRNA) and protein expression in chicken tissues. The coding sequence (CDS) of *Selu* contained 387 bases with a typical mammalian selenocysteine insertion sequence (SECIS) located in the 3'-untranslated region. The deduced amino acid sequence of chicken SelU contains 224 amino acids with UAA as the stop codon. Like all SelU genes identified in different species, chicken SelU contains one well-conserved selenocysteine (Sec) at the 85th position encoded by the UGA codon. The SECIS element was with the conserved denosine (–AAA–) rather than the motif cytidine (–CC–) motif. Moreover, the expression pattern of *Selu* mRNA in muscle, liver, kidney, heart, spleen, lung, testis, and brain was analyzed with real-time quantitative PCR in young male chickens fed a Se-deficient corn-soybean meal basal diet supplemented with 0.0 and 0.3 mg Se/kg in the form of sodium selenite. We found that the abundance of *Selu* mRNA in muscle, liver, kidney, heart, spleen, and lung was downregulated ($P < 0.05$) by Se deficiency. However, it was not affected by dietary Se concentrations in testis and brain. Furthermore, protein abundance of SelU in these seven tissues was consistent with the mRNA abundance. Hence, we suggest

that *Selu* might play an important role in the biochemical function of Se in birds.

Keywords Chicken · Selenium · Selenocysteine insertion sequence · Selenoprotein U

Abbreviations

BD	Basal diet
EST	Expressed sequence tag
PCR	Polymerase chain reaction
Q-PCR	Real-time quantitative PCR
Se	Selenium
SECIS	Selenocysteine insertion sequence
SelU	Selenoprotein U

Introduction

Selenium (Se) has been shown to be an essential component of all living organisms [1]. It was discovered in 1817 by Swedish chemist and doctor Jöns Jakob Berzelius [2]. Se is present in selenoproteins in the form of selenocysteine (Sec), the 21st amino acid. Sec is inserted co-translationally in response to UGA codons, a stop signal in the canonical genetic code. About 25 Sec-containing proteins have been identified in eukaryotes [3], but distribution among taxa varies greatly. The majority of selenoproteins have homologs in which Sec is replaced by cysteine (Cys), even in genomes lacking the Sec-containing gene. Most members of the selenoprotein family have been identified using bioinformatics approaches. The functions of many of them are still unknown, and there is evidence of the existence of some further forms of selenoproteins that have not yet been identified.

Selenoprotein U (SelU), encoded by *Selu*, was found in fugu as well as in other fish, sea urchins, a green alga, and

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diatom [4]. Surprisingly, SelU homologs in other species, including humans, contain Cys instead of Sec. And, its specific functions are still unknown. Chicken SelU is believed to be an important member of the avian selenoprotein family, as defined using bioinformatics approaches.

It has long been known that the tissue expression hierarchy under different Se availability correlated with the biological importance of selenoproteins in specific tissues [5, 6], so we cloned chicken *Selu*, analyzed the characteristics of its nucleotide and amino acid sequences, and investigated the tissue-specific distribution and regulation by dietary Se concentrations. Furthermore, we also investigated whether SelU was differentially expressed in many chicken tissues.

Materials and Methods

Chicken and Diets

All procedures used in the present study were approved by the Institutional Animal Care and Use Committee of China Agricultural University. A total of 50 1-day-old male broiler poulters (Dafa Zhengda Poultry Co., Ltd., Beijing, China) were allotted into two dietary treatment groups ($n = 25$). The basal diet (BD) [7] was composed of corn and soybean meal produced in the Se-deficient area of Sichuan, China, and was not supplemented with Se (–Se). Another experimental diet was supplemented with Se (as sodium selenite, Sigma-Aldrich, St. Louis, MO) at 0.3 mg/kg (+Se). Over the entire experimental period, the chickens were allowed ad libitum consumption of feed and water. The chickens were maintained either on a Se-deficient diet or on sodium selenite for 28 days. At the end of experiment, the animals were killed and eight tissues (liver, kidney, heart, lung, spleen, testis, muscle, and brain) were collected and immediately frozen at $-80\text{ }^{\circ}\text{C}$.

Molecular Cloning of Chicken *Selu*

Liver sample was immediately collected to prepare the RNA mixture for *Selu* cloning by reverse transcriptase PCR (RT-PCR). The specific primers for chicken *Selu* were designed based on the sequence of expressed sequence tag (EST) clone, C10orf58 (GenBank Accession: NM_001193518), by using Primer Premier 5 (PREMIER Biosoft International, Canada). One primer was 5'-GGAATTCATATGTCTTTCCTACCTGACTT-3' and the other was 5'-CGCGCTCGAGGCTTTCAA CCGACTGTC-3'. They were paired successively with the 3' AP reverse primer in semi-nested PCR procedures using the Taq DNA polymerase kit (TaKaRa, Cat. no. DR001AM). The thermal cycling program included an initial melting at $94\text{ }^{\circ}\text{C}$ for 5 min, followed by 30 cycles of $94\text{ }^{\circ}\text{C}$ for 30 s, $58\text{--}60\text{ }^{\circ}\text{C}$ for 30 s, and $72\text{ }^{\circ}\text{C}$ for 45 s, and a final extension at $72\text{ }^{\circ}\text{C}$ for 10 min. The amplified DNA product was analyzed by gel

electrophoresis, recovered, and ligated to PMD18-T vector (TaKaRa, Cat. no. D101) by T/A cloning. After blue/white screening and sequencing (Invitrogen Company, Shanghai, China), the confirmed sequences were consisted with the sequence of messenger RNA (mRNA) NCBI Reference Sequence, NM_001193518.

Predicting the Chicken *Selu* SECIS Element

The selenocysteine insertion sequence (SECIS) element is the characteristic feature of selenoprotein mRNA; in the presence of this element, the UGA codon can be translated into Sec. To confirm that the novel RNA sequence was *Selu* mRNA and to identify the SECIS element, we used the SECISearch engine (<http://sebastian.crg.es/>, SECISearch3) [8] to analyze the primary sequence and secondary structure of chicken *Selu* mRNA.

Sequence Analysis, 3D Molecular Modeling, and Phylogenetic Analysis

The complementary DNA (cDNA) sequence and deduced amino acid sequence of chicken SelU were analyzed using the BLAST algorithm (<http://www.ncbi.nlm.nih.gov/blast>) and the Expert Protein Analysis System (<http://www.expasy.org/>). The 3D structure and biological function of chicken SelU were predicted using the I-TASSER server (<http://zhanglab.ccmb.med.umich.edu/I-TASSER/>) [9, 10]; the evolutionary and tree homology analysis were constructed using the DNAMAN 6 (Lynnon Biosoft, Canada), and MEGA5 [11] softwares.

Real-Time Quantitative PCR Analysis of Selenoprotein mRNA Levels

To determine effects of dietary Se on the mRNA expression of these genes, we isolated total mRNA from muscle, liver, kidney, heart, spleen, lung, testis, and brain (50 to 100 mg tissue) of five most representative chicks from each group. The relative mRNA expression of chicken *Selu* was quantified by real-time quantitative RT-PCR (qRT-PCR) (ABI 7900HT, Applied Biosystems, USA; QuantiTect SYBR Green RT-PCR kit, QIAGEN, Cat. no. 204243, Hilden, Germany) using the $\Delta\Delta\text{Ct}$ method and β -actin gene (*Actb*) as the internal reference gene. For the qRT-PCR analysis of *Selu* mRNA, the forward primer was 5'-GATGCTTTCAGGCTTCTTCC-3' and the reverse primer was 5'-CTGTCTTCTGCTCCAATCA-3'. The forward primer for *Actb* was 5'-CCGCTCTATG AAGGCTACGC-3'; the reverse primer was 5'-CTCTCGGC TGTGGTGGTGAA-3'.

The $\Delta\Delta\text{Ct}$ values representing relative mRNA levels in all the tissues were calculated by first subtracting the average ΔCt of muscle from chicks fed from the ΔCt of all the tissues,

and then converting the results to fold differences by raising $-\Delta\Delta C_t$ to the power of two ($2^{-\Delta\Delta C_t}$). Univariate analysis of general linear model followed by Bonferroni *t* test (SPSS for Windows 13.0, USA) was used to test the effects of tissue type, Se level, and their interaction on the mRNA abundance.

Western Blot Analyses

For Western blot analysis, chicken tissues were rapidly ground in liquid nitrogen. The resulting powders were reconstituted in ice-cold RIPA buffer containing 1 mmol/l phenylmethanesulfonyl fluoride (PMSF) and a cocktail of protease inhibitors (1:100 dilution). Samples were centrifuged at 4 °C for 15 min at 12,000g. Supernatants were recovered, and total protein was determined using a BCA protein assay kit. A 50- μ g portion of protein or equal proportion of concentrated supernatant was subjected to sodium dodecyl sulfate/polyarylamide gel electrophoresis (SDS-PAGE), and the blotted following standard methods. Non-specific binding to the membrane was blocked by 5 % (w/v) dry non-fat milk in PBS/0.05 % (v/v) Tween-20 (PBST) at room temperature for 1 h in a covered container. Blots were incubated overnight at 4 °C with rabbit polyclonal anti-SelU antibody (1:500, made by our lab), and rabbit polyclonal anti- β -actin (1:1000, Biosynthesis Biotechnology, Beijing, China) diluted in 5 % BSA. Membranes were washed with PBST and incubated with a secondary goat anti-rabbit IgG-HRP antibody (1:10,000) obtained from ZSGB-Biotechnology (Beijing, China) diluted in 5 % (w/v) dry non-fat milk in PBST for 1 h at room temperature. Finally, membranes were washed with PBST, developed using enhanced chemiluminescence reagent (Millipore, MIT, USA), and quickly dried, and the signal was detected on film (Hyperfilm ECL; Amersham) using a chemiluminescence (ECL Western blotting analysis system; Amersham imager 600, Amersham Biosciences, Piscataway, NJ).

Statistical Analysis

Data analysis was performed by using SPSS statistical software for windows (version 13, SPSS, Chicago, IL, USA). When a main effect ($P < 0.05$) was identified by one-way analysis of variance, mean comparisons were followed. Data are presented as means \pm standard error of the mean (SEM). Differences were considered to be significant at $P < 0.05$.

Results

Sequence and Structure of Chicken *Selu*

Based on the reference chicken sequences obtained by in silico cloning, a genomic DNA fragment of about 964 bp including the coding sequence (CDS) to the poly(A) tail of

Selu was cloned by RT-PCR (GenBank accession no. NM_001193518.1). The characteristic of chicken *Selu* cDNA and its SECIS element are illustrated in Fig. 1. The *Selu* CDS had 675 bases with an in-frame TGA triplet; the sequence theoretically encoded 224 amino acid residues and the Sec was the 85th residue (Fig. 1a). The chicken *Selu* had 74 % CDS homology to human *Selu*. According to the generally used classification method [12] and SECISearch program analysis, a SECIS element, with the conserved denosine (–AA– or –AAA–) rather than the motif cytidine (–CC–) motif in the apical loop, was found in the 3'-untranslated region of the cDNA (mRNA), similar to that found in most lower species [13]. Figure 1b compares the *Selu* SECIS elements in chick, mouse, rat, human, cow, zebrafish, and some lower animals. The SECIS element had the –AAA– motif in *Gallus gallus* (chick), *Ictalurus punctatus* (channel catfish), *Danio rerio* (zebrafish), and *Taeniopygia guttata* (hybrid). In contrast, the SECIS element had the –AA– motif in *Salmo salar* (atlantic salmon), *Cavia porcellus* (Guinea pig), and *Oncorhynchus mykiss* (rainbow trout); the SECIS element had the –CC– motif in *Homo sapiens* (human), *Xenopus tropicalis* (frog), and *Oryctolagus cuniculus* (rabbit).

Homology and Phylogeny Analyses

The homology and phylogeny of chicken *Selu* were analyzed in 17 species (Fig. 2). The chicken SelU protein was aligned with 16 other species (Fig. 2b): *D. rerio* (zebrafish), *Oryzias latipes* (rice fish), *Salmo salar* (atlantic salmon), *Oncorhynchus mykiss* (rainbow trout), *T. guttata* (hybrid), *I. punctatus* (channel catfish), *H. sapiens* (human), *Mus musculus* (mouse), *Bos taurus* (cattle), *Sus scrofa* (pig), *Cavia porcellus* (Guinea pig), *Oryctolagus cuniculus* (rabbit), *Pan troglodytes* (Chimpanzee), *Rattus norvegicus* (rat), and *X. tropicalis* (frog). These proteins shared 64 % identity overall, with a highly conserved thioredoxin-like domain containing a UXXC/CXXC motif across all the SelU proteins.

The amino acid sequence of chicken SelU shares 74, 71, and 92 % identity with human SelU, zebrafish SelU, and hybrid SelU, respectively (Table 1). The nucleotide identity range of CDS was varied from 68 to 89 % between chicken and other animal *Selu* from mammals to aquatic invertebrates (Table 1).

The phylogeny by MEGA5 software used *G. gallus* and 15 other animals: *Canis lupus*, *Sus scrofa*, *Cavia porcellus*, *H. sapiens*, *P. troglodytes*, *M. musculus*, *R. norvegicus*, *Oryctolagus cuniculus*, *X. tropicalis*, *D. rerio*, *I. punctatus*, *Oryzias latipes*, *Oncorhynchus mykiss*, *Salmo salar*, and *Volvox carteri f. nagariensis* (Fig. 2b). The SelU of the 16 species were genetically clustered, and of the euteleostomis, chicken and frog had the shortest distance.

A

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ATGTCTTCTCTACCTGACTTTGGGATCTTCACCATGGGCATGTGGTCGGTTGGTCTCGG 59
AGCCGTTGGCGCAGCCATAACAGGGATTGTGCTTGCCAACACTGACTTATTTTTGTCCA 118
AGCCAGAAAAAGCAACGCTGGAATTTTTGGAGGCGATAGAGCTAAAAACTTTGGGAT 175
CAGAACCAGAACATTCAAAGCAAGTGAAGTGTGGAAGAAGAAATGGTGCAGTGATCA 232
TGGCTGTGCGAAGACCTGGGTGATTTTTTGTGCAGAGAGGAGGCTTCTGAGCTTCTCTC 290
TCTGAAACCTCAGCTGTCCAAGCTGGGTGTTCTCTCTATGCTGTTGTGAAAGAGAAG 348
ATAGGGACCGAAGTGGAGGATTTTCAGCATTATTTCCAAGGAGAAATCTTTCTAGATGA 407
AAAGAGAAGCTTCTATGGCCACGCAAGCGAAAAATGATGCTTTCAGGCTTCTTCCGC 465
ATTGGAGTCTGGCAAAATTTCTCCGTGCTTGAAAAATGGTTATAGTGGCAACCTGGA 524
AGGAGAAGGGTTCACCCTGGGAGGAGTGTATGTGATTGGAGCAGGAAGACAGGGTGT 581
TCTATTGGAGCATCGTGAGAAAAGAAATTTGGTGACAAAAGTCAGCCTTCCATCTGTCCTTG 640
AAGCTGCCGAGAAGATAAAGCCACAGGCTTCATAAgctgaaataattgcatgttttctgacgcagcctg 713
aaacgtagaacacatcgtttc tgaatgtgcaatttaattgc tcttaac tattactgatc attgcc gaggaagattc tttc aaatgtaataac t 814
gaaaaccctcctggagttttgttgc tttgctgtcttccaggttc agagttc agctgacctttg TGTCTGACTGTATTAATGA 901
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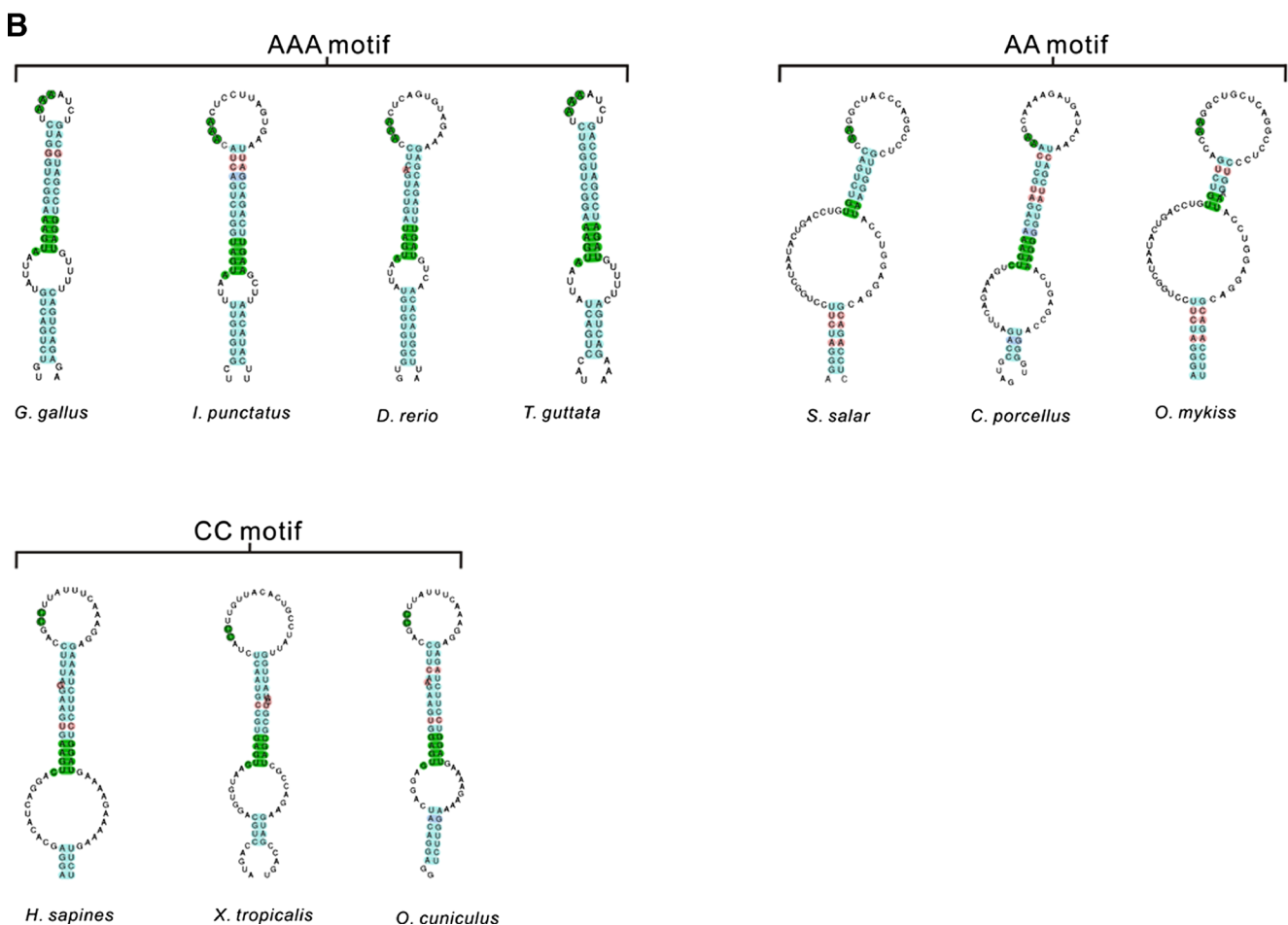


Fig. 1 Chicken *Selu* cDNA and its selenocysteine insertion (SECIS). **a** The cDNA sequence of chicken *Selu* cloned by RT-PCR. The sequence in *uppercase* is the coding sequence from ATG to TAA (in **bold**), and the framed TGA at 85th triplet from the initiation codon encodes selenocysteine (Sec). The *underlined letters in bold* in the 3'-untranslated region indicate that the highly conserved nucleotides are in the SECIS. **b** The SECIS structures were predicted by the SECISearch

program with appropriate patterns. The highly conserved nucleotides are in *bold*. Accession numbers of the sequences mentioned above are NM_001193518 (*G. gallus*), NM_001201270 (*I. punctatus*), NM_001193525 (*D. rerio*), NM_001193526 (*T. guttata*), NM_001193547 (*Salmo salar*), NM_003466089 (*Cavia porcellus*), NM_001193546 (*Oncorhynchus mykiss*), NM_032333 (*H. sapiens*), NM_001097222 (*X. tropicalis*), NM_002720973 (*Oryctolagus cuniculus*)

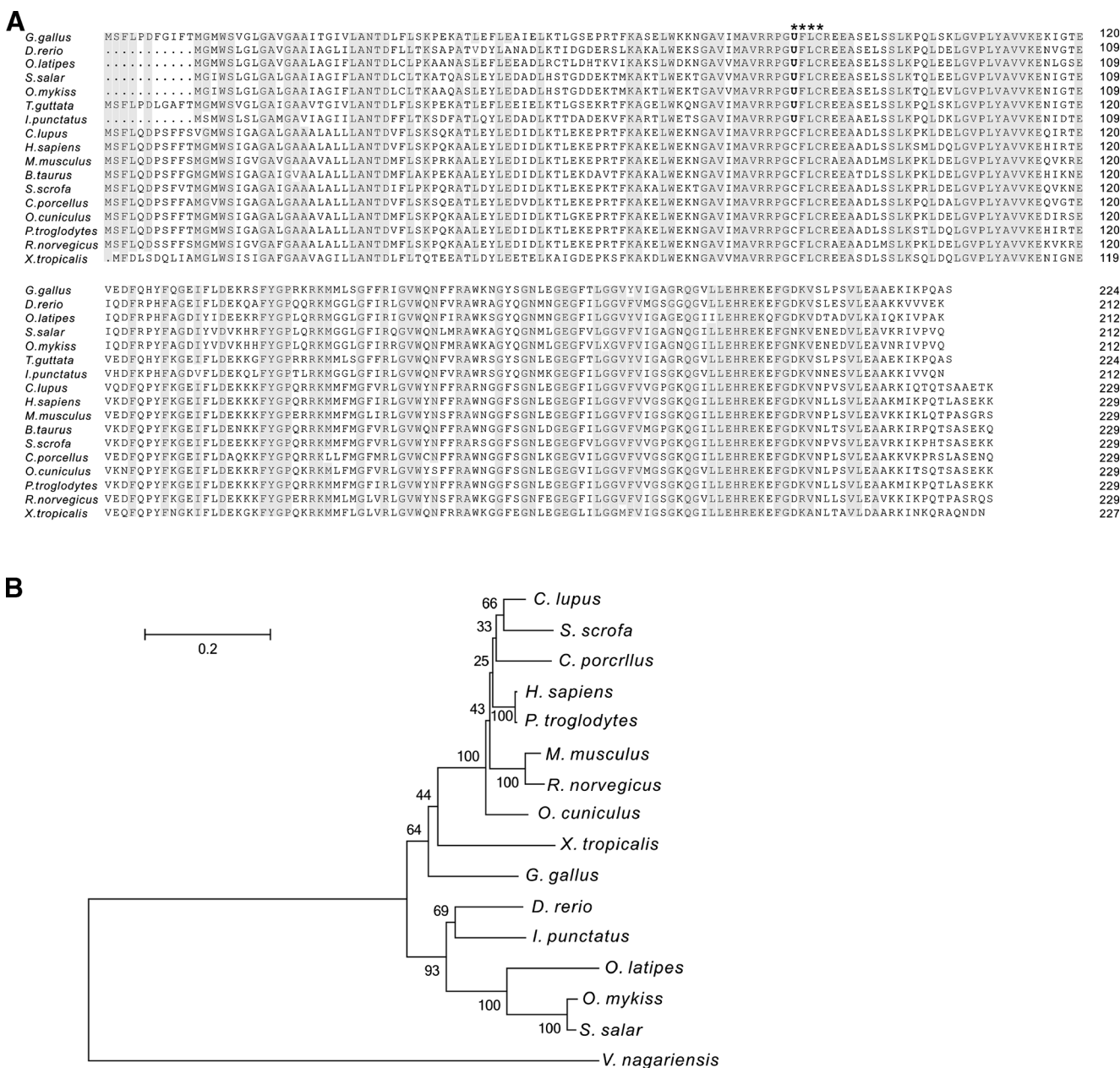


Fig. 2 Alignment and phylogeny analyses of SelU based on the amino acid sequences. **a** The conserved XFLC motif (UFLC in the chicken) is indicated by the asterisks. The gray-colored letters indicate conserved amino acid residues across the 17 species. **b** The phylogenetic analyses were conducted using MEGA5 and encompassed 16 SelU proteins. The

bootstrap consensus tree generated by the neighbor-joining method with 500 replicates and pairwise deletion options was taken to represent the evolutionary history of the 16 proteins. The statistics and frequency are presented at each of the nodes, and the length of the distance scale bar at the bottom of the panel defines 0.2 of the genetic distance

The Predicted 3D Structure of Chicken SelU

Predicting the protein secondary structure is generally considered to be the first step in protein structure prediction. The 3D structure and biological function of chicken SelU were predicted using the I-TASSER server (Fig. 3). The predicted 3D molecular modeling of the Sec⁸⁵→Cys variant of chicken SelU is shown in Fig. 3. The structure of chicken SelU consists of a seven

stranded α -helices with seven extended β -sheets. The molecule is characterized by a $\alpha_1(9-31)$ - $\beta_1(55-56)$ - $\beta_2(63-65)$ - $\alpha_2(66-70)$ - $\beta_3(75-80)$ - $\alpha_3(87-103)$ - $\beta_4(108-112)$ - $\alpha_4(116-123)$ - $\beta_5(131-133)$ - $\alpha_5(137-143)$ - $\alpha_6(147-166)$ - $\beta_6(183-188)$ - $\beta_7(192-198)$ - $\alpha_7(208-217)$ secondary structure pattern, wherein β_2 and β_5 are parallel strands forming three classical β - α - β motifs. The predicted active site Sec (U) is located in the loop (residues 85) between β_3 and α_3 .

Table 1 Sequence(seq) homology of SelU cDNA and deduced amino acid (AA) of chicken, mouse, rat, human, cow, salmon, frog, zebrafish, and zebra finch

cDNA seq (AA seq)	<i>M. musculus</i>	<i>R. norvegicus</i>	<i>H. sapiens</i>	<i>B. taurus</i>	<i>S. salar</i>	<i>X. tropicalis</i>	<i>D. rerio</i>	<i>T. guttata</i>
<i>G. gallus</i>	84 % (72 %)	72 % (74 %)	74 % (74 %)	72 % (71 %)	68 % (67 %)	71 % (69 %)	70 % (71 %)	89 % (92 %)
<i>M. musculus</i>	100 % (100 %)	92 % (94 %)	85 % (88 %)	82 % (85 %)	67 % (63 %)	71 % (69 %)	72 % (67 %)	72 % (72 %)
<i>R. norvegicus</i>	92 % (94 %)	100 % (100 %)	85 % (87 %)	82 % (83 %)	66 % (62 %)	71 % (69 %)	72 % (68 %)	72 % (73 %)
<i>H. sapiens</i>	85 % (88 %)	85 % (87 %)	100 % (100 %)	88 % (88 %)	67 % (64 %)	72 % (72 %)	71 % (70 %)	72 % (75 %)
<i>B. taurus</i>	82 % (85 %)	82 % (83 %)	88 % (88 %)	100 % (100 %)	68 % (63 %)	71 % (70 %)	72 % (70 %)	72 % (73 %)
<i>S. salar</i>	67 % (63 %)	66 % (62 %)	67 % (64 %)	68 % (63 %)	100 % (100 %)	66 % (65 %)	75 % (67 %)	68 % (66 %)
<i>X. tropicalis</i>	71 % (69 %)	71 % (69 %)	72 % (72 %)	71 % (70 %)	66 % (65 %)	100 % (100 %)	70 % (67 %)	71 % (69 %)
<i>D. rerio</i>	72 % (68 %)	72 % (68 %)	71 % (70 %)	72 % (70 %)	75 % (67 %)	70 % (67 %)	100 % (100 %)	71 % (71 %)

Parentheses indicate the homology of AA sequences between two different species

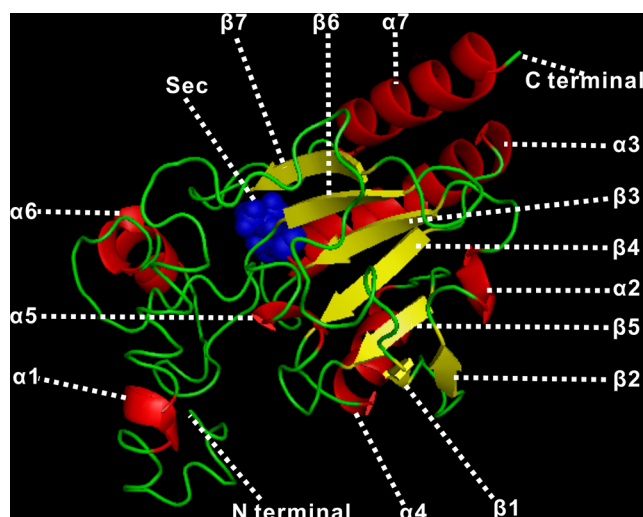


Fig. 3 The 3D structure of chicken SelU. α and β indicate α -helix and β -sheet, respectively. The active site Sec residue, located in the loop between $\beta 3$ and $\alpha 3$ in a pocket on the protein surface

Expression Pattern of *Selu* mRNA and Protein Level of SelU in Chicken Tissues

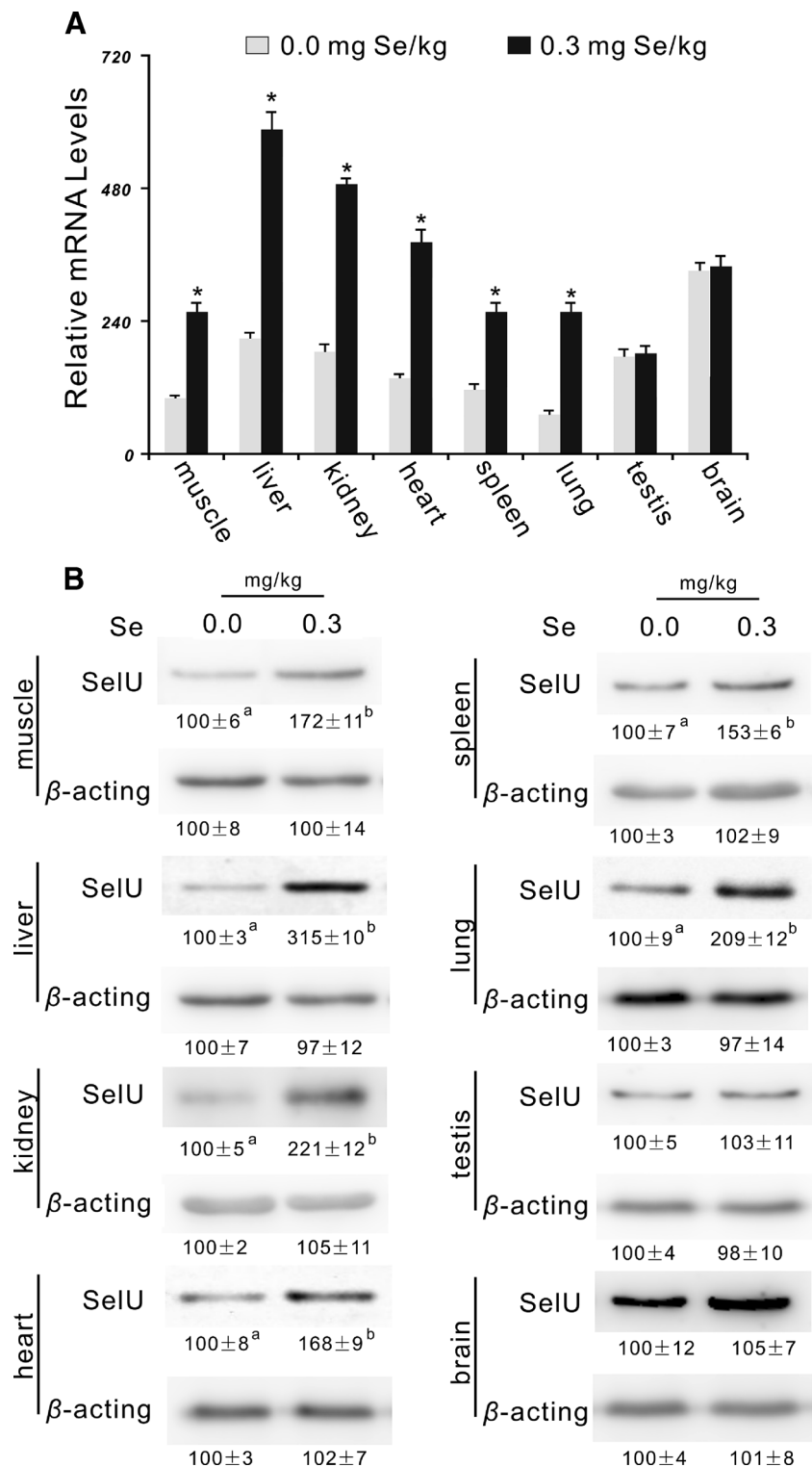
The -Se chicks had lower ($P < 0.05$) mRNA levels of *Selu* (Fig. 4a) in muscle, liver, kidney, heart, spleen, and lung than +Se chicks. However, dietary Se concentration (in Se-deficient BD supplemented with 0.0 and 0.3 mg Se/kg) did not affect mRNA levels of *Selu* in testis and brain. The -Se chicks had lower abundance of ($P < 0.05$) SelU (Fig. 4b) in muscle, liver, kidney, heart, spleen, and lung than +Se chicks. However, dietary Se concentration (in Se-deficient BD supplemented with 0.0 and 0.3 mg Se/kg) did not affect protein levels of SelU in testis and brain.

Discussion

As an essential trace element, Se is important for animal and human health. The three classical Se deficiency diseases in chickens include exudative diathesis (ED), nutritional muscular dystrophy (NMD), and nutritional pancreatic atrophy (NPA). Se protects against ED and muscle degeneration and increases reproductive performance in poultry [7, 14, 15]. In addition, the recent report confirmed the negative effects of Se deficiency on chicken immune organs [16]. In a word, Se supplementation is necessary for poultry growth performance.

Se exerts its biological function as selenoproteins in living organisms. There are at least 25 selenoproteins in the chicken selenoproteome [17]. Although the function for many identified selenoproteins is still not confirmed, such as SelU, most selenoproteins suggest their biological function to be involved in antioxidative or redox-related reactions [18]. The *Selu* gene was first identified from the ESTs database of human and puffer fish by the computational method [4]. The

Fig. 4 Effects of dietary Se concentration on (a) *Selu* mRNA abundance and (b) protein level of SelU in muscle, liver, kidney, heart, spleen, testis, lung, and brain of chickens fed a basal selenium-deficient diet (BD) plus 0.0 and 3.0 mg Se/kg for 4 weeks. Data are presented as means \pm SEM (standard error of the mean), $n = 6$ (a) or 4 (b). Within the same tissue, asterisk ($P < 0.05$) shows differences under different dietary Se concentrations. Means showing different superscript letter are different ($P < 0.05$)



method led to the discovery of a novel selenoprotein family (SelU) in puffer fish, whereas its human counterpart contained Cys. In addition, Sec-containing homologs exist in other chicken, fish, sea urchin, and so on. In our study, the *in silico* method was applied to predict the CDS-containing cDNA of

chicken *Selu*, and the sequence was confirmed by RT-PCR. Consistent with the *Selu* SECIS elements in channel catfish, zebrafish, and hybrid, the chicken *Selu* SECIS element had –AAA– in the apical loop. In fact, the SECIS element with the –AAA– or –AA– motif appears to be universal in the *Selu*

genes of lower animals. To explain the evolutionary meaning of forms and motifs in the *Selu* SECIS element, phylogenetic analysis of a greater number of species is required.

The SelU family is widely distributed across the eukaryotic lineage, either as Sec- or as Cys-containing proteins, but lacks the counterpart in prokaryotes. The scattered and taxa-specific distribution of Sec and Cys forms of a SelU. Sec is located in SelU proteins close to a conserved Cys such that the two residues form a motif that resembles the U_{xx}C/C_{xx}C motif that is present in various thiol-dependent redox proteins. Similar motifs are also present in a number of eukaryotic selenoproteins, including SelP [19], SelW [20, 21], SelV, SelT [22], SelM [23], and SelH [24]. The 3D structure of SelU protein also suggests that chicken SelU might have the same functions like thiol-dependent redox proteins due to the conserved motifs [18]. The fact that selenoproteins are distributed discretely at very different taxonomic levels raises the question of whether Sec loss or Sec gain is favored by evolution. Arguments exist in favor of both possibilities. Replacement of Sec by Cys is plausible because it yields a protein with diminished, but still functional, catalytic activity. In addition, Sec-containing homologs exist in chicken, zebrafish, rice fish, atlantic salmon, rainbow trout, and hybrid. These results cast doubts on the scattered phylogenetic distribution of selenoprotein genes, suggesting a quite dynamic Sec/Cys evolutionary exchange.

The most essential selenoproteins are less affected by Se status. In other words, not all selenoproteins are equally affected when Se becomes limiting [25]. Exploring these aspects of chicken *Selu* will lead to a deeper understanding of its regulation by Se status and shed light on its biological importance in specific tissues. Tissue and temporal expressions of the *Selu* during embryogenesis were addressed in the zebrafish model. The *Selu* was widely expressed in all embryonic tissues from all stages, demonstrating ubiquitous expression of the *Selu* [4]. In our experiment, chicken tissue samples of muscle, liver, kidney, heart, spleen, lung, testis, and brain were tested by qRT-PCR and western blots. The chicken *Selu* mRNA expression was high in liver followed by kidney. The dietary Se supplementation upregulated *Selu* gene expression in the chicken, and the liver was the most responsive tissue when dietary Se content was low. However, the expressions of *Selu* in testis and brain were not affected by Se deficiency. Chicken SelU is a 25-kDa selenoprotein containing one Sec encoded by UGA codon. SelU was determined in tissues by Western blots with a polyclonal antibody against a recombinant protein corresponding to full-length chicken SelU protein. What is more, expression of SelU mRNA and protein expression were consistent in these tissues. Thus, SelU levels in all tissues of chicken except the testis and brain were sensitive to Se status. It is of interest that the abundance of SelU in testis and brain was preserved even under conditions of Se deficiency, with SelU probably

expressed in high level in brain. Compared with other avian selenoproteins [26–28], SelU in the present study was more sensitive to Se deficiency in most tissues. This indicates that SelU could potentially be a molecular biomarker of Se status. Se deficiency causes irreversible changes in the neuronal cells and brain injury. Evidence from clinical studies revealed that Se deficiency leads to cognitive impairment, seizures, Parkinson's disease, and Alzheimer disease [29]. It is speculated that SelU is the major selenoperoxidase responsible for the prevention of brain injury, via its peroxide scavenging and antioxidant functions.

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