CHUGOKUGAKUEN J. 2007
Vol. 6, pp. 23-27
Copyright©2006 by Chugokugakuen

CHUGOKUGAKUEN Journal
http://www.cjc.ac.jp/

Original Article

Genomic structure and sequence of a chicken homolog (chNthl 1) of Escherichia coli endonuclease Ⅲ with those of the adjacent parts of chTsc2 and chSlc9a3r2 genes

Shuji Seki[1], Altaf H. Sarker[2], Takashi Nakamura[3]
Yuichi Seki[4] and Shogo Ikeda[5]

[1] *Department of Human Nutrition, Faculty of Contemporary Life Science, Chugokugakuen University, Okayama 700-0197, Japan,*
[2] *Department of Cellular and Molecular Biology, Life Science Division, Lawrence Berkeley National Laboratory, University of California, California, USA,*
[3] *Department of Molecular Biology, Okayama University Graduate School of Medicine and Dentistry, Okayama 700-8558, Japan,*
[4] *Tsuyama Medical Examination Center of Chugoku Occupational Health Association, Tsuyama 708-0001, Japan,*
[5] *Department of Biological Chemistry, Okayama University of Science, Okayama 700-0005, Japan*

Genomic cloning and sequencing of a chicken homolog (chNthl1) for *Escherichia coli* endonuclease Ⅲ, that is involved in pyrimidine base excision repair, were performed. The sequence covered the entire chNthl1 gene consisting of six exons and five introns spanning 4.9kb with 5' flanking (6.6kb) and 3' flanking (3.1kb) regions. The boundaries between exon and intron follow the GT/AG rule. The sequenced 5' flanking region lacks typical TATA and CAAT boxes, but contains a CpG island having putative binding sites for several transcription factors such as Ests 1 and Sp1. The chNthl1 gene lies immediately adjacent to the tuberous sclerosis 2 (chTsc2) gene in a 5'-to-5' orientation. Downstream of the chNthl1 gene, the gene for the regulatory factor 2 (chSlc9a3r2) of the solute carrier family 9 (sodium/hydrogen exchanger), isoform A3, lies in a 3'-to-3' orientation. The genomic organization of the chNthl1 gene and the relative orientation of the gene with the adjacent genes of chTsc2 and chSlc9a3r2 are similar to those in human and mouse genomes.

Key Words: chicken Nthl1 gene, genomic cloning, endonuclease Ⅲ homolog, chicken

Introduction

Oxidative DNA damage produced by endogenously and exogenously generated reactive oxygen species has been implicated in cell death, mutagenesis, carcinogenesis, and aging [1]. Thymine residue of DNA is known to be especially susceptible to oxidative DNA damage, and the major detectable, oxidative product is thymine glycol [2]. Repair of the lesion is generally initiated by the DNA glycosylase activity of such enzyme as endonuclease Ⅲ (endo Ⅲ) of *Escherichia coli* (*E. coli*) which is followed by the AP lyase activity of the same enzymes [1, 3]. Endo Ⅲ was also reported to be involved in the repair of other damaged pyrimidine derivatives such as 5, 6-dihydrothymine, 5-hydroxy dihydrothymine, 5-hydroxycytosine, 5-hydroxyuracil, uracil glycol, beta-ureidobutyric acid, 5-hydroxy-5-methyl hydantoin and urea [1, 3, 4] which are cytotoxic or mutagenic, otherwise they are repaired properly [1, 3, 4, 5].

Enzyme activities functionally homologous to endo

[1] Corresponding author.
Shuji Seki
Department of Human Nutrition, Faculty of Contemporary Life Science, Chugokugakuen University, Okayama 700-0197, Japan

III have been reported from both prokaryotic and eukaryotic cells [1, 6, 7, 8, 9]. Previously, we have cloned and characterized the mouse homolog (mNthl1) and human homolog (hNTHL1) of endo III and determined their genomic organization [10, 11, 12, 13]. The human NTHL1 or mouse Nthl1 gene has been shown to lie immediately adjacent to the tuberous sclerosis (TSC2 or mTsc2) gene in a 5'-to-5' orientation [12, 14], and adjacent to the gene for the regulatory factor 2 of the solute carrier family 9, isoform A3 (OCTS2-SLC9A3R2 or mSlc9a3r3) in a 3' -to-3' orientation [12, 13, 15]. The cDNA for the chicken homolog (chNthl1) of *E. coli* endonuclease III has also been cloned and characterized [16].

In the present paper, the genomic organization and the sequence of a 14.6kb genomic fragment containing the chNthl1 gene and partial sequences of the neighboring genes (chTsc2 and chSlc9a3r2 genes) are reported.

Materials and Methods

Materials

The reagents used in the present experiments were obtained as described previously [11, 12]. The chicken Nthl1 cDNA was cloned by screening a chicken fibroblast cDNA library (Clontech Lab., Inc., Palo Alto, CA 94303, USA) with the ^{32}P-labeled mouse Nthl1 cDNA [11], and then recloned by screening another embryonic cDNA library (Stratagene, La Jolla, CA 92037, USA) and characterized, as described previously [16]. Chicken spleen genomic library in Lambda FIXII and *E. coli* strain XL1-Blue MRA (P2) were obtained from Stratagene.

Southern blot analysis of chNthl1 genomic DNA

High molecular weight genomic DNA was isolated from chicken liver as described [17]. Five micrograms of the DNA digested with restriction enzymes were electrophoresed on a 0.5% agarose gel at 7V for 16 hours, and transferred onto a positively charged nylon membrane (Hybond N$^+$; Boehringer Mannheim Biochem., IN) as described [11, 17]. The chNthl1 cDNA fragment was excised from the plasmid containing chNthl1 cDNA [16] by treating with EcoRI and XhoI, gel-purified, and labeled with [^{32}P] dCTP (3,000Ci/mmol) by the random priming method. The blotted membrane was hybridized with the ^{32}P-labeled probe. Hybridization was carried out at 68℃ for 12h in 6x SSC (1x SSC: 150mM NaCl, and 15mM sodium citrate, pH7.0), 5x Denhardt's solution, 0.5% SDS, denatured salmon testes DNA at 100μg/ml and the labeled cDNA probe at 5$\times$ 10^{5}cpm/ml. After washing at room temperature for 10min in 2x SSC and 0.1% SDS and then at 68℃ for 1h in 1x SSC and 0.1% SDS with twice changes, the membrane was air dried, and exposed on a Kodak X-Ray film for autoradiography.

Cloning of the chNthl1 gene

The genomic library constructed using chicken spleen genomic DNA and cloning vector Lambda FIXR II T7/T3 (Stratagene) was screened using the same DNA probe as that used for the Southern blot analysis. Among 16 positive clones obtained, the clone 2 (designated as chNthl1G-2) having a 15kb insert was selected for further analysis. The 15kb fragment was subcloned into the plasmid vector pBluescript KS (−) for sequencing.

DNA sequencing and sequence analysis

The nucleotide sequence of the insert of the subclone in the pBluescript KS (−) was determined by the dideoxy chain termination method [18] using

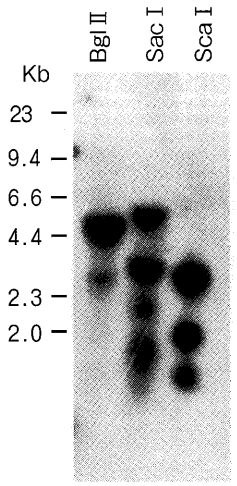


Fig. 1 Southern blot analysis of the chNthl1 gene. Chicken liver genomic DNA was digested with the restriction enzymes indicated on the top. Five micrograms of DNA per lane were subjected to electrophoresis on 0.5% agarose gel, and then transferred onto a nylon membrane. The blots were hybridized with the ^{32}P-labeled chNthl1 cDNA. Numbers in the left column indicate the size markers in kilobase pairs.

either M13 universal primers or oligonucleotide primers synthesized according to the sequences determined using AmpliTaq FS Ready Mix (Perkin-Elmer) by an Applied Biosystem Model 373A DNA sequencer. The nucleotide sequence data reported in this paper have been submitted to the DDBJ/GenBank/EMBL Data Bank under the accession number AB358957.

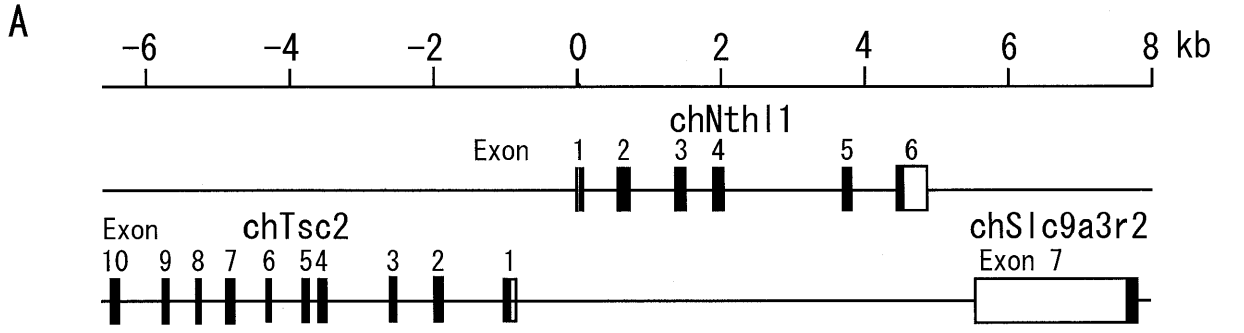
Results and Discussion

Southern Blot Analysis

The rough chNthl1 gene structure was studied by Southern blot analysis of chicken liver DNA digested with restriction enzymes and hybridized with chNthl1 cDNA as a probe. The results showed that the hybridizing fragments detected corresponded to the restriction enzyme fragments deduced from the sequence of the chNthl1 gene, although minor unidentified fragments were also observed (Fig. 1). The origins of the 3.6kb minor hybridizing fragment in the BglII-digested DNA (lane 1) and the 2.3kb and 1.5kb fragment in the SacI-digested DNA (lane 2) were not exactly known.

Cloning and Sequencing of chNthl1 gene

A chicken spleen genomic library was screened with randomly [^{32}P]-labeled chNthl1 cDNA as a probe. Sixteen positive clones were isolated from 1 $\times 10^6$ independent clones. Among them, one clone containing the entire region of chNthl1 gene (chNthl1G-2 clone) was analyzed. The chNthl1G-2 clone was isolated and digested with a restriction enzyme, SacI. The digestion produced four restriction fragments of 4.5, 3.5, 3.0 and 1.7kb in size. These fragments were inserted into the pBluescript KS (−) plasmid vectors and sequenced. By compar-



B

5' - -3'

```
                ( 1)  ATG・・(Exon 1:>64bp)・・CGCAG ( 64) gtacggggcg ( 457bp)・・
・・・cctcctgcag ( 65) GGAGC・・(Exon 2:221bp)・・CGCAG (285) gtaatggcac ( 875bp)・・
・・・tttcccccag (286) GTTAT・・(Exon 3:171bp)・・GGAGG (456) gtaagcgtgg ( 546bp)・・
・・・ggttttccag (457) AACAA・・(Exon 4:160bp)・・GATAG (616) gtaagttgaa (1219bp)・・
・・・cccttcctag (617) CTGTG・・(Exon 5:106bp)・・CCCAG (722) gtgaggtgtg ( 624bp)・・
・・・ctcactgcag (723) GGATC・・(Exon 6:>124bp)・・TGA (846) acccatgacg ・・
```

Fig. 2 The structure and exon-intron organization of the chicken Nthl1 (chNthl1) gene and its flanking regions. In order to tentatively assign a base position in the gene and in the flanking regions, the adenine residue (A) in the translation initiation codon of the chNthl1 gene was designated as position +1 and the 5' neighboring residue C as position −1. With this as a starting point, positive numbers are given to positions downstream from the starting point and negative numbers to those upstream. (A) From the top: scale in kilobases, a diagram of chNthl1 gene, a 5' region of chicken tuberous sclerosis 2 gene (Tsc2) and a 3' region of the chicken gene for the regulatory factor 2 (Slc9a3r2) of the solute carrier family 9 (sodium/hydrogen exchanger), isoform A3. The Nthl1 gene lies adjacent to the Tsc2 gene in a head-to-head orientation and adjacent to the Slc9a3r2 in a tail-to-tail orientation. The exons are numbered from the 5' end of the gene. Filled and open boxes represent translated and untranslated regions, respectively. (B) Nucleotide sequences of the exon-intron boundaries of the chNthl1 gene. Sequences of introns are given in lowercase letters, and those of exons in uppercase letters. On both sides of the parentheses for the number and size of each exon, the first and last five nucleotides are shown. The nucleotide numbers of the first and last residue of each exon are shown in parentheses before and after the first and last five nucleotides, respectively. The size of each intron is shown in parentheses on the right side.

ing the sequence with chNthl1 cDNA sequence, we defined the exon/intron structure of chNthl1 gene (Fig. 2). The gene consists of six exons and five introns, with a total length of 4,932bp. All exon/intron junctions follow the GT/AG rule [19]. The translation initiation and termination codons (ATG and TAA) are located in exons 1 and 6, respectively (Fig. 2). To tentatively assign a base position in the gene and the flanking regions, the adenine residue in the translation initiation codon of chNthl1 gene was assigned to position +1 and the 5' neighboring residue cytosine to position −1, and positive and negative numbers were given to positions in the down stream and in the upstream sequences from the starting positions, respectively. Besides the chNthl1 gene, the chNthl1G-2 clone contained the 5'-flanking region of 6.65kb and the 3'-flanking region of 3.06kb.

Sequence analysis of the 5'-flanking region by comparing it with the mouse tuberous sclerosis 2 (mTsc2) mRNA sequence reported by Kim et al. [20] revealed that chNthl1 gene is lying immediately adjacent to the chTsc2 gene and in a 5'-to-5' orientation with the chTsc2 gene. The exon 1 (155bp) of chNthl1 gene and the exon 1 (73bp) of Tsc2 gene both lie within the 1155bp-sequence.

The 5' flanking region of chNthl1 gene lacks typical TATA and CAAT boxes, but contains a GC-rich region (a region between −1 and −360) having putative binding sites for several transcription factors such as Ets and Sp1. Ikeda et al. [21] showed that the short spacer sequence of 63bp between the mouse Nthl1 and Tsc2 genes shows bidirectional promoter activity essential for the transcription of both genes. The 63-bp sequence is well conserved among several mammalian species and contains two Ets-transcription factor binding sites in opposite directions. Although there are several Ets-transcription factor binding sites in the 5' flanking region of chNthl1 gene, the spacer sequence between the chNthl1 and chTsc2 genes is more than ten times longer than that in the mouse genome, and the sequence homology between them is too low to discuss as bidirectional promoter of chNthl1 and chTsc2 genes.

Sequence analysis of the 3'-flanking region of 3.06kb by comparing it with the chicken Slc9a3r2 mRNA (data base accession no. XM_414851) and human SLC9A3R2 mRNA [12, 13] revealed that chNthl1 gene is lying immediately adjacent to the chSlc9a3r2 gene and in a 3'-to-3' orientation with the chSlc9a3r2 gene. The exon 6 (480bp; the last exon containing a stop codon) of chNthl1 gene and the exon 7 (2272bp; the last exon containing a stop codon) of chSlc9a3r2 gene both lie within the 3306bp-sequence.

Acknowledgements and notes. This work had been conducted largely in Department of Molecular Biology, Institute of Cellular and Molecular Biology, Okayama University Medical School, Okayama 700-8558, Japan. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture and in part by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, Sports and Culture of Japan (Repair, Recombination and Mutagenesis, 08280101).

The nucleotide sequence data reported in this paper have been deposited with the GSDB, DDBJ, EMBL, and NCBI nucleotide databases under the Accession Number AB358957.

References

1. Friedberg EC, Walker GC and Siede W: DNA Repair and Mutagenesis. ASM Press, Washington DC (1995).
2. Cathcart R, Schwiers E, Saul RL and Ames BN: Thymine glycol and thymidine glycol in human and rat urine: a possible assay for oxidative DNA damage. Proc Natl Acad Sci USA (1984) **81**, 5633-5637.
3. Krokan HE, Standal R and Slupphaug G: DNA glycosylases in the base excision repair of DNA. Biochem J (1997) **325**, 1-16.
4. Roldan-Arjona T, Anselmino C and Lindahl T: Molecular cloning and functional analysis of a schizosaccharomyces pombe homologue of Escherichia coli endonucleaseIII. Nucleic Acids Res (1996) **24**, 3307-3312.
5. Zuo S, Boorstein RJ and Teebor GW: Oxidative damage to 5-methylcytosine in DNA. Nucleic Acids Res (1995) **23**, 3239-3243.
6. Demple B and Harrison L: Repair of oxidative damage to DNA: enzymology and biology. Annu Rev Biochem (1994) **63**, 915-948.
7. Bacchetti S and Benne R: Purification and characterization of an endonuclease from calf thymus acting on irradiated DNA. Biochim Biophys Acta (1975) **390**, 285-297.
8. Kim J and Linn S: Purification and characterization of UV endonuclease I and II from murine plasmacytoma cells. J Biol Chem (1989) **264**, 2739-2745.
9. Doetsch PW, Helland DE and Haseltine WA: Mechanism of action of a mammalian DNA repair endonuclease. Biochemistry (1986) **25**, 2212-2220.
10. Ikeda S, Biswas T, Roy F, Izumi T, Boldogh I, Kurosky A, Sarker AH, Seki S and Mitra S: Purification and characterization of a human homolog (hNTH1) of Escherichia coli endonuclease III: Direct identification of lys-212 as the nucleophilic residue. J Biol Chem (1998) **273** (34), 21585-21593.
11. Sarker AH, Ikeda S, Nakano H, Terato H, Ide H, Akiyama K, Tsutsui K, Bo Z, Kubo K, Yamamoto K, Yasui A, Yoshida MC and Seki S: Cloning and characterization of a mouse homologue (mNthl1) of Escherichia coli endonucleaseIII. J Mol Biol (1998)

282, 761-774.
12. Imai K, Sarker AH, Akiyama K, Ikeda S, Yao M, Tsutsui K, Shohmori T and Seki S: Genomic structure and sequence of a human homologue (NTHL1/NTH1) of Escherichia coli endonuclease Ⅲ with those of the adjacent parts of TSC2 and SLC9A3R2 genes. Gene (1998) **222**, 287-295.
13. Seki S, Sarker AH, Mitani K, Yao M, Nakamura T, Akiyama K, Ikeda S, Seki Y and Tsutsui K: The human gene for the regulatory factor 2 (SLC9A3R2/E3KARP) of the solute carrier family 9 (sodium/hydrogen exchanger), isoform A3. J Biochem Mol Biol and Biophys (2000) **4**, 157-163.
14. Hilbert TP, Chuang W, Boorstein RJ, Cunningham RP and Teebor GW: Cloning and expression of the cDNA encoding the human homologue of the DNA repair enzyme, Escherichia coli endonuclease Ⅲ. J Biol Chem (1997) **272**, 6733-6740.
15. European Chromosome 16 Tuberous Sclerosis Consortium, 1993: Identification and characterization of the tuberous sclerosis gene on chromosome 16. Cell (1993) **75**, 1305-1315.
16. Seki S, Sarker AH, Nakamura T, Seki Y and Ikeda S: cDNA cloning and analysis of the chicken homolog of E. coli endonuclease Ⅲ. Chugokugakuen J (2006) **5**, 23-27.
17. Sambrook J, Eritsch EF and Maniatis T: Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, Plainview, NY (1989).
18. Sanger F, Nicklen S and Coulson AR: DNA sequencing with chain-terminating inhibitors. Proc Nat Acad Sci USA (1977) **74**, 5463-5467.
19. Breathnach R and Chambon P: Organization and expression of eukaryotic split genes coding for proteins. Annu Rev Biochem (1981) **50**, 349-383.
20. Kim KK, Pajak L, Wang H and Field LJ: Cloning, developmental expression, and evidence for alternative splicing of the murine tuberous sclerosis (TSC2) gene product. Cell Mol Biol Res (1995) **41**, 515-526.
21. Ikeda S, Mochizuki A, Sarker AH and Seki S: Identification of functional elements in the bidirectional promoter of the mouse Nthl1 and Tsc2 genes. Biochem Biophys Res Commun (2000) **273**, 1063-1068.

Accepted March 30, 2007.