Isolation and Linkage Mapping of Coding Sequences from Chicken Cosmids by Exon Trapping

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ABSTRACT : We performed exon trapping in order to locate functional genes on chicken chromosomes (GGA) and to identify functional gene sequences from chicken cosmids. Sequence analysis of 100 clones revealed 17 putative exons, five of which were identified with known sequences in a gene database search: thymopoietin beta (TMPO), U5 snRNP-specific 40 kDa protein (HPRP8BP), dihydropyridine receptor alpha 1 subunit (CACNLIA3), cystein string protein (CPS) and C15orf4. We attempted to map the genes to chicken chromosomes by using FISH and linkage analysis. The chromosomal localizations were GGA1 (TMPO), GGA10 (C15orf4), GGA23 (HPRP8BP) and GGA28 (CPS) by FISH and linkage analysis, while that of CACNLIA3 was predicted to be on a microchromosome by FISH but not by linkage analysis. Comparative mapping analyses between chickens and humans for the genes revealed both known and new synteny. The syntenic conservation between GGA1 and human chromosome (HSA) 12q23 (TMPO) and between GGA10 and HSA15q25 (C15orf4), were consistent with a recent publication, while two new syntenies were observed between GGA28 and HSA20q13.3 in CPS and between GGA23 and HSA1p34-35 in HPRP8BP. The information of presently mapped genes can contribute as anchor markers based on functional genes and the construction of a comparative map. (Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 3 : 309-312)

Key Words : Exon Trapping, Chicken, Linkage Mapping, FISH, Comparative Mapping

INTRODUCTION

Exon trapping is a rapid and efficient technique for identifying expressed gene sequences, based on the selection of functionally splice sites in genomic DNA (Church et al., 1994). Although there are other current methods for identifying genes, exon trapping has an advantage in that it does not require any prior knowledge of the tissue-specific transcriptional status and can be easily performed on a complex genome (Comincini et al., 1997).

Chicken linkage maps have been constructed during the past decade (Groenen et al., 2000; Lee et al., 2002). Comparative maps among chickens, humans and mice have also been constructed by using orthologous genes and anonymous loci (Schmid et al., 2000). Subsequently, a global image of the chromosomal construction among them has been obtained. However, a more comprehensive comparative map is now required in order to identify genes responsible for disease (Yoshizawa et al., 2003) and also to investigate chromosomal evolution, especially for microchromosomes. The incrementation of functional genes mapped to the chromosome will contribute to the construction of a high-resolution comparative map.

Our work is the first application of exon trapping in chickens to map functional genes to chicken chromosomes. The functional genes obtained by exon trapping were mapped to chicken chromosomes by FISH and linkage analysis, then the homologous regions between chickens and humans were investigated.

MATERIALS AND METHODS

Exon trapping

Exon trapping was performed using chicken genomic cosmid library (Clontech, CA). The whole procedure is based on the Exon Trapping System (GIBCO BRL, MD). Twenty single DNA pools from each five cosmids (total 100 cosmids) were digested with a combination of PstI and PvuII restriction endonucleases (New England BioLabs, MA), purified and ligated to pSPL3 trapping vector. The ligated products were transformed into HB101 E. coli cells. Plasmid DNA was isolated after growing in liquid culture. The recombinant plasmid DNA was transfected into 60% to 80% confluent COS-7 cells (Riken Cell Bank, Tsukuba, Japan) by using Effectene Transfection Reagent (QIAGEN, CA). The cells were incubated for 40 h after the transfection.

RNA was extracted by Sepasol-RNAI (nacalai tesque, Japan) and cDNA synthesis was performed. In order to eliminate vector-only and false positive products, BstXI restriction endonuclease was added directly to the reactions overnight. Secondary PCR amplification was performed by SD2 and SA4 primer (GIBCO BRL, MD). The product was run by 2% agarose gel electrophoresis and then the adequate size of PCR products (160 bp to 450 bp) were recovered from the gel using Mate Gelpure DNA
purification Kit (ISC BIO EXPRESS, UT). Finally, the products were ligated to the pGEM-T Easy Vector (Promega, WI) and transformed into JM109.

Characterization of trapped exons and sequence analysis

White single colonies were picked up and used for PCR amplification with SD2 and SA4 primers. Positive clones with an insert larger than the 153 bp that derived from self-splicing of the vector, were sequenced by using SequiTherm EXCEL II DNA sequencing kits (Epicentre Technologies, Madison, WI) with dyed vector primers and analyzed on a LI-COR automatic sequencer (model 4200L, LI-COR, NE). Sequences were analyzed by using BLAST search. The sequences of the trapped exons have been deposited at the DDBJ database under the accession numbers AB114863-AB114867.

FISH

FISH was performed basically according to Matsuda and Chapman (1995). Lymphocytes were isolated from the blood of female adult chicken, and transferred to 5 ml AMNIO-MAX-C100 medium (GIBCO BRL, MD) with 0.06% glutamine and 50 µg/ml HA15 (Murex). The lymphocytes were cultured for 66 h at 39°C. Colcemid (0.04 µg/ml) was added 2 h before harvesting the cells. The DNA probes were labeled by nick translation kit (Roche, Swiss) and the hybridized probes were stained with avidin-FITC (Roche, Swiss). The slides were stained with 1 µg/ml propidium iodide for observation.

Identification of polymorphisms and linkage analysis

Partial genomic sequences flanking cosmid vector were determined by sequencing with cosmid vector primers. Primers were designed based on the genomic sequences to amplify from the region flanking cosmid vector to the region of trapped exons. Primers within exon sequences were also designed. All primers amplified successfully were listed in Table 1. The PCR reactions were carried out with 1 ng of purified cosmid DNA as a template in a volume of 50 µl of 1× reaction buffer; 400 µM dNTPs; 0.5 µM of each primer; and 2.5 U of LA Taq polymerase (Takara Shuzo Co., Tokyo, Japan). An initial denaturation period of 1 min at 94°C was followed by 30 cycles for 10 s at 98°C, 15 min at 68°C and a final extension period of 10 min at 72°C.

The PCR products were digested with several restriction endonucleases to detect polymorphisms between parents of the Kobe University (KU) resource family. The KU family had been established in previous study (Lee et al., 2002). The resource family consists of 380 backcross chicks produced by crossing a single male of the White leghorn WL-F line to eight F1 females produced by crossing the male and a Fayoumi OPN line female. A subset of 55 backcross chicks from one F1 female was used for linkage analysis in this study. These polymorphisms were analyzed with previously published information of KU linkage map (Lee et al., 2002). Linkage groups were determined by two-point analysis under linkage criterion of p<0.05 as a G-statistic for independence and Kosambi function, using Map Manager QTXPb12 (Manly et al., 2001).

RESULTS AND DISCUSSION

In total, 100 clones were analyzed for the presence of an insert by PCR. The products ranged in size from 160 to 500 bp. After a selection based on size, 34 clones were sequenced with the PCR products directly as templates. The lengths of trapped exons were from 24 bp to 327 bp. The DNA sequences were analyzed by using BLAST search (http://www.ncbi.nlm.nih.gov/blast/). Seventeen clones possessed the putative trapped exons contained in sequences derived from the intron of the pSPL3 trapping vector.
Five sequences out of 17 clones were identified as known genes. Table 2 summarizes the results. Three were the genes for human thymopoietin beta (TMPO, M1), U5 snRNP-specific 40 kDa protein (HPRP8BP, S21) and dihydropyridine receptor alpha 1 subunit (CACNLIA3, J1). Two were expressed sequence tags (EST) for chickens (A1, F8); these EST sequences were reanalyzed by BLAST search. The EST AJ395662 was similar to rat cystein string protein (CPS) and the EST BI390001 to human C15orf4. The trapped sequence of TMPO was included in three exons, while the other four gene sequences were consistent with a single exon for each gene. Additionally, 12 clones were not similar to any entry in the database.

The parent cosmids of five clones were identified by using the PCR method with the exon specific primers (Table 1). Five cosmids were used for mapping to chicken chromosomes by FISH. Consequently, one clone was mapped to chicken chromosome (GGA) 1q, while four on chicken microchromosomes could not be identified by chromosomal numbers (Table 3).

We therefore attempted to locate these genes on the chicken chromosome by linkage analysis using the Kobe University (KU) resource family (Lee et al., 2002). Polymorphisms for each gene between parents of the KU family were detected as follows. PCR was performed for parental cosmid DNA with primers of cosmid vector and trapped-exon sequences (Table 1). The flanking genomic sequence of TMPO revealed a coding sequence, so that the primer was designed within the exon sequence.

Using these primers, we conducted PCR reactions for genomic DNA of the KU family parents as templates. The PCR product of CPS revealed null allele in male chickens, and that of HPRP8BP exhibited size differences between parents. For TMPO and C15orf4, several restriction endonucleases were used to detect the polymorphisms. Consequently, MspI digestion yielded polymorphic bands for TMPO PCR products, and DraI digestion yielded polymorphic bands for C15orf4 PCR products. These polymorphisms were analyzed for the KU resource family to locate the genes. The benefit of this strategy is the possibility of a wide screening of spacer or intron regions within cosmid inserts to detect the nucleotide polymorphisms in the reference family.

As a result of linkage analysis, four genes were located on chicken chromosomes (Figure 1 and Table 3). The TMPO gene was mapped at 142.2 cM from the distal end of GGA1p. The C15orf4 was on GGA10 at the 46.4 cM position, HPRP8BP at the terminal end of GGA23 and CPS at the terminal end of GGA28q. The human chromosome (HSA) localizations of these genes have been mapped at 12q23 (TMPO), 15q25 (C15orf4), 1p34-35 (HPRP8BP) and 20q13.3 (CPS) and CACNLIA3 was at 1q31-32, although the position of this gene could not be located by the chicken linkage map (Table 3).

The syntenies for TMPO (GGA1p vs. HSA12q23) and C15orf4 (GGA10 vs. 15q25) are consistent with a recent publication of a comparative genome map of humans and chickens (Schmid et al., 2000). However, the comparative
map did not illustrate the synteny between chicken GGA28 and HSA20q shown for CPS and there is no functional gene on GGA23 in the map. Consequently, in this study, the synteny between GGA23 and HSA1p34-35 exhibited for HPRP8BP is a new finding. Therefore, the chromosomal localizations and the synteny would contribute to a more detailed comparative map between chickens and humans.

In this study, we found that exon trapping is a useful and effective approach for isolating and mapping functional genes in chickens. Additionally, we presented a strategy to locate the exon trapped genes on the linkage map. These techniques will be very useful to develop the functional gene markers located on the chicken linkage map. The approach would be used to identify responsible genes within the candidate region for chicken disease or economic traits (Yonash et al., 1999; Yoshizawa et al., 2003). As a result, the information of presently mapped genes could contribute as anchor markers based on functional genes and to the construction of a comparative map between chickens and other species.

**IMPLICATIONS**

Full sequencing of the chicken genome is underway by both the whole genome shotgun method and clone-based sequencing. A linkage map is an essential guide for navigation, allowing the location of any gene or other landmark in the chromosomal DNA. Exon trapping is a valuable tool for identifying expressed gene sequences. Functional genes that are mapped using this strategy would contribute as anchor markers and to the construction of a comparative map between chickens and other species.

**REFERENCES**


