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The Murine *cot* Proto-oncogene: Genome Structure and Tissue-specific Expression

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We cloned and analyzed the murine *cot* proto-oncogene and examined its tissue-specific expression in fetal, newborn and adult mice. Genomic *cot* DNA consists of eight exons, spanning more than 25 kb, and all intron-exon borders are well conserved as compared to the human homolog. Analysis of the full-length *cot* cDNA revealed that it contained an open reading frame of 1,401 nucleotides, like human *cot* proto-oncogene. The sequence identity between murine and human *cot* gene is 84.4% at the nucleotide level and 93.9% at the deduced amino acid level. On northern blot analysis of poly (A)$^+$ RNA, the *cot* message was detected at 2.9 kb in size. Expression of the *cot* gene was observed in many tissues from fetal to adult mice, though the level of expression was low in all tissues examined.

Key words: Murine *cot* proto-oncogene — Full-length cDNA — Genomic mapping — Tissue-specific expression

Originally the *cot* oncogene was isolated from SHOK cells transformed by transfection with genomic DNA of human thyroid carcinoma cell line,[1,2] and its oncogenicity was presumed to be due to a rearrangement of the carboxyl-terminal region and its higher expression. Characterization of the *cot* gene product with specific antibodies revealed that Cot protein is serine/threonine kinase, and it is predominantly localized in cytoplasm (Aoki *et al.*, submitted),[3] though the physiological functions of the Cot kinase are still unknown. Thus far, four categories of proto-oncogenes encoding serine/threonine kinase have been reported; *mos*,[4-6] *raf*,[7,8] *pim*,[9,10] *atk* (*rac*).[11,12] However, the *cot* proto-oncogene is distinct from these genes and encodes a unique protein.[13] The roles of Cot kinase in the signal transduction pathway for cell growth and/or differentiation are clearly of interest. As a first step to clarify the functions of Cot kinase, it is indispensable to know which tissues and what kind of cells express this kinase at a high level. In this study, we molecularly cloned the murine *cot* proto-oncogene and examined its tissue-specific expression by northern blot analysis.

MATERIALS AND METHODS

Southern blot analysis with the mouse genomic DNA High-molecular-weight genomic DNA was extracted from testis and liver of adult mice. Portions (10 μg) of the purified DNA were digested with *Eco* RI, *Bam* HI or *Eco* RI and *Bam* HI endonucleases (Takara), then the digests were electrophoresed on 0.7% agarose gel and transferred onto nylon membranes (Hybond-N, Amersham). Following UV irradiation, the membrane was prehybridized and hybridized in aqueous buffer (6× SSC, 5×Denhardt's solution, 0.5% SDS, 0.1 μg/ml of sonicated salmon sperm DNA) and washed under a less stringent condition (see "Results"). The 1.1 kb human *cot* cDNA which covered exons 3 to 8 of human *cot* oncogene[2] was labeled with the random-primed DNA labeling kit (Boehringer Mannheim) and [α-^{32}P]dCTP (~3,000 Ci/mmol; Amersham Japan), then used as a probe. After washing, the membrane was exposed to Fuji X-ray film for three days at −70°C.

Screening of the genomic library The library constructed by Clontech Laboratories, Inc. was screened for the isolation of mouse genomic *cot* DNA (cat. # ML 1030j, genomic DNA from Balb/c mouse liver). Screening was performed with the same probe and the same hybridization/washing condition as for genomic Southern blot analysis. Out of 5.8×10^5 phages, we obtained six positive clones through the secondary screening. These phage DNAs were purified and insert fragments were subcloned into pUC 18, 19 or pBluescript II SK(−) vectors. The genomic map was constructed based on the results of endodigestions and sequencing.

DNA sequence and computer analysis Sequencing was performed by the dideoxy chain termination method with T7 Sequencing™ kit (Pharmacia) and [α-^{35}S]dATPαS (~3,000 Ci/mmol; Amersham Japan). Obtained sequences were analyzed with UWGCG programs.

Cloning of the full-length murine *cot* cDNA First, we screened the oligo (dT)-primed cDNA library constructed by Stratagene (cat. #935303, cDNA from thymus of female C57 Black 6×CBA mouse). Two genomic fragments, 0.45 kb *Eco* RI-*Sal* I fragment (probe A) and 0.3 kb *Pst* I-*Hin*d III fragment (probe B), were used as probes. Screening was performed under the most stringent condition; hybridization at 65°C in the

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above-mentioned aqueous buffer and washing at 52°C in 0.1×SSC/0.1% SDS. Out of 7.8×10^5 phages, we obtained six positive clones, but only one was authentic *cot* cDNA. The insert fragment was digested with endonucleases, subcloned into pBluescript II SK(−) vectors and sequenced.

The 5′ end cDNA was cloned by the one-sided PCR method with *cot* specific primers and anchored primers.[14, 15] The oligonucleotide sequences used for the cloning are shown in Fig. 1. Oligonucleotides B and C from the anti-sense strand against the *cot* gene were used as primers, while oligonucleotide A from the sense strand was used as a probe. These oligonucleotides were synthesized with a Cyclone™ Plus DNA Synthesizer (MilliGen/Biosearch) and purified on an OLIGO-PAK™ column (MilliGen/Biosearch). Anchored primers 1 and 2 were kindly given by Dr. O. Ohara. The single-stranded cDNA was stretched from oligonucleotide C primer, using Mo-MLV (Moloney murine leukemia virus) reverse transcriptase (BRL) and 1 μg each of adult thymus, ovary or newborn digestive tract mRNA as templates. Then $(dA)_n$ tailing was performed at the 3′ end of the synthesized cDNA with terminal deoxynucleotidyl transferase (Takara). Polymerase chain reaction (PCR) amplification with *Taq* polymerase (Perkin Elmer Cetus) was performed on a DNA thermal cycler (Perkin Elmer Cetus) under the following conditions. The first round of the PCR cycle with $(dA)_n$ tailed cDNA, oligonucleotide C and anchored primer mixture (1 and 2 were mixed at 1:5 molar ratio) was run at 94°C, 30 s, 45°C, 30 s, 72°C, 60 s for 10 cycles and then 94°C, 30 s, 55°C, 30 s, 72°C, 60 s for 30 cycles. For the second round of PCR, 10 μl of the first round product was treated with oligonucleotide B and anchored primer 2, then amplified at 94°C, 30 s, 55°C, 30 s, 72°C, 60 s for 30 cycles. The amplified *cot* cDNA was identified by Southern blot analysis with the oligonucleotide A probe which was end-labeled with T4 polynucleotide kinase (Takara) and [γ-^{32}P]ATP (~3,000 Ci/mmol; Amersham Japan). The cDNAs corresponding to the hybridized sizes were extracted from agarose gels, subcloned into pBluescript II T vectors,[16] then sequenced.

Oligonucleotide A : GGAGAACCTTTATGCAAGTG (214 - 233)
Oligonucleotide B : GCTCATACACTCCTGGCTCT (254 - 235)
Oligonucleotide C : CTGGATACATGGTCATCAGA (278 - 259)
Anchored primer 1 : TAAGATCTAATACGACTCACTATAGGGAAGCTTTTTTTTTTTTTTTT
Anchored primer 2 : TAAGATCTAATACGACTCACTATAG

Fig. 1. Oligonucleotide sequences used for the one-sided PCR. The numbers in parenthesis indicate the nucleotide locations on the *cot* cDNA.

Determination of the 3′ structure of human *cot* proto-oncogene We have already reported the restriction map for human genomic *cot* clone.[2] Using the isolated cDNA of human *cot* proto-oncogene as a probe (Aoki *et al.*, submitted), the loci of undefined 3′ exons were mapped and confirmed by sequencing.

Northern blotting analysis Cellular RNAs were extracted by the AGPC method[17] from various tissues of fetal (14 days old), newborn (0 to 3 days after birth) and adult (3 month old) mice. Poly $(A)^+$RNAs were purified with Oligotex™-dT30 (Japan Synthetic Rubber Co., Ltd.). Poly $(A)^+$RNAs (1.5 μg or 1.0 μg) were electrophoretically separated on 1.0% formaldehyde gel with a standard size marker (RNA ladder: BRL) and transferred onto the nylon membrane. After UV fixation, the membrane was stained with methylene blue[18] to visualize the size marker, then prehybridized and hybridized at 65°C in GMC buffer (George M. Church's buffer; 0.25 *M* Na_2HPO_4 (pH 7.2), 1.0% BSA, 1 m*M* EDTA, 7.0% SDS).[19] The probe was 2.5 kb murine *cot* cDNA fragment labeled with the random-primed system. After the hybridization, the membrane was washed at 52°C in 0.1×SSC/0.1% SDS, then exposed to Fuji X-ray film for 10–12 days at −70°C. As the positive control of the northern blotting, we re-hybridized the filters with 1.0 kb glyceraldehyde-3-phosphate dehydrogenase (G3-PDH) cDNA (Clontech Laboratories Inc., cat. #9805-1), under the same conditions described above.

RESULTS

Isolation of genomic *cot* DNA In order to isolate the murine *cot* proto-oncogene with a human *cot* cDNA as a probe, less stringent conditions were tested for hybridization and washing. Portions of 10 μg of mouse genomic DNA were digested with endonucleases and processed for Southern blot analysis using a 1.1 kb cDNA fragment of the human *cot* oncogene as a probe. Among several trials under different conditions, hybridization at 56°C in standard aqueous buffer and washing at 52°C in the 0.5× SSC/0.1% SDS solution resulted in a good resolution (Fig. 2). Under these conditions, background signals were observed over the lanes, whereas clear bands were detectable at 7.5 kb and 3.3 kb after *Eco* RI digestion, and at 10 kb, 6.4 kb and 5.7 kb (weakly hybridized) after *Bam* HI digestion. Then we screened a genomic library under the same conditions.

After screening 5.8×10^5 phages, we obtained six positive clones (G1 to G6). The insert fragments of these clones (11 to 15 kb in length) were subcloned into pUC or pBluescript vectors. To identify the *cot* gene, parts of the fragments cross-hybridized with the human *cot* probe were sequenced. Thus we could define six exons corresponding to human exons 3 to 8, and the constructed

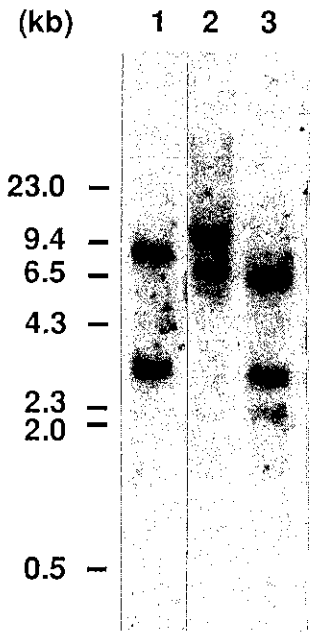

Fig. 2. Southern blot analysis for the genomic *cot* gene. Samples of 10 μg of genomic DNA digested with *Eco* RI (lane 1), *Bam* HI (lane 2) and *Bam* HI & *Eco* RI (lane 3) were analyzed. Hybridization was performed under the less stringent condition. The probe was a 1.1 kb cDNA fragment of the human *cot* oncogene and the standard marker was lambda DNA digested with *Hind* III. The film was exposed for 3 days at -70°C.

genomic map was consistent with the result of Southern blot analysis mentioned above.

Cloning of full-length *cot* cDNA and complete genomic mapping Since the extreme 3′ structure of the *cot* proto-oncogene was not yet determined, we tried to clone the full-length *cot* cDNA with murine *cot* DNA probes and complete the genomic map. First, the thymus cDNA library was screened, because our preliminary result showed that *cot* message was highly expressed in thymus tissues. Using probes of genomic fragments which were free from repetitive sequences, 7.8×10^5 phages were screened under the most stringent condition and six positive clones were obtained. Sequence analysis of these clones, however, showed that only one clone (CZ-21) was authentic *cot* cDNA, and the other five clones had a part of the *cot* cDNA but were rearranged with other cDNAs. CZ-21 clone contained 2,425 nucleotides of murine *cot* sequences, including 1,401 nucleotides covering the entire coding region corresponding to the human *cot* proto-oncogene (Aoki *et al.*, submitted), and 42 and 982 nucleotides of the 5′ and 3′ noncoding regions, respectively (Fig. 3).

In order to determine the 5′ structure, we cloned the 5′ end cDNA by the one-sided PCR method, with anchored primers[15] and anti-sense oligonucleotides specific to the murine *cot* mRNA. Reverse transcriptase PCR was performed with mRNAs extracted from adult thymus, ovary and newborn digestive tract as templates. Amplified *cot*

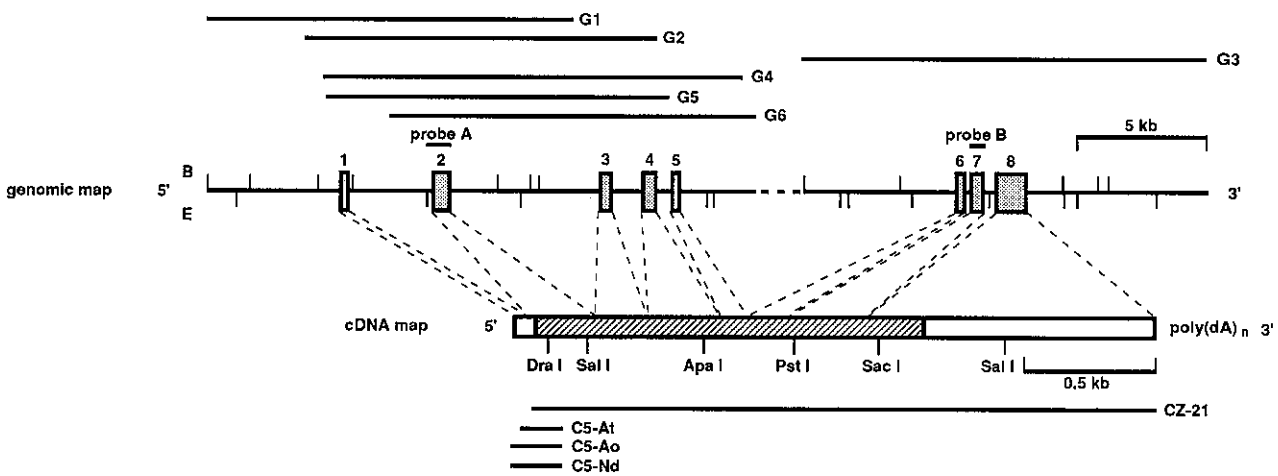

Fig. 3. Genomic and cDNA map of the murine *cot* proto-oncogene. (Upper half) By using 1.1 kb of human *cot* cDNA as a probe, six clones (G1 to G6) were isolated from the genomic library and exons 2 to 7 were identified. Exons 1 and 8 were defined using full-length cDNA. The genomic map was constructed based on the results of endodigestions and sequence analysis. Restriction sites of *Bam* HI (B) and *Eco* RI (E) are shown. Exons are indicated by shaded boxes. The dotted line indicates a part of the intron absent in these clones. Probes A and B were used for screening of the cDNA library. (Lower half) The full-length cDNA was analyzed with CZ-21 (obtained from the library) and C5-At, C5-Ao and C5-Nd (cloned by one-sided PCR), then the cDNA map was constructed. Hatched areas indicate the coding regions. With the full-length cDNA, complete genomic mapping was accomplished.

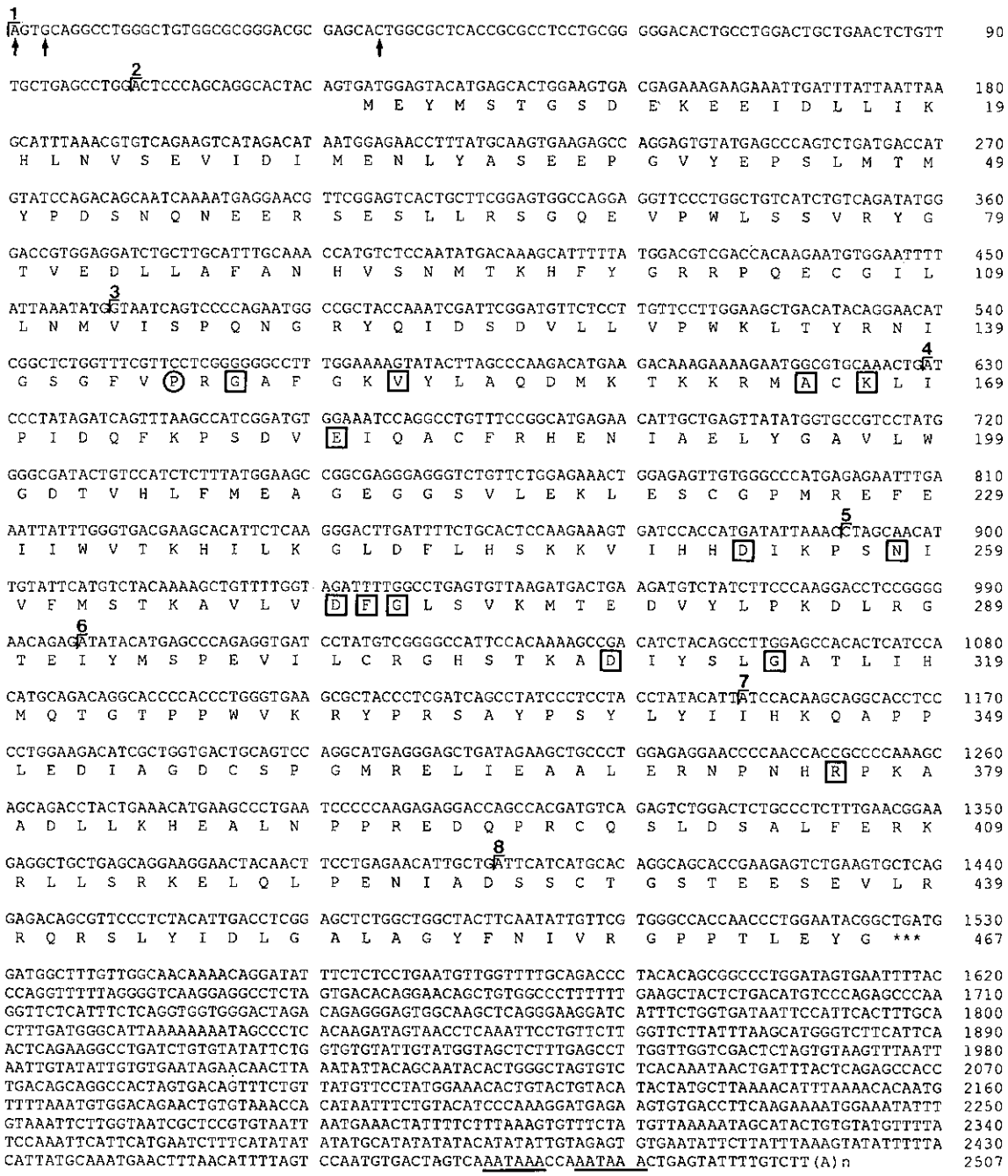

Fig. 4. The nucleotide and deduced amino acid sequences of the murine *cot* cDNA. The first nucleotide sites of the three isolates (C5-Ao, C5-Nd and C5-At) are indicated by arrows. Each exon boundary is also shown by ⌈. The stop codon is indicated by ***, and polyadenylation signals are underlined. Boxed amino acids are commonly conserved among the kinases, whereas the circled amino acid P (proline) is unique to Cot kinase.

cDNAs were identified by Southern blot analysis (data not shown), and then cloned into pBluescript vectors. Three clones, C5-At, C5-Ao, C5-Nd, were obtained from adult thymus, ovary and newborn digestive tract and each contained 47, 82 and 79 nucleotides of the further upstream region (Fig. 3).

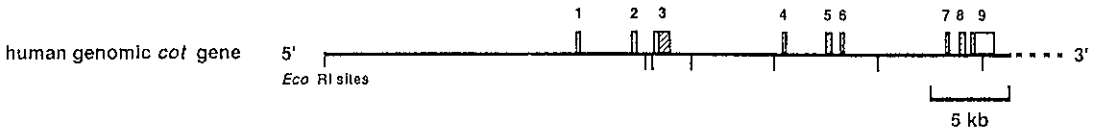

Fig. 5. The genome structure of the human *cot* proto-oncogene. With the newly isolated human *cot* proto-oncogene as a probe, the 3′ structure (the end site of exon 8 and the locus of exon 9) were defined. Hatched boxes indicate the coding regions.

With these full-length cDNAs, we defined the loci of other exons and identified two more exons. The genomic map was constructed based on the results of endodigestions and sequencing analysis (Fig. 3). Consequently, the mouse *cot* proto-oncogene was proved to consist of 8 exons, spanning more than 25 kb on the genome. Although a part of the intron was still absent in these genomic fragments (shown by dotted line in Fig. 3), we could identify all *cot* exons.

Fig. 4 shows the nucleotide sequence of murine *cot* cDNA and the deduced amino acid sequence. The 144th amino acid residue was proline (P), as in human Cot protein, although most other kinases are known to possess a glycine (G) residue at this position.[13]

Comparison of murine and human *cot* proto-oncogenes Before we cloned the murine *cot* gene, the human *cot* proto-oncogene had already been isolated (Aoki *et al.*, submitted). Therefore, we determined the 3′ genomic structure of the human *cot* proto-oncogene, which had not been defined.[2] The human *cot* proto-oncogene was shown to consist of nine exons, and the end of exon 8 and the locus of exon 9 were determined here (Fig. 5). When the murine *cot* gene was compared with the human homolog, it was revealed that they were well conserved; each intron-exon border of coding exons was completely conserved and the sequence homology was high (84.4% at the nucleotide level and 93.9% at the deduced amino acid level)(Table I). Even in exons 2 and 8 which did not contain the kinase conserved regions, the homology of deduced amino acids was still high, and the 3′ untranslated region also showed a moderate homology.

Tissue-specific expression of *cot* mRNA The *cot* mRNA expression in various tissues of fetal, newborn and adult mice were examined by northern blot analysis using the isolated murine *cot* cDNA as a probe (insert fragment of CZ-21). As shown in Fig. 6, the *cot* mRNA was detected at 2.9 kb in size (upper panels), but the control G3PDH mRNA was seen at 1.4 kb (lower panels). Although the expression of *cot* mRNA was generally low (because it took 10–12 days exposure to get clear signals), many tissues expressed the *cot* message: brain, liver, digestive tract and placenta of 14-day fetus (Fig. 6A), brain, lung, liver, digestive tract, thymus and kidney of newborn mice (Fig. 6B), and brain, submandibular gland, thymus, heart, liver, spleen, stomach, colon, skeletal muscle, kidney and ovary of adult mice (Fig. 6C). The *cot* messages were particularly highly expressed in adult submandibular gland, thymus, spleen and newborn digestive tract. Table II summarizes the result of northern blot analysis, suggesting that the *cot* mRNA was constantly expressed from embryonal or neonatal stages to the adult stage in brain, lung, liver, digestive tract, and kidney. In thymus and stomach, the *cot* expression was elevated in the adult stage compared with the newborn stage.

Table I. The Sequence Homology between Murine and Human *cot* Genes

Murine *cot* gene		Definition		Identity with human *cot* gene (%)	
Exon No.	Length(nt)			DNA level	Amino acid level
1	102	5′UTR		—	
2	22			69.6	
	336			86.0	88.4
3	168			84.5	91.1
4	262			89.7	96.6
5	107	ORF	KD	90.7	94.3
6	153			90.2	100.0
7	247			89.1	94.0
8	128			84.4	92.9
	982	3′UTR		75.6	
Total	2507			84.4	93.9

UTR, untranslated region; ORF, open reading frame; KD, kinase domain.

DISCUSSION

We have completely mapped and analyzed the murine *cot* proto-oncogene. It was revealed that the borders of coding exons are conserved between the murine and the human *cot* genes, but are unique compared to those of other proto-oncogenes of the serine-threonine kinase group, *raf*, *mos* and *pim*. Furthermore, comparison of the deduced amino acid sequences revealed that this gene is well conserved not only inside the kinase domain but also outside the kinase domain and the 3′ untranslated region. As to the human Cot kinase, it was shown that two types of Cot proteins were produced by alternative initiation mechanisms. They possessed different half-lives and, if overexpressed, exhibited different transforming ability

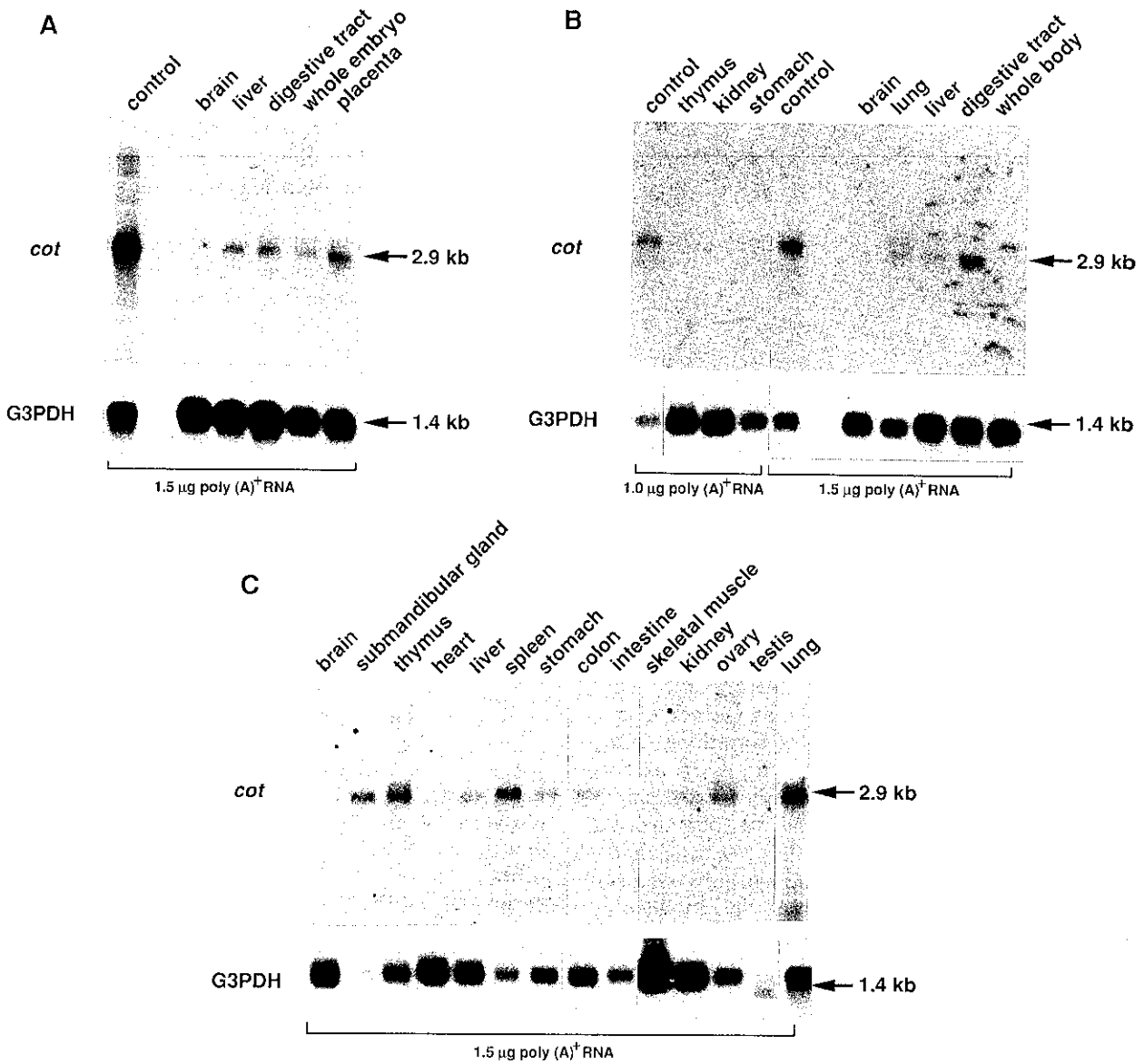

Fig. 6. Tissue-specific expression of the *cot* mRNA. Northern blot analysis were performed with poly $(A)^+$RNA isolated from various tissues in embryo (A), newborn (B) and adult (C) mice, and with 2.5 kb murine *cot* cDNA fragment as a probe. The upper panels show the *cot* expression, but the lower panels control G3PDH gene. The 2.9 kb *cot* mRNA was detected in many tissues, though its expression seemed to be low in general. On the control lanes (A) and (B), adult spleen poly $(A)^+$RNA was applied to compare the expression level. The films were exposed at $-70°C$ (10–12 days for *cot* mRNA detection, but 16 h for G3PDH mRNA detection).

(Aoki *et al.*, submitted). These Cot proteins were proved to be translated from the ATGs in exon 3 (corresponding to the murine exon 2) and both of the ATG sites were conserved in the murine *cot* gene. Therefore, the murine Cot protein may have similar alternative initiation mechanisms for its translation. On the other hand, the 3′ untranslated regions of both murine and human *cot* genes were high in A/T content (~65%) and there were some AT sequences.[20] The mRNAs of certain proto-oncogenes, cytokines and lymphokines are known to contain AU sequence(s) in the 3′ untranslated region and this motif was considered to confer instability. It is probable that the low content of *cot* mRNA is caused by similar post-transcriptional instability.

Table II. Summary of *cot* mRNA Expression

Tissues	Fetus	Newborn	Adult
Brain	+	+	+
Submandibular gland			+++
Thymus		+	+++
Lung		++	++
Heart			+
Liver	++	++	++
Spleen			+++
Stomach		−	++
Intestine			−
Colon	(++)	(+++)	++
Skeletal muscle			+
Kidney		+	+
Overy			++
Testis			−
Placenta	++		

The relative expression level is shown based on the result of northern blot analysis, as compared to the adult spleen expression, +++. Minus means that no signal was detected. Parenthesized results were obtained from digestive tract mRNA which included both intestinal and colonic mRNA.

We cloned the 5′ end cDNA by the one-sided PCR method and three different-sized cDNAs were isolated. Although we tried to confirm the transcriptional initiation site by S1 protection analysis with 5 μg of poly $(A)^+$RNA as a template and uniformly labeled single-stranded DNA as a probe, no signal was obtained. As shown in Fig. 5, the human *cot* gene has two exons in the 5′ untranslated region, but we identified only one exon in this region of mouse genomic DNA. The Sp1 binding motif and Rb control element (RCE) consensus sequence were present in the 5′ flanking region. However, it is still possible that another upstream exon is present and further study will be necessary to confirm the 5′ structure of the *cot* mRNA.

By northern blot analysis, we detected the 2.9 kb *cot* mRNA. Because the sequencing of the full-length cDNA revealed that there were 2,507 nucleotides, the addition of the poly $(A)^+$ tail should give an mRNA of around this size. An additional weakly hybridized signal was observed at 3.2 kb in several tissues, such as thymus, lung, liver and ovary. Although we examined by the reverse transcriptase-PCR method whether this longer mRNA was formed through insertions in the 2.9 kb *cot* mRNA, we have not obtained any result supporting this possibility.

The northern blot analysis of the *cot* gene showed that many fetal, newborn and adult tissues expressed the *cot* messages. Investigations such as immunohistological observation and *in situ* hybridization should make it possible to identify precisely the nature of the cells, and give us a clue to resolve the biological function of the Cot kinase. On the other hand, we have examined the *cot* expression in many human cell lines and two of them, Alexander cells (derived from hepatoma) and Hos cells (derived from osteosarcoma), highly expressed the *cot* mRNA (Aoki *et al.*, submitted). These cell lines will be useful to analyze how the Cot kinase functions in signal transduction systems. Furthermore, the construction of animal models which have gained the null-allele of *cot* locus would be another approach to analyze the *cot* functions.

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Addendum: The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL and Gen Bank Nucleotide Sequence Databases with the accession number D13759.

REFERENCES

1) Higashi, T., Sasai, H., Suzuki, F., Miyoshi, J., Ohuchi, T., Takai, S., Mori, S. and Kakunaga, T. Hamster cell line suitable for transfection assay of transforming genes. *Proc. Natl. Acad. Sci. USA*, **87**, 2409–2413 (1990).
2) Miyoshi, J., Higashi, T., Mukai, H., Ohuchi, T. and Kakunaga, T. Structure and transforming potential of the human *cot* oncogene encoding a putative protein kinase. *Mol. Cell. Biol.*, **11**, 4088–4096 (1991).
3) Aoki, M., Akiyama, T., Miyoshi, J. and Toyoshima, K. Identification and characterization of protein products of the *cot* oncogene with serine kinase activity. *Oncogene*, **6**, 1515–1519 (1991).
4) Watson, R., Oskarsson, M. and Vande Woude, G. F. Human DNA sequence homologous to the transforming gene (*mos*) of Moloney murine sarcoma virus. *Proc. Natl. Acad. Sci. USA*, **79**, 4078–4082 (1982).
5) Schmidt, M., Oskarsson, M. K., Dunn, J. K., Blair, D. G., Hughes, S., Propst, F. and Vande Woude, G. F. Chicken

homolog of the *mos* proto-oncogene. *Mol. Cell. Biol.*, **8**, 923–929 (1988).
6) Sagata, N., Oskarsson, M., Copeland, T., Brumbaugh, J. and Vande Woude, G. F. Function of c-*mos* proto-oncogene product in meiotic maturation in *Xenopus* oocytes. *Nature*, **335**, 519–525 (1988).
7) Bonner, T. I., Kerby, S. B., Sutrave, P., Gunnell, M. A., Mark, G. and Rapp, U. R. Structure and biological activity of human homolog of the *raf/mil* oncogene. *Mol. Cell. Biol.*, **5**, 1400–1407 (1985).
8) Bonner, T. I., Oppermann, H., Seeburg, P., Kerby, S. B., Gunnell, M. A., Young, A. C. and Rapp, U. R. The complete coding sequence of the human *raf* oncogene and the corresponding structure of the c-*raf*-1 gene. *Nucleic Acids Res.*, **14**, 1009–1015 (1986).
9) Selton, G., Cuypers, H. T., Boelens, W., Robanus-Maandag, E., Verbeek, J., Domen, J., van Beveren, C. and Berns, A. The primary structure of the putative oncogene *pim*-1 shows extensive homology with protein kinases. *Cell*, **46**, 603–611 (1986).
10) Padma, R. and Nagarajan, L. The human *PIM*-1 gene product is a protein serine kinase. *Cancer Res.*, **51**, 2486–2489 (1991).
11) Jones, P. F., Jakubowica, T., Pitossi, F. J., Maurer, F. and Hemmings, B. A. Molecular cloning and identification of a serine/threonine protein kinase of the second-messenger subfamily. *Proc. Natl. Acad. Sci. USA*, **88**, 4171–4175 (1991).
12) Bellacosa, A., Testa, J. R., Staal, S. P. and Tsichlis, P. N. A retroviral oncogene, *akt*, encoding a serine-threonine kinase containing an SH2-like region. *Science*, **254**, 274–277 (1991).
13) Hanks, S. K. and Quinn, A. M. Protein kinase catalytic domain sequence database: identification of conserved features of primary structure and classification of family members. *Methods Enzymol.*, **200**, 38–62 (1991).
14) Ohara, O., Dorit, R. L. and Gilbert, W. One-sided polymerase chain reaction: the amplification of cDNA. *Proc. Natl. Acad. Sci, USA*, **86**, 5673–5677 (1989).
15) Ishizaki, J., Ohara, O., Nakamura, E., Tamaki, M., Ono, T., Kanda, A., Yoshida, N., Teraoka, H., Tojo, H. and Okamoto, M. cDNA cloning and sequence determination of rat membrane-associated phospholipase A_2. *Biochem. Biophys. Res. Commun.*, **162**, 1030–1036 (1989).
16) Marchuk, D., Drumm, M., Saulino, A. and Collins, F. S. Construction of T-vectors, a rapid and general system for direct cloning of unmodified PCR products. *Nucleic Acids Res.*, **19**, 1154 (1991).
17) Chomczynski, P. and Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.*, **162**, 156–159 (1987).
18) Herrin, D. L. and Schmidt, F. W. Rapid, reversible staining of northern blots prior to hybridization. *Bio-Techniques*, **6**, 196–200 (1988).
19) Church, G. M. and Gilbert, W. Genomic sequencing. *Proc. Natl. Acad. Sci. USA*, **81**, 1991–1995 (1984).
20) Shaw, G. and Kamen, R. A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. *Cell*, **46**, 659–667 (1986).