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C3G, a Guanine Nucleotide Exchange Factor Bound to Adapter Molecule c-Crk, Has Two Alternative Splicing Forms

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Two types of C3G cDNA were isolated from mouse 3T3-L1 adipocyte cDNA library. A 114-bp sequence in the middle of C3G cDNA is deleted in the short type cDNA. By RT-PCR analysis, it was found that these two types of C3G mRNA existed in all the mouse tissues. Sequence comparison revealed 88% nucleotide sequence identity between mouse and human C3G cDNA. Comparison of mouse C3G cDNA with the human genome database suggested that this 114-bp sequence comprised an entire exon, and it is confirmed by PCR analysis using mouse genomic DNA and cDNA template. These results indicate that two C3G mRNAs and proteins result from alternative RNA splicing.

Key Words: **C3G; alternative splicing; adapter molecule; guanine nucleotide exchange factor**

C3G is a guanine nucleotide exchange factor, which was isolated by its ability of binding to the SH3 domain in adapter molecule c-Crk (1, 2). In C3G molecule, the SH3 domain binding region is located in the middle of the molecule and a CDC25 homologous sequence catalyzing the guanine nucleotide exchange reaction at its C-terminal (1, 2). The function of C3G molecule's N-terminal is not well understood. Recently it was reported that $p130^{Cas}$, a docking molecule, binds to the N-terminus of C3G molecule via its SH3 domain (3). As a signal molecule, C3G can catalyze guanine-nucleotide exchange reaction for Rap1, but not other Ras-family G proteins (4). Activation of C3G occurs through its membrane recruitment (5, 6). This membrane recruitment is believed to be mediated by c-Crk, not by direct interaction of C3G molecule with cell membrane components (5, 6). Thus, the SH3-domain binding region in middle of the molecule is crucial for the activation of C3G during signal transduction process. Our recent studies imply that C3G, through the interaction with c-Crk, is involved in signal transduction for IGF-1 receptor during 3T3-L1 cell differentiation (7).

As a primary mediating molecule for C3G activation, c-Crk mRNA has two alternative splicing forms, which result in two types of c-Crk protein (8). The large form, c-CrkII, contains one SH2 domain and two SH3 domains with a regulatory phosphorylation tyrosine residue in between the two SH3 domains, whereas the small from, c-CrkI, lacks the C-terminal SH3 domain and the regulatory phosphorylation tyrosine residue. In normal cells, c-CrkI is usually present at much lower level than c-CrkII. Elevated expression of c-CrkI form will lead to the cell transformation (8, 9). It appears that c-CrkI, structurally reassembling to the viral oncoprotein v-Crk, lacks the functional control regulation of c-CrkII. The physiological relevance of the expression of c-CrkI is not clear. Nevertheless, the alternative splicing of c-Crk leads to the change of its functional regulations.

In this report, we identified two mouse C3G mRNA forms from screening library. Further analysis indicated that these two forms of C3G mRNA resulted from different exon splicing.

MATERIALS AND METHODS

Cell culture. The 3T3-L1 preadipocytes were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% calf serum and allowed to reach confluence. Differentiation of two-day postconfluent preadipocytes (designated as day 0) was initiated with 1 μg/ml insulin, 1 μM dexamethasone (DEX) and 0.5 mM 3-iso-butyl-1-methylxanthine (MIX) in DMEM supplemented with 10% fetal bovine serum (12, 13). After 48 h (day 2), the culture medium was replaced with DMEM supplemented with 10% fetal bovine serum and 1 μg/ml insulin for additional 48 h, and the cells were then fed every other day with DMEM containing 10% fetal bovine serum. The cytoplasmic triglyceride droplets are visible by day 4 and the cells are fully differentiated by day 6.

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    1 CCGCCGCGCC GCCGCCCGCC CGCCGGCCGG CGGCTCGCGG CCCCGGAGCG GCGGCTGCGC
                                                                  M
   61 GGGAGGTGGA GCGGCGGAGT GGCAGGCCCG GTGCGGGCTG GAGCGCGGGC GGCGAGCGCA
  121 CGAGCCGCGG CCGCGCGGAG CCCGCCATGA CGGCGCGAGT GTGAGGCGCG GCGAGCACCG
  181 GTCCAGTAGA GCGGCGGGCG CGCCGGCCTC TTATCCCGCA GCGGCCGGGC CGGGCCCTGA
  241 TGAGCAGCGG CCTCGGCCTC CGGCGCAGCC CGGAAATGTC CGGCAAGATC GAGAAAGCAG
        S  S  G  L  G  L  R  R  S  P  E  M  S  G  K  I  E  K  A  D
  301 ACTCTCAGCG TTCTCATCTC TCCTCCTTCA CCATGAAGCT GATGGACAAA TTCCACTCTC
        S  Q  R  S  H  L  S  S  F  T  M  K  L  M  D  K  F  H  S  P
  361 CCAAAATCAA GAGAACACCA TCCAAGAAGG GAAAGCCAGC CGAGGTGTCG AAGATTCCTG
        K  I  K  R  T  P  S  K  K  G  K  P  A  E  V  S  K  I  P  E
  421 AGAAGCCTGT GAGCAAAGAG GCAAGAGACA GATTTCTACC AGAGGGCTAC CCTATCCCCT
        K  P  V  S  K  E  A  R  D  R  F  L  P  E  G  Y  P  I  P  L
  481 TGGATCTGGA GCAGCAGGCA GTAGAATTTA TGTCCACCAG TGCTGTGGCT TCCAGGTCTC
        D  L  E  Q  Q  A  V  E  F  M  S  T  S  A  V  A  S  R  S  Q
  541 AGAGGCAGAA GAACCTGTGC TGGCTGGAGG AGAAAGAGAA GGAGGTTGTC AGTGCCTTGC
        R  Q  K  N  L  C  W  L  E  E  K  E  K  E  V  V  S  A  L  R
  601 GCTACTTTAA GACCATTGTG GACAAAATGG CCATTGATAA GAAGGTTCTG GAGATGCTCC
        Y  F  K  T  I  V  D  K  M  A  I  D  K  K  V  L  E  M  L  P
  661 CGGGGTCAGC CAGCAAGGTG CTGGAGGCCA TCTTACCCCT GGTGCAGACT GACCCTCGGA
        G  S  A  S  K  V  L  E  A  I  L  P  L  V  Q  T  D  P  R  I
  721 TCCAGCACAG CTCAGCCCTC TCCTCCTGTT ATAGCCGAGT ATACCAGAGC CTCGCCAACC
        Q  H  S  S  A  L  S  S  C  Y  S  R  V  Y  Q  S  L  A  N  L
  781 TTATCCGATG GTCTGACCAG GTGATGCTAG AGGGCGTGAA CTCAGAAGAT AAGGAGATGG
        I  R  W  S  D  Q  V  M  L  E  G  V  N  S  E  D  K  E  M  V
  841 TGACAACTGT GAAGGGCGTT ATCAAAGCTG TCCTGGACGG AGTGAAGGAG CTAGTGAGGC
        T  T  V  K  G  V  I  K  A  V  L  D  G  V  K  E  L  V  R  L
  901 TAACCATTGA GAAGCAGGGG CGGCCATCGC CAACAAGCCC AGTGAAGCCC AGTTCCCCAG
        T  I  E  K  Q  G  R  P  S  P  T  S  P  V  K  P  S  S  P  A
  961 CCAGCAAGCC TGATGGCCAG CCTGAGCTCC CTCTGACAGA CCGAGAAATG GAGATTCTGA
        S  K  P  D  G  Q  P  E  L  P  L  T  D  R  E  M  E  I  L  N
 1021 ACAAGACGAC AAGTGTGTCA CCATCTGCTG AACTGCTCCC AGACTCCACC AGTGAAGAGG
        K  T  T  S  V  S  P  S  A  E  L  L  P  D  S  T  S  E  E  V
 1081 TCGCACCCCC CAAGCCCCCT TTACCTGGCA TCCGGGTGGT TGATAACAGT CCACCAGCAT
        A  P  P  K  P  P  L  P  G  I  R  V  V  D  N  S  P  P  A  L
 1141 TACCACCCAA GAAAAGGCAG TCTGCTCCAT CCCCCACTCG GGTGGCTGTG GTAGCCCCAA
        P  P  K  K  R  Q  S  A  P  S  P  T  R  V  A  V  V  A  P  M
 1201 TGAGTCGGGC TACCAGTGGC TCCAGTTTGC CTGTTGGAAT CAATAGGCAG GACTTTGATG
        S  R  A  T  S  G  S  S  L  P  V  G  I  N  R  Q  D  F  D  V
 1261 TTGAATGTTA CACCCAGAGG CGCCTGTCAG GAGGCAGCCG CTCCTGCGGT GGTGAGTCTC
        E  C  Y  T  Q  R  R  L  S  G  G  S  R  S  C  G  G  E  S  P
 1321 CTCGCCTGTC CCCCTGCAGC AGCACAGGCA AGCTCAGCCG CTCAGACGAG CAGCTGTCCT
        R  L  S  P  C  S  S  T  G  K  L  S  R  S  D  E  Q  L  S  S
 1381 CCCTGGACAG GGATAGTGGG CAGTGCTCAC GGAACACAAG CTGTGAAACA CTAGATCACT
        L  D  R  D  S  G  Q  C  S  R  N  T  S  C  E  T  L  D  H  Y
 1441 ACGACCCCGA CTATGAATTC CTCCAGCAAG ATCTCTCCAA TGCAGACCAG ATCCCTCCAC
        D  P  D  Y  E  F  L  Q  Q  D  L  S  N  A  D  Q  I  P  P  Q
 1501 AGGCAGCCTG TAACCTCAGC CCTCTGCCGG AGTCCCTGGG GGAATCTGGG CCTCCATTTC
        A  A  C  N  L  S  P  L  P  E  S  L  G  E  S  G  P  P  F  L
 1561 TTGGCCACCC TTTCCAGCTG CCTTTGGGCA GCTGTCTGCA GCAGGAGGGA CAGCAGACAG
        G  H  P  F  Q  L  P  L  G  S  C  L  Q  Q  E  G  Q  Q  T  D
 1621 ACACTCCACC TGCCCTTCCG GAGAAGAAGC GTAGGAGCGC AGTCTCCCAG ACCACGGACA
        T  P  P  A  L  P  E  K  K  R  R  S  A  V  S  Q  T  T  D  S
 1681 GCTCTGGCTG CAGGGTGTCC TATGAGCGAC ACCCCTCACA GTATGACAAC ATCTCAGAGG
        S  G  C  R  V  S  Y  E  R  H  P  S  Q  Y  D  N  I  S  E  G
 1741 GTGACCTGCA GAACCCAGTC CCAGTCCAGC CTGTGCCCTA CCCACCCTTT GCTGCTGTCC
        D  L  Q  N  P  V  P  V  Q  P  V  P  Y  P  P  F  A  A  V  L
 1801 TGCCCTTTCA GCAGGGAGCT TCCTCTGCCT CTGCTGAGTT TGTGGGTGAT TTCAGTGTTC
        P  F  Q  Q  G  A  S  S  A  S  A  E  F  V  G  D  F  S  V  P
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FIG. 1. Nucleotide and amino acid sequences of mouse C3G cDNA. (A) The amino acid sequence is shown under the nucleotide sequence using the standard single-letter code. The boxed nucleotide and overshadowed amino acid sequences indicate the region, which is deleted in the short type of C3G cDNA. (B) Comparison of human and mouse C3G cDNA. The exons of human C3G cDNA were obtained from search human genome database with human C3G cDNA. Human C3G cDNA exon 3 (overshadow) corresponds to the deleted sequence in mouse cDNA. The bold and underlined nucleotide sequences are two pairs of primers (P1-P1′and P2-P2′) used for PCR analysis.

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1861  CTGAGTTGGC GGGTGACACA GAGAAGCCAC CTCCTCTACC AGAGAAGAAG AACAAGCACA
        E  L  A  G  D  T  E  K  P  P  P  L  P  E  K  K  N  K  H  M
1921  TGCTGGCCTA CATGCAACTG CTGGAGGACT ACTCAGAGCC ACAGCCCTCC ATGTTCTACC
        L  A  Y  M  Q  L  L  E  D  Y  S  E  P  Q  P  S  M  F  Y  Q
1981  AGACACCGCA GAGTGAGCAC ATCTACCAGC AGAAGAACAA GATGCTCATG GAGGTGTACG
        T  P  Q  S  E  H  I  Y  Q  Q  K  N  K  M  L  M  E  V  Y  G
2041  GCTTCAGCGA GTCCTTCTGC GGTAGTGATT CCACGCAGGA GCTGGCCCCT CCACCCGCTC
        F  S  E  S  F  C  G  S  D  S  T  Q  E  L  A  P  P  P  A  L
2101  TGCCCCCCAA GCAACGGCAG CTGGAACCAC CATCTGGGAA GGACGGACAT CCCAGAGATC
        P  P  K  Q  R  Q  L  E  P  P  S  G  K  D  G  H  P  R  D  P
2161  CTTCGGTCAG CAGTGCATCT GGGAAGGACA GCAGAGAAAA TGGGGAAAGG TCCCCAAAGT
        S  V  S  S  A  S  G  K  D  S  R  E  N  G  E  R  S  P  K  S
2221  CACTGGATGG TCTGGAGTCA GCGCAGTCAG AAGAGGAAGT GGATGAACTG TCCCTCATTG
        L  D  G  L  E  S  A  Q  S  E  E  E  V  D  E  L  S  L  I  D
2281  ACCACAATGA AATTATGGCC AGGCTGACAC TCAAGCAAGA GGGTGATGAT GGGCCAGATG
        H  N  E  I  M  A  R  L  T  L  K  Q  E  G  D  D  G  P  D  V
2341  TTCGTGGTGG TTCAGGAGAC ATCTTACTGG TCCATGCTAC TGAGACAGAC AGAAAAGACC
        R  G  G  S  G  D  I  L  L  V  H  A  T  E  T  D  R  K  D  L
2401  TGGTGCTGTA CTGTGAAGCC TTCCTGACCA CCTACAGGAC CTTCATCAGC CCGGAGGAGC
        V  L  Y  C  E  A  F  L  T  T  Y  R  T  F  I  S  P  E  E  L
2461  TCATCAAGAA GCTGCAGTAC CGGTACGAGA AGTTCTCTCC CTTTGCTGAC ACGTTCAAGA
        I  K  K  L  Q  Y  R  Y  E  K  F  S  P  F  A  D  T  F  K  K
2521  AGCGAGTGAG CAAGAACACA TTCTTTGTGC TGGTTCGAGT GGTGGATGAG CTCTGCCTGG
        R  V  S  K  N  T  F  F  V  L  V  R  V  V  D  E  L  C  L  V
2581  TGGAGCTGAC AGAGGAGATC CTGAAGCTGC TGATGGAGCT GGTCTTCCGC CTAGTGTGCA
        E  L  T  E  E  I  L  K  L  L  M  E  L  V  F  R  L  V  C  S
2641  GCGGAGAGCT CAGCCTGGCC AGAGTCCTCC GGAAGAACAT TCTGGACAAG GTGGACCAGA
        G  E  L  S  L  A  R  V  L  R  K  N  I  L  D  K  V  D  Q  K
2701  AGAAGCTGCT CAGGTGTGCC CATTCCGACC AGCCTCTGGC AGCCAGGGGT GTTGCAGCCA
        K  L  L  R  C  A  H  S  D  Q  P  L  A  A  R  G  V  A  A  R
2761  GGCCAGGAAC CTTGCATGAT TTCCACAGCC ACGAGATAGC TGAGCAGCTG ACACTGCTGG
        P  G  T  L  H  D  F  H  S  H  E  I  A  E  Q  L  T  L  L  D
2821  ATGCCGAGCT TTTCTACAAG ATAGAGATTC CTGAAGTTTT GCTTTGGGCC AAAGAGCAGA
        A  E  L  F  Y  K  I  E  I  P  E  V  L  L  W  A  K  E  Q  N
2881  ATGAGGAGAA GAGTCCCAAT CTGACCCAGT TCACAGAGCA CTTCAACAAC ATGTCCTACT
        E  E  K  S  P  N  L  T  Q  F  T  E  H  F  N  N  M  S  Y  W
2941  GGGTGCGGTC CATCATCATG CTGCAGGAGA AGGCCCAGGA CCGGGAGAGG CTGCTCCTCA
        V  R  S  I  I  M  L  Q  E  K  A  Q  D  R  E  R  L  L  L  K
3001  AGTTCATCAA GATCATGAAG CACCTGCGCA AGCTCAACAA CTTCAACTCC TACCTGGCCA
        F  I  K  I  M  K  H  L  R  K  L  N  N  F  N  S  Y  L  A  I
3061  TCCTCTCAGC ACTAGACTCG GCCCCCATCC GCAGACTGGA GTGGCAGCGG CAGACCTCAG
        L  S  A  L  D  S  A  P  I  R  R  L  E  W  Q  R  Q  T  S  E
3121  AGGGCCTGGC TGAGTACTGC ACATTGATTG ACAGCTCATC CTCCTTCCGA GCCTACCGGG
        G  L  A  E  Y  C  T  L  I  D  S  S  S  S  F  R  A  Y  R  A
3181  CTGCTCTCTC AGAGGTGGAG CCCCCATGTA TCCCATACCT AGGTCTGATT CTGCAAGACC
        A  L  S  E  V  E  P  P  C  I  P  Y  L  G  L  I  L  Q  D  L
3241  TGACCTTCGT TCACCTGGGA AACCCAGATT ATATTGACGG GAAAGTAAAC TTCTCCAAGC
        T  F  V  H  L  G  N  P  D  Y  I  D  G  K  V  N  F  S  K  R
3301  GGTGGCAACA GTTCAACATA TTGGACAGCA TGCGGTGCTT CCAGCAGGCG CACTATGAAA
        W  Q  Q  F  N  I  L  D  S  M  R  C  F  Q  Q  A  H  Y  E  I
3361  TCCGGAGAAA CGACGACATC ATAAATTTCT TCAATGACTT CAGTGACCAC CTGGCCGAGG
        R  R  N  D  D  I  I  N  F  F  N  D  F  S  D  H  L  A  E  E
3421  AAGCCCTGTG GGAACTCTCT CTGAAGATCA AGCCTAGGAA CATAACAAGG CGGAAAACAG
        A  L  W  E  L  S  L  K  I  K  P  R  N  I  T  R  R  K  T  D
3481  ACCGCGAAGA GAAGACCTAG GAGCAGGCTG GCTGTGAGAA CGCTCAAGCA GCCAGGAGAG
        R  E  E  K  T  *     (1086 AA)
3541  GACCTGGACC ATCCCAGCCT CAGCCTGTCC ATGCCTGGCT GTGTGGCAGC ACCTGAGTCT
3601  CCTGGCCGGT CTCCTGCCTC CTTCCCTATG GCTAAAGTCC CAGGGTTCAC AATGAGCTGG
3661  CTGGCAGGCC TCAGGGCTGG CTTGTGTACT GGTGGCCCCT GGTCTGGTTT TGTTTTCTGT
3721  TTTCTGTCAG TGCTCCCTCC TGTTCCCTCC TCTCCCCTGC G    (3761bp)
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FIG. 1—*Continued*

FIG. 1—*Continued*

Isolation of mouse C3G cDNA clones. The human C3G cDNA was labeled with [α-^{32}P]dATP by random-priming methods (10) and used as probe to screen ~1 million λ phage plaques of a day 5 3T3-L1 adipocyte (mouse cell line) cDNA library (11). The hybridization was carried out in 20% formamide, 4× SSC (standard saline citrate), 1× Denhardt's solution, 1% SDS, 50 μg/ml salmon sperm DNA, 0.5 mg/ml sodium pyrophosphate, and 50 mM sodium phosphate, pH 7.0, at 42°C overnight, and washing in 0.1% SDS and 0.1× SSC 3 times at 50°C.

Isolation of DNA and PCR analysis. Chromosomal DNA from one 10-cm dish of confluent 3T3-L1 preadipocytes was isolated by the method of Wigler *et al.* (14). One microgram genomic DNA was used as template for PCR analysis with primers as described under Results. The DNA amplification was carried out in 50 μl containing 10 mM KCl, 8 mM $(NH_4)_2SO_4$, 10 mM Tris–HCl, pH 9.0, 1.5 mM $MgCl_2$, 200 μM dNTPs, 1 μM each primers and 2.5 units of Taq DNA polymerase. The reaction was conducted for 30 cycles (94°C for 1 min, 54°C for 0.5 min and 72°C for 1 min). The products were analyzed by agarose or polyacrylamide gel electrophoresis.

Isolation of mRNA and RT-PCR analysis. Total RNA was isolated from 2-day postconfluent 3T3-L1 preadipocytes, day 6 3T3-L1 adipocytes and mouse tissues using TRIZOL reagent (GibcoBRL), and total mRNA was isolated from RNA using Polytract mRNA isolation reagent (Promega). One microgram total mRNA was reversed transcribed in 20 μl mixture containing 2 mM oligo (dT_{18}), 1 mM dNTPs, 50 mM Tris–HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT and 200 units MMLV reverse transcriptase at 42°C for 1 h. The reaction was terminated by incubating at 75°C for 10 min and cDNA equivalent for 50 ng mRNA was used for PCR analysis.

C3G protein analysis. Total cellular protein was extracted from 3T3-L1 cells and used for Western blot analysis. 3T3-L1 cell monolayer was washed twice with ice-cold phosphate-buffered saline (PBS), pH 7.5 and then lysed directly in boiling 1× Laemmli SDS sample buffer containing 20 mM dithiolthretol. Cellular protein was subjected to 8% SDS–PAGE and transferred to Immobilon-P membrane. C3G protein was detected with antibody (Santa Cruz) against C3G and visualized by ECL (enhanced chemiluminescence).

RESULTS

Cloning of mouse C3G. Human C3G was isolated by screening expression library with SH3 domains of c-Crk as probe (1, 2). Using human C3G cDNA as probe the mouse C3G cDNA was isolated by screening 3T3-L1 adipocyte cDNA library. Sequence comparison revealed 88% nucleotide sequence identity between human and mouse C3G cDNA at coding region, but less sequence homology at 5′ and 3′ untranslated region. From 3T3-L1 adipocyte cDNA library, two types of mouse C3G cDNA were identified (Fig. 1A). The long type C3G cDNA contains an extra 114-bp sequence in the coding region over the short type cDNA. The multiple C3G protein bands detected by C3G antibody on Western blot may represent this mRNA variance (Fig. 3C). To ascertain whether this 114-bp sequence represents an alternative mRNA splicing or an aberration of library construction, both mouse and human C3G cDNAs were compared with human genomic sequence database to locate the exon sequences. Database search revealed that on human genomic sequence this 114-bp sequence represents one exon (Fig. 1B). Due to the high degree of homology between mouse and human C3G cDNA sequence, it is likely that this 114-bp sequence is also a single-exon in mouse genomic sequence.

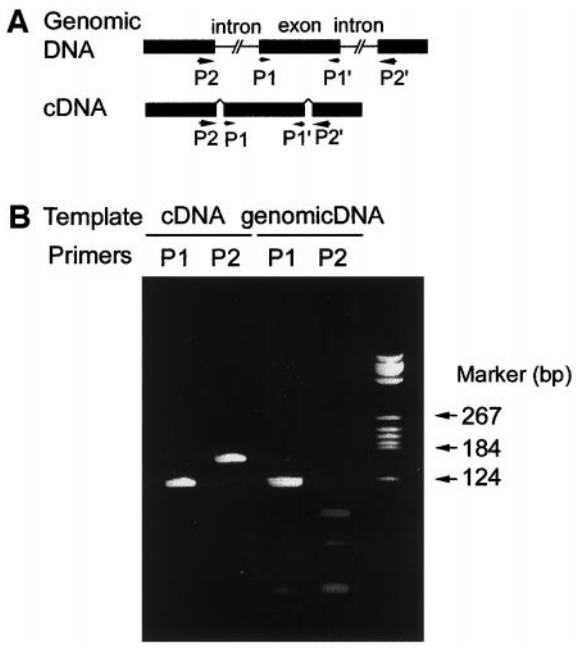

FIG. 2. PCR analysis of mouse C3G genomic gene exon structure. (A) Schematic diagram of PCR using genomic DNA or cDNA as template. Primers P1, P1′, P2 and P2′ are indicated in Fig. 1B. (B) PCR result. *P1* and *P2* indicate the primer pairs of P1-P1′ and P2-P2′ respectively.

Alternative splicing for C3G mRNA. To prove this 114-bp sequence in mouse C3G also represents one exon, PCR analysis was conducted using mouse genomic DNA template and primers designed as that one pair (P1-P1′) is on the inside ends of 114-bp region and the other pair (P2-P2′) is on the outside ends (Figs. 1B and 2A). As shown in Fig. 2B, using C3G cDNA template both pairs of primers produced PCR products. As expected, a 114-bp DNA fragment was produced by P1 pair primers and a 156-bp fragment was produced by P2 pair primers. However, using mouse genomic DNA template, only the pair of primers at the inside ends (P1 pair) produced the 114-bp DNA fragment, the same as using cDNA template, whereas the outside pair of primers produced no DNA fragment around this size. This result indicated that the cDNA sequences corresponding to the outside pair of primers were located far away in the genomic DNA, while the sequences corresponding to the inside primers were in one exon. Thus, in genomic DNA this 114-bp cDNA sequence is single exon, and the cDNA sequences adjacent to this region were located in the different exons.

Two forms of C3G mRNA in most tissues. mRNA alternative splicing could be ubiquitous, such as c-CrkII and c-CrkI which exist in many tissues (5, 8, 15), or tissue specific, such as mammalian STE20-like kinase 3 (MST3), which has a brain-specific alternative spliced form MST3b (16). To find whether the alternative splicing process for C3G mRNA is a tissue specific or ubiquitous process, total mRNA from different mouse tissues were isolated and the C3G transcripts were analyzed using RT-PCR. As shown in Fig. 3A, C3G mRNA in all the tissues tested (brain, adipose, heart, kidney, liver, lung, muscle and spleen) had two spliced forms. Thus, alternative splicing for C3G mRNA is not a tissue specific process. Unlike c-Crk alternative splicing process, in which the level of c-CrkII is much higher than that of c-CrkI (15), both long and short forms of C3G mRNA were present in the tissues at a similar level. However, the expression levels of total C3G mRNA in various tissues were very different, high in brain, heart, liver and muscle, and low in adipose, kidney and spleen. Interestingly, 3T3-L1 preadipocyte differentiation also affected the C3G mRNA splicing process. In preadipocytes, the long C3G mRNA was the predominant form of C3G mRNA (Fig. 3B), while the short C3G mRNA was barely detectable if PCR was carried out in more cycles (result not shown). However, as the cells differentiated into adipocytes, the level of short C3G mRNA increased and two types of C3G mRNA were equally presented in

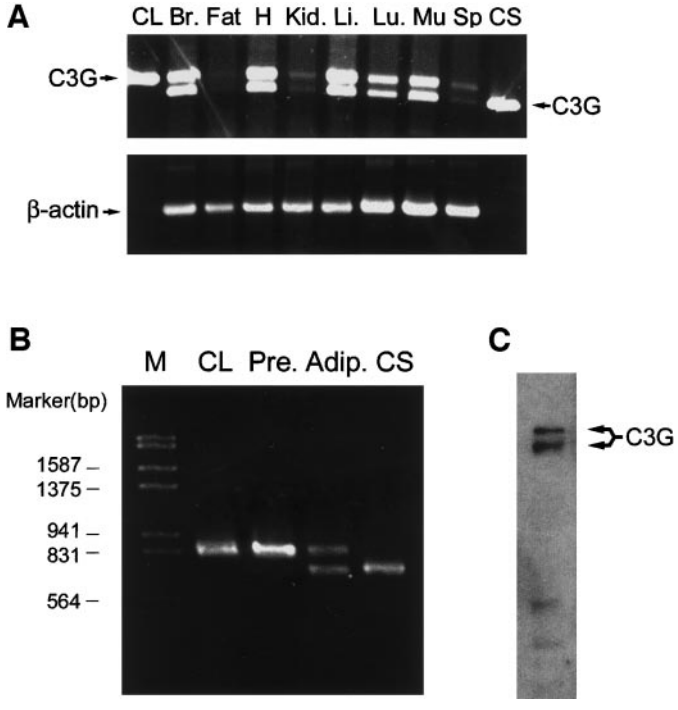

FIG. 3. RT-PCR analysis of C3G mRNA in mouse tissues and 3T3-L1 cell. (A) Two types of C3G mRNA in mouse tissues. The forward primer is 5′ACTCTCAGCGTTCTCATCTC corresponding to the cDNA sequence from bp 301 to 320 and the complement primer is 5′GCTGGTGGACTGTTATCAAC corresponding to the sequence from bp 1119 to 1138. *CL* refers to control for long form C3G mRNA and *CS* to control for short form mRNA. *Br, Fat, H, Kid, Li, Lu, Mu,* and *Sp* refer to RT-PCR analysis of mRNA from brain, adipose, heart, kidney, liver, lung, muscle and spleen respectively. (B) C3G mRNA in 3T3-L1 preadipocytes and adipocytes. *CL* and *CS* are the same as in A. *Pre.* refers to 3T3-L1 preadipocyte and *Adip.* to 3T3-L1 adipocyte. (C) Western blot of C3G protein in 3T3-L1 adipocytes.

adipocytes. Thus, in mature differentiated tissue cells both types of alternative spliced C3G mRNA existed in similar amount.

DISCUSSION

Protein molecules involved in signal transduction often have several variants by alternative RNA splicing. This alternative splicing increases the structural and possibly functional complexity for signal molecules. For C3G protein, the domain of guanine nucleotide releasing factor is located at the C-terminal and c-Crk's SH3 domain binding motif is in the middle of the molecule (1, 2). So far, the function of C3G's N-terminal is not clear. The alternative splicing for C3G RNA results in a deletion of 38 amino acids in the nonfunctional N-terminal region. Thus, the functional implication of this RNA alternative splicing is not known.

The change of C3G RNA splicing during 3T3-L1 preadipocyte differentiation is a little puzzling. Since the difference between these two C3G mRNAs are not big enough for them to be resolved on Northern blot, their relative amounts were only determined by RT-PCR. It should be pointed out in preadipocytes the short C3G mRNA still exists. Thus, the change of RNA splicing during differentiation may only reflect a quantitative change not a fundamental change in the RNA splicing process.

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