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Delineation of genomic deletion in cardiomyopathic hamster

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Abstract Cardiomyopathic hamster is a representative animal model for autosomal recessive cardiomyopathy. We have previously shown that the transcript of δ-sarcoglycan is missing in the heart of cardiomyopathic hamster due to genomic deletion. Here we define the normal genomic region deleted in cardiomyopathic hamster, which spans about 30 kb interval and includes the two first exons of the δ-sarcoglycan gene. RNA blot analysis using genomic DNA fragments covering the entire deletion as probes failed to detect any transcript other than δ-sarcoglycan in normal hamster heart, suggesting that δ-sarcoglycan is the only transcript defective in the heart of cardiomyopathic hamster.

Key words: Cardiomyopathy; Muscular dystrophy; Dystrophin; Sarcoglycan; Deletion; Cardiomyopathic hamster

1. Introduction

Cardiomyopathy is a clinical set of heart diseases which manifests congestive heart failure or sudden death, causing severe morbidity and mortality throughout the world [1]. Pathologically, cardiomyopathy is defined as a primary degenerative disease of myocardium and is categorized into hypertrophic and dilated cardiomyopathies according to its gross appearance [2]. Recent progress in human genetics has suggested and indeed identified several causative genes for this group of diseases. One of the best studied forms of cardiomyopathy is familial hypertrophic cardiomyopathy, the majority of which is autosomal dominant trait and is caused by mutation of genes encoding sarcomeric proteins such as β-myosin heavy chain [3]. Another insight for the molecular basis of cardiomyopathy has come from studies on hereditary muscular dystrophy, which is in some cases accompanied by cardiomyopathy [4].

In skeletal and cardiac muscles, dystrophin, the protein product of the Duchenne muscular dystrophy (DMD) gene, binds actin and dystrophin-associated glycoprotein complex (DGC) [4–7]. By linking intracellular cytoskeleton and extracellular matrix, DGC plays a pivotal role in protecting muscles against mechanical stress generated by contraction [5,6]. More recently, genetic defects for any sarcoglycans (SGs), a subcomplex of DGC, have been associated with various forms of autosomal recessive limb-girdle muscular dystrophy (LGMD); mutations of α-, β-, γ-, and δ-SG genes result in LGMD2D, LGMD2E, LGMD2C, and LGMD2F, respectively with concomitant loss of all the other SG proteins [5,6]. The incidence of cardiomyopathy in DMD and LGMD is about 30% [8] and 10% [9], respectively. Significant but low incidence of cardiomyopathy in muscular dystrophic patients suggests that mutation of some of the causative genes for muscular dystrophy is more likely associated with cardiomyopathy, or that there might be another modifier gene causing cardiomyopathy in the genetic background of muscular dystrophy.

Cardiomyopathic hamster (CM hamster) is a representative animal model for autosomal recessive cardiomyopathy and muscular dystrophy [10,11]. CM hamster serves as a good model for studying cardiomyopathy associated with muscular dystrophy, because the penetrance of cardiomyopathy is 100% in this animal model [10,11]. From the well known BIO14.6 [10], several sublines have been isolated, including UMX7.1, TO-2, and CHF146 [11–13]. Not only in BIO14.6 [14,15] but also in UMX7.1 and TO-2 [15], genomic deletion in the 5′ end of the δ-SG gene has been recently identified with consequent loss of its transcript and protein product. Thus, CM hamster could be considered an animal model for LGMD2F. We have previously demonstrated that an identical genomic region is deleted in BIO14.6, UMX7.1, and TO-2, the 3′ end of which is located at 6.1 kb 5′ upstream from the second exon of the δ-SG gene (Exon 2) [15]. We have also elucidated how the deficiency of δ-SG protein causes disruption of DGC leading to instability of sarcolemma [15]. Very recently, indeed, damage of structural integration of skeletal and cardiac sarcolemma of BIO14.6 has been directly demonstrated by means of an in vivo tracer assay using Evans blue dye [16]. In this present study, we defined the entire genomic interval deleted in CM hamster including CHF146, and searched for potential genes other than δ-SG which are located in the genomic deletion interval and are expressed in normal hamster heart, in order to elucidate whether or not δ-SG is the only transcript defective in the heart of CM hamster.

2. Materials and methods

2.1. Animals

BIO14.6 and TO-2 hamsters were purchased from Bio Breeders (Fitchburg, MA, USA). CHF146 and its congenic normal CHF148 hamsters from Canadian Hybrid Farms (Nova Scotia, Canada). UMX7.1 hamsters were kindly provided by Dr. Jasmin of the University of Montreal. Unrelated normal Golden hamsters were purchased from SLC (Shizuoka, Japan). All the animals used in the present study were male, aged 4–6 months old, and were deeply anesthetized before the experiments.

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Abbreviations: CM hamster, cardiomyopathic hamster; DMD, Duchenne muscular dystrophy; LGMD, limb-girdle muscular dystrophy; DGC, dystrophin-associated glycoprotein complex; SG, sarcoglycan; Exon 1A, exon 1A of δ-SG; Exon 1B, exon 1B of δ-SG; Exon 1C, exon 1C of δ-SG; Exon 2, exon 2 of δ-SG; 5′ BP, 5′ deletion breakpoint; 3′ BP, 3′ deletion breakpoint; bp, base pair; kb, kilo base

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2.2. Isolation of genomic DNA clones of normal hamster encompassing the genomic deletion interval for CM hamster

The nucleotide sequences of the genomic region flanking the deletion breakpoints (Fig. 2B: −510∼−1 and +1∼+150) were determined by analyzing the genomic DNA fragment of CM hamster digested with *Pst*I and *Spe*I which shows restriction fragment length polymorphism [15]. λFIXII genomic library of Golden hamster (Stratagene, La Jolla, CA, USA) was screened by hybridization with the PCR product amplified with a primer set of 5′-TGCCTAGGAGTCAGTAGTTCAC-3′ (5bp-F1)/5′-AAAACCCATTCATAGTGGAAG-3′ (5bp-R1) (Fig. 3: 5′-Conserved). By using initially isolated clones as probes, the same library was sequentially screened. Overlapping clones covering the genomic deletion interval for CM hamster, some of which had been previously isolated [15], were assembled to create a restriction map (Fig. 1). DNA sequencing on both strands was carried out by using a 373S automated DNA sequencer (PE Applied Biosystems, Foster City, CA, USA).

2.3. Identification of a novel first exon of δ-SG gene

Potential first exons of normal hamster δ-SG were identified by the 5′ RACE method by using a Marathon cDNA Amplification kit (Clontech, Palo Alto, CA, USA). Briefly, double-stranded cDNA of Golden hamster heart was synthesized with a primer specific to Exon 2 (ex2-R2: 5′-ACTTTGAGAATCCAGATGGTCATG-3′), ligated with a Marathon adapter, and amplified with a primer set of a Marathon adaptor primer and a nested primer locating in Exon 2 (ex2-R1: 5′-CAGGCATCTTTTCCTCCAGC-3′). The amplified products were cloned into pBluescript (Stratagene) and sequenced on both strands. A putative transcription initiation site of a novel first exon of δ-SG (Exon 1C) was assigned based on the longest clone isolated. The 5′ upstream sequence of Exon 1C was determined by analyzing the corresponding genomic DNA clone of normal hamster. The TFSEARCH program (http://pdap1.trc.rwcp.or.jp/research/db/TFSEARCH.html) was used to predict potential binding sites for transcription factors.

2.4. Genomic PCR

Genomic DNA was isolated from hamster liver by the standard SDS/proteinase K method [15]. Genomic DNA fragments corresponding to Exon 1B, Exon 1C, or Exon 2 were amplified by PCR with the following primer sets: 5′-GTGCAGGCAGGGCCTGCTCAC-3′ (ex1B-F)/5′-CTCTCCTCTGTTTCACAGAG-3′ (ex1B-R), 5′-AGTGAAGGGACCAGGTGGAC-3′ (ex1C-F)/5′-GCATATATAGCATGGTCTTC-3′ (ex1C-R), or 5′-CCACAGGAGCACCATGCCCAGCTC-3′ (ex2-F)/ex2-R2, respectively. The genomic deletion breakpoints near Exon 1A and Exon 2, which were designated here as 5′ deletion breakpoint (5′ BP) and 3′ deletion breakpoint (3′ BP), respectively (Fig. 1), were determined by comparing the nucleotide sequence of the polymorphic *Pst*I/*Spe*I genomic DNA fragment of CM hamster with the corresponding sequences of normal genomic DNA clones (Fig. 2B). The genomic regions corresponding to 5′ upstream from 5′ BP (Fig. 3: 5′-Conserved), surrounding 5′ BP (Fig. 3: 5′-BP/Deletion), surrounding 3′ BP (Fig. 3: Deletion/3′-BP), or encompassing 5′ BP and 3′ BP (Fig. 3: 5′-BP/3′-BP) were amplified by the following primer sets: 5bp-F1/5bp-R1, 5′-TTTCCTCTGAGAAGTGTGTCC-3′ (5bp-F2)/5′-GATAGGATTTCTCTGTATTG-3′ (5bp-R2), 5′-AACCGTTGACTATTTATGCC-3′ (3bp-F)/5′-GCATTACACCACACGTTCAC-3′ (3bp-R1), or 5bp-F2/5′-CTCAAATGAGCTAGTGCCAGG-3′ (3bp-R2), respectively (Fig. 2B). PCR was carried out by using a GeneAmp PCR system 9700 (PE Applied Biosystems) under the following conditions: preheating at 94°C for 2 min and then 35 cycles of 94°C for 20 s, 55°C for 30 s, and 72°C for 1 min. *TaKaRa LA Taq* (TaKaRa, Shiga, Japan) was chosen as a thermo-stable polymerase.

2.5. Reverse transcription-PCR (RT-PCR)

cDNAs randomly synthesized from 10 μg of total RNA prepared from cardiac muscle, skeletal muscle, and stomach of Golden or BIO14.6 hamsters were subjected to RT-PCR [15]. δ-SG transcripts spliced with Exon 1A, Exon 1B or Exon 1C were amplified with the common reverse primer (ex2-R1) and the following three distinct forward primers; 5′-GAATTTGGAGACAGCTGCAC-3′ (ex1A-F), ex1B-F, or ex1C-F, respectively. RT-PCR targeting Exon 2 was performed with a primer set of ex2-F/ex2-R1. The amount of template cDNA for RT-PCR was normalized with respect to that of GAPDH (glyceraldehyde 3-phosphate dehydrogenase). The reaction conditions were as followed: preheating at 94°C for 2 min and then 35 cycles of 94°C for 20 s, 65°C for 30 s, and 72°C for 1 min.

2.6. RNA blot analysis

The procedure for RNA blot analysis was as described previously [17]. Poly(A)$^+$ RNA was purified from hamster heart using MACS reagent (Miltenyi Biotec, Auburn, CA, USA) according to the manufacturer's instructions. Transcripts for δ-SG in the hearts of Golden or BIO14.6 hamsters were scanned with hamster cDNA containing all the protein coding region of δ-SG (Fig. 5A). δ-SG transcripts spliced with Exon 1A, Exon 1B, or Exon 1C were identified with the PCR products amplified with primer sets of ex1A-F/5′-CTTTTTAGTCTTAATCCTCT-3′, ex1B-F/ex1B-R, or ex1C-F/ex1C-R, respectively (Fig. 5B). The restriction fragments of the cloned normal genomic DNAs encompassing the deletion interval for CM hamster (Fig. 5D) were also used as probes for screening potential exons located in the genomic deletion interval (Fig. 5C). ^{32}P-labelled probes were prepared with Prime-It II Random Primer Labelling kit (Stratagene). Data analysis was performed by using a BAS 5000 Imaging Analyzer (Fuji Film, Tokyo, Japan).

3. Results and discussion

3.1. Genomic structure of 5′ upstream region of normal hamster δ-SG gene

We isolated overlapping genomic DNA clones of normal hamster covering 58 kb 5′ upstream from Exon 2, about 40 kb region of which was presented in Fig. 1. In addition to Exon 1A and Exon 1B, which were previously identified and designated as 'alternative' and 'authentic' first exons of δ-SG, respectively [15], the 5′ RACE technique identified Exon 1C, a novel first exon of δ-SG comprising 133 bp. Neither TATA box nor Sp-1 binding sites [18] were found in the 5′ upstream genomic sequence of Exon 1C (Fig. 2A). Putative binding sites for several transcription factors were found in the 5′ upstream sequence of Exon 1C but none of them was specific to cardiac muscle (Fig. 2A) [19]. The splice-donar sequences (gt in Fig. 2A) were in accordance with the prediction by the Splice Site Prediction Program (http://www-hgc.lbl.gov/projects/splice.html) with a maximum score of 1.0. In normal hamster, Exon 1B and Exon 1C were located at 24.7 kb and 21.2 kb 5′ upstream from Exon 2, respectively, both of which resided within the genomic deletion interval for CM hamster (Fig. 1). Exon 1A, which is conserved in CM hamster [15], was not found within 58 kb 5′ upstream region of Exon 2 (Fig. 1).

3.2. Identification of the genomic interval deleted in CM hamster

The primer set of 5bp-F2/3bp-R2 amplified no genomic DNA fragments from normal hamster (Golden and CHF148) but 361 bp genomic DNA fragments from all the CM hamsters (BIO14.6, UMX7.1, TO-2, and CHF146) (Fig. 3: 5′-BP/3′-BP). These data could be explained by the fact

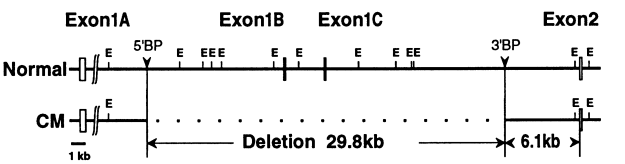

Fig. 1. Genomic structure of the 5′ upstream region of δ-SG gene in normal and CM hamsters. Exon 1A, Exon 1B, Exon 1C and Exon 2 are depicted as boxes. The genomic interval deleted in CM hamster is indicated by a dotted line. 5′ BP, 5′ deletion breakpoint; 3′ BP, 3′ deletion breakpoint. E, *Eco*RI.

that the primer-annealing sites in genomic DNA, which are distantly separated in normal hamster, locate very close to each other in CM hamster due to the genomic deletion described in this study (Figs. 1 and 2B). The nucleotide sequencing of the PCR product common to all the CM hamsters revealed that each of the two deletion breakpoints, 5′ BP or 3′ BP, was identical for all the CM hamsters (Fig. 2B). The genomic deletion interval common to all the CM hamsters was calculated as 29.8 kb, because 5′ BP and 3′ BP were located at 35.9 and 6.1 kb 5′ upstream from Exon 2 of normal hamster, respectively (Fig. 1). Genomic PCR with the primer sets of 5bp-F2/5bp-R2 and 3bp-F/3bp-R1 detected 276 bp and 180 bp bands in normal hamster, respectively, but no corresponding bands in CM hamster, which confirmed the 5′ and 3′ ends of the genomic deletion interval for CM hamster (Fig.

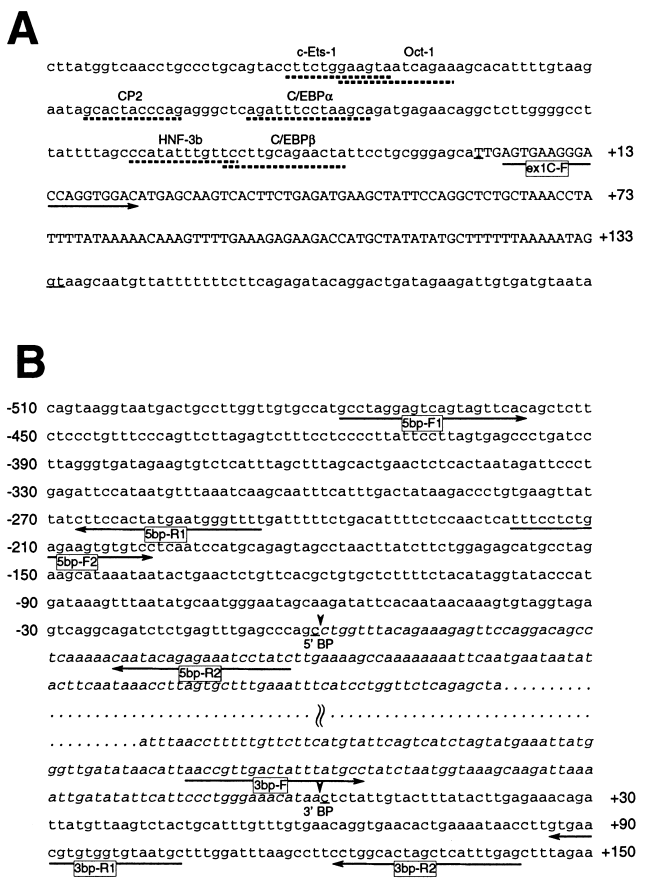

Fig. 2. A: Nucleotide sequence of normal hamster genomic DNA surrounding Exon 1C. The nucleotide sequences corresponding to Exon 1C or its surrounding introns are represented in capital or lowercase letters, respectively. The putative transcription initiation site (T) is numbered as +1. The splice-donar sequences (gt) are underlined and putative binding sites for transcription factors are indicated with dashes. B: Nucleotide sequences of normal hamster genomic DNA surrounding 5′ BP and 3′ BP. The nucleotide sequences deleted in CM hamster are italicized. 5′ BP or 3′ BP, which are shared by BIO14.6, UMX7.1, TO-2, and CHF146, are indicated by arrowheads. The conserved nucleotides in CM hamster closest to 5′ BP or 3′ BP are underlined and numbered as −1 and +1, respectively. The primers used for PCR are located by arrows. The nucleotide sequences for normal hamster genomic DNA surrounding 5′ BP or 3′ BP, CM hamster genomic DNA spanning 5′ BP and 3′ BP, and normal hamster genomic DNA including Exon 1C have been deposited in DDBJ, EMBL, and GenBank databases with accession numbers AB020230, AB020231, AB020232, and AB020233, respectively.

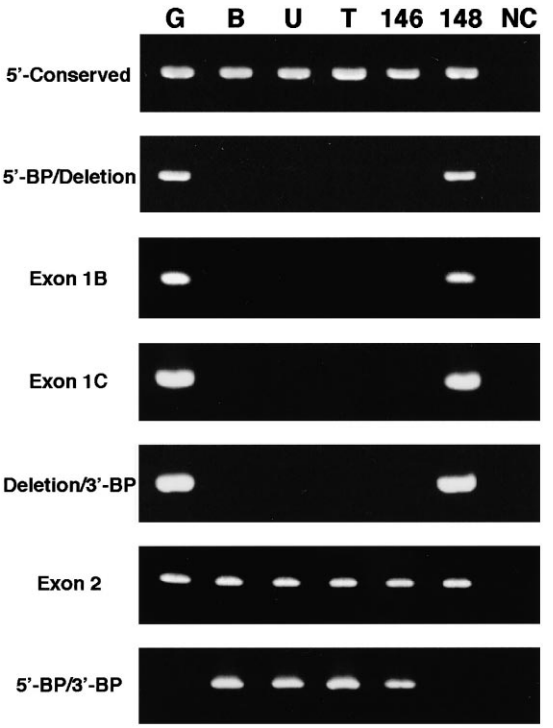

Fig. 3. Detection of selective genomic regions of normal and CM hamsters by PCR. The sizes of PCR products for 5′ Conserved, 5′ BP/Deletion, Exon 1B, Exon 1C, Deletion/3′ BP, Exon 2, and 5′ BP/3′ BP are 232, 276, 115, 116, 180, 150, and 361 bp, respectively. G, Golden; B, BIO14.6; U, UMX7.1; T, TO-2; 146, CHF146; 148, CHF148. NC, negative control without template DNA.

3: 5′-BP/Deletion and Deletion/3′-BP). In all the CM hamsters, it was confirmed by PCR that 5′ upstream regions from 5′ BP and Exon 2 were conserved (Fig. 3: 5′-Conserved and Exon 2) and that Exon 1B and Exon 1C were deleted (Fig. 3: Exon 1B and Exon 1C). We demonstrated that CHF148, congenic normal hamster which was established by backcrossing of unrelated normal Golden hamster to CHF146, completely conserves the genomic interval deleted in CM hamster (Fig. 3: CHF148), which was also confirmed by genomic Southern blot analysis (data not shown).

It is well known that genomic deletion in cancerous or neuromuscular diseases is frequently associated with tandem repeat sequences on both sides of the deletion, which causes inter- and/or intra-chromosomal rearrangement [20,21]. However, no tandem repeat sequences were found in the vicinities of 5′ BP and 3′ BP of the normal hamster genome (Fig. 2B). The molecular basis of the genomic deletion in CM hamster requires further study.

3.3. *Relative expression levels of δ-SG transcripts spliced with the three distinct first exons in various tissues of normal and CM hamsters*

The amount of RT-PCR products targeting to Exon 2 is considered to reflect the total amount of functional δ-SG transcripts, because the protein coding region of δ-SG gene starts from Exon 2 [15]. In normal hamster, δ-SG, like β-SG [5,6], is expressed abundantly in smooth muscle (stomach) as well as in striate (cardiac and skeletal) muscles (Fig. 4: Normal; Exon 2), which was in marked contrast to α- and γ-SG predominantly expressing in striate muscles [5,6]. Relative expres-

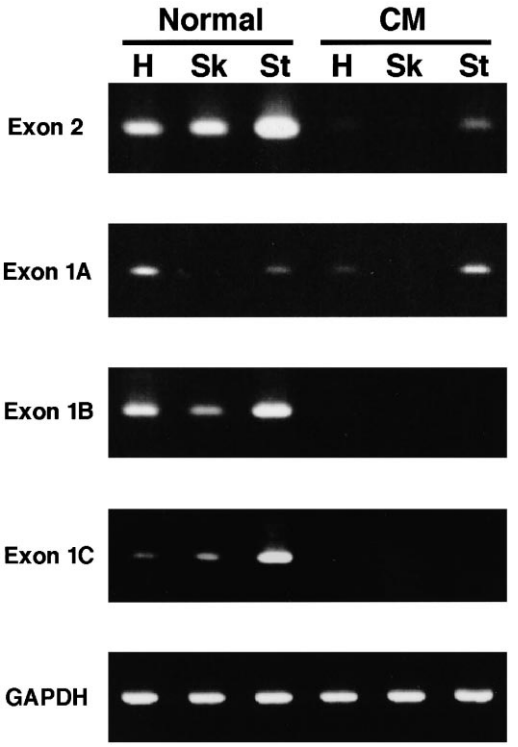

Fig. 4. RT-PCR analysis of δ-SG transcripts in various tissues of normal and CM hamsters. The sizes of PCR products for Exon 1A, Exon 1B, Exon 1C, Exon 2, and GAPDH were 305, 288, 303, 150, and 452 bp, respectively. Normal, normal Golden; CM, cardiomyopathic BIO14.6. H, heart; Sk, skeletal muscles; St, stomach.

sion levels of δ-SG transcripts spliced with Exon 1A, Exon 1B, and Exon 1C were quite different among cardiac, skeletal, and smooth muscles of normal hamster (Fig. 4: Normal; Exon 1A, Exon 1B, and Exon 1C). In cardiac muscle, preference of the first exons of δ-SG transcripts was ordered from Exon 1B, Exon 1A down to Exon 1C. In skeletal muscle, on the other hand, δ-SG transcript with Exon 1C was comparable in amount to that with Exon 1B, while δ-SG transcript with Exon 1A was undetectable. Expression levels of δ-SG transcripts in smooth muscle were in the following order: Exon 1B, Exon 1C, and Exon 1A. In all the tissues of normal hamster tested, Exon 1B was considered to be the major first exon for δ-SG transcript.

Next, we analyzed δ-SG transcripts in CM hamster by RT-PCR. In cardiac or skeletal muscles of CM hamster, faint or no bands corresponding to Exon 2 were detected by RT-PCR, respectively (Fig. 4: CM; Exon 2), which was in good agreement with the previous data shown by RNA blot analysis [15]. As was predicted from the genomic deletion of Exon 1B and Exon 1C, none of the transcripts for δ-SG spliced with these two first exons were detected in any tissue of CM hamster (Fig. 4: CM; Exon 1B and Exon 1C). It was noteworthy that a detectable band for Exon 2 was amplified by RT-PCR in smooth muscle (stomach) of CM hamster (Fig. 4: CM; Exon 2). The δ-SG transcripts expressing in smooth and cardiac muscles of CM hamster were considered to be spliced with the conserved Exon 1A (Fig. 4: CM; Exon 1A). It is well known that smooth muscle of CM hamster is little affected [11], which could be partially explained by the appreciable amount of δ-SG transcript present in smooth muscle of CM hamster.

3.4. *Screening of potential exons which locate in the genomic interval deleted in CM hamster and express in the heart of normal hamster*

RNA blot analysis using a full-length cDNA for δ-SG [15] as a probe detected 9.5 kb and 1.4 kb predominant transcripts for δ-SG in normal hamster heart, both of which were missing in the heart of CM hamster (Fig. 5A). These two predominant bands were hybridized with the Exon 1B-specific probe (Fig. 5B: 1B), showing that both of the transcripts for δ-SG were spliced with Exon 1B. The probe specific to Exon 1A, which was conserved in CM hamster, did detect two faint transcripts (11.0 kb and 9.5 kb) using 30 μg poly(A)$^+$ RNA of both normal and CM hamster hearts (Fig. 5B: 1A). However, the amount of these two transcripts was too scarce to be detected by the full-length δ-SG probe in 5 μg poly(A)$^+$ RNA of normal and CM hamster hearts (Fig. 5A). The δ-SG transcript spliced with Exon 1C could be detected by RT-PCR (Fig. 4: Normal; Exon 1C) but not by RNA blot analysis using the probe specific to Exon 1C (Fig. 5B: 1C). Taken together with the data by RT-PCR, Exon 1B was the major first exon of δ-SG in hamster heart, deletion of which was considered to cause dramatic reduction of δ-SG transcript in the heart of CM hamster.

We searched for potential exons which locate in the genomic interval deleted in CM hamster and express in the heart of normal hamster. For this purpose, 12 restriction fragments of the cloned normal genomic DNAs encompassing the deletion interval for CM hamster were used as probes for RNA

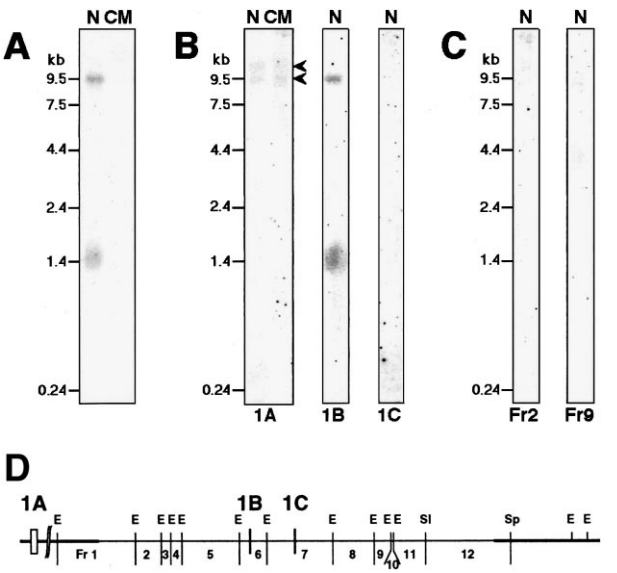

Fig. 5. A: RNA blot analysis using a full-length cDNA for δ-SG. Five μg of poly(A)$^+$ RNA from hamster heart per lane. Molecular markers (kb) are indicated in the left. N, normal Golden; CM, cardiomyopathic BIO14.6. B: Detection of δ-SG transcripts spliced with Exon 1A, Exon 1B, and Exon 1C by RNA blot analysis. Thirty μg of poly(A)$^+$ RNA from hamster heart per lane. Two faint bands detected by Exon 1A-specific probe are indicated by arrowheads. 1A, Exon 1A; 1B, Exon 1B; 1C, Exon 1C. C: Screening of potential exons within the genomic deletion interval for CM hamster by RNA blot analysis. Representative data using Fr2 and Fr9 as probes are shown. Thirty μg of poly(A)$^+$ RNA from normal hamster per lane. D: Nomenclature of the genomic DNA probes to screen potential exons. Fr1 through Fr12 are the restriction genomic DNA fragments of normal hamster, which encompass the genomic deletion interval for CM hamster depicted as a thin line. 1A, Exon 1A; 1B, Exon 1B; 1C, Exon 1C. E, *Eco*RI; Sl, *Sal*I; Sp, *Spe*I.

blot analysis (Fig. 5D: Fr1–Fr12). Among the 12 genomic probes, only Fr6 and Fr7 detected transcripts in normal hamster heart, which were identical to the ones detected by Exon 1B- and Exon 1C-specific probes (Fig. 5B: 1B, 1C), respectively (data not shown). The other 10 genomic probes could not detect any transcript, even if up to 30 μg of poly(A)$^+$ RNA was subjected to the analysis. The representative data using Fr2 and Fr9 were shown in Fig. 5C. These data strongly suggested that δ-SG is the only transcript encoded in the genomic interval deleted in CM hamster, which is virtually missing in the heart of CM hamster.

3.5. *δ-SG: a novel candidate causative gene for autosomal recessive cardiomyopathy*

Very recently, mice with null mutation of α- or γ-SG genes were generated by a gene targeting technique [22,23], both of which develop muscular dystrophy. Cardiac involvement in these mouse models was very interesting. No histopathological changes were observed in the heart of α-SG deficient mice [22] but mice deficient in γ-SG developed prominent cardiomyopathy [23], which clearly demonstrates that mutation in some of the SG genes by itself could cause cardiomyopathy at least in mice. In hamster, mutation of the δ-SG gene is considered to cause cardiomyopathy, because δ-SG seems to be the only transcript defective in the heart of CM hamster. In humans, there are several unequivocal case reports on cardiomyopathy associated with LGMD where SG proteins are missing [9,24], although mutation in one of the SG genes remains to be elucidated. Taken together, some of the SG genes seem to be strong candidate genes causing autosomal recessive cardiomyopathy.

From the standpoint of gene therapy [25], identification of cardiomyopathic patients with mutated SG genes is important for the following reasons, even if they share a minor population of human hereditary cardiomyopathy. First, recessive disease could be rescued in principle by transferring a normal counterpart of the mutated gene into an affected organ, because, unlike in the case of dominant disease, the protein product of the transfected normal cDNA is less likely to be interfered by the mutated gene product of the disease [25]. Second, packaging of functional cDNAs for the SGs into a gene-transfer vector is physically suitable [25], because the protein coding regions of cDNAs for all the SGs are less than 1 kb [5,6]. Indeed, muscular dystrophy of CM hamster was successfully restored by adenoviral-mediated somatic gene transfer [26]. In conclusion, δ-SG gene could be a novel candidate gene for autosomal recessive cardiomyopathy, for which CM hamster would be a promising animal model to develop gene-transfer technique.

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