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Striated Muscle Preferentially Expressed Genes α and β Are Two Serine/Threonine Protein Kinases Derived from the Same Gene as the Aortic Preferentially Expressed Gene-1*

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Aortic preferentially expressed gene (APEG)-1 is a 1.4-kilobase pair (kb) mRNA expressed in vascular smooth muscle cells and is down-regulated by vascular injury. An APEG-1 5′-end cDNA probe identified three additional isoforms. The 9-kb striated preferentially expressed gene (SPEG)α and the 11-kb SPEGβ were found in skeletal muscle and heart. The 4-kb brain preferentially expressed gene was detected in the brain and aorta. We report here cloning of the 11-kb SPEGβ cDNA. SPEGβ encodes a 355-kDa protein that contains two serine/threonine kinase domains and is homologous to proteins of the myosin light chain kinase family. At least one kinase domain is active and capable of autophosphorylation. In the genome, all four isoforms share the middle three of the five exons of APEG-1, and they differ from each other by using different 5′- and 3′-ends and alternative splicing. We show that the expression of SPEGα and SPEGβ is developmentally regulated in the striated muscle during C2C12 myoblast to myotube differentiation in vitro and cardiomyocyte maturation in vivo. This developmental regulation suggests that both SPEGα and SPEGβ can serve as sensitive markers for striated muscle differentiation and that they may be important for adult striated muscle function.

Vascular smooth muscle cells (VSMCs) play an important role in regulating vascular tone by contraction and relaxation under normal physiological conditions. Their excessive growth and extracellular matrix secretion, however, significantly contribute to various occlusive vascular diseases such as arteriosclerosis and restenosis after balloon angioplasty (1, 2). Although the molecular mechanisms regulating these VSMC phenotypic modulations have not been fully elucidated, one theory holds that VSMCs are not terminally differentiated and that their phenotype can change from a quiescent and contractile state to a proliferative and synthetic state in vascular pathogenesis (1, 3).

In contrast to the plastic phenotype of VSMCs, the striated skeletal muscle cells and cardiomyocytes undergo terminal differentiation during perinatal and neonatal stages (4–6). In skeletal muscle, the terminal differentiation of myoblasts into myotubes is marked by the permanent withdrawal from the cell cycle and the induction of muscle-specific structural genes by myogenic transcriptional factors (e.g. MyoD, myogenin, Myf5, and MRF4) (4, 7). In the heart, the maturation of cardiomyocytes from hyperplasia to hypertrophy takes place soon after birth, and they also permanently withdraw from the cell cycle (6, 8, 9). Multiple myofibrillar protein genes are regulated during maturation and terminal differentiation of striated muscles. Much of this regulation involves switches of various myofibrillar proteins from the fetal/neonatal isoforms to the adult isoforms. Isoforms of these myofibrillar proteins may derive from multigene families or from alternative splicing and/or use of different promoters in the same gene (for review, see Ref. 10). For instance, the cardiac troponin T (cTnT) is expressed in the human heart as four isoforms (cTnT1 through cTnT4) generated by alternative splicing of two 5′ exons. The cTnT3 is the dominant adult isoform in the heart, whereas the other three isoforms are expressed in the fetal heart (11). It is interesting that the fetal cTnT4 isoform is also re-expressed in failing adult hearts (11).

Aortic preferentially expressed gene (APEG)-1 was originally cloned by differential mRNA display as a 1.4-kb mRNA preferentially expressed in adult VSMCs (12). It is a single copy gene, and analysis of its promoter demonstrates that a 2.7-kb mouse APEG-1 5′-flanking sequence confers VSMC-specific reporter gene expression (13). The expression of APEG-1 mRNA rapidly disappears in primary culture of VSMCs at early passages (12). In the rat carotid artery balloon injury model APEG-1 mRNA is significantly decreased 2 and 4 days after injury but recovers slightly after 8 days (12). On the basis of these observations we propose that APEG-1 serves as a sensitive marker specific for VSMC differentiation.

We report here the identification of three additional APEG-1 isoforms expressed in the striated muscles and in the brain. These isoforms, termed striated muscle (S)PEGα, SPEGβ, and brain (B)PEG, were identified by using the 5′-end portion of APEG-1 cDNA in tissue Northern blot analysis and were de-
rived from the same gene locus as APEG-1. The largest 11-kb SPEG β isoform was cloned and characterized. The SPEG proteins contained a functional serine/threonine kinase domain. In the heart, the SPEG α and SPEG β were expressed only after birth, as the APEG-1 was down-regulated. This isoform switch from APEG-1 to SPEG α and SPEG β correlated with the timing of neonatal heart maturation. The developmental regulation of both SPEG α and SPEG β suggested that they can serve as sensitive differentiation markers for the striated muscles and that they may be important for adult striated muscle function.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Reagents**—C2C12 myoblasts were obtained from the American Type Cell Culture Collection, and 293T cells were obtained from Dr. Hamid Band (Dana Farber Cancer Institute, Boston) (14). C2C12 cells were grown in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 20% fetal calf serum (HyClone), 4 mM l-glutamine, penicillin (100 units/ml), and streptomycin (100 µg/ml) in a humidified incubator (37 °C, 5% CO₂). 293T cells were grown similarly except that they received 10% fetal calf serum and were maintained in a 10% CO₂ incubator. C2C12 cells were differentiated into myotubes in Dulbecco’s modified Eagle’s medium supplemented with 1% insulin (HyClone).

SPEG cDNA Cloning and Sequencing—A mouse APEG-1 cDNA 5'-end probe that contained the open reading frame was used to screen an adult mouse heart 5'-stretch plus cDNA agt11 library (CLONTECH). Positive clones were purified, and cDNA inserts were excised by EcoRI digestion and ligated into plasmid vectors. Subsequent screening of the same cDNA library was carried out with the newly obtained cDNA fragments that extended at either the 5'- or the 3'-end. Both 5'- and 3'-RACE was performed according to the protocols from the manufacturer (Life Technologies, Inc.). The primers used in 5'-RACE experiment are as follows: 5'-GGTGGACAGGCTCTATTACC-3', 5'-CT- GGGTCTCCGCACATGGC-3', and 5'-GATGATCACTCATCGGAGAT- CTCCAG-3'. The primers used in 3'-RACE were 5'-GGGGAGTTGGATCCTC- TCCAG-3' and 5'-GCGGCGGACAGCAGACCTT-3'.

**GENOMIC LIBRARY SCREENING AND SEQUENCE ANALYSIS**—A 129/SvJ mouse genomic library in αFII vector (Stratagene) was screened by a mouse SPEG cDNA 5' probe. The genomic DNA was sequenced by the dyeoxy chain termination method with primers designed from the mouse SPEG cDNA sequence to determine the exon-intron junctions. The intron sizes were determined by polymerase chain reactions (PCR) with primers in the flanking exons and by direct sequencing.

DNA Extraction and Northern Blot Analysis—Total RNA from cultured cells was prepared by the RNeasy Mini Kit (Qiagen). Tissue RNA was extracted from adult male Harlan Sprague-Dawley rats or from adult male Balb/c mice (Taconic Farms) as described (12). Rat hearts at post-coitum) and from rats 2, 14, and 28 days after birth. Ten different developmental stages were obtained from fetuses (17–19 days after transfection cells were prepared by the RNeasy Mini Kits (Qiagen). Tissue RNA was extracted from adult male Harlan Sprague-Dawley rats or from adult male Balb/c mice (Taconic Farms) as described (12). Rat hearts at post-coitum) and from rats 2, 14, and 28 days after birth. Ten different developmental stages were obtained from fetuses (17–19 days after birth). The RNA was fractionated on 1.3% formaldehyde-agarose gels and transferred to nylon membranes. The membranes were blocked by 5% nonfat milk in TBST (12.5 mM Tris-HCl, pH 7.6, 75 mM NaCl, and 0.1% Tween 20) and incubated with appropriate primary antibodies (1:2000) monoclonal antibody to FLAG (M2) (Sigma), to myosin heavy chain (MY-32) (Sigma), to α-tubulin (DM 1A) (Sigma), and affinity-purified polyclonal antibody to APEG-1/1SPEG. After three washes in TBST, the blots were incubated with horseradish peroxidase-conjugated secondary antibodies (1:4000), and chemiluminescent results were obtained by exposure to x-ray films.

**Immunohistochemical Analysis**—Adult mouse hearts were fixed in methyl Carnoy’s fixative (60% methanol, 30% chloroform, 10% acetic acid) for 3 h and embedded in paraffin. Heart sections were processed for immunostaining essentially as described (16). In brief, sections were incubated with an affinity-purified antibody to APEG-1/USPEG that was generated in rabbit against a FLAG peptide-tagged APEG-1 fusion protein (see below) and negative control staining, the same antibody was preabsorbed with the FLAG-APEG-1 fusion protein (1:1 by weight) at 4 °C overnight before incubation. After washing, a biotinylated goat antibody to rabbit IgG (Vector Laboratories) and an avidin-horseradish peroxidase were sequentially applied to the sections. Staining results were developed with a peroxidase DAB kit (Vector Laboratories).

**Immunofluorescent Staining**—Differentiated C2C12 cells (for 5 days) grown on glass coverslips and mouse heart sections (see above) were used for staining as described (12). The C2C12 cells were fixed for 4% paraformaldehyde. The heart sections and C2C12 cells were incubated with both the affinity-purified rabbit antibody to APEG-1/USPEG and a mouse monoclonal antibody to desmin (Sigma) at 4 °C overnight. After washing, an Alexa 488-conjugated goat antibody to mouse IgG and an Alexa 594-conjugated goat antibody to rabbit IgG were applied to the sections and coverslips at room temperature for 2 h before washing. Localization of desmin and SPEG protein signals was observed by fluorescent microscopy.

**RESULTS**

**Identification of BPEG, SPEGα, and SPEGβ**—By using a full-length APEG-1 cDNA probe, we identified four different isoforms in adult rat and mouse tissues by Northern analysis (Fig. 1, A and B, respectively). In addition to the 1.4-kb APEG-1 mRNA, two messages of 9 and 11 kb were found in adult tissues containing striated muscles such as skeletal muscle and heart (Fig. 1). A 4-kb message was also seen in the brain and aorta (Fig. 1). The 9- and 11-kb messages were not identified previously by an APEG-1 3' cDNA probe obtained by differential mRNA display (data not shown and also Fig. 5A, IV). We termed the 9- and 11-kb messages SPEGα and SPEGβ, respectively, for their preferential expression in the striated muscle, and the 4-kb message BPEG for its expression in the brain. Since we have shown that APEG-1 is a single copy gene in the genome (13), we speculated that these isoforms were products of the same gene locus as APEG-1.

**Cloning and Analysis of SPEGβ cDNA**—To obtain the SPEGβ cDNA sequence, we screened an adult mouse heart cDNA library using an APEG-1 cDNA 5'-end probe. An 11-kb SPEGβ cDNA contig was assembled from several cDNA library screenings and both 5'- and 3'-RACE (Fig. 2A). We repeated the 5'-RACE twice using different sets of primers and confirmed the same 5'-end of the SPEGβ cDNA 4 kb not shown. An open reading frame was identified between two frames 144 and 9299 with no other upstream ATG initiation codon. Multiple step codons were also found in all three reading frames in the 5'-untranslated region (data not shown). The deduced 3262-residue SPEGβ peptide sequence (Fig. 2B) had a predicted molecular mass of 355 kDa. The APEG-1 peptide sequence was found at residues 855–965 of the SPEGβ protein with the
except its last two amino acids (Fig. 2B). A PROSITE data base search revealed that the SPEGβ protein contained two putative serine/threonine kinase catalytic sites and one ATP-binding site (Fig. 2B) (17). A BLAST search in the nonredundant protein data base identified a partial human KIAA1297 protein (18) with a 75% identity to the SPEGβ protein, suggesting that the KIAA1297 is a human counterpart of the SPEGβ. Furthermore, significant homology was identified between the SPEGβ and proteins of the myosin light chain kinase (MLCK) family including MLCK, titin, twitchin, projectin, and death-associated protein kinases. By using ProDom and PFam data base analyses (19, 20), we found that the homology between SPEGβ and these proteins was not limited to the kinase domains but extended to multiple immunoglobulin and fibronectin structural domains (Fig. 2B and 3A). These domains have been implicated in homophilic or heterophilic interaction of other myosin-binding proteins (21). We hypothesized that the SPEGβ and potentially the other three isoforms (APEG-1, BPEG, and SPEGα) were part of the functionally and structurally diverse MLCK protein family (22).

Activity of the SPEG Kinase Domain—We compared the kinase domains of SPEGβ and several other homologous proteins (Fig. 3A). This region is defined to contain 11 subdomains and is conserved among the serine/threonine kinases (23, 24). The internal putative kinase domain of SPEGβ (SPEG-i) showed a 37% identity (98 of 268 residues) to the consensus sequence of the other kinases (Fig. 3A). In addition, all but one of the 15 conserved residues within the kinase domain are identified in the internal kinase domain of SPEGβ (Fig. 3A, see asterisks) (23, 24). Due to the divergence in the ATP-binding region (subdomains I and II) and subdomain VII in the carboxyterminal putative kinase domain of SPEGβ (SPEG-c), there was only a 24% identity (64 of 268 residues) to the other kinases (Fig. 3A). We therefore chose to test the biochemical activity of the internal putative kinase domain of SPEGβ. A SPEG minikinase plasmid that expressed a FLAG-tagged SPEG internal kinase domain was constructed. The sequence of the SPEG minikinase included 29 serine and threonine residues, and in an in vitro kinase assay this minikinase was capable of autophosphorylation (Fig. 3B). As a positive control we used a non-related FLAG-tagged Akt, which is also a serine/threonine protein kinase, in the same experiment. This FLAG-Akt fusion protein showed autophosphorylation (Fig. 3B) as previously reported (25).

Genomic Analysis of the SPEGs—We previously reported that APEG-1 is a single copy gene (13). We also found that sequences corresponding to the first and the last exons of APEG-1 were absent in the 11-kb SPEGβ cDNA sequence, suggesting that SPEGβ was produced by another upstream promoter and by alternative splicing in a tissue-specific manner. To study how all four isoforms were transcribed from a single copy gene, we obtained additional genomic DNA flanking the APEG-1. From the genomic DNA clones we identified multiple SPEGβ exons both upstream and downstream of APEG-1 (Fig. 4). The identified exon-intron junctions (Fig. 4) were all in agreement with the consensus 5′-GT and 3′-AG sequences (26). In addition, the first exon of APEG-1 was located in an ~9-kb SPEGβ intron, and the last APEG-1 exon was within another downstream intron (Fig. 4). The APEG-1 promoter (13) was located within the ~8-kb intronic sequence between the first exon of APEG-1 and the immediate upstream SPEGβ exon.

Northern Analysis of the APEG-1 and Its Isoforms by Exon-specific cDNA Probes—To elucidate further the use of different exons by the four different isoforms, we performed a series of Northern blot analyses with various APEG-1 and SPEG cDNA probes (Fig. 5A). The last exon of APEG-1 hybridized only to the APEG-1 and BPEG messages (Fig. 5A, IV). This exon contained an in-frame stop codon at its 5′-end and a polyadenylation signal, and was likely the last exon of the BPEG as it is of the APEG-1 (13). All four isoforms (APEG-1, BPEG, SPEGα, and SPEGβ) were recognized by a cDNA probe derived from the middle three exons of APEG-1 (Fig. 5A, III). This result was consistent with our initial finding (Fig. 1) and implied that these three exons were used by all isoforms, since excluding any of the three exons would have caused a shift in the reading frame. The probe derived from the first APEG-1 exon hybridized to the 1.4-kb APEG-1 and the 9-kb SPEGα (Fig. 5A, II), suggesting that these two isoforms initiated their transcription from the intronic APEG-1 promoter. The immediate upstream SPEGβ exon hybridized only to the 4-kb BPEG and the 11-kb SPEGβ (Fig. 5A, I), indicating that the transcription for these two isoforms initiated from at least one other promoter upstream of the APEG-1. We had also tested a SPEGβ cDNA probe further upstream of the APEG-1 that gave results similar to those in panel I of Fig. 5A (data not shown). Two cDNA probes corresponding to the two putative SPEG kinase domains downstream of APEG-1 hybridized to both SPEGα and SPEGβ (Fig. 5A, V and VI), demonstrating that these two isoforms contained both kinase domains and that neither the APEG-1 nor the BPEG did. Taken together, we hypothesized that the selective use of two transcription start sites and alternative splicing in tissue-specific manners gave rise to the four different isoforms (Fig. 5B). Besides their tissue
distribution, the distinction between SPEG and SPEG\(b\) and between APEG-1 and BPEG was their 5'end promoter selection. Moreover, alternative splicing differentiated SPEG\(b\) from BPEG and SPEG\(a\) from APEG-1. However, we could not exclude the possibility that SPEG\(b\) and BPEG used different promoters due to their distinct tissue distribution.

**Induction of SPEG Expression during C2C12 Myoblast Differentiation**—We previously showed that APEG-1 is a sensitive marker for VSMC differentiation (12). We speculated that SPEG expression is also regulated by striated muscle differentiation, and we examined the expression of SPEG\(a\) and SPEG\(b\) during C2C12 cell differentiation from myoblasts to myotubes (Fig. 6). In the rapidly growing C2C12 myoblasts, both SPEG\(a\) and SPEG\(b\) were minimally expressed (Fig. 6A). Increased expression of the 9-kb SPEG\(a\) could be detected when the C2C12 cells became confluent (Fig. 6A). A significant induction of SPEG\(a\) mRNA was observed when these cells were induced to differentiate and formed myotubes. The 11-kb SPEG\(b\) message, however, remained at a minimal level (Fig. 6A). We next confirmed the induction of SPEG protein by an affinity-purified antibody to APEG-1/SPEG in Western blot analysis. This polyclonal antibody was generated in rabbit against a FLAG-tagged APEG-1 fusion protein. It cross-reacted with the SPEG\(a\), SPEG\(b\), and BPEG because of their shared peptide
sequences. We found that an ∼250-kDa protein was induced by the C2C12 differentiation (Fig. 6B). The size of this identified protein was in agreement with what we had predicted for the SPEGα protein (residues 855–3262 of SPEGβ, Fig. 2B). To assess further the association between SPEGα induction and C2C12 differentiation, we examined the expression of skeletal myosin heavy chain (Fig. 6B), a differentiation marker for skeletal muscle (27). We found that myosin heavy chain was expressed as early as 1 day and continued to increase through 5 days of differentiation, showing a concordant expression pattern between SPEGα and myosin heavy chain. The induction of SPEGα protein in differentiated C2C12 myotubes was further demonstrated by immunofluorescent staining of these cells by C.-M. Hsieh, S. Fukumoto, M. D. Layne, K. Maemura, H. Charles, A. Patel, M. A. Perrella, and M.-F. Lee, unpublished results.
promoter was used by both APEG-1 and SPEG
APEG-1 and last
sequences of several
SPEG
 correlates on the
size (excluding poly(A) tail length, in kilobase) of each isoform is indi-
cated by dashed lines.
The shaded boxes had not been fully characterized and were shown as

Fig. 5. Northern analysis of APEG-1, BPEG, SPEG, and SPEGα. A, Northern analysis of mouse tissues using APEG-1 isoform-specific probes. Six probes (dashed lines) were generated from different regions of APEG-1 and SPEG cDNAs and used to hybridize with identical blots (I–VI) containing total RNA (10 μg/lane) from mouse brain (B), heart (H), skeletal muscle (S), and liver (L). B, generation of APEG-1, BPEG, and SPEG mRNAs by two transcription initiation sites and alternative splicing. The same upstream transcription initiation site may be used by APEG-1 and BPEG, whereas the intronic APEG-1 probe was used by both APEG-1 and SPEGα. The APEG-1 and SPEGα mRNAs used the last exon of APEG-1, whereas the SPEGα and SPEGβ ended with another downstream exon. The predicted mRNA size (excluding poly(A) tail length, in kilobase) of each isoform is indicated on the right.

the antibodies to APEG-1/SPEG and desmin (Fig. 6). We observed that SPEGα, as recognized by the antibody to APEG-1/SPEG, was found only in differentiated C2C12 myotubes but not in the surrounding individual cells. The expression pattern of the SPEGα protein in the differentiated and undifferentiated myotubes was identical to that of the desmin protein, a sarcomeric protein expressed in differentiated striated muscles. Thus, SPEGα may serve as a sensitive marker for skeletal muscle differentiation at both the mRNA and the protein levels.

SPEGα/β and APEG-1 Expression during Maturation and Terminal Differentiation of the Heart—To test whether the induction of SPEGα and SPEGβ also occurred during cardiomyocyte maturation, we isolated RNA from rat heart at different developmental stages and performed Northern analysis (Fig. 7A). By using a probe that recognized all four isoforms, we detected strong APEG-1 and minimal BPEG expression in fetal hearts (17–19-day rat fetuses). Neither SPEGα nor SPEGβ was detected at this stage, which suggested that APEG-1 was the predominant embryonic isoform expressed in the heart, despite its preferential expression in adult VSMCs (Fig. 1). After birth, the APEG-1 mRNA decreased significantly in neonatal hearts, and the two SPEG messages began to increase (Fig. 7A). Two and four weeks after birth, the APEG-1 expression decreased further to less than 20% of its prenatal level (Fig. 7B). In the contrary, the two SPEG messages continued to increase, and together they reached close to 70% of the prenatal APEG-1 expression level at 4 weeks (Fig. 7B). The time course of the isoform switch from APEG-1 to SPEGα and SPEGβ showed a strong correlation with neonatal cardiomyocyte maturation (28). This finding was consistent with the aforementioned induction of SPEGα during skeletal muscle differentia-
tion in vitro and implied a functional importance of SPEGα and SPEGβ in the maturation and terminal differentiation of cardiomyocytes.

SPEG Proteins Colocalized with Desmin to the Sarcomeric Z Bands in Cardiomyocytes—We immunohistochemically stained the mouse heart to localize the distribution of SPEG proteins. The antibody to APEG-1/SPEG used in this study recognized all isoforms. An 18 S rRNA hybridization was used to show loading of RNA. B, quantitative analysis of APEG-1 and SPEGα and β expression during heart maturation. The expression levels of APEG-1 and SPEGα and β were first normalized by their corresponding 18 S rRNA signals. APEG-1 expression in the embryo heart was then set as 100% and used to compare with the expression of APEG-1 (solid columns) and the SPEGα and β (white columns) at different stages of heart maturation.

![Fig. 7. Isoform switch from APEG-1 to SPEGα and SPEGβ during neonatal heart maturation.](Image)

**DISCUSSION**

We identified three additional isoforms of APEG-1 termed SPEGα, SPEGβ, and BPEG. SPEGα and SPEGβ were demonstrated to be serine/threonine kinases (Fig. 3B) derived from the same gene locus as APEG-1 by both differential promoter usage and alternative splicing (Fig. 5). Our results suggest that SPEGα and SPEGβ expression, like APEG-1 expression in VSMCs, serve as sensitive markers for striated muscle cell differentiation (Figs. 6 and 7). SPEGα was up-regulated during C2C12 myotube formation at both mRNA and protein levels. This implicated SPEGα as a differentiation marker for skeletal muscle cells. Interestingly, the induced SPEGα expression and the low level of SPEGβ in differentiated C2C12 myotubes were contrary to their respective expression levels in adult skeletal muscle in vivo (Fig. 1). This may reflect that C2C12 myotubes, despite their extensive fusion and occasional spontaneous contraction, were not as terminally differentiated as skeletal muscle in vivo. Moreover, both SPEGα and SPEGβ were expressed at similar levels in adult heart. This would argue that SPEGβ is also a marker for striated muscle differentiation and may represent a differentiation state of striated muscle later than that represented by SPEGα. Indeed, in the embryonic stem cell to embryoid body differentiation model, SPEGα expression was detected 2 days prior to SPEGβ expression (7 and 9 days after differentiation, respectively) (data not shown).
APEG-1 Isoforms in Striated Muscle

Since APEG-1 expression is down-regulated by vascular injury (12), we had speculated that SPEG expression in the heart might also be suppressed by cardiomyopathy. However, we detected little change in SPEG expression in a cardiac hypertrophy and dilated cardiomyopathy animal model (data not shown) (29). It is interesting that the APEG-1 mRNA level was increased by 2-fold in the hypertrophic heart (data not shown). Although this increase may have been a result of increased vascularization in cardiomyopathy, it was also possible that the cardiomyocytes re-expressed APEG-1 in response to hypertrophic cardiomyopathy. The latter is consistent with our observation that APEG-1 was the dominant embryonic isoform in the failing heart (11, 30, 31).

The SPEG proteins are homologous to the MLCK family, which includes titin, twichin, and MLCK (Fig. 3A (22)). We found that the protein sequences of APEG-1 and SPEGαβ shared significant homology with those of telokin and MLCK, respectively. Both APEG-1 and telokin are proteins that contain an immunoglobulin-like domain (Fig. 2B and (32)) and are a small portion of a larger muscle kinase (SPEGαβ and MLCK, respectively). telokin is also transcribed from a promoter located within a 3′-end intron of MLCK (33), which is similar to the genomic organization of APEG-1 and SPEG (Fig. 4). Telokin can accelerate myosin light chain dephosphorylation and relaxation (34), reduces MLCK binding to myosin filaments, and modulates the oligomeric state of MLCK (35). These direct functional modulations of MLCK by telokin, however, may not apply to APEG-1 and SPEG proteins due to their differences in developmental expression and adult tissue distribution (Figs. 1 and 7). Since APEG-1 was expressed in embryonic hearts and later switched to SPEGs and SPEGαβ during neonatal heart maturation (Fig. 7), it is still possible that the APEG-1 and SPEG proteins interact with similar proteins.

Although many proteins of the MLCK family contain a calcium/calmodulin-binding domain that regulates their kinase activity, we did not find any amphipathic α-helical structure (36) within the SPEG protein sequence that might bind calcium/calmodulin. The presence of immunoglobulin, fibronectin type III, and kinase domains in SPEG proteins is reminiscent of the much larger protein titin. The immunoglobulin and fibronectin type III domains have been implicated in homophilic and heterophilic interaction between proteins (21). Since SPEG proteins and the amino terminus of titin are colocalized to the sarcomeric Z bands (Fig. 8 (37)), it is interesting to speculate whether an interaction between SPEG proteins and titin exists in striated muscle cells. Future studies will also focus on understanding the biological significance of the various isoforms of APEG-1 and whether the isoform switch from APEG-1 to SPEGα and SPEGβ is important for the terminal differentiation of the striated muscle cells.

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