An Alternate Promoter Directs Expression of a Truncated, Muscle-specific Isoform of the Human Ankyrin 1 Gene*

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Ankyrin 1, an erythrocyte membrane protein that links the underlying cytoskeleton to the plasma membrane, is also expressed in brain and muscle. We cloned a truncated, muscle-specific ankyrin 1 cDNA composed of novel 5' sequences and 3' sequences previously identified in the last 3 exons of the human ankyrin 1 erythroid gene. Northern blot analysis revealed expression restricted to cardiac and skeletal muscle tissues. Deduced amino acid sequence of this muscle cDNA predicted a peptide of 155 amino acids in length with a hydrophobic NH₂ terminus. Cloning of the corresponding chromosomal gene revealed that the ankyrin 1 muscle transcript is composed of four exons spread over ~10 kilobase pairs of DNA. Reverse transcriptase-polymerase chain reaction of skeletal muscle cDNA identified multiple cDNA isoforms created by alternative splicing. The ankyrin 1 muscle promoter was identified as a (G+C)-rich promoter located >200 kilobase pairs from the ankyrin 1 erythroid promoter. An ankyrin 1 muscle promoter fragment directed high level expression of a reporter gene in cultured C2C12 muscle cells, but not in HeLa or K562 (erythroid) cells. DNA-protein interactions were identified in vitro at a single Sp1 and two E box consensus binding sites contained within the promoter. A MyoD cDNA expression plasmid transactivated an ankyrin 1 muscle promoter fragment/reporter gene plasmid in a dose-dependent fashion in both HeLa and K562 cells. A polyclonal antibody raised to human ankyrin 1 muscle-specific sequences reacted with peptides of 28 and 30 kDa on immunoblots of human skeletal muscle.

Erythrocyte ankyrin, ankyrin 1, is the prototype of a family of homologous proteins that are involved in the local segregation of integral membrane proteins within functional domains on the plasma membrane (1–4). This important cellular localization of membrane proteins may be provided by the relative affinities of the many different isoforms of ankyrin for target proteins. This specialization appears to have evolved through the tissue-specific, developmentally regulated expression of multiple protein isoforms. The molecular mechanisms by which ankyrin has acquired distinct isoforms with specialized function(s) are beginning to be revealed. The isoform diversity of ankyrin arises from both different gene products and from differential, alternative splicing of the same gene product (5–9).

In humans, the cDNAs for three ankyrin proteins have been cloned and their gene products studied. These ankyrins share similar antigenic sites and domain structures, differing in a number of ways such as their cellular patterns of expression and their relative affinities of binding to spectrin and band 3. Ankyrin binding has been described for a variety of proteins including membrane skeleton proteins, ion transport proteins, and cell adhesion molecules (1, 4).

Ankyrin 1, first discovered in preparations of erythrocyte membranes, provides the principal linkage between the spectrin-actin based erythrocyte membrane skeleton and the plasma membrane (1, 10–12). The primary structure of human ankyrin 1, deduced from cDNA clones obtained from a reticulocyte cDNA library, encodes a mature protein of 1881 amino acids (7, 8). Ankyrin 1 has been identified in erythrocyte tissue, brain, and muscle (7, 8, 13–17). The major form of ankyrin 1, ~210 kDa, is composed of three domains, an 89-kDa NH₂-terminal domain composed of 24 conserved repeats known as cdc 10/ankyrin repeats that contain the binding site for band 3; a 62-kDa domain that contains the binding sites for spectrin and vimentin; and a 55-kDa COOH-terminal regulatory domain (1, 2, 4). Complex patterns of alternative splicing have been identified in the region encoding the regulatory domain (5, 13, 14, 17). The precise role(s) of the regulatory domain is unknown, but it does appear to modulate spectrin and band 3 binding (18, 19). Defects of ankyrin 1 have been implicated in approximately half of all patients with hereditary spherocytosis (20, 21).

Initial studies in muscle immunolocalized ankyrin to the sarcolemma adjacent to the Z lines co-distributed with spectrin, as well as at the neuromuscular junction, and at the muscle triads (22–26). Studies performed in muscle cells suggested that ankyrin accumulation and assembly into the membrane was determined by a control mechanism operative at the posttranslational level, triggered near the time of cell fusion and onset of terminal differentiation (27). Northern blot analyses by Birkenmeier and colleagues using an erythroid ankyrin 1 cDNA probe encoding the regulatory domain identified multiple transcripts in murine skeletal muscle RNA (13, 28). These transcripts ranged in size from 1.6 to 3.5 kb, compared with the 7.5 and 9 kb ankyrin 1 transcripts observed on Northern blots of erythrocyte RNA (29, 30). Northern blot analysis of RNA from chicken myotubes using an ankyrin 1 cDNA fragment as probe also identified a small 3.6-kb transcript (31).

Isoform diversity in different muscle cell types is frequently determined by the presence of muscle type-specific isoforms (32, 33). These isoforms may be encoded by separate genes, may be generated by alternative splicing of a given gene, or
may be controlled by specific regulatory elements in or around a given gene at different times. For example, numerous iso-
forms of spectrin with varying patterns of cellular localization and developmental expression have been identified in muscle
cells (3, 34–36). These isoforms are the products of separate
genes or alternative splicing of individual genes (3, 36, 37).
Recent studies have identified two populations of ankyrin 1 in
muscle cells (38). One population was identified at the sarco-
lemma using an antibody to the spectrin binding domain of
ankyrin. This localization is similar to previous observations. A
second population was identified at the M and Z lines using an
antibody to sequences identified previously in neural isoforms
of ankyrin 1, also present in muscle (38).

This report describes the cloning of a novel, truncated, mus-
cle-specific ankyrin 1 isoform, characterization of its corre-
sponding genomic structure, study of its pattern of expression, and identification of its promoter. Because of similarities de-
tected on Western blotting, the isoform described here is likely to be the one detected at the sarcoplasmic reticulum, providing
additional evidence that two populations of ankyrin 1 are pres-
ent in muscle. These observations extend the molecular basis of
ankyrin 1 isoform diversity to include the use of an alternate
NH2 terminus and a tissue-specific alternate promoter.

MATERIALS AND METHODS

RNA Preparation and Northern Blot Analyses—Total RNA was pre-
pared from human skeletal muscle, or from the human tissue culture
cell lines RD (human rhabdomyosarcoma, embryonal, ATCC 136-CCL),
K562 (chronic myelogenous leukemia in blast crisis with erythroid
characteristics, ATCC CCL 243), and HeLa (epithelioid carcinoma,
cervix, ATCC CCL 2) as described previously (39). Multiple-tissue
Northern blots containing 2 μg of poly(A)+ mRNA per tissue were
obtained from CLONTECH (Palo Alto, CA). A human β-actin cDNA
probe was used as a control for loading in Northern blot analyses (40).

cDNA and Genomic DNA Cloning—An 838-bp cDNA fragment
was generated by 5’ RACE and PCR using primers A and B (Table I) with
human skeletal muscle cDNA as template. This fragment, which con-
tains the entire coding region of the human ankyrin 1 muscle-specific
cDNA (see below) was used as the hybridization probe to screen a random- and oligo(dT)-primed human skeletal muscle cDNA library in
Agt1 (CLONTECH). A human ankyrin 1 cDNA fragment, pAnk15 (8),
containing the 3’ end of the human ANK1 muscle transcript was used
as a hybridization probe to screen a human genomic DNA library. The
library is a Charon 4A bacteriophage library containing fragments of
genomic DNA partially digested with AluI and HaelII with EcoRI
linkers added. For both library screens, selected recombinants
hybridized to the screening probes were purified, subcloned, and ana-
yzed by standard techniques.

Rapid Amplification of cDNA Ends (RACE)1—5’ RACE was per-
duced as described (41, 42). 1 μg of human skeletal muscle mRNA was
reverse transcribed using primer C (see Table I). Single-stranded oli-
gonucleotide ligation and PCR amplification were carried out using
primer D and primers A and B, respectively. Amplification products
were subcloned and sequenced.

Primer Extension Analyses—The transcription start site of the mus-
cle-specific ankyrin 1 cDNA isoform was determined using primer ex-
tension analysis. Primers E or F (see Table I) were used in primer
extension reactions as described elsewhere (43). Templates in these
reactions were 20 μg of total RNA from the human cell lines RD and
HeLa, or 10 μg of DNA.

Cell Culture—The tissue culture cell lines C2C12 (murine muscle
myoblast, ATCC 1772-CRL), RD, K562, and HeLa were used to study
expression of the putative promoter of the muscle-specific isoform of
the ankyrin 1 gene. C2C12, RD, and K562 cells were maintained in RPMI
1640 medium with 10% fetal calf serum. HeLa cells were maintained in
Eagle’s minimal essential medium with 10% fetal calf serum. C2C12
cells were maintained as myoblasts for all experiments described.

Preparation of Promoter-Reporter Plasmids—Test plasmids were
prepared by inserting a 2.1-kb fragment of the 5’-flanking ankyrin 1

1 RACE, rapid amplification of cDNA ends; HS, hereditary spherocy-
tosis; PCR, polymerase chain reaction; bp, base pair(s); kb, kilobase
pair(s).
muscle-specific genomic DNA upstream of the firefly luciferase reporter
gene in the plasmid pGL2B (Promega, Madison, WI). Serial truncations of
this 2.1-kb fragment in the pGL2B plasmid were constructed using
convenient restriction enzyme sites or PCR amplification. Test plas-
mids were sequenced to exclude cloning or PCR-generated artifacts.

Transplant Transfections and Transactivation Assays—All plasmids
were transfected using Qiagen columns (Qiagen, Inc., Chatsworth,
CA) and at least two preparations of each plasmid were tested. 105
K562 cells were transfected by electroporation with a single pulse of 300
V at 960 uF with 20 μg of test plasmid and 0.5 μg of pCMVβ, a
mammalian reporter plasmid expressing β-galactosidase driven by
the human cytomegalovirus immediate early gene promoter (CLONTECH).
105 C2C12 or HeLa cells were transfected with 2.0 μg of test plasmid
and 0.25 μg of the pCMVβ plasmid by lipofection using 4 μl of Lipo-
fectAMINE (Life Technologies, Inc.). Twenty-four hours after transfe-
c tion, cells were harvested and lysed, and the activity of both luciferase
and β-galactosidase activity was determined in cell extracts. All assays
were performed in triplicate. Differences in transfection efficiency were
determined by co-transfection with the pCMVβ plasmid. For transacti-
vation assays, K562 and HeLa cells were transfected using 5 and 1 μg
of reporter plasmid, respectively, and varying amounts of a MycD cDNA
expression plasmid, pMyoD (EMBL no. X56677), and reporter gene
activity were assayed as above.

Gel Mobility Shift Analyses—Nuclear extracts were prepared from
RD, C2C12, K562, and HeLa cells by hypotonic lysis, followed by high
salt extraction of nuclei as described by Andrews and Faller (44).
Binding reactions were carried out as described (45, 46). Competitor
oligonucleotides were added at molar excesses of 10- or 100-fold. Re-
sulting complexes were separated by electrophoresis through 6% poly-
acylamide gels at 21 °C.

Immunoblot Analyses—A rabbit-specific polyclonal antibody was
raised to a synthetic peptide, ISPBRVBRRVFLKGN, conjugated to
keyhole limpet hemocyanin and bovine serum albumin (Immuno-Dy-
namics, La Jolla, CA). The sequence of this peptide is contained in
the novel, muscle-specific region of ankyrin 1. After 12 weeks, anti-peptide
antisera was collected, then affinity purified on a column to which the
synthetic peptide had been covalently linked. Human erythocyte mem-
brane and skeletal muscle homogenates were prepared as described
previously (47, 48). These erythroid and muscle fractions were sepa-
rated by SDS-polyacrylamide gel electrophoresis on a 4–20% gel and
either stained with Coomassie Blue or transferred onto nitrocellulose
and immunoblotted. Immunoblotting was performed as described
elsewhere (49).

Computer Analyses—Computer-assisted analyses of derived nucleo-
tide and predicted amino acid sequences were performed utilizing
the sequence analysis software package of the University of Wisconsin
Genetics Computer Group (UW GCG; Madison, WI) (50) and the BLAST
algorithm, National Center for Biotechnology Information (Bethesda,
MD) (51).

RESULTS

Identification of Novel Ankyrin 1 Sequences in Muscle—
Northern blot analyses of human skeletal muscle RNA with
human ankyrin 1 erythroid cDNA probes encoding the repeat-
domain (pAnk58), the spectrin-binding domain (pAnk37),
or the regulatory domain (pAnk15) (8) yielded hybridization sig-
nals of 2.3 and 1.6 kb only when the regulatory domain probe
was used (see below). These results are in contrast to Northern
blot analyses of human erythroid RNA using these probes
where hybridization signals of 7.3 and 9.0 kb are seen with all
three domain-specific probes (7, 8). To identify the molecular
basis of these truncated transcripts, we performed 5’ RACE
using oligonucleotide primers A (sense, linker) and B and C
(antisense, both in the 3′-untranslated region of the erythroid
cDNA), with total human skeletal muscle RNA as a template.

This 838-bp skeletal muscle RACE product was
identified using the BLAST algorithm, National Center for Biotechnology Information (Bethesda, MD) (51).

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(antisense, both in the 3′-untranslated region of the erythroid
cDNA), with total human skeletal muscle RNA as a template.

This 838-bp skeletal muscle RACE product was
used as probe to screen a human skeletal muscle cDNA library. Eight clones that hybridized to the screening probe were iso-
lated after primary screening of a human skeletal muscle cDNA library. Three clones were purified, subcloned and se-
quenced (Fig. 1). The clones varied in size from 1096 to 2543 bp. All three clones contained 5′-untranslated sequence, an open reading frame of 528 bp (the same identified in the RACE cDNA library. Three clones were purified, subcloned and se-
quences of clones 6 and A47 are denoted by solid and dashed lines, respectively.

The predicted initiator methionine is located at positions 251–253 (Fig. 2A). The sequences around this translation start site match important consensus sequences, specifically, there is an A in position –3 from the predicted initiator methionine (53). A termination codon is located 150 bp upstream of the predicted initiator methionine; no additional ATGs are present in the intervening 150 bp. Deduced amino acid sequence of the open reading frame predicts a peptide of 155 amino acids with a predicted molecular mass of 17.6 kDa and a pI of 6.5. Sec-
ondary structure predictions of the muscle-specific protein predict the presence of two domains, a highly charged NH2-termi-
nal domain followed by a COOH-terminal domain composed of alternating α helix and β sheet. These sequences do not contain the membrane binding domain, the spectrin/fodrin binding do-
main, and most of the regulatory domain found in the erythroid ankyrin 1 gene transcript.

Two overlapping genomic DNA clones were isolated that contained the entire ankyrin 1 muscle cDNA sequence. Analy-
ysis of its structure revealed that this transcript is composed of 4 exons spread over ~10 kb of DNA (Fig. 3, top). There is a novel exon one, followed by sequences present in the erythroid cDNA transcripts encoded by exons 40, 41, and 42. This novel exon 1, labeled 39a in Fig. 3, is located in intron 39 of the erythroid gene. The first and fourth exons contain untranslated sequences; the 5′-untranslated region is 250 bp in length. Comparison of the exon/intron boundaries with reported consensus sequences reveals that the aggt rule was not violated at any splice junction (54, 55). There are no AG dinucleotides within the 15 bp upstream of the 3′ (acceptor) splice junctions. The coding sequences for the hydrophobic domain of the ankyrin 1 muscle protein are contained entirely within the novel exon one.

Additional RACE products identified three additional mus-
cle-specific isoforms generated by alternative splicing (Fig. 4, isoforms 2–4). Comparison of these isoforms with the genomic organization demonstrate that these four isoforms vary in their usage of exon 41. The function of these isoforms is unknown. In erythroid and neural tissue, complex patterns of alternative splicing of exon 41 have been observed (5). However, the pat-
ters of alternative splicing observed in muscle cDNA differ from those observed in erythroid and neural cDNA isoforms.

**Expression of the Novel Ankyrin 1 Exon Is Restricted to Cardiac and Skeletal Muscle Tissue**—Northern blot analysis using the 838-bp ankyrin 1 muscle cDNA RACE product de-
tected abundant mRNAs of 2.3 and 1.6 kb in cardiac and skeletal muscle tissues (Fig. 5). Signals of 3.7 and 7.0 kb were also detected, but in lesser amounts compared with 2.3 and 1.6 kb. These signals may represent ankyrin 1 muscle transcripts generated by alternative splicing, or, for the 7.0-kb signal, cross-hybridization with the erythroid ankyrin 1 isoform.

Identification of the Ankyrin 1 Gene Muscle Promoter—The nucleotide sequence of the 5′-flanking genomic DNA upstream of the human ankyrin muscle cDNA transcription start site is shown in Fig. 6. Inspection of the sequences reveals features characteristic of a muscle-specific gene promoter including lack of consensus CCAAT sequences and a high G + C content (61%, between nucleotides –245 and +18).

To investigate if this 5′-flanking DNA was capable of direct-
ing expression of a reporter gene in cultured cells, transient transfection assays were performed. A test plasmid containing a DNA fragment from about –2100 to –14 fused to a luciferase reporter gene was transfected into muscle (C2C12), erythroid (K562), or nonerythroid (NIH3T3) cells. The relative luciferase activity was determined 24 h after transfection and compared with the activity obtained with pGL2B, a negative control, the promoterless plasmid, and pGL2P, a positive control, the lucif-
erase reporter gene under control of the SV40 early promoter. As shown in Fig. 7, the muscle ankyrin 1 gene promoter plas-
mid, p-2100, directed high level expression of the luciferase reporter gene in muscle cells, but not in erythroid and noner-
throid cells. Deletional analysis of this 21-bp ankyrin 1 gene promoter fragment identified a 170-bp minimal promoter frag-
ment, p-184, that directed ankyrin 1 gene muscle-specific ex-
pression. This DNA fragment contains two E boxes and an Sp1 site, a combination shown to be adequate for expression of a minimal promoter in other muscle-specific genes.

**The Human Ankyrin 1 Gene Muscle Promoter Contains Binding Sites for Sp1 and MyoD**—Consensus sequences for a num-
ber of potential DNA-binding proteins, including Sp1, GATA-1, and two E boxes were present in the ankyrin 1 gene muscle
FIG. 2. Nucleotide sequence with predicted amino acid sequence of human ankyrin 1 muscle cDNA. A, a composite nucleotide sequence shown was determined from clones l2, l6, and RACE products. The initiation codon and the termination codons are double underlined.
promoter (Fig. 6). E boxes are binding sites for members of the MyoD family of basic helix-loop-helix transcription factors that are important in controlling muscle-specific gene expression.

To determine if nuclear proteins could bind these sites in vitro, double-stranded oligonucleotides containing the corresponding ankyrin 1 muscle sequences (Sp1 G + H; E box left I + J; E...
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box-right K + L; GATA M + N; Table I) or control sequences (Sp1 O + P (56, 57); E box Q + R (58); GATA-1 S + T (59)) were prepared and used in gel shift analyses.

When either of the E box site-containing oligonucleotides were used in gel shift analyses, a single retarded species was observed in RD (muscle) nuclear extracts (Fig. 8). This species migrated at the same location as a control oligonucleotide containing the left E box of the murine creatine kinase gene (58). For both ankyrin 1 E boxes, the single species was effectively competed by an excess of homologous unlabeled oligonucleotide, the other ankyrin 1 muscle E box oligonucleotide, and the creatine kinase control oligonucleotide. Nuclear extracts from K562 and HeLa cells did not bind either of the ankyrin 1 E box oligonucleotides or the control creatine kinase E box oligonucleotide.

When oligonucleotides containing the ankyrin 1 muscle promoter Sp1 site or a control, high affinity Sp1 binding site (56, 57) were used in gel shift analyses, major and minor complexes were observed in RD nuclear extracts (Fig. 9A). The ankyrin 1 muscle complexes are both competed by an excess of homologous unlabeled oligonucleotide and the control Sp1 oligonucleotide. The ankyrin 1 muscle promoter Sp1 oligonucleotide competed most, but not all of the complex formed by the control Sp1 oligonucleotide in RD extracts. Similar results were obtained when K562 and HeLa extracts were used with these oligonucleotides in gel shift analyses (not shown).

An oligonucleotide containing the ankyrin 1 muscle promoter GATA motif did not form any complexes in gel shift analyses when RD, K562, or HeLa extracts were used. This oligonucleotide did not compete the complexes formed by an oligonucleotide containing the erythroid ankyrin 1 promoter GATA-1 sequence (Fig. 9B) (59).

**MyoD Transactivates the Human Muscle Ankyrin 1 Gene Promoter in Heterologous Cells**—None of the ankyrin 1 muscle promoter fragments directed expression of a reporter gene in K562 or HeLa cells, but the addition of MyoD by co-transfection conferred promoter activity to these fragments. Co-transfection of 1 µg of the ankyrin 1 minimal muscle promoter/reporter plasmid, p-184, and increasing amounts of a MyoD cDNA expression plasmid into HeLa cells resulted in increasing promoter activity with increasing amounts of MyoD plasmid (Fig. 10, top). Similar results were observed in co-transfection experiments in K562 cells (Fig. 10, bottom). The ability of MyoD to transcriptionally activate the ankyrin 1 muscle promoter in these cells which do not contain this muscle-specific factor, correlates with the inability of the ankyrin 1 muscle promoter to function in these cells.

**Immunoblotting**—Immunoblots of human erythrocyte membrane ghosts and skeletal muscle homogenates using the affinity-purified anti-peptide antibody 2401, raised against sequences unique to the ankyrin 1 muscle isoform, detected bands of 28 and 30 kDa in skeletal muscle (Fig. 11). Longer exposures revealed a band at 70 kDa in both skeletal muscle and erythrocyte membranes and a band at 210 kDa in erythrocyte membranes. A polyclonal antibody raised to ankyrin 2.1 from erythrocyte membranes (kindly supplied by Jon S. Morrow) detected bands of 205 and 210 kDa in erythrocyte membranes (Fig. 11). Long exposure demonstrated a band of 210 kDa in skeletal muscle. The identity of the 70-kDa band detected in skeletal muscle homogenates and erythrocyte membranes is unknown, but it was highly reproducible and was identified in immunoblots of RD cells (not shown).

**Computer Analyses**—When compared with sequences present in available data bases, significant homology was demonstrated only between the novel human ankyrin 1 muscle-specific gene sequence and a corresponding murine sequence. The identity between the translated sequence of the human ankyrin 1 muscle isoform and the translated murine sequence was 91% with a similarity of 94%. Searching using only the highly charged 73 amino acid NH2 terminus also failed to reveal any significant homologies.

**DISCUSSION**

The diversity of the numerous ankyrin family isoforms appears to be critical for specific cellular functions. Of the three ankyrin family proteins cloned, ankyrin 1 is considered to have the most limited pattern of expression, with expression restricted to erythroid, muscle and neural tissue. Despite these “limitations,” the ankyrin 1 erythroid cDNA has at least 15 different transcripts generated by alternative splicing and/or alternative polyadenylation (5). The identification of a muscle-
tissue-specific isoform with multiple transcripts generated by alternative splicing under the control of an alternate, tissue-specific promoter adds to this diversity. Interestingly, truncated isoforms of ankyrin 3, the ankyrin isoform with the widest pattern of tissue distribution, have been localized to the cytoplasm and Golgi apparatus of kidney and muscle cells as well as to the lysosomes of macrophages (6, 60–62). These truncated isoforms, however, lack only the NH2-terminal membrane-binding domain.

The regulation of truncated, tissue-specific isoforms of the ankyrin 1 gene by the use of an alternate promoter is similar to that observed in MCL1/3 or dystrophin gene transcripts (63–68). In dystrophin, five autonomous promoters direct the transcription of respective alternate first exons in a cell-specific and developmentally controlled manner (63). Two of these promoters direct the expression of transcripts encoding only the COOH terminus of dystrophin, utilizing exons 56–79 or exons 63–79, respectively, in a manner similar to muscle ankyrin 1, which utilizes exons 40–42. Remarkably, like ankyrin-1, the tissue-specific promoters of dystrophin may be remote (>100 kb) from each other.

The functions of the two populations of ankyrin 1 in muscle are unknown. The co-localization of the 210-kDa ankyrin iso-
form with spectrin at the sarcomere suggests a role for ankyrin in providing a linkage between the membrane skeleton to the plasma membrane, as it does in the erythrocyte. The truncated ankyrin 1 isoform lacking the membrane and spectrin binding

FIG. 9. Gel shift analyses of the Sp1 and GATA-1 consensus binding sites in the human ankyrin 1 muscle promoter. A, gel shift analyses were performed with [32P]ATP-labeled, double-stranded oligonucleotides containing the Sp1 consensus binding site of the ankyrin 1 muscle promoter and a control oligonucleotide containing a high affinity Sp1 binding site and nuclear extracts from RD muscle cells. Protein-DNA complexes migrating at the same location were obtained using both double-stranded oligonucleotides. Unlabeled double-stranded oligonucleotides added in excess to the binding reactions effectively competed the protein-DNA complexes. Similar results were obtained when K562 and HeLa nuclear extracts were used with these oligonucleotides in gel shift analyses (not shown). B, gel shift analyses were performed with [32P]ATP-labeled, double-stranded oligonucleotides containing the GATA-1 consensus binding site of the ankyrin 1 muscle promoter and a control oligonucleotide containing the GATA-1 binding site of the ankyrin 1 erythroid promoter and nuclear extracts from K562 erythroid cells. A protein-DNA complex was obtained only when the control oligonucleotide was used. Homologous, unlabeled ankyrin 1 erythroid GATA-1 control oligonucleotide added in excess to the binding reaction effectively competed the control protein-DNA complex in K562 cells, but the ankyrin 1 muscle GATA-1 oligonucleotide did not. In addition, the ankyrin 1 muscle GATA-1 oligonucleotide did not form any protein-DNA complexes when RD muscle cell, or HeLa cell nuclear extracts were used in gel shift analyses (not shown).
domains localized to the Z and M lines of internal myofibrils and was highly enriched in the sarcoplasmic reticulum (38). The hydrophobic NH2 terminus of the truncated ankyrin 1 isoform could insert into the sarcoplasmic reticulum membrane, with the COOH terminus serving as a ligand for myosin proteins. The specificity of the truncated ankyrin 1 for different protein ligands could be provided by the isoforms generated by alternative splicing. The antibody used to immunolocalize the truncated muscle ankyrin isoform was raised to sequences shared by ankyrin 1 neural and muscle cDNA isoforms (5, 38). There were similarities detected on immunoblots of skeletal muscle using this antibody and our muscle-specific antibody 2401. Together, these data suggest that the isoform described here is likely to be the same one detected at the sarcoplasmic reticulum. The sequence of this isoform does not match any others in available data bases, suggesting that this may represent a novel class of proteins.

Defects in ankyrin 1 are the most common cause of typical hereditary spherocytosis (HS) in humans. Interestingly, kindreds with HS and co-segregating myopathic manifestations have been described, including two brothers with HS, a movement disorder and myopathy (69), and a three-generation Russian kindred with co-segregating HS and hypertrophic cardiomyopathy (70). It is tempting to speculate that these patients have a mutation in the very 3′ end of the ankyrin 1 gene in the region that is common to both erythroid and muscle gene in the region that is common to both erythroid and muscle expression of a gene that is expressed in both cell types (71–74). It will be important to identify the factors that control cardiac- and skeletal muscle-expressed in both cell types (71–74). It will be important to identify the factors that control cardiac- and skeletal muscle-specific expression of ankyrin 1, as this information may aid in the identification of the defects in HS patients with co-segregating skeletal muscle or cardiac myopathic symptoms. One potential regulatory factor is GATA-4, a member of the GATA family of transcription factors expressed in cardiac and foregut potential regulatory factor is GATA-4, a member of the GATA family of transcription factors expressed in cardiac and foregut.

Identification of the defects in HS patients with co-segregation of skeletal muscle or cardiac myopathic symptoms. One potential regulatory factor is GATA-4, a member of the GATA family of transcription factors expressed in cardiac and foregut potential regulatory factor is GATA-4, a member of the GATA family of transcription factors expressed in cardiac and foregut.

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