Multiple promoters direct expression of three AKAP12 isoforms with distinct subcellular and tissue distribution profiles

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Summary

A Kinase Anchoring Protein 12 (AKAP12; also known as SSeCKS and Gravin), is a multivalent anchoring protein with tumor suppressor activity. Although expression of AKAP12 has been examined in a number of contexts, its expression control remains to be elucidated. Here we characterized the genomic organization of the AKAP12 locus, its regulatory regions, and the spatial distribution of the proteins encoded by the AKAP12 gene. Using comparative genomics and various wet-lab assays, we show that the AKAP12 locus is organized as three separate transcription units that are governed by non-redundant promoters coordinating distinct tissue expression profiles. The proteins encoded by the three AKAP12 isoforms (designated α, β, and γ) share >95% amino acid sequence identity but differ at their N-termini. Analysis of the targeting of each isoform reveals distinct spatial distribution profiles. An N-terminal myristoylation motif present in AKAP12α is shown to be necessary and sufficient for targeted expression of this AKAP12 isoform to the endoplasmic reticulum, a novel subcellular compartment for AKAP12. Our results demonstrate heretofore unrecognized complexity within the AKAP12 locus and suggest a mechanism for genetic control of signaling specificity through distinct regulation of alternately targeted anchoring protein isoforms.
Introduction

Transduction of intra- and extra-cellular signals is a tightly regulated process requiring the coordination of multiple signaling pathways to direct the appropriate physiological response to these signals. Correct spatial assembly or targeting of signaling components confers specificity to activated signaling cascades. In most cases, such targeting appears to be regulated by adaptor, scaffolding, and anchoring proteins that facilitate the assembly of multiprotein signaling complexes at various regions of the cell (1). The A Kinase Anchoring Protein (AKAP) family of proteins is a prototypic class of anchoring proteins that coordinate the co-localization of the cAMP-dependent protein kinase (PRKA) with its physiological substrates (2). Many members of this family also scaffold other signaling proteins, such as PRKC and PP2B (3,4). The composition and expression of these complexes are thus important determinants of a cell’s response to stimuli.

AKAP12 is the official gene symbol of a group of orthologous proteins that includes human gravin/AKAP250 and the rodent Src-Suppressed C Kinase Substrate (SSeCKS). As its name implies, AKAP12 binds the regulatory subunit of PRKA via a C-terminal amphipathic helix similar to that of other AKAPs (5). The ability to bind PRKC (6), calmodulin (7), and PP2B (8) in addition to PRKA suggests that AKAP12 functions to assemble a multiprotein signaling complex at sites of its localization. Recent work has demonstrated that AKAP12 can assemble such a complex at the plasma membrane in association with the β-adrenergic receptor (8). However, previous studies have demonstrated cytosolic distribution of AKAP12 in addition to membrane association (9), suggesting that AKAP12 has multiple functions in different cellular locales.

While several interacting partners for AKAP12 have been identified, the downstream targets of AKAP12-moderated signaling remain undefined. Although the mechanisms of AKAP12 function are still being investigated, several studies have implicated AKAP12 in the control of cytoskeletal architecture and cell-cycle progression (10,11). Whereas experimental overexpression of AKAP12 results in cell
cycle arrest (11), endogenous expression is severely attenuated in transformed cells (12). Consistent with a role in growth control, many mitogenic stimuli transiently increase AKAP12 expression (13,14). More recently, oxygen tension has been shown to be an important regulator of AKAP12 expression in astrocytes during formation of the blood-brain barrier (15). Collectively, these studies highlight the importance of control of AKAP12 expression in determining cell function.

In the present study, we have characterized the AKAP12 gene to establish a foundation for further study of AKAP12 regulation. Interestingly, the AKAP12 locus encodes for three separate mRNAs (AKAP12α, β, γ) that are regulated by unique promoters and differentially expressed in various organs. While the proteins encoded by each isoform are highly similar, a myristoylation motif present in AKAP12α targets the protein to the endoplasmic reticulum. Deletion or mutation of the myristoylation motif results in cytosolic distribution similar to that of the other isoforms. Our data illustrate the complexity of the AKAP12 locus and demonstrate a novel mechanism of genetic control of spatial and temporal signal compartmentalization through transcriptional regulation of alternately targeted AKAP isoforms.
Experimental Procedures

Cell Culture

A7r5 rat aortic smooth muscle cells, Rat-2 rat fibroblasts, NRK rat kidney epithelial cells, Clone-9 rat liver epithelial cells, GC-2spd(ts) mouse spermatocytes, and Cos-7 rat kidney fibroblasts were all purchased from American Type Culture Collection. NIH-3T3 mouse fibroblasts were kindly provided by Dr. Wang Min (Yale University School of Medicine). A7r5 and 3T3 cells were maintained in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, 100 µg/ml streptomycin, and 250 ng/ml amphotericin B. Rat-2, NRK, GC-2, and Cos-7 cells were maintained in DMEM supplemented with 10% FBS. Clone-9 cells were maintained in Ham’s F12 media supplemented with 10% FBS. GC-2 cells were maintained at 32°C; all other cells were maintained at 37°C.

5’ RACE/Transcription start site mapping

Two independent assays were used to define the 5’ boundaries of the three AKAP12 isoforms. 5’ RACE was performed using the FirstChoice RLM-RACE kit (Ambion) per manufacturer’s directions and primers specific for each isoform. Rat testis cDNA served as the template for the α and γ isoforms and rat kidney cDNA was used for the β isoform. In all cases, a single band was amplified (data not shown) and subcloned into pBluescript II SK+ for sequencing. At least two independent clones were sequenced for each isoform. Transcription initiation start site mapping using RNAse protection was performed as previously described (16) using isoform-specific riboprobes.

Comparative Genomics

Identification of conserved regions around the promoter regions of the three AKAP12 isoforms was performed using VISTA (http://www-gsd.lbl.gov/vista/index.shtml) (17). Briefly, either the entire
locus or 10 kb regions surrounding exons 1, 3, or 4 of human and rat AKAP12 were downloaded with the aid of the UCSC Genome Browser (http://genome.ucsc.edu/) (18). Each exon was then annotated before submitting the sequences to VISTA for comparison. For comparison of the entire locus a window length of 75 base pairs was used to determine percent identity. A window length of 50 base pairs was used in comparison of the promoter regions. We acknowledge the Human Genome Sequencing Consortium (19), the Mouse Genome Sequencing Consortium (20), and the Rat Genome Sequencing Project Consortium (21) for making available early draft sequence that was used in this report.

PAC library screening

The RPCI-31 rat bacteriophage P1 artificial chromosome (PAC) library (22) was screened with a probe to exon 5 of AKAP12 to obtain a template for cloning the AKAP12 promoters. The exon 5 probe was amplified from rat cDNA using primers as follows: Exon5Forward-5’GATACGGATCCCCAGGATGGGGAAGCTGA; Exon5Reverse-5’GATACAAGCTTTTCCTTGCTCTTCTTCTTGG. Briefly, radiolabeled probe was hybridized for two hours to five membranes of the PAC library using standard hybridization techniques followed by copious washing to remove unbound probe. Positive clones were visualized following autoradiographic exposure (Kodak X-Omat AR, Rochester, NY). Four PAC clones were identified and were screened by PCR using primers to exons 1 and 3 to confirm the PACs contained the full locus. Two clones, RPCI-31 311H10 and RPCI-31 489O7, were found to contain the full AKAP12 locus. Initial sequencing in the promoter regions of both clones did not reveal any differences; subsequent cloning was only performed using the 489O7 clone.

Reporter Constructs

Promoter constructs for the AKAP12α promoter were designed to encompass conserved regions identified with VISTA. All constructs were amplified from the 489O7 PAC clone using PfuUltra High-Fidelity polymerase (Stratagene) with restriction enzyme-clamped primers and then cloned into Mulu-
XhoI digested pGL3-Basic (Promega). Promoter constructs for the AKAP12β promoter were similarly designed to encompass conserved regions identified with VISTA. To clone the -396, -646, -2300, and -5000 constructs, an XbaI fragment encompassing exon 3 was excised from the 489O7 PAC clone and inserted into pBluescript II SK+(Stratagene). Restriction enzymes were then used to subclone these inserts into Smal cut pGL3-Basic as follows: -396- 5′-NcoI-SanDI-3′, blunted; -646- 5′-PvuII-SanDI-3′, blunted; -2239- 5′-Af III-SanDI-3′, blunted; -5399- 5′-XbaI-SanDI-3′, blunted. Insert orientation was determined by PCR. The short constructs +1, -120, and -250 were amplified as above using the -2239 reporter as template with restriction enzyme clamped primers and sequenced to confirm identity. The promoter constructs for AKAP12γ were amplified as for AKAP12α using restriction clamped primers and cloned into MluI-XhoI digested pGL3-Basic. The putative AKAP12γ enhancer was amplified by PCR and cloned into SalI-BamHI digested pGL3-Promoter (Promega). All PCR-generated inserts were sequenced to confirm integrity, with the exception of the long AKAP12α reporter constructs, -2032, -4304, and -4764. These inserts were end sequenced only; however, multiple independent constructs were tested to ensure no aberrant changes had occurred in the non-sequenced regions.

Luciferase Reporter Assays

Cells were seeded in 24-well dishes, allowed to adhere overnight, and then transfected with luciferase reporter constructs using Lipofectamine2000 (Invitrogen). The amount of each reporter used was adjusted to account for size variations between the constructs, with the largest construct set as the upper limit at 450 ng/well. pBluescript II SK+ was added to equalize the amount of DNA transfected per condition. The renilla luciferase reporter pRL-TK (Promega) was co-transfected at 50 ng/well to normalize for transfection efficiency between conditions. All constructs were transfected in triplicate and assayed 48 hours post transfection as previously described (16). Data presented is a representative experiment of at least three independent experiments. Error bars represent S.E.M.
Nuclear Extracts and Gel Shifts

Nuclear Extracts from Rat-2 fibroblasts were prepared using the NE-PER Nuclear and Cytoplasmic Extract Kit (Pierce) per manufacturer’s instructions. For examination of USF-1 binding to the E boxes within the AKAP12α and β minimal promoter, gel shifts were performed as follows. Nuclear extracts were incubated on ice for 10 minutes in gel shift buffer (40 mM KCl, 5 mM HEPES, pH 7.9, 2 mM EDTA, 0.4 mM MgCl₂, 2 mM spermidine, 0.2 mM DTT, 1 μg/μl BSA, and 50 ng/μl poly dI:dC) with or without the indicated competitors. End-labeled probes for the AKAP12α and β promoters were then added and the reaction was incubated for 20 minutes at room temperature. For supershifts, either rabbit IgG or rabbit anti-USF-1 (Santa Cruz) were added as indicated and all reactions were further incubated at room temperature for 20 minutes. Nucleoprotein complexes were resolved on nondenaturing 4% polyacrylamide gels and then visualized following autoradiographic exposure.

Tissue isolation, RT-PCR, and Northern Blotting

Indicated tissues were isolated from either C57BL/6 mice or Sprague-Dawley rats, snap frozen in liquid nitrogen, and homogenized with a tissue homogenizer (Polytron) in Trizol (Invitrogen). Total RNA was then purified according to manufacturer’s directions. For RT-PCR, cDNA was prepared from total RNA using the FirstStrand cDNA synthesis kit (Amersham) per manufacturer’s directions. The 5’ end of the α, β, and γ transcripts was then amplified using a reverse primer to exon 5 and forward primers to exons 1, 3, or 4, respectively. For analysis of human AKAP12γ expression, human multiple tissue blots were purchased from Clontech and hybridized with a probe from exon 4 of human AKAP12.

Recombinant AKAP12 Constructs

Construction of FLAG-tagged AKAP12 constructs was performed in several steps. First, the full length AKAP12 cDNA from pUHD10-3-SSeCKS (23) was subcloned into EcoRI-XhoI cut pBluescript II SK+ to create pBS-AKAP12α. Next, the stop codon was replaced with an in frame XbaI site and the
3’UTR was removed, allowing in frame fusion to the C-terminus. The AKAP12β and AKAP12γ constructs were created by exchanging the 5’XhoI-SwaI fragment of AKAP12α with the 5’ regions of either β or γ created by PCR amplification using either the primer AKAP12β5’For-
5’GATAGGTCGACTGCTCTGAGGATAGTTAGG or AKAP12γ5’For-
5’GATAGTGGACTGCTCTGAGGATAGTTAGG and AKAP125’Rev-
5’GATAGGTCGACTGCTCTGAGGATAGTTAGG. Rat lung and testis cDNA were used as templates, respectively. The full-length cDNAs were then subcloned into pCMV-3xFLAG-14 (Sigma) to create pAKAP12α-FLAG, pAKAP12β-FLAG, and pAKAP12γ-FLAG.

Full-length AKAP12-EGFP fusions were created by excising AKAP12 cDNAs from pBS-
AKAP12α, β, or γ with XhoI and SacII and subcloning into pEGFP-N3 (Clontech). AKAP12α-EGFP 5’ deletion constructs were constructed by cloning different regions of the first two AKAP12 exons using restriction enzyme clamped primers. Mutation of the myristoylation site in full length and deletion AKAP12α constructs was performed by amplifying a new 5’ end with the primer AKAP12MyrMut-
5’GATACTCGAGCCACCATGcGcGAGGCAGTTCCACC where the lower case letter indicates the changed nucleotide (AKAP12 G2A mutation). Preservation of reading frame in all fusion constructs was confirmed by sequencing.

**Western blotting**

Cell lysates were prepared in “crack” lysis buffer (10% glycerol, 2% SDS, 0.5 mM EDTA, 24 mM Tris-HCL, pH 7) containing 1X protease inhibitor cocktail and 1X phosphatase inhibitor cocktail II (Sigma). Lysates were passed ten times through a 22 gauge needle to shear genomic DNA and protein concentration determined with the DC protein assay (Biorad). For expression of FLAG-tagged AKAP12, constructs were transfected into Cos-7 with FuGENE6 (Roche) and lysates were prepared 48 hours post-transfection. Lysates were resolved on denaturing 5% polyacrylamide gels, transferred to nitrocellulose membranes (Amersham), blocked, and incubated with either rabbit anti-SSeCKS (9) or mouse anti-
FLAG-M2 (Sigma) antibodies overnight as indicated. Blots were then incubated with the appropriate horseradish peroxidase-conjugated secondary antibody and visualized by chemiluminescence.

Fluorescence microscopy

AKAP12-EGFP constructs were transfected into Cos-7 cells with FuGENE6. Cells were visualized 48 hours post-transfection using a BX51 Fluorescence Microscope and a 60X water-immersion lense (Olympus). In some experiments, either the endoplasmic reticulum, golgi apparatus, or lysosomes were labeled using ER-Tracker Blue-White DPX, BODIPY TR C5-ceramide complexed to BSA, or LysoTracker Red DND-99, respectively, (Molecular Probes) per manufacturer’s directions. Images were processed with Photoshop (Adobe).

Results

AKAP12 encodes three different mRNAs-The availability of high quality drafts of the human, mouse, and more recently, rat genomes is accelerating the study of gene regulation. While finalized drafts of these genomes were not available for the majority of this study, early release of unassembled sequence allowed us to examine the AKAP12 gene in these three species. To begin our characterization, we searched the non-redundant database and trace archives at NCBI for genomic sequences encompassing and flanking the AKAP12 locus. We were able to obtain sequence in this fashion that spanned from the upstream flanking gene, MTHFD1, through the entire AKAP12 locus, to the downstream flanking gene ZBTB2. We then assembled the AKAP12 gene structure by tiling previously described AKAP12 mRNAs available in the non-redundant database against the genomic sequence (Figure 1A).

Three isoforms of AKAP12 mRNAs were revealed during tiling that differ in the positioning of their 5’ exons within the genomic landscape. We propose that these three isoforms be named AKAP12α,
AKAP12β, and AKAP12γ to reflect both their timeline of discovery and position in the AKAP12 gene. The majority of described mRNAs belong to one class, AKAP12α. This class includes SSeCKS and Gravin and is represented in all three species. AKAP12β and AKAP12γ, each have a single representative in human and mouse, with the latter corresponding to the testis-restricted transcript described by Camus et al. (24). We searched the EST database at NCBI and the AKAP12 genomic sequence to identify orthologous transcripts in mouse and rat and human and rat, respectively. Although a potential human ortholog for AKAP12γ was identified within the human AKAP12 locus, support for expression cannot be gleaned from the EST database. Interestingly, the 5’ end of the human ortholog of AKAP12γ does not align with its rodent counterparts, but is spatially conserved in the context of the AKAP12 gene (discussed below).

**Organization of the AKAP12 gene** - The AKAP12 gene is contained in a moderate sized locus that spans approximately 100 kb and is composed of six exons (Figure 1A). The unusually large 3’ exons, exons 5 and 6, contain almost the entire protein coding region and 3’ untranslated region. Splicing of the isoform specific 5’ exons, exons 1 through 4, to these large exons generates the full length mRNA of each isoform (Figure 1A). CpG islands characteristically found in the promoter regions of genes encompass exons 1, 3 and 4 (data not shown) which suggested to us that the three isoforms represented distinctly regulated transcripts. In order to rule out alternative splicing as the mechanism that generates the three isoforms, we performed RACE to define the 5’ boundary of each isoform. Despite minor extension of the 5’ UTR of each AKAP12 isoform (data not shown), the terminal end of each isoform remained in the same position within the AKAP12 locus, indicating the three isoforms are not generated by alternative splicing (data not shown). RNAse protection mapping of the transcriptional start sites of each isoform supported our RACE data (data not shown). AKAP12α and AKAP12β have strictly defined transcriptional start sites (TSS). In contrast, AKAP12γ has multiple TSS dispersed throughout the 5’ flanking region of exon 4 (data not shown).
Cross-species comparison is rapidly emerging as a tool to identify important regulatory regions in large spans of genomic DNA, operating under the assumption that conservation reflects functional significance (25). As a first step towards identifying the promoters of the three AKAP12 isoforms, we compared the entire rat AKAP12 locus with the corresponding human locus (Figure 1B). This comparison gave us a detailed view of the entire array of putative conserved regions within the AKAP12 locus. As expected, peaks representing regions of high similarity were observed at the positions of the AKAP12 exons. Clusters of conserved non-coding elements flank exons 1, 3, and 4 in the putative promoter regions of the α, β, and γ isoforms respectively, supporting our hypothesis that each isoform is independently regulated. Numerous conserved regions are also scattered throughout the introns but concentrate in the regions flanking the second exon. Whether these regions correspond to novel genes within the AKAP12 locus, unidentified AKAP12 exons, or intronic enhancers that regulate AKAP12 expression is unclear at this time.

The AKAP12α Promoter- Using the conserved regions identified by cross-species comparison as a guide, we constructed several luciferase reporters encompassing various amounts of the 5’ region flanking exon one to characterize the AKAP12α promoter (Figure 2A). These constructs were tested in multiple cell lines to determine important regions regulating basal promoter activity. Although normalized promoter activity varies with cell line, the activity profile of the AKAP12α promoter constructs is fairly consistent (Figure 2B). The shortest construct, corresponding to the first conserved region upstream of the TSS, contains sufficient sequence to support maximal activity in all lines. Inclusion of additional conserved regions 5’ of this sequence does not significantly enhance basal activity. Interestingly, inclusion of distal flanking sequence >600 and >2300 base pairs upstream of the TSS
suppresses promoter activity in the Clone9 and A7r5 cells, but does not affect the activity of the promoter in the two kidney cell lines, Rat-2 and NRK. Based on these results, it appears that the AKAP12α promoter may be subdivided into three regions: a basal promoter immediately adjacent to the TSS (-173 to +1), a putative modulator region (-588 to -174), and a negative control region (-4764 to -589).

**INSERT FIGURE 2 HERE**

The region corresponding to the basal AKAP12α promoter is highly conserved between rat, mouse, and human (Figure 2C). Using the TRANSFAC database of known transcription factor binding sites (www.gene-regulation.com), we identified multiple potential cis-regulatory elements in the rat AKAP12α promoter. To minimize the chance that these identified sites represent false positives, we used conservation in human and mouse as our threshold for inclusion. The AKAP12α promoter lacks a TATA-like element, but contains multiple GC boxes, recognition sites for the stimulating protein (Sp) family of transcription factors, which could partially explain the strong activity of this promoter. A unique poly-thymidine tract occurs just upstream of a consensus site for p300 and the TSS. The opposite end of this region contains the GC boxes centered around an E Box, a cis-element that is known to be a binding site for a wide array of basic Helix-Loop-Helix transcription factors. The remainder of conserved regions of the basal promoter did not align well with sites in the database.

To determine if the cis-elements identified in our scan contribute to the basal activity of the AKAP12α promoter, we constructed further deletion reporter constructs within the basal promoter. Deletion beyond -173 nearly abolished basal promoter activity in all cases, indicating that the entire region is required for maximal activity (data not shown). Since our deletions would not allow us to assess the contribution to maximal activity of the cis-elements contained in the basal promoter, we circumvented this limitation by replacing each element with a sequence that differs from the consensus or by deleting them (Figure 2D). Our analysis focused on the p300 site, poly T tract, and E Box. The activity of these
altered constructs was then compared to that of the wild type promoter in Rat-2 cells. Replacement or deletion of these cis-elements reduced promoter activity to varying degrees, indicating that all contribute to the maximal activity of the basal promoter (Figure 2E). This is in keeping with our deletion data. These sites are good candidates for future studies focused on determining the transcription factors that regulate AKAP12α expression (see below).

The AKAP12β promoter- We approached characterization of the AKAP12β promoter in the same fashion as the AKAP12α promoter. We again used conserved regions to direct our cloning of reporter constructs to test for promoter activity of the 5' region flanking exon 3, the initiating exon of AKAP12β (Figure 3A). Although not as robust as the AKAP12α promoter, the reporter constructs encompassing the AKAP12β promoter display a remarkably similar pattern of activity to its upstream counterpart (Figure 3B). Again, the shortest construct, corresponding to the first conserved region upstream of the TSS, is sufficient to support maximal promoter activity. Similar to the AKAP12α promoter, further addition of upstream sequence does not increase promoter activity. The distal region 5’ of -2239 suppresses promoter activity in Clone9 and A7r5 cells, but does not affect AKAP12β promoter activity in the two kidney cell lines tested. The AKAP12β promoter thus appears to be similarly organized as the AKAP12α promoter with a basal promoter (-120 to +1), a region that may function as a modulator (from -2239 to -121), and a negative control region (-5399 to -2240).

INSERT FIGURE 3 HERE

The basal AKAP12β promoter is not as well conserved as the AKAP12α promoter. While the corresponding sequence of the human, rat, and mouse AKAP12α basal promoter are nearly contiguous, the alignment of the AKAP12β basal promoter in these three species is punctuated by multiple segments unique to the human sequence (Figure 3C). The presence of these extra sequences is intriguing given
their occurrence in the control region of a gene and suggests that there may be species-specific regulation of AKAP12β. Aside from these insertions, the remainder of the basal promoter region is moderately conserved, with the exception of four highly homologous regions that stand out in the alignment. Given their positioning inside of the basal promoter, we rationalized that these regions may correspond to conserved transcription factor binding sites so we again searched the TRANSFAC database. Aside from the presence of a near consensus TATA box, our search identified elements similar to those found in the AKAP12α promoter. The TATA box residing just upstream of the TSS is flanked on its 5’ end by GC box. Another GC box resides in the distal region of the basal promoter, downstream of a well conserved E Box.

Due to the lack of other highly conserved regions within the basal promoter, these four conserved elements may represent the core sequence of the AKAP12β promoter. As further deletion of the sequence of the basal promoter completely abolishes promoter activity (data not shown), we chose instead to construct basal promoter constructs in which we exchanged the sequence of each conserved region with that of a sequence divergent from the consensus of each element (Figure 3D). Replacement of each element significantly impacted on promoter activity, indicating that each of these elements is necessary for full AKAP12β promoter activity (Figure 3E). The factors that bind these elements are thus likely determinants of AKAP12β expression.

The AKAP12γ promoter- The region flanking exon 4, the first exon of the AKAP12γ transcripts, is the least conserved of the three AKAP12 promoter regions (Figure 4A). This lack of conservation nullified the scheme we used above in cloning the AKAP12α and AKAP12β promoters. Furthermore, when we mapped the TSS, multiple start sites were found dispersed over a 150 nucleotide stretch (data not shown). Since none of the TSS appears to predominate and the region encompassing them does not appear to be coding, we designed our promoter reporter constructs to encompass all of the TSS. We designed two constructs in this fashion that encompass different amounts of 5’ flanking sequence in an
attempt to locate the basal promoter of AKAP12γ. Initial tests in the cell lines used for the other promoters suggested that no promoter resided in this region as the constructs were not active above a promoter-less control (data not shown). However, given that expression of the endogenous AKAP12γ transcripts is restricted to testis, these lines are unlikely to express the appropriate factors to control AKAP12γ promoter activity. We therefore obtained one of the few spermatid cell lines available, the murine GC-2 spd(ts) line (GC-2), and re-tested these constructs for activity. Although these constructs displayed some activity in this line, it was not tissue-specific activity as the reporters were also weakly active in another murine cell line, NIH-3T3 (Figure 4B). It appears, then, that the basal promoter of AKAP12γ is not very strong, which may be an aid to preventing leaky expression in other tissues.

**INSERT FIGURE 4 HERE**

As mentioned above, the position of the first exon of human AKAP12γ does not overlap that of its rodent counterparts, but instead lies almost 1000 base pairs upstream (Figure 4A). The initial report identifying the presence of the mouse transcript demonstrated that the murine transcript is tightly restricted to the testis, where it is expressed only in cells in the late stages of spermatogenesis (24). To ascertain whether what we have termed human AKAP12γ is indeed the ortholog of this transcript, we examined the expression of this transcript in 16 tissues using a probe to exon 4 of the human AKAP12 gene. Consistent with this transcript being the ortholog of the testis restricted murine AKAP12γ, hybridization of this probe to poly A selected mRNA only occurred in testis (data not shown). Taken with the multiple TSS of rat AKAP12γ and the weak activity of the AKAP12γ promoter reporter constructs, this finding suggests that the promoter region of AKAP12γ is not strictly defined like that of other promoters and may in fact contain multiple weak promoters and exons that give rise to distinct AKAP12γ transcripts.
Since the AKAP12γ promoter appears insufficient to support tissue-specific promoter activity, we hypothesize that the tight regulation of this transcript may be controlled by a nearby spermatid-specific enhancer. This hypothesis may also explain why there are multiple start sites and different initiating exons in different species. To test this hypothesis, we scanned the conserved regions flanking exon 4 for potential binding sites for SRY and SOX-5, two transcription factors important in sex determination and expression of spermatid-restricted gene expression. A highly conserved near-consensus site for these factors was found in a conserved region (-2712 to -2176) upstream of exon 4 (Figure 4C). Heterologous luciferase reporter constructs containing this conserved region upstream of an SV-40 promoter are more active than a control reporter, demonstrating that this region functions as an enhancer (Figure 4D). However, this enhancer activity is also seen in a non-spermatid line, indicating that this region can function to increase activity but does not confer testis-restricted expression. Despite our inability to demonstrate cell-restricted expression of the AKAP12γ promoter, our characterization demonstrates that the AKAP12γ promoter is under the control of its own promoter and provides a basis for future studies of the mechanism of testis-restricted expression of this transcript.

USF-1 binds the E boxes of the AKAP12α and AKAP12β minimal promoters: The high degree of similarity between the α and β minimal promoters suggests that they may be regulated by similar factors. For example, E boxes in both promoters appear necessary for maximal activity (Figure 2E and Figure 3E). To determine if similar factors bind both promoters at their respective E boxes, we assessed the binding of Rat-2 nuclear extracts to the minimal promoters by gel shift. Both promoters are strongly bound by proteins contained in the nuclear extracts (Figure 5). Although a wide array of transcription factors bind the E box motif, the positioning of the E boxes near the TSS and our findings that their alteration reduced minimal promoter activity suggested to us that the Upstream Stimulatory Factor, USF-1, may be an important regulator of the α and β promoters. Whereas a non-specific IgG had no effect on the migration of the nucleoprotein complexes of either promoter, an antibody against USF-1 supershifted
a portion of the nucleoprotein complex seen bound to both promoters indicating that USF-1 can bind to both promoters (Figure 5).

Binding to either promoter was competed by unlabeled wildtype promoter. Interestingly, competition with E box mutant constructs competed the majority of binding and resulted in the formation of a faster-migrating nucleoprotein complex that represents only the E box nucleoprotein complex. This complex could be supershifted by addition of USF-1 antibody, indicating that USF-1 binds to these promoters through the E box (data not shown). Together, these data indicate that multiple proteins bind both the α and β minimal promoters and that USF-1 binds specifically through the E boxes found in both promoters.

Differential Expression of the three AKAP12 isoforms- The presence of three nearly identical AKAP12 transcripts each under the control of a separate promoter implies that each transcript fulfills a unique requirement. The highly restricted expression of AKAP12γ suggests that the expression of the three transcripts may be partitioned among tissues. However, the similarity between the α and β promoters implies that they may have comparable regulation. To determine if the other two isoforms are expressed in a tissue specific manner, we isolated 12 organs from C57/B6 mice and extracted total RNA. Following reverse transcription, we performed semi-quantitative RT-PCR using isoform-specific primers to assess the expression profile of each isoform. In support of the initial report (24), AKAP12γ expression was only detected in testis (Figure 5A). In contrast to this highly-restricted profile, AKAP12α is nearly ubiquitously expressed, with liver being the sole organ in which expression was not detected. The AKAP12β isoform is also broadly expressed, but to a lesser extent. Thus, despite their similarity, the α and β promoters have distinct regulation and expression profiles.

Expression of the three isoforms overlaps in several organs. AKAP12α and AKAP12γ are both expressed in the testis, whereas AKAP12α and AKAP12β expression overlap in several organs, including
bladder and lung. While we cannot rule out that these transcripts are differentially expressed within the cells of each organ, it appears unlikely that the transcripts are solely present to fulfill tissue-specific roles.

**INSERT FIGURE 6 HERE**

Expression of the three isoforms in cultured cells is similar to that in organs. In culture, AKAP12α and AKAP12β are broadly expressed and are expressed in all of the cell lines used in this study (data not shown). Similar to its tight restriction to the testis, we have yet to identify a cell line that expresses AKAP12γ (data not shown). The overlapping expression of AKAP12α and AKAP12β in multiple organs and cell lines suggests that the proteins encoded by these two transcripts may have different roles within cells. Interestingly, AKAP12 migrates as a doublet in western blots of cell lysates from a number of cell lines, including several of those used in this study (data not shown). To investigate the idea that the bands of this doublet represent the α and β isoform, we constructed FLAG-tagged constructs of the three rat isoforms to examine the apparent molecular weight of each transcript. These constructs were transfected into Cos-7 cells and then separated side-by-side with endogenous AKAP12 by SDS-PAGE. A myosin marker that migrates at approximately 250 kDa was used to re-align the blot following antibody incubation. AKAP12α-FLAG co-migrated with the upper band of the doublet while both AKAP12β and AKAP12γ co-migrated with the lower band (Figure 5B).

*A myristoylation motif in AKAP12α directs a localization pattern distinct from the other isoforms*- The differential migration of the three AKAP12 isoforms is likely to be a reflection of the differences in the proteins encoded by each transcript. The three proteins are highly similar, and like their corresponding transcripts, differ from each other only at the 5' end. In rat, AKAP12α encodes a 1687 amino acid protein that contains 88 amino acids at the N-terminus not found in either of the other isoforms. The rat AKAP12β transcript encodes a 1607 amino acid protein with 8 unique N-terminal
amino acids whereas the rat AKAP12γ transcript encodes a protein 1582 amino acids in length with no unique sequence due to translation initiation from a methionine present in all three transcripts. Interestingly, only the AKAP12α protein contains the previously described myristoylation motif (9), suggesting that the 5’ prime regions of the three isoforms may direct the proteins to different cell compartments.

As the available AKAP12 antibodies do not discriminate between isoforms, we fused each isoform to EGFP to examine their subcellular distribution. The AKAP12β- and AKAP12γ-EGFP were similarly distributed in the cytoplasm and tended to concentrate around the nucleus (Figure 6A). This distribution pattern is similar to the endogenous pattern detected previously by indirect immunofluorescence (9). In contrast, AKAP12α-EGFP localized to the periphery of vesicles concentrated around the nucleus and distributed more diffusely around the cytoplasm. All three fusions displayed concentrations in structures similar to membrane ruffles in a subpopulation of transfected cells (Figure 6A and data not shown for AKAP12α). Fusion of only the N-terminal 88 amino acids of AKAP12α was sufficient to target EGFP to these vesicles (Figure 6B).

**INSERT FIGURE 7 HERE**

In addition to the myristoylation motif, this AKAP12α amino terminus also contains a conserved region similar to a peroxisomal targeting motif (data not shown). To determine which of these sites mediates vesicular targeting, we fused either the myristoylation motif or the AKAP12α N-terminus without the myristoylation sequence to EGFP. Deletion of the myristoylation motif abolished targeting whereas the first fifteen amino acids corresponding to the myristoylation sequence recapitulated targeting to these structures (Figure 6B). A similar result was obtained when we mutated the myristoylation site (G2A) (data not shown). In the context of the full length AKAP12α protein, deletion of the myristoylation motif or mutation of the myristoylation site abolished vesicular targeting and resulted in a
localization pattern similar to the other two isoforms (Figure 6C). Differential targeting of the AKAP12α protein is thus conferred by the myristoylation motif which supercedes targeting information common to all three isoforms.

Myristoylation is most often associated with facilitating anchoring to the plasma membrane. Since a minor pool of all three isoforms appears to localize to the plasma membrane, it does not appear that myristoylation is a requirement for localization of AKAP12 to the plasma membrane. Furthermore, myristoylation in the case of AKAP12α does not appear to enhance association with the plasma membrane, but rather localizes the protein to the periphery of vesicles. To better understand the role of myristoylation in targeting AKAP12α, we stained cells transfected with the AKAP12α-EGFP construct for organelle-specific stains to identify the vesicular structures. Staining of the Golgi apparatus and lysosomes did not coincide with AKAP12α-EGFP expression (data not shown). A stain for the membrane of the endoplasmic reticulum, however, completely overlapped the vesicles where AKAP12α-EGFP was localized (Figure 6D). Myristoylation of AKAP12α appears then to direct localization to ER-derived vesicles.
Discussion

Our characterization of the AKAP12 gene is a foundational study that provides a framework for future examination of the regulation of AKAP12 expression. The AKAP12 gene is contained in a complex locus that, while only containing six exons, encodes for three separate isoforms. These isoforms are not alternate splice variants but rather independent transcripts under the control of separate promoters. As a reflection of their independent regulation, the three isoforms differ in their expression in various organs. Although the proteins encoded by the three AKAP12 transcripts are highly similar, differences in the amino terminus of at least one isoform regulate the localization of the protein. Specifically, a myristoylation motif uniquely present in the AKAP12α protein is both necessary and sufficient to target localization to vesicles of the endoplasmic reticulum. Due to the high degree of similarity, our results suggest that the three AKAP12 isoforms perform similar functions in distinct subcellular domains.

The three promoters of AKAP12 allow for independent control of each isoform. In addition to regulating distinct tissue expression profiles, these promoters may afford isoform-specific regulation by external stimuli, such as growth factors. AKAP12 expression has been shown to increase following stimulation with a number of growth-regulating factors, including serum (14), angiotensin II (13), platelet derived growth factor (26), and retinoic acid (27). In order to understand the role of increased AKAP12 expression in a cell’s response to stimuli, these and other studies examining AKAP12 expression should be revisited to identify the specific isoform involved in each response. Indeed, the AKAP12 isoforms appear to be distinctly regulated by external stimuli as serum induction selectively increases AKAP12β expression². In light of their different localization, such distinct regulation suggests that each isoform plays a distinct role in regulating cell function spatially.

AKAP12 is a member of the AKAP family of anchoring proteins that directs assembly of a multiprotein signaling complex that can include PRKA, PRKC, Calmodulin, PP2B, CyclinD1, and the β-adrenergic receptor (28). The role of AKAP12 in regulating cell functions such as growth is likely to be
dependent upon assembly of such multiprotein complexes in discrete subcellular compartments, thereby facilitating the co-localization of these signaling molecules with the appropriate substrates. In the present study, we have demonstrated that the multiple AKAP12 isoforms are differentially targeted to distinct subcellular compartments. We expected the myristoylation motif present in AKAP12α to direct targeting to the plasma membrane. However, AKAP12α was localized primarily to the endoplasmic reticulum. Some myristoylated proteins are similarly targeted to the endoplasmic reticulum and it appears that other amino acids in this motif determine which membrane the protein is targeted to (29). For example, proteins containing a palmitoylation site or a polybasic region adjacent to the myristoylation site, both of which the AKAP12α motif lacks, are targeted to the plasma membrane, whereas proteins lacking either of these features are targeted to the endoplasmic reticulum and endosomes (30). Since a small pool of all three isoforms localized to membrane ruffle-like structures, it appears that the membrane targeting sequences are common to all three proteins. Further study of AKAP12 targeting will be necessary to define these and other elements regulating the subcellular distribution of each isoform.

Generation of three differentially targeted anchoring proteins from one gene illustrates a mechanism to achieve compartmentalization of generic signals to multiple subcellular domains. This mechanism affords increased protein diversity and regulatory complexity for the dynamic signaling networks required by higher organisms. Among the AKAP family, generation of differentially targeted proteins from one gene is not unique to AKAP12. For example, AKAP7 (formerly known as AKAP18), utilizes alternate promoters and alternate splicing to generate proteins with different targeting motifs (31). Compartimentalization of PRKA to a subset of its hundreds of known substrates by genetically encoded variants of AKAP12 establishes a link between the genome and control of signaling specificity. Given the different signaling requirements of the diverse cell types in an organism, it will be important to determine how the differential expression of these alternately targeted anchoring proteins impacts on cell identity, fate determination, and differentiation.
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Footnotes

1 Abbreviations that appear in this report are: AKAP, A kinase anchoring protein; DMEM, Dulbecco’s modified Eagle’s medium; EGFP, enhanced green fluorescent protein; EST, expressed sequence tag; FBS, fetal bovine serum; GC-2, GC-2spd(ts) mouse spermatocytes; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; PRKA, protein kinase A; PRKC, protein kinase C; PP2B, protein phosphatase 2B; RACE, rapid amplification of cDNA ends; SSeCKS, src-suppressed C kinase substrate; TSS-, transcriptional start site; UTR, untranslated region.

2 J.W.S and J.M.M., unpublished observation.
Figure Legends

Figure 1. **Organization and conservation of the AKAP12 locus.** A. Generic gene structure of mammalian AKAP12. The arched lines connecting the exons represent the splicing of the three mRNAs encoded by AKAP12. All three mRNAs include exons 5 and 6. Sequence of the mRNAs for the rat AKAP12 isoforms have been deposited in GenBank: rAKAP12α- accession number AY695056, rAKAP12β- accession number AY695057, rAKAP12γ- accession number AY695058. B. VISTA plot of the AKAP12 locus detailing conserved regions between rat and human. Conserved regions are represented by peaks, where peak width represents the size of the conserved region and peak height is the percent identity between rat and human sequence. The positions of the exons is indicated by the black boxes on the upper axis and the dark shaded regions within the plot. The gray boxes on the upper axis indicate the positions of repetitive and low complexity elements. The gray shaded regions indicate clusters of conserved regions that represent the promoter regions of the three AKAP12 isoforms. Clusters of conserved regions also flank exon 2. Several of these regions contain open reading frames, suggesting the presence of yet-to-be identified AKAP12 exons or an additional isoform.

Figure 2. **The AKAP12α promoter.** A. VISTA plot of the promoter region of AKAP12α. Exon positions are indicated as described in Figure 1. The positions of the promoter constructs used in this study are indicated below the lower axis. The annotated sequence of the rat AKAP12α promoter has been deposited in GenBank (accession number AY695059) B. The activity of the AKAP12α promoter constructs in four rat cell lines. The activity of the AKAP12α reporter constructs was normalized to the average activity of a promoter-less control (Con) in each line to generate relative promoter activity. C. Alignment of the rat basal AKAP12α promoter with mouse and human sequence. Potential transcription factor sites are indicated above the sequence. The positions of three conserved elements tested for contribution to AKAP12α promoter activity are boxed. D. Sequence of the three conserved regions
replaced in the -173 AKAP12α reporter construct E. Activity of the altered -173 reporter constructs compared to the original construct.

Figure 3. **The AKAP12β promoter.** A. VISTA plot of the promoter region of AKAP12β. Exon positions are indicated as described in Figure 1. The positions of the promoter constructs used in this study are indicated below the lower axis. The annotated sequence of the rat AKAP12α promoter has been deposited in GenBank (accession number AY695060) B. The activity of the AKAP12β promoter constructs in four rat cell lines. The activity of the AKAP12βα reporter constructs was normalized as described in Figure 2. C. Alignment of the rat basal AKAP12β promoter with mouse and human sequence. Potential transcription factor sites are indicated above the sequence. The positions of four conserved elements tested for contribution to AKAP12β promoter activity are boxed. D. Sequence of the four conserved regions replaced in the -120 AKAP12β reporter construct E. Activity of the altered -120 reporter constructs compared to the original construct.

Figure 4. **The AKAP12γ promoter.** A. VISTA plot of the promoter region of AKAP12γ. Exon positions are indicated as described in Figure 1. The position of the human AKAP12γ 5’ exon is indicated by the gray shaded box. The positions of the promoter constructs used in this study are indicated below the lower axis. The conserved region tested for enhancer activity is circled. The annotated sequence of the rat AKAP12α promoter has been deposited in GenBank (accession number AY695061) B. The activity of the AKAP12γ promoter constructs in two mouse cell lines. The activity of the AKAP12γ reporter constructs was normalized as described in Figure 2. C. Partial multiple species alignment of the upstream conserved region (-2711 to -2175 relative to the 3’ most TSS) tested for enhancer activity. D. Enhancer activity of the -2711/-2175 region in two mouse cell lines. The activity of the enhancer construct (Enh) was normalized to the average activity of an enhancer-less control (Con).
Figure 5. **USF-1 binds to the E boxes in the α and β promoters.** Gel shifts were used to examine binding to the AKAP12α and β promoters. Incubation of radiolabeled probes from either promoter with Rat-2 nuclear extracts (4 µg) results in the formation of a large nucleoprotein complex. A portion of this complex is supershifted by USF-1 antibody, but not non-specific IgG. A smaller nucleoprotein complex representing only the E box bound proteins is observed when excess unlabeled E box mutant probe is pre-incubated with the nuclear extracts.

Figure 6. **The three AKAP12 isoforms have different expression and migration profiles.** A. Semi-quantitative RT-PCR was used to examine expression of the three AKAP12 isoforms in multiple mouse tissues. Expression of GAPDH was used to control for quality of cDNA synthesis. Tissue abbreviations: Bl- bladder; Br- brain; Ht- heart; In- small intestine; Ki- kidney; Li- liver; Lg- lung; Sk- skeletal muscle; Sp- spleen; Ts- testis; Ut- uterus. B. Migration of the three AKAP12 isoforms was measured against endogenous AKAP12 by Western blot. Lysates of Cos-7 cells transfected with FLAG-tagged AKAP12 expression constructs and GC-2 cell lysates were resolved on a 5% denaturing polyacrylamide gel, transferred to nitrocellulose, and probed with anti-FLAG, or anti-AKAP12 antibodies, respectively. The arrows indicate the positions of AKAP12α (upper) and AKAP12β/γ (lower) proteins.

Figure 7. **Differential targeting of AKAP12α is regulated by a myristoylation motif** A. The three AKAP12 isoforms are targeted to different regions of the cell. Cos-7 cells expressing EGFP-tagged AKAP12α (Panel 1), AKAP12β (Panel 2), or AKAP12γ (Panel 3). B. The N-terminus of AKAP12α is sufficient for targeting. Cos-7 cells expressing EGFP fused with either amino acids 1-88 (Panel 1) or 1-15 (Panel 2), of AKAP12α show similar distribution as the full length AKAP12α. Deletion of the myristoylation motif (Δ1-15) abolishes targeting (Panel 3). C. The myristoylation motif is required for distinct targeting of AKAP12α. The distinct localization pattern of full length AKAP12α (Panel 1) is lost when the myristoylation motif (amino acids 1-15) is deleted (Panel 2) and the protein is localized in a
similar manner as AKAP12β (Panel 3). D. AKAP12α localizes to the endoplasmic reticulum. Cos-7 cells transfected with AKAP12α-EGFP were stained with ER-Tracker Blue-White DPX to visualize the endoplasmic reticulum. Images of AKAP12α-EGFP were captured on the green channel (Panel 1). Images of the endoplasmic reticulum were captured on the blue channel and then pseudocolored red to ease visualization (Panel 2). The red and green channels were then merged to demonstrate co-localization, indicated by yellow signal (Panel 3). Size bars in all panels indicate 10 μM.
Figure 1
Figure 2

A

% Identity

Construct

AKAP12 Exon 1

1 kb

B

Relative promoter activity

Construct

Cell line

Rat-2

NRK

Clone9

A7r5

C

-173

Rat

GTGGCGGAGTCTCTGTCTAGCTGCTTAGGGGGGAGA---

Human

.C..A.C.C.........CG..A...G....C...GTC

Mouse

..T.........C........A...........

-133

Rat

TGCTGCAAGGCGTGCT-GGGCTGTGCTCATGTGATGAAG

Human

..G.........G...........

Mouse

..A...........

-93

Rat

GGAGGGAAAAAC---AAGGAGGGGGGAGAGCAGCTAGAGG

Human

..C........CGGGG...........C......G...

Mouse

..A...........

-53

Rat

TGCTGCTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT

Human

..C...........

Mouse

..A...........

-13

Rat

GCCACGAGCGCTCTCTCATCTCTACAGCTCGGGGTACGT

Human

..G...........T.......G..G.T..CG....

Mouse

..G...........G.....GT...
Figure 3

A

AKAP12 Exon 3

% Identity

Construct

-5399

100

75

50

-396

-120

2239

2250

646

596

1 kb

B

Relative promoter activity

Construct

Cell line

Rat-2

NRK

Clone9

A7r5

C

-121 E Box

Rat AGGG[AGT]GACTGCCCCCCCGGTGTGCAGCCAAGTCGGG
Human [..T..CGG..CTT..CGG..]
Mouse [..T..CGG..]

-81

Rat ACAGAGGTGTGGGGCT
Human [..A..T..CGC..CCCAGAGTCCTGGAAGACTAGCAAGG
Mouse [..]

-66 GC Box

Rat GGCTAGC---TG TGCTGGTGGGTCTCTGGG
Human [..C..CGG..CT..CGG..]
Mouse [..C..CGG..]

-43 TATA Box

Rat GCGTGGCGGGGCTCAGCTATTAGCTGAGAAGCG
Human [..T..CGG..CG..]
Mouse [..T..CGG..]

-4 E Box

Rat CTTCACCAGCGGCCAG-----TGCTGCACTCTCTAGG
Human [..T..CG..]
Mouse [..T..CG..]

D

TATA

Con NCTATAAAAAR
WT CCTATAATTA
Mut CCTACCTTA

GC Box

Con GGGGGG
WT GGGYGGG
Mut AAAYGGG

E

Normalized RLU's

0 2 4 6

WT TATA GC Box 1 GC Box 2 E Box
**Figure 4**

A

![Diagram showing AKAP12 Exon 4 and Exon 5 with a scale of 1 kb.](image)

% Identity

B

![Bar chart showing Relative Promoter Activity for different conditions and cell lines.](image)

C

| Rat    | AAGAACCTA----ATGGGGAGTATGGGTGTTAAGTCTT |
|--------|----------------------------------------|
| Human  | .TG.TT.--------.AA.A..A--.AA..TA------ |
| Mouse  | ...G..T..ATGGG............G............T..T.. |
|        | -2321                                  |
| Rat    | TGAAAGTACGTAGAG-----------------TCAAAAAGACAAA |
| Human  | .A.....GTAGA.A.ATGACTTTTCTTT        |
| Mouse  | ........GGGTA........207N------------ |
|        | -2292                                  |
| Rat    | CTTGATGTCC--CTCCCATCACA--CTCTGCTCAAGGC-C|
| Human  | .G..G..AT..TG....T.AGTG-C.T.C....T.A.A.|
| Mouse  | ...----CA.TG..GC--------------------|
|        | -2258                                  |

D

![Bar chart showing Relative Activity for different conditions and cell lines.](image)
**Figure 5**

|                  | AKAP12α -173 to +1 | AKAP12β -121 to +1 |
|------------------|---------------------|---------------------|
| Extract          | - + + + + +        | - + + + + +        |
| IgG              | - - + - - -        | - - + - - -        |
| USF-1 Ab         | - - - + - -        | - - - + - -        |
| WT Comp.         | - - - - + -        | - - - - + -        |
| E Box Mut. Comp. | - - - - - +        | - - - - - +        |

- Supershift ➔
- Multi-protein complex ➔
- E-Box complex ➔

- Non-specific ➔
- Free probe ➔
- Free probe ➔
Figure 6

A

| Bl | Br | Ht | In | Ki | Li | Lg | Sk | Sp | St | Ts | Ut |
|----|----|----|----|----|----|----|----|----|----|----|----|
| AKAP12α |    |    |    |    |    |    |    |    |    |    |    |
| AKAP12β |    |    |    |    |    |    |    |    |    |    |    |
| AKAP12γ |    |    |    |    |    |    |    |    |    |    |    |
| GAPDH    |    |    |    |    |    |    |    |    |    |    |    |

B

[Image of immunoblot analysis]
Multiple promoters direct expression of three AKAP12 isoforms with distinct tissue and subcellular distribution profiles
Jeffrey W. Streb, Chad M. Kitchen, Irwin H. Gelman and Joseph M. Miano

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