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The Relationship between SMN, the Spinal Muscular Atrophy Protein, and Nuclear Coiled Bodies in Differentiated Tissues and Cultured Cells

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The spinal muscular atrophy protein, SMN, is a cytoplasmic protein that is also found in distinct nuclear structures called "gems." Gems are closely associated with nuclear coiled bodies and both may have a direct role in snRNP maturation and pre-RNA splicing. There has been some controversy over whether gems and coiled bodies colocalize or form adjacent/independent structures in HeLa and other cultured cells. Using a new panel of antibodies against SMN and antibodies against coilin-p80, a systematic and quantitative study of adult differentiated tissues has shown that gems always colocalize with coiled bodies. In some tissues, a small proportion of coiled bodies (<10%) had no SMN, but independent or adjacent gems were not found. The most striking observation, however, was that many cell types appear to have neither gems nor coiled bodies (e.g., cardiac and smooth muscle, blood vessels, stomach, and spleen) and this expression pattern is conserved across human, rabbit, and pig species. This shows that assembly of distinct nuclear bodies is not essential for RNA splicing and supports the view that they may be storage sites for reserves of essential proteins and snRNPs. Overexpression of SMN in COS-7 cells produced supernumerary nuclear bodies, most of which also contained coilin-p80, confirming the close relationship between gems and coiled bodies. However, when SMN is reduced to very low levels in type I SMA fibroblasts, coiled bodies are still formed. Overall, the data suggest that gem/coiled body formation is not determined by high cytoplasmic SMN concentrations or high metabolic activity alone and that a differentiation-specific factor may control their formation.

Key Words: **gems; nucleus; RNA splicing; motor neurons; coilin.**

INTRODUCTION

Childhood spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by degradation of the anterior horn cells of the lower spinal cord resulting in progressive symmetrical atrophy of the voluntary muscles of the limb and trunk. SMA has been divided into three clinical subgroups, based on age of onset and severity [30]. A decrease in production of functional SMN (survival of motor neurons) protein has been shown to cause SMA [9, 17, 18, 37]. SMN is a ubiquitously expressed 40-kDa protein, present in both nucleus and cytoplasm and localized within nuclei of cultured cell lines in prominent dot-like structures termed "gemini of coiled bodies" or "gems" [19]. The SMN protein is encoded by two almost identical genes, telomeric SMN (SMN-1) and centromeric SMN (SMN-2). The two gene copies differ by 11 nucleotides, one of which is in the coding region (exon 7), but there are no amino acid differences [18, 29]. Both SMN-1 and SMN-2 produce an alternatively spliced isoform lacking exon 5, but SMN-2 undergoes a further alternative-splicing event as a result of one of the 11 nucleotide changes, producing isoforms lacking exon 7 or both exons 5 and 7 [14, 22, 29]. SMN is an essential protein, since a knockout of the single mouse SMN gene is embryonic lethal [39] and examples in man of genetic deletions affecting both SMN-1 and SMN-2 genes are unknown. Survival of severe Type I SMA patients is thought to be due to very low levels of expression from SMN-2, while milder SMA patients achieve intermediate SMN levels through gene conversion or increased SMN-2 copy number [10, 16, 43].

SMN colocalizes in gems with SMN-interacting protein-1 (SIP-1), forming a 300-kDa complex which also contains Sm core proteins B/B′, D, E, F, and G and small nuclear ribonucleoproteins (snRNPs) U1 and U5 [20]. Although the exact function of SMN is still unclear, a role in spliceosomal RNA processing has been suggested [11, 19–21]. A known RNA-binding motif $(YxxG)_3$, found in human eukaryotic initiation factor 4B, heterogeneous nuclear ribonucleoprotein A/B and hnRNP40, has been identified at the C-terminus of SMN [42]. A further RNA-binding element has been identified at the N-terminus in exon 2a, with RNA-binding modulatory domains identified in exons 2b and 3 [2, 21]. SMN interacts with fibrillarin, a 34-kDa

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rRNA-binding protein, and possibly also with hnRNP U, a nuclear protein involved in transportation and processing of nuclear RNAs [19]. It has been suggested that the SMN/SIP-1 complex binds to the newly transcribed snRNA and is involved in its transportation into the cytoplasm, binding of the Sm core protein, forming a snRNP, and reentry into the nucleus [33]. Mature snRNPs may undergo a further maturation step within the nucleolus when they return to the nucleus [23, 41]. Mature snRNPs migrate to interchromatin granules for spliceosome assembly [28, 35]. The role of coiled bodies within the snRNP biosynthesis/maturation pathway is not precisely defined. They may act as storage sites for mature snRNPs and aid their transportation to the interchromatin granules [28, 35, 36], but there is also evidence that coiled bodies are involved in snRNP maturation and transport out of the nucleolus [3, 23, 41]. It has also been suggested that coiled bodies mediate snRNA U1 and U2 transcription through storage and release of the snRNA specific transcription factor, PTF, as well as the TATA-box binding protein (TBP) and hypophosphorylated RNA polymerase II [38].

Most studies of gems and coiled bodies have been performed on cultured cells and the original studies on HeLa cells revealed SMN and coilin-p80, a coiled body marker protein, in adjacent nuclear structures [19]. More recently, however, the two proteins were found to colocalize in other cultured cell lines and HeLa strains, suggesting that gems and coiled bodies may be the same structure, at least under some circumstances [24, 25, 28, 29]. In the present study, we address the questions of whether gems and coiled bodies are found in all cell types of mammalian tissues, whether they occur as separate nuclear structures and whether coiled body formation is dependent on the presence of SMN. The results suggest that, although high cytoplasmic SMN levels or high transcriptional activity may influence gem formation, additional cell-specific factors may be required for assembly of both coiled bodies and gems.

MATERIALS AND METHODS

Expression of cloned SMN cDNA. Full-length SIP1 and SMN cDNAs were cloned into pET32c. Transformed bacteria were induced with 1 mM IPTG for 16 h at 37°C. Expressed fusion protein was purified from inclusion bodies by sequential extraction with increasing concentrations (2, 4, 6, 8 M) of urea in phosphate-buffered saline (PBS), followed by 8 M urea with 1% 2-mercaptoethanol (2-ME). The extract with 8 M urea + 1% 2-ME was further purified by gel filtration on Sephadex G-15 (Pharmacia) to remove the 2-ME before His-tag affinity chromatography (Novagen).

Production of antibodies. Monoclonal antibodies were produced by immunization of BALB/c mice and fusion of spleen cells with Sp2/0 myeloma cells as described elsewhere [32]. Both the sera and the hybridoma culture supernatants were screened by ELISA, Western blot (40-kDa band on HeLa total protein extract), and immunofluorescence microscopy (nuclear gems in COS-7 and HeLa cell lines). Hybridoma cell lines were cloned to homogeneity by limiting dilution. Ig subclass was determined using an isotyping kit (Serotec). Polyclonal antibodies against SMN were produced by immunization of a New Zealand White rabbit with full-length recombinant SMN. Rabbit polyclonal 204 [3] and mouse monoclonal 5P10 [1] antibodies against coilin-p80 were a generous gift from Dr. Angus Lamond (University of Dundee, UK).

SDS–PAGE and Western blotting. SDS–PAGE and Western blotting were carried out as described elsewhere [31]. The respective protein bands were visualized following development with a biotin/avidin/diaminobenzidine detection system for mouse mAbs (Vectastain; Vector Laboratories) or with peroxidase-conjugated rabbit anti-(mouse Ig) (DAKOpatts) and a chemiluminescent system (SuperSignal; Pierce).

Immunohistochemistry. COS-7 and skin fibroblast cell lines were plated and fixed as described elsewhere [9]. Primary skin fibroblasts from asymptomatic carriers (control) and type I SMA patients were obtained and grown in vitro as described elsewhere [9]. Human, rabbit, and pig tissues were embedded in tissue freezing medium and frozen in isopentane cooled in liquid nitrogen. Cryostat sections (5 μm) were mounted on microscope slides and fixed for 1 min with 50% acetone/50% methanol followed by a PBS wash. Mabs were diluted 1 + 4 with PBS and protein localization was revealed using FITC-conjugated horse anti-(mouse Ig). Nuclear localization was confirmed using an ethidium bromide or DAPI counterstain. For double labeling experiments, sections were incubated with the mouse mAb for 1 h, followed by the rabbit polyclonal antibody (diluted 1 in 250 PBS) for 1 h. The mAb was detected using a TRITC-conjugated goat anti-mouse (Sigma: diluted 1 in 80 PBS) and the polyclonal antibody was detected using a FITC-conjugated goat anti-rabbit (DAKO: diluted 1 in 50). TRITC and FITC (L4) filter sets and a 63× oil immersion objective were used with the Leica DMRB photomicroscope. The TRITC filter was also used for ethidium bromide fluorescence and a UV filter for DAPI fluorescence. Images were captured using an integrating camera and frame grabber under standard and comparable conditions.

RESULTS

Monoclonal Antibody Characterization.

A new panel of monoclonal antibodies against SMN (MANSMA1-5) was produced by the hybridoma method and screened for recognition of SMN on Western blots and nuclear gems in COS-7 or HeLa cells. Figure 1a shows that all 5 mAbs recognized a 40-kDa SMN band in total protein extracts of HeLa, though MANSMA4 and 5 were weaker. Antibody binding sites on SMN were mapped using subconstructs of the full-length SMN expressed in *Escherichia coli.* All five mAbs recognized full-length SMN (not shown), but only four of them recognized an isoform lacking exon 5 (Fig. 1b). This shows that mAb MANSMA3 requires exon 5 for binding. None of the mAbs recognized a recombinant protein encoded by exons 6 and 7 only (results not shown), showing that the other four mAbs recognize epitopes encoded by the first four exons.

Distribution of p80 and SMN in Primary Skin Fibroblasts and COS-7 Cell Lines

SMN and p80 were initially reported either to be in adjacent structures or, occasionally, to form indepen-

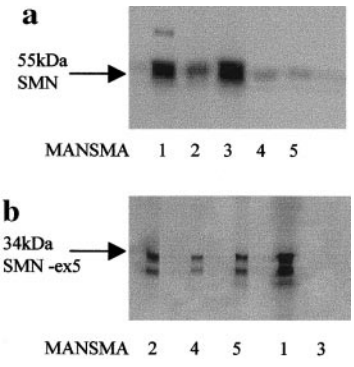

FIG. 1. Characterization of a panel of five anti-SMN monoclonal antibodies by Western blotting. (a) A HeLa cell total protein extract was separated as a strip on SDS–PAGE. The Western blot was probed with MANSMA1-5 using a miniblotter. All five mAbs recognize the 38-kDa SMN band. MANSMA1 also cross-reacts with a higher M_r band in HeLa. (b) The recombinant 34-kDa isoform lacking exon 5 was run as a strip on SDS–PAGE. Using a miniblotter, the Western blot was probed with mAbs as follows: Lane 1, MANSMA2; lane 2, MANSMA4; lane 3, MANSMA5; lane 4, MANSMA1; lane 5, MANSMA3. All mAbs except MANSMA3 recognize the 34-kDa band.

dent coiled bodies and nuclear gems in HeLa cells [19]. Subsequent studies suggested complete colocalization of p80 and SMN in another HeLa cell line [25]. Double-label experiments for SMN and p80 were performed using MANSMA1 and Ab204 or anti-SMN Rb sera and mAb 5P10 on cultured human primary fibroblasts from control and SMA type I patients. In control fibroblasts, gems and coiled bodies were often seen as independent nuclear bodies (Fig. 2a). As expected, nuclear gems were completely absent from type I SMA fibroblasts, but coiled bodies were still formed in apparently normal numbers (Fig 2a). This shows that coiled body formation does not require SMN.

Coiled bodies and gems were counted in COS-7 cells overexpressing full-length SMN protein and in untransfected COS-7 cells (Table 1). Untransfected COS-7 cells normally contained one or two gems per nucleus (Fig. 2b), though some cells contained up to six gems, and 86% of nuclear bodies contained both SMN and p80 (Table 1). An additional 10% of the bodies contained only p80 and 4% only SMN (Table 1). In COS-7 cells overexpressing the full-length SMN protein, the average number of gems per nucleus more than doubled (Table 1) and some cells contained very high numbers (Fig. 2b). Most of the nuclear bodies produced by SMN transfection contained both SMN and p80 (Fig. 2b) and the proportion of independent gems and coiled bodies was little changed (Table 1).

SMN Total Protein Human and Rabbit Tissue Levels

SMN was present in all human and rabbit tissues tested by Western blotting (Figs. 3a–3c). SMN levels were similar in all rabbit tissues, including heart and spleen (Figs. 3a and 3b). As reported by Burlet *et al.* [7], SMN levels were higher in human fetal lung and skeletal muscle than in the adult tissues (Fig. 3c). Figure 3c shows that the difference between adult and fetal tissues is less marked in brain [not studied in 7].

RNA studies on human skeletal muscle [14] identified three SMN isoforms lacking exon 5 or exon 7 or exons 5 and 7. The isoforms lacking exon 5 or exons 5 and 7 are 4 and 5 kDa smaller and should be resolved from the 40-kDa SMN by SDS–PAGE. Such isoforms could have been distinguished from both N-terminal and C-terminal degradation products of SMN using our exon-5-specific mAb, MANSMA3. However, Fig. 3c shows that no lower M_r bands were detected in total protein extracts from either adult or fetal human tissues, suggesting that isoform protein does not normally accumulate in significant amounts.

Distribution of SMN and Coilin p80 in Mammalian Tissues

Various rabbit tissues were examined for the expression of SMN in the form of nuclear gems using mAbs MANSMA1-3 (Table 2). In brain and spinal cord, most cell types have some or all nuclei with gems, blood vessels being a notable exception (Fig. 4b). Large neuronal cells have more gems on average than glial cells or smaller neurons. Motor neurons of the spinal cord have an increased number of gems, which are larger than in other cell types and tend localize around the nucleolus (Figs. 4a and 4b, 45 of 217 motor neuron gems counted were perinucleolar).

Outside the central nervous system, gems were found in epithelial cell types from liver, pancreas, duodenum, and esophagus. Gems were also present in skeletal muscle, liver parenchymal cells (Fig. 4a), and islets of Langerhans (Table 2). In all nuclei with gems, no significant difference in numbers of gems was found using the exon-5-specific mAb, MANSMA3, compared with MANSMA1 or 2 (Table 2). This shows that there are no gems that are composed mainly or exclusively of SMNΔex5.

Surprisingly, we were not able to detect gems in any cell types in heart (Fig. 4b), tongue, spleen, skin (Fig. 4b), trachea, and lung alveoli, although gems were common in one very specific region of the lung, the epithelial cells of the pulmonary bronchi (Table 2). Gems were absent from both endothelial cells and smooth muscle of blood vessels in all tissues examined (Table 2, Fig. 4b). There is no correlation between total SMN levels on Western blots (mainly cytoplasmic) and the presence or abundance of nuclear gems (cf. Figs. 3 and 4).

Nuclear gems and coiled bodies display similar distribution patterns in cultured cell lines, suggesting an associated function [2, 19, 40].The anti-coilin-p80 mAb

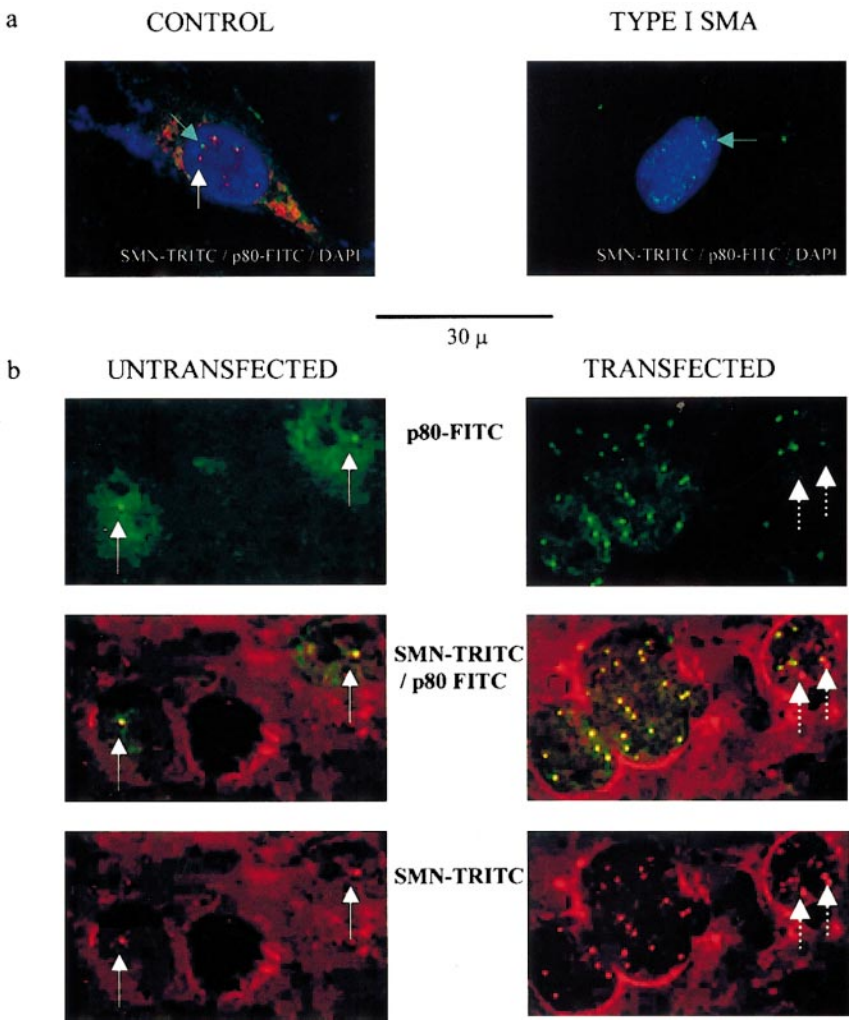

FIG. 2. Distribution of SMN and coilin-p80 by double label in (a) control and Type I SMA skin fibroblasts and (b) untransfected COS-7 cells and COS-7 cells overexpressing full-length SMN. SMN (red) was localized using MANSMA1 and a TRITC–goat anti-(mouse Ig) second antibody. Coilin-p80 (green) was localized using mAb 5P10 and a FITC–goat anti-(rabbit Ig) second antibody. A DAPI counterstain was used to identify fibroblast nuclei. On the fibroblasts, some of the nuclear gems are indicated by white arrows and coiled bodies are indicated by green arrows. SMN- and p80-containing bodies are indicated by the blue arrows on the untransfected COS-7 cells. Independent nuclear gems are indicated on the transfected COS-7 cells (broken arrow).

was used to determine whether coiled bodies and gems are expressed in the same cell types in rabbit (Table 2), human, and pig tissues (Figs. 4a–4b, Table 3). All cell types tested displaying nuclear gems also contained coiled bodies (Fig. 4a, Table 3). The cell types lacking nuclear gems also lacked coiled bodies (Fig. 4b). The number of coiled bodies per nucleus was comparable with that found for gems (Table 2). As with gems, the coiled bodies identified in motor neurons of the spinal cord were larger than in other cells (Fig. 4b, cf. surrounding cells). It is important to remember that gem/coiled body counts on tissues in Tables 2 and 3 are performed on 5-μm sections. The total numbers *per nucleus* will therefore be much higher and disproportionately higher in larger neuronal cell nuclei (multiply by nucleus diameter). In cultured cell lines, all nuclear bodies within a nucleus can be seen by using different focal planes, so counts are not comparable with tissue section nuclei.

TABLE 1

Gem and Coiled Body Density and Colocalization Frequency in Nuclei of COS-7 Cells and COS-7 Cells Overexpressing Full-Length SMN

Cell type	Nuclear bodies/ total cells	p80/SMN	p80	SMN
COS-7 (normal)	201/92	173 (86%)	21 (10%)	7 (4%)
COS-7 (transfected)	198/35	169 (85%)	11 (6%)	18 (9%)

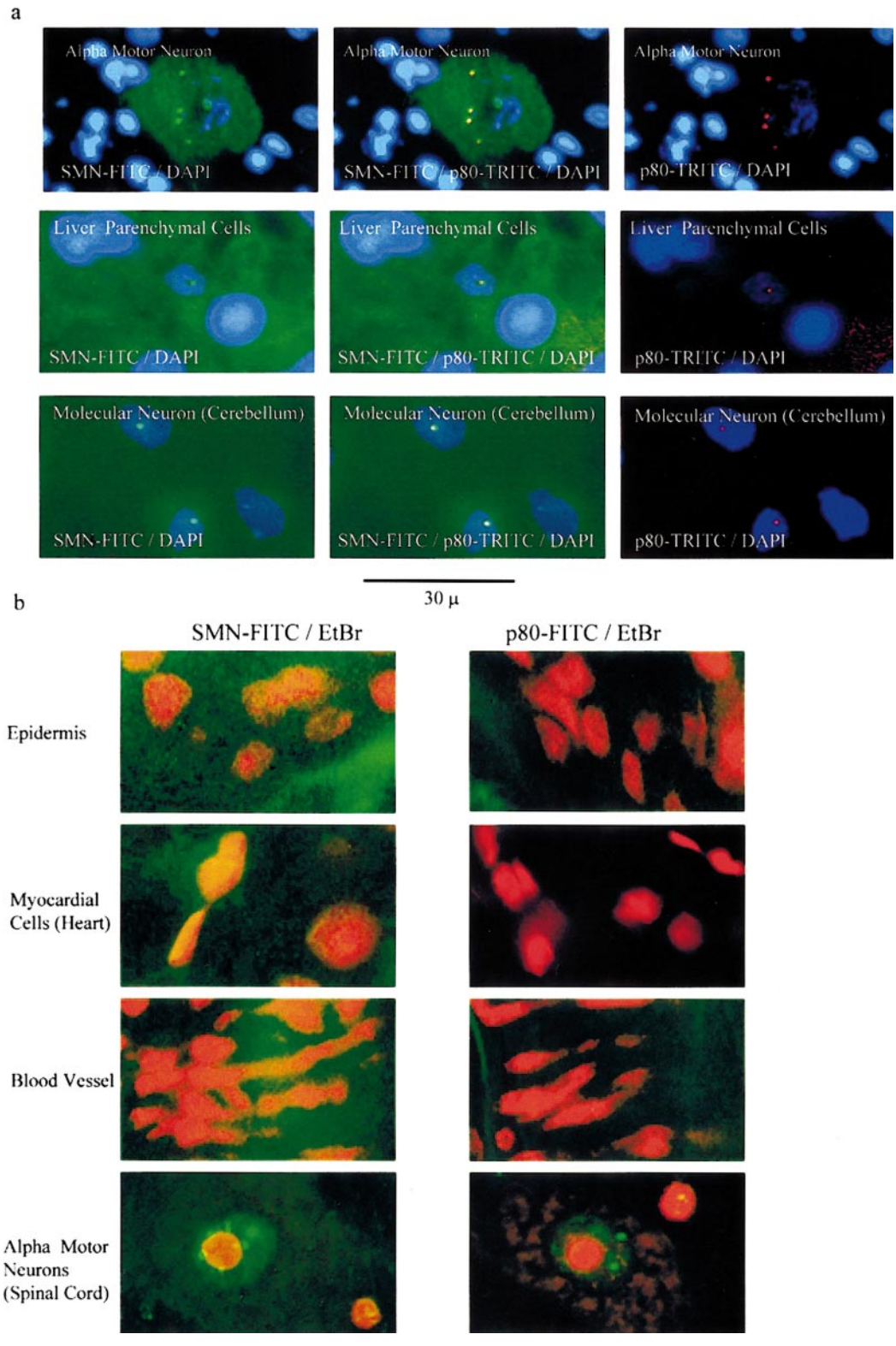

FIG. 4. Distribution of SMN and coilin-p80 by double label in different pig and rabbit tissues. (a) Colocalization of SMN and p80 in pig spinal cord (top), liver parenchyma (middle), and molecular neurons of the cerebellum (bottom) using a DAPI counterstain for nuclei. SMN (green) was localized using MANSMA1 and a FITC-labeled goat anti-(mouse Ig) secondary and coilin-p80 (red) was identified using anti-p80 rabbit serum (204) and a TRITC-labeled goat anti-(rabbit Ig) secondary. DAPI staining of the spinal cord motor neuron nucleus (a, top) is less intense than surrounding cells and only the nucleolus is clearly stained; the four gems/coiled bodies in the cell shown are at the edge of the nucleus. (b) Presence of SMN and p80 in rabbit α motor neurons of the spinal cord and absence of both proteins from rabbit epidermis, myocardium, and blood vessels, using an ethidium bromide counterstain for nuclei. SMN (left) and coilin-p80 (right) were localized using MANSMA1 and anti-p80 mAb (5P10), respectively, and a FITC–horse anti-(mouse Ig) secondary antibody.

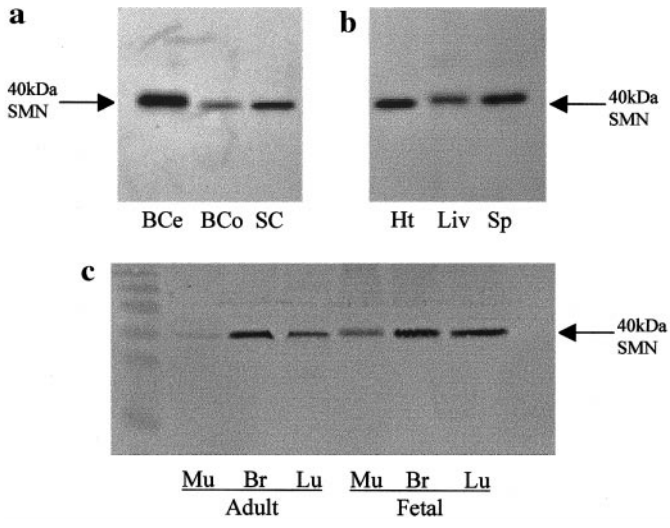

FIG. 3. Relative concentrations of SMN in different rabbit and human tissues by Western blotting. Protein extracts from different tissues for SDS–PAGE were adjusted to the same total protein concentration before electrophoresis on 12% acrylamide gels and equal loading was checked again by Coomassie blue staining of a separate gel. (a, b) Rabbit tissues are cerebellum (BCe), cortex (BCo), spinal cord (SC), heart (Ht), liver (Liv), and spleen (Sp). The mAb was MANSMA1. (c) Human tissues are fetal or adult skeletal muscle (Mu), brain (Br), or lung (Lu). The mAb was MANSMA1.

Double-label experiments on pig tissues using an anti-coilin-p80 polyclonal antibody (Ab204) (3) and MANSMA1 were performed to determine the precise relationship between coiled bodies and gems. In pig skeletal muscle, liver epithelial and parenchymal cells (Fig. 4a), spinal cord (Fig. 4a), cerebellum (Fig. 4a), cerebral cortex and hippocampus, SMN, and coilin-p80 staining were found to be largely colocalized, except that a small proportion (<10%) of nuclei in some tissues contained structures which stained only for p80 (Table 3).

DISCUSSION

Using a new panel of monoclonal antibodies against the SMN protein and previously described monoclonal and polyclonal antibodies against coilin-p80 [1, 3], we have performed the first systematic and quantititative immunolocalization study of adult mammalian tissues and have identified a conserved nuclear gem/coiled body distribution pattern (Tables 1–3). Many cell types were found to lack visible gems and these cell types also lacked coiled bodies. It is unclear whether these nuclear structures are completely absent or simply too small to detect by light microscopy. This observation suggests that visible gems or coiled bodies are not essential for RNA splicing and is consistent with the view that coiled bodies and gems are storage sites for snRNP components of the spliceosome, while RNA splicing by mature spliceosomes takes place in other regions of the nucleus, e.g., the perichromatin fibrils [28, 35]. Where they do occur, gems and coiled bodies appear to be colocalized or independent of one another, rather than form the adjacent structures ("gemini") observed in one strain of HeLa cells [19]. Colocalization is consistent with more recent observations in other cell lines and HeLa strains [25], suggesting that the gemini affect is a characteristic of specific cultured cell lines. This idea is supported by our findings in COS-7 cells and cultured skin fibroblasts, where staining patterns vary between the differing cell lines (Figs. 2a and 2b). These findings are in complete contrast with those found in tissue sections. In rabbit, pig, and human tissues, independent gems were not observed, although 10% of coiled bodies did not contain SMN (Table 3). Cultured cells containing independent coiled bodies and gems may lack a linker protein which is always present in sufficient quantity in tissues. Fibrillarin, the rRNA-binding protein that is a coiled body constituent and also binds directly to SMN [19], could be a candidate. SIP-1 is not involved since we have found that it always colocalized with SMN (results not shown).

Within the central nervous system, gem/coiled bodies were identified in all cell types except blood vessels. The gem/coiled bodies within motor neurons of the spinal cord were unusual in both their larger size and their more frequent positioning adjacent to the nucleolus. Gem/coiled bodies were also identified within epithelial cell types from the liver, pancreas, duodenum, and esophagus, as well as skeletal muscle, islets of Langerhans, and liver parenchymal cells. As previously reported by Burlet *et al.* [7], we found that SMN protein levels were higher in human fetal than in postnatal tissues (Fig. 3b). Burlet *et al.* [7] also observed gems in fetal human thymus, kidney, lung, brain, and skeletal muscle, but they did not report any fetal cell types lacking gems. The widespread presence of nuclear gems in fetal lung contrasts with their very localized distribution in adult lung. Our preliminary studies suggest that this is a real difference between adult and fetal tissues and that the results presented here are not inconsistent with that earlier study.

The absence of nuclear gems in the adult heart and other tissues shows that the presence of high cytoplasmic SMN levels is not sufficient for visible gem formation. There is also no evidence from tissue studies that gems form only in cells with high metabolic or transcriptional activity. This idea arose from the fact that both rapidly dividing cultured cells and metabolically active, though nondividing, neuronal cells have a large number of gems. However, gems/coiled bodies are present in skeletal muscle nuclei, but not in cardiac muscle; in lung, gems are formed in one layer of a particular type of bronchiole, but not in other airways

TABLE 2

Gem Density (per 5-μm Section) in Nuclei of Different Rabbit Cell Types Using Three Different mAbs

	MANSMA1	MANSMA2	MANSMA3
CNS			
1. Hippocampal formation			
CA1 pyramidal cells	1.85 (1–3; 74)	1.47 (0–3; 78)	1.30 (0–3; 69)
CA2 pyramidal cells	1.40 (0–3; 56)	1.17 (1–3; 70)	1.31 (0–3; 64)
CA3 pyramidal cells	2.38 (1–4;131)	1.78 (1–3;132)	2.08 (1–4; 94)
Subiculum pyramidal cells	1.79 (1–4; 70)	1.84 (0–3; 59)	1.65 (0–3; 71)
Subiculum granular neurons	0.85 (0–2; 54)	0.86 (0–2; 56)	0.88 (0–2; 52)
Dentate gyrus granular neurons	1.22 (1–3; 50)	1.00 (0–2; 45)	1.07 (0–2; 73)
Ependymal cells	0.67 (0–1; 28)	0.81 (0–1; 31)	0.81 (0–1; 32)
Nonneuronal cells	0.77 (0–2; 32)	0.88 (0–2; 38)	0.81 (0–2; 63)
2. Cerebellum			
Granular neurons	1.12 (1–2; 82)	0.97 (0–3;163)	0.93 (0–2; 70)
Molecular neurons	1.02 (0–2;114)	0.97 (0–2;140)	0.96 (0–2; 58)
Purkinje cells	1.13 (0–4;117)	1.16 (1–4;123)	1.09 (0–4;106)
3. Cortex			
Molecular neurons	1.12 (0–3;145)	1.01 (0–3;137)	0.98 (0–3; 95)
4. Spinal cord			
Motor neurons (anterior horn)	2.07 (1–4; 54)	2.65 (1–7; 61)	2.25 (0–6; 45)
Ependymal cells	1.00 (0–2; 40)	1.00 (0–1; 35)	0.93 (0–2; 37)
Nonneuronal cells	0.85 (0–1; 21)	0.90 (0–1; 30)	0.88 (0–1; 25)
Non-CNS cells with gems[a]			
1. Skeletal muscle			
Muscle nuclei	0.44 (0–2; 71)	0.47 (0–2; 64)	0.41 (0–2; 57)
2. Esophagus			
Stratified squamous epithelium	0.97 (0–2; 75)	0.88 (0–2; 61)	0.82 (0–2; 57)
3. Duodenum			
Simple columnar epithelium	0.85 (0–1;116)	0.83 (0–2; 94)	0.94 (0–2; 51)
4. Liver			
Parenchymal cells	0.89 (0–2; 75)	0.97 (0–3; 70)	0.95 (0–3; 60)
Simple columnar epithelium	0.71 (0–1; 47)	0.91 (0–2; 41)	0.60 (0–2; 47)
Simple cuboidal epithelium	0.86 (0–2; 52)	0.85 (0–1; 56)	0.82 (0–1; 50)
5. Pancreas			
Islets of Langerhans	0.37 (0–1;121)	0.37 (0–1;100)	0.39 (0–1; 80)
Simple columnar epithelium	0.79 (0–2;100)	0.75 (0–2; 85)	0.72 (0–2;100)
Simple cuboidal epithelium	1.02 (0–2;105)	1.00 (0–2; 62)	1.02 (0–2;100)
6. Testis			
Spermatogonia/Sertoli cells	0.95 (0–2; 84)	0.93 (0–2; 70)	0.89 (0–2; 58)

Note. The average number of gems per section is followed, in parentheses, by the range and the number of gems counted.

[a] Non-CNS cells without gems: 1. Skin dermis and epidermis. 2. Smooth muscle in tongue, esophagus, duodenum, trachea and blood vessels. 3. Myoepithelium in tongue, buccus and the stomach body. 4. Myocardial cells in the heart. 5. Blood vessel endothelium in hippocampus, cerebellum, cortex, spinal cord, skeletal muscle, heart, tongue, esophagus, duodenum, pancreas, liver, spleen, lung, testis and eye. 6. Stomach parietal and peptic cells. 7. Spleen parenchymal cells. 8. Primary and secondary spermatocytes and spermatozoa in the testis.

or blood vessels; in epidermis, gems are absent from both the dividing cells of the basal layer and the differentiated cells of the upper layers; and in active secretory cells of the pancreas, gems were absent from acinar cells but present in islet cells. There is also no correlation with nuclear size, since neurons and cardiomyocytes both have large nuclei, but only neurons have gems/coiled bodies. These and similar data suggest that additional cell-specific factors may be required for gem and coiled body formation; these may be differentiation-specific protein components of gems/coiled bodies, as yet unidentified. There are over 30 protein components of gem/coiled bodies and many have not been fully characterized [23, 24]. The high degree of cell specificity of gem/coiled body formation and its reproducibility does not support the view that gem/coiled bodies are formed in response to local and transient increases in transcriptional activity. Although SMN and SIP-1 may be regarded as constituents of coiled bodies and although overexpression of SMN in COS cells induces p80-containing nuclear structures, SMN does not appear to be essential for coiled body formation. Thus, SMN may be absent in up to 10% of coiled bodies in some tissues (Table 3) and coiled bodies still form when SMN is almost undetectable in Type I SMA fibroblasts (Fig. 2a).

In view of a report of a shorter SMN protein found only in nuclei [12], we used an exon-5-specific mAb to

TABLE 3

Gem and Coiled Body Density (per 5-μm Section) and Colocalization Frequency in Nuclei of Different Cell Types

	0	1	2	3	4	Total cells counted	Total nuclear bodies counted	Cells with p80 but no SMN
1. Molecular neurons (cortex)								
SMN	40	126	35	7	0	208	217	
p80	24	142	35	7	0	208	233	16 (7.7%)
2. Molecular neurons (cerebellum)								
SMN	27	77	14	4	0	105	117	
p80	26	78	14	4	0	105	118	1 (1.0%)
3. Granular neurons (cerebellum)								
SMN	28	170	36	4	0	238	254	
p80	17	181	36	4	0	238	265	11 (4.6%)
4. Purkinje cells (cerebellum)								
SMN	4	47	29	18	2	100	167	
p80	4	47	29	18	2	100	167	0 (0%)
5. Motor neurons (spinal cord)								
SMN	5	28	32	36	3	94	212	
p80	5	28	32	36	3	94	212	0 (0%)
6. Nonneuronal cells (spinal cord)								
SMN	53	50	38	0	0	143	126	
p80	47	56	38	0	0	143	132	6 (4.2%)
7. Ependymal cells								
SMN	17	86	4	0	0	107	94	
p80	13	90	4	0	0	107	98	4 (3.7%)
8. Skeletal muscle nuclei								
SMN	96	39	11	0	0	146	61	
p80	96	42	13	0	0	146	68	7 (4.8%)
9. Parenchymal cells (Liver)								
SMN	31	99	12	2	0	144	129	
p80	24	105	13	2	0	144	137	8 (5.6%)
10. Columnar epithelial cells (Liver)								
SMN	11	57	0	0	0	68	57	
p80	10	58	0	0	0	68	58	1 (1.5%)
11. Cuboidal epithelial cells (Liver)								
SMN	4	49	2	0	0	55	53	
p80	2	51	2	0	0	55	55	2 (3.6%)

Note. Adult pig tissue sections were double labeled for SMN (MANSMA1) and coilin p80 (pAb 204). For each tissue, the number of nuclei containing 0, one, two, three or four nuclear bodies which stain for SMN or p80 is shown.

determine whether the alternatively spliced isoforms lacking exon 5 or both exon 5 and 7 are preferentially incorporated into gems/coiled bodies. No smaller band was seen at 34 kDa on Western blots, but the proportion of SMN in nuclear gems is quite small. However, the identical staining pattern observed with exon-5-specific MANSMA3 (Table 2) shows that gems do not consist solely of SMN lacking exon 5. There is no evidence in the literature for significant amounts of authenticated SMN splicing isoforms at the protein level.

The observation that gem/coiled bodies are larger, more numerous, and more likely to be associated with the nucleolus in motor neurons than in most other cell types may be significant for the pathogenesis of SMA, since motor neurons are specifically affected in SMA. However, if gem/coiled bodies are nonessential storage sites, then a need for higher total levels of SMN in motor neurons seems more likely. The presence of a specific Sm protein in neurons, SmN instead of SmB core protein [13], could be relevant.

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