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The snRNP core protein SmB and tissue-specific SmN protein are differentially distributed between snRNP particles

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ABSTRACT

The SmN protein is a tissue specific component of the small nuclear ribonucleoprotein particle which is closely related to the ubiquitously expressed SmB protein but is expressed only in the brain and heart. To investigate the function of SmN, its localisation within different snRNP particles was investigated using a range of anti-snRNP monoclonal antibodies. SmN and SmB were found to exhibit different patterns of association with snRNP particles in two cell lines, ND7 and F9 which express SmN. In both cases, SmN was found to be present in the U-2 snRNP but was excluded from the U-1 snRNP whereas SmB was present in both U-1 and U-2 snRNPs. Data from transfected 3T3 mouse fibroblasts cell lines artificially expressing a low level of SmN also confirm this observation. In contrast, SmN was found to be an integral component of both the U-1 and U-2 snRNPs in both 3T3 cells artificially expressing high levels of SmN and in adult rat brain which has a naturally high level of SmN expression. Taken together, the results suggest that the pre-U1 snRNP particle has a lower affinity for SmN than for SmB. Thus, SmN expressed at low levels incorporates into U2, but SmN expressed at high levels incorporates into both U1 and U2 snRNPs and replaces SmB. The significance of these effects is discussed in terms of the potential role played by SmN in constitutive and alternative splicing pathways in neuronal cells.

INTRODUCTION

The Sm proteins were originally defined on the basis of autoantibodies present in sera from patients with systemic lupus erythematosus (SLE) (1) or in the MRL mouse (2). Subsequently the proteins were shown to play a critical role in the process of RNA splicing (3), with the Sm polypeptides SmD (16kDa) and SmB (28 kDa) being common to the U1, U2, U4/6 and U5 snRNPs which catalyse the splicing process (4). In agreement with the requirement for RNA splicing in all cell types, these Sm proteins are ubiquitously expressed in all tissues.

Recently however, both our laboratory (5) and others (6) have reported the existence of the first tissue specific Sm protein. This protein, SmN (29 kDa) is closely related to the protein SmB, but was found to be expressed only in brain, heart and embryonal carcinoma cell lines and not in other tissues or cell types (5,6). SmN, exhibits 92.5% homology at the amino acid level to SmB (7) but is encoded by a distinct gene to that which encodes both SmB and the closely related SmB′ protein which is expressed in human cells but is completely absent from rodent tissues and cell lines (8). At present, it is unclear why three such homologous snRNP proteins exist and why SmN should almost totally replace SmB in neurons (9,10).

On the basis of its unique expression pattern, SmN has been postulated to play a role in alternative splicing, the process whereby distinct mRNAs are produced from the same primary transcript in different cell types (for review see 11). Thus for example the correlation between the expression of SmN and the ability of specific tissues/cell types to splice the calcitonin/CGRP transcript to produce CGRP mRNA (5,6,9,12–14) led to the suggestion that SmN was involved in catalysing CGRP-specific splicing. However certain discrepancies in this correlation (9,10) suggest that SmN may be involved in other neuron specific splicing events.and furthermore that SmN may be neither required for or is sufficient for regulating the alternative splicing of the calcitonin/CGRP transcript (15). Interestingly, the gene encoding SmN in humans, SNRPN, has recently been mapped to chromosome 15 (15q12) (16) which is the critical region for Prader–Willi Syndrome (PWS), which results in developmental abnormalities and obesity due to a paternal deletion or two copies of the mother's chromosome 15 (17) This observation, together with data from mapping the mouse homologue, Snrpn, reveals that the gene is expressed exclusively from the paternal allele and is therefore maternally imprinted (18). Moreover, a murine

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model of PWS (19), suggested an important functional role for SmN, as a lack of SmN expression due to similar chromosomal deletions resulted in a Prader–Willi-Syndrome-like phenotype.

In order to affect the process of alternative splicing, SmN must influence the activity of the different snRNPs which bind to distinct regions of a transcript undergoing splicing. Initial studies showed that SmN was indeed associated with snRNPs since it could be immunoprecipitated either with anti-Sm antibodies or antibodies against the 5′ cap structure of the snRNAs (6). However, since these antibodies recognise components common to all the snRNPs, these findings did not determine whether SmN was restricted to particular snRNPs with specific functions in the splicing process. We have therefore performed a study of the localisation of SmN in the snRNPs using antibodies against components specific to the U1 or U2 snRNPs and extracts from the F9 (embryonal carcinoma) (20,21) and ND7 (neuronal) (22) cell lines, 3T3 and SmN transfected 3T3 mouse fibroblast cells (32), and adult rat brain, each of which express different levels of SmN.

MATERIALS AND METHODS

Patients and sera

Sera from patients with SLE (diagnosed using the ARA criteria (23)), were determined as having anti-RNP autoantibodies by counterimmunoelectrophoresis (CIE) with rabbit thymus extract using standard reference sera. Anti-Sm-positive sera were similarly detected, and quantitated using a commercially available ELISA (SHIELD DIAGNOSTICS, Dundee UK Ltd).

Monoclonal antibodies

The monoclonal antibodies KSm2, KSm5 (24) and K8.43 were derived from MRL mouse spleen cell hybridomas. KSm2 binds an epitope restricted to Sm D, KSm5 to a proline-rich epitope (PPGMRPP) (25) restricted to SmB/B′ and N, and K8.43 is specific for the U1-restricted 70 kD polypeptide (Williams 1986 and MR Stocks *et al.*, unpublished). The control isotype-matched antibody OX-12 was a gift of Dr A.F.Williams, Oxford University. The anti B′′ monoclonal 4G3 (26) was a gift of Dr W.J.Van Venrooij, University of Nijmegen, and is specific for the U2-restricted SmB′′ protein which is unrelated to SmN or SmB.

Cells and tissues

F9 and ND7 cell lines (D.Horn, University College London) were maintained at 37°C and 4% CO_2. F9 were grown in methionine-free DMEM with 5% foetal calf serum.

Leibowitz L15 medium replaced DMEM for the growth of ND7 cells. Brain tissue was derived from a Wistar rat.

Immunoprecipitation

The basic immunoprecipitation protocol of Steitz. J.A. (27)was used in all experiments. Protein A Sepharose (PAS) beads (CL-4B, Bioprocessing Ltd. CO. Durham) were washed, diluted 1:5 in NET-2 buffer (50mM Tris, 150 mM NaCl, 0.1% NP40 v/v) and incubated overnight at 4°C with monoclonal antibody ascites or SLE serum (0.2ml bead suspension + 40μl antibody). The beads were centrifuged 10 sec at 13000 r.p.m. and washed 3 times with NET-2.

In the case of the rat tissue immunoprecipitation, the protocol was modified in order to detect specifically SmN and SmB by probing the blot with the KSm5 antibody. The KSm5 antibody was covalently cross-linked to the SPA-sepharose using 0.5% glutaraldehyde at 4°C for 30min. This minimised the appearance of mouse IgG onto the blot which could then be probed with an anti-mouse IgG Horse Raddish Peroxidase (HRP) conjugate (ICN 67-428-1). Beads were then washed five times in NET-2 buffer.

F9 cells (1×10^7) were labelled for 24 hrs with 100 μCi L-[^{35}S]methionine (SJ 1015 in-vivo cell labelling grade, Amersham, UK). Both cell lines (ND7, F9) were pelleted, washed, diluted in 2 mls NET-2 buffer, sonicated on ice (3×30sec, MSE soniprep 150) and then centrifuged at 15000 rpm for 30 minutes at 4°C. In order to preclear nonspecific background, the sonicate was incubated for 30 minutes at 4°C with 100 μl of a 20% suspension of PAS beads in NET-2, spun briefly at 13000 rpm and the beads discarded. The resulting precleared supernatant was used in immunoprecipitations. Whole rat tissue samples (2g brain/liver tissue in 5ml NET-2) were homogenised and treated as cell lysates as described above.

Eighty microlitres of precleared cell/tissue supernatant were added to the antibody-coated PAS beads, diluted with 0.5ml NET-2 buffer and mixed for 1 hour at RT. The mixture was then spun at 13000 rpm, the beads washed 5 times with NET-2 and then extracted by boiling (3 minutes) in 0.3ml SDS sample buffer (10% glycerol, 70 mM SDS, 250 mM Tris, and 200 mM dithiothreitol, pH 6.8).

Gel electrophoresis and Western blotting

Samples were applied to 12.5% or 15% SDS-polyacrylamide gels (28) (30% acrylamide: 0.8% bisacrylamide) and electroblotted onto nitrocellulose (29). Antigens were detected on the blots with anti-Sm positive human sera (Figs.2,3,4) diluted 1 in 100 with 0.5% w/v casein/PBS for 1h followed by anti-human IgG (H and L) horseradish peroxidase conjugate (ICN 67-416-1). Total cell samples (Figs.3 and 4) and rat tissue immunoprecipitates (Fig.5) were probed with KSm5. Electroblots of SDS-gel electrophoretograms of radiolabelled F9 cells were visualised using Kodak b-max film for 14 days at −70°C.

Resolving SmN, SmB and B′′ proteins

In order to resolve these three snRNP-associated proteins, ND7 total cell protein samples were run on five different 12.5% gels which had varying acrylamide and bisacrylamide ratios. The acrylamide and bisacrylamide compositions (w/v) of the gels used were 1.) 30%:0.5%, 2.) 30%:1%, 3.) 30%:2%, 4.) 34%:1% and 5.) 24%:1% respectively.

Densitometry of Western blots

Quantitative analysis of the levels of SmN and SmB in rat brain and F9 and ND7 cell lines was performed by averaging values from 5 scans on a Shimadzu dual wavelength TLC scanner (CS-930) of three dilutions of total cell/tissue samples on blots probed with KSm5.

RESULTS

snRNP localisation of SmN in rodent cell lines

Immunoprecipitations from [^{35}S-Met]-labelled rodent embryonal carcinoma cell line F9 (which expresses SmN (30)) were done in order to examine the polypeptide composition of the U-1 and U-2 snRNPs. This was performed using anti-Sm monoclonal antibodies reactive with various snRNP polypeptides (KSm2, anti-SmD; KSm5, anti-SmB,B′,N; KSm4 anti-SmD,B,B′,N), (24,25), the K8.43 monoclonal antibody,which reacts with the U1-specific

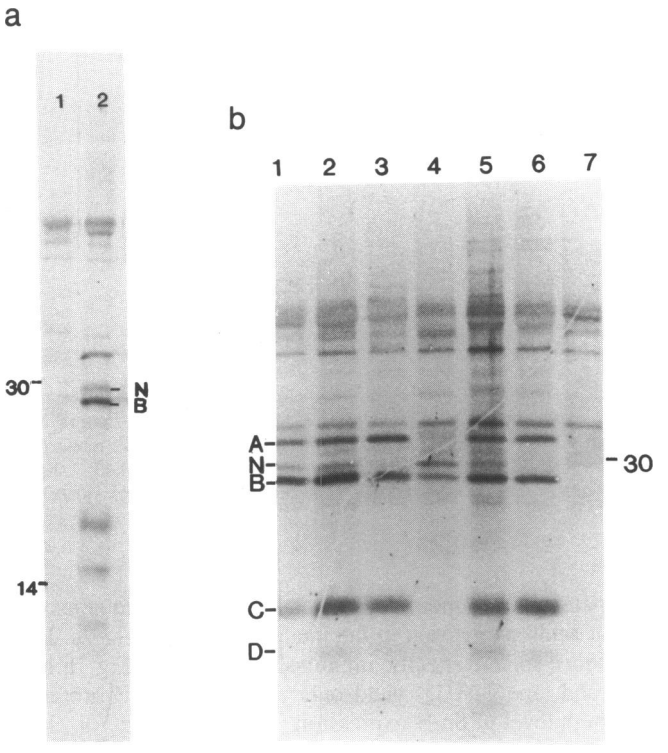


Figure 1. a. Autoradiograph of L-[^{35}S]methionine-labelled F9 embryonal carcinoma cell line immunoprecipitation. Lanes 1, OX-12 non anti-Sm monoclonal; 2, KSm2 anti-Sm D monoclonal. **b.** Autoradiograph of L-[^{35}S]methionine-labelled F9 embryonal carcinoma cell snRNP polypeptides immunoprecipitated with: Lanes 1, KSm4; 2, KSm5; 3, K8.43; 4, 4g3 monoclonal; 5, anti-Sm positive SLE sera; 6, anti-RNP SLE sera; 7, OX-12 non anti-Sm monoclonal.

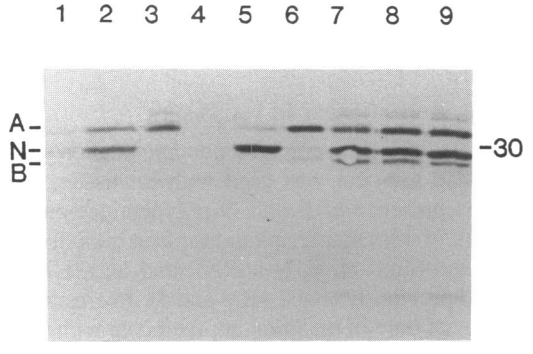


Figure 2. Immunoblot with anti-Sm-positive SLE sera of snRNP polypeptides immunoprecipitated from ND7 cells using the indicated antisera. Lanes: 1, ND7 total cell extract; 2, anti-Sm positive SLE sera; 3, anti-(U1)RNP serum (no anti-Sm activity); 4, OX-12 non anti-Sm monoclonal; 5, 4g3 anti-B'' (U2) monoclonal; 6, K8.43 anti-p67 monoclonal; 7, KSm5; 8, KSm4; 9, KSm2.

p67 protein,the anti-U1 RNP SLE serum and the 4g3 antibody which reacts with the U2 specific B'' protein (26). Each of the monoclonals used in this study had previously been shown to be specific and not to cross react with other proteins (unpublished data).

The KSm2 monoclonal antibody binds the common 16 kDa SmD polypeptide and hence immunoprecipitates the total snRNP population. KSm2 immunoprecipitated, from F9, (Fig.1a) the

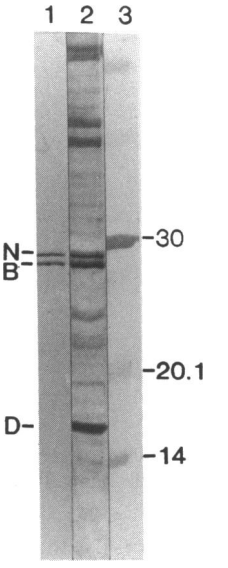


Figure 3. Immunoblot of F9 total cell extract; Lanes 1, probed with KSm5 (anti-N/B/B' specific monoclonal); 2, probed with anti-Sm positive SLE sera; 3, low molecular weight markers.

33 kDa A protein, a 29 kDa band, the 28 kDa SmB protein, C (22kDa) and D (16 kDa). KSm5 (anti-B,B',N) immunoprecipitated an identical pattern of proteins from F9 as KSm2. The K8.43 antibody immunoprecipitated (Figs.1b lane 3.) a similar set of polypeptides except that the 29 kDa band was absent. The SLE serum precipitated A, SmB, C, SmD and the 29kDa polypeptide from F9 (Fig.1B, lane 5). The selectivity of 4g3 antibody for the U-2 snRNP particle was demonstrated by the lack of the (U1) snRNP- specific A and C proteins in the immunoprecipitate (FIG.1B lane 4). SmB and the 29 kDa protein were present however in the immunoprecipitate. The 29kDa polypeptide was thus detectable in the U-2 and total snRNP populations but absent from the U-1 snRNP. As expected, neither the 29 kDa protein or the other snRNP or Sm proteins were immunoprecipitated with the control OX-12 monoclonal antibody (FIG.1b, lane 7), confirming that they were being specifically immunoprecipitated by the antibodies to snRNP components. In contrast, the higher molecular weight protein (larger than the A protein, Fig. 1b)) present in the immunoprecipitates with the anti-snRNP sera were also detected with the OX-12 antibody confirming that they were non-specific.

In order to characterise the 29kDa band as the N polypeptide, the same experiment was conducted with the neuronal cell line ND7 (22) which also expresses SmN (9), and the antigenic components of the immunoprecipitated snRNP particles were defined by immunoblotting. Nitrocellulose blots of immunoprecipitates from the ND7 cell line were probed with an anti-Sm SLE serum to detect the presence of antigenic components of snRNP particles. As expected, the KSm2, KSm4 and KSm5 immunoprecipitates from ND7 cells (Fig.2, lanes 9,8,7), contained the SmB and 29 kDa bands and both these bands reacted with the SLE serum confirming the identity of the 29 kDa band as SmN. The SLE serum (lane 2) immunoprecipitated the 29kDa more intensely than B, although the same serum reacted with B on the immunoblot of other immunoprecipitates (tracks 7,8,9). Immunoprecipitates with the U-1 specific antibody K8.43 (Fig.2 lane 6) lacked the 29 kDa SmN band as expected although the

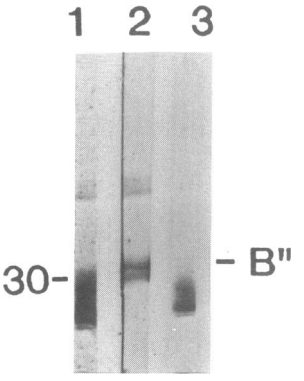


Figure 4. Immunoblot of ND7 total cell extract. Lanes: 1, probed with KSm5; 2, probed with 4g3 anti-B'' monoclonal; 3, probed with anti-Sm positive SLE sera.

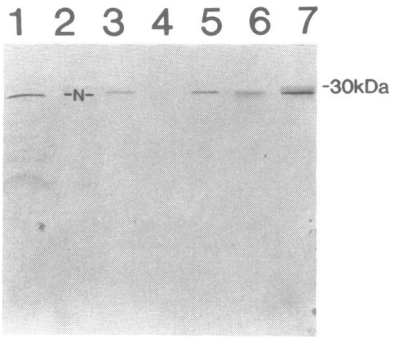


Figure 5. Immunoblot of snRNP polypeptides immunoprecipitated from rat brain tissue with: Lanes: 1, brain total protein; 2, blank; 3, 4g3 anti-B'' monoclonal antibody; 4, OX-12 negative control; 5, anti-RNP SLE sera; 6, K8.43; 7, KSm2 anti-SmD monoclonal antibody. The blot was probed with the KSm5 monoclonal antibody.

SmB band was present. Conversely, the U2 specific monoclonal antibody 4g3 immunoprecipitated the 29 kDa protein but not SmB (Fig 2, lane 5). This data confirms that in Fig 1b indicating that the 29 kDa SmN protein is present in the U-2 snRNP but absent from the U-1 snRNP and shows that as expected, this band bound anti-Sm antibodies in the SLE serum. Further confirmation that the 29kDa band corresponds to SmN was provided by showing that it is detected by both the anti-Sm SLE serum (Fig.2, and Fig.3, lane 2) and by the SmN/B/B·-specific monoclonal antibody KSm5 (Fig.3 lane 1). However,due to the similarity in size between the SmN and U2 specific SmB— protein (29 and 28.5 kDa respectively) (31), further investigation was performed in order to prove the identity of the 29 kDa polypeptide as SmN rather than B''.

Resolving SmN and B''

An SDS-polyacrylamide gel incorporating a novel ratio of acrylamide to bisacrylamide of 30:0.5% (Fig.4), was found to resolve SmN and SmB from the B'' protein in ND7 cell extract, at a price of lower resolution between SmN and SmB. The SLE serum (Fig.4,lane 3) did not react with the (U2) snRNP restricted B'' protein in ND7 cell extract whereas 4g3 (Fig.4, lane 2) visualised the B'' polypeptide as a band migrating slower than SmN and SmB. Hence the 29 kDa protein in the ND7 immunoprecipitate using 4g3 (Fig.2 lane 5) clearly corresponds to SmN and not to SmB'' which does not react with the anti-Sm SLE serum.

Data presented here therefore shows that SmN is a constituent of the (U2) snRNP but not the (U1) snRNP in the cell lines examined. This rather unexpected finding that SmN may differentially segregate between different snRNP particles was further investigated.

snRNP localisation of SmN transfected 3T3 cells

To further examine the snRNP particle distribution of SmN, immunoprecipitations were performed on mouse 3T3 fibroblasts (which lack endogenous SmN (9)), and on 3T3 cells which had been stably transfected with the mouse SmN cDNA clone 201 and which show SmN expression (32). Two different stably transfected cell lines expressing different levels of SmN (3T3-MSN=high, 3T3-7SNa=low) were derived from transfections of 3T3 cells with an EcoR1 fragment containing the complete coding region of SmN subcloned in the sense orientation into the M5Gneo vector or the pJ7 vector.

The parental 3T3 mouse fibroblast cells clearly demonstrate a lack of SmN expression in the total cell sample (Fig.7a. lane 3) and hence snRNP specific immunoprecipitations (Fig.7a.lane 1 (U2) and lane 2 (U1)) yield only the 28kDa SmB protein.

At a high level of SmN expression (3T3-MSN, Fig.7.a., lane 7) it is clear that artificially expressed SmN is incorporated into the snRNP particles as it is immunoprecipitated indirectly with the KSm2 anti-SmD monoclonal (lane 6). SmN was immunoprecipitated in both (U1) and (U2) snRNPs in this cell line (lanes 5 and 4 respectively).

At lower levels of artificial SmN expression (3T3-7SNa, Fig.7.b., lane 1), the transfected SmN is incorporated into the (U2) snRNP particle (lane2). However, despite the deliberate overloading of the (U1) snRNP specific K8.43 immunoprecipitate lane which yields a strong band corresponding to SmB, SmN is not clearly detectable when compared to the levels in the (U2) immunoprecipitate.

snRNP localisation of SmN in rat tissues

A (U1) and (U2) snRNP-specific immunoprecipitation and KSm5 antibody blotting protocol was used with rat tissues to detect specifically the presence of B and N polypeptides only. Fig.5 shows the result of immunoprecipitations of rat brain tissue using the range of anti-Sm, anti-(U1) snRNP and anti-(U2) snRNP antibodies used previously (Figs. 1B,2 and 4). Interestingly SmN was detectable not only in the immunoprecipitate with the KSm2 and the U2-specific 4g3 antibody, but was also in that with the U-1 specific 4g3 antibody (Figure 5). In contrast, it was not detected in the immunoprecipitate with the control OX-12 antibody, confirming that this effect was specific to anti-snRNP antibodies.

As expected, in view of the very low levels of SmB in the brain, no SmB was detectable in snRNP particles in these experiments (Figure 5). In contrast, however, SmB was readily detectable in both U1 and U2 particles when rat liver extracts were used (data not shown).

Quantitative analysis of relative expression of SmN and SmB

The discrepancy in U-1 snRNP localisation of SmN between brain and cell lines prompted us to question whether there was insufficient SmB expressed in the brain to produce functional (U1) snRNPs, thus necessitating production of N-containing (U1)

a

	SmN	SmB
BRAIN	85 %	15 %
F9	54 %	46 %
ND7	37 %	63 %

b

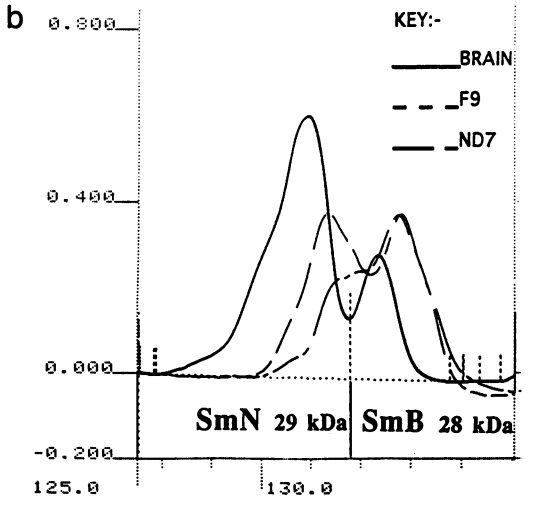


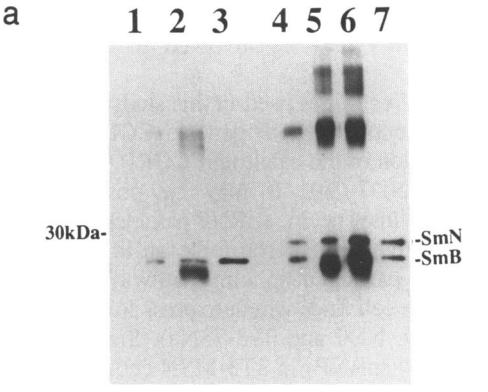
Figure 6. a. Quantitative analysis of the relative expression of SmN and SmB in rat brain, F9 and ND7 cell lines. Densitometer data obtained from averages of 5 scans of KSm5-probed Western blots of total tissue/cell extract covering three different sample concentrations. **b.** Output from typical single densitometer scan of KSm5-probed Western blot of total cell/tissue extracts.

snRNPs. Densitometry of KSm5-probed Western blots of F9, ND7, and adult brain total protein extracts (Fig.6) revealed the levels of SmN relative to SmB are: F9 = 54% N:46% B; ND7 = 37% N: 63% B and brain = 85% N:15% B.

DISCUSSION

Anti-Sm autoantibodies have been used to study the localisation of SmN in the small nuclear ribonucleoprotein particles of embryonal carcinoma cell line F9, a neuroblastoma cell line ND7, and adult rat brain. Here we have shown that the tissue-specific SmN protein in cell lines exhibits differences in terms of its U-1 snRNP localisation in comparison to the highly homologous SmB protein. Data presented here suggests that SmN does in fact behave differently to SmB, and therefore may not be a typical core component of SnRNPs in cell lines expressing it. Indeed, previous studies (4,10) referred to SmN as a neuronal specific form of the SmB core protein that is common to all the (U1) to (U6) SnRNPs. This was due to initial immunoprecipitation experiments of PC12 cells (6) in which SmN was immunoprecipitated using antibodies against the CAP structure of U-RNAs. SmN was also precipitated using an anti-(U1) RNP sera which suggested it was a component of the (U1) snRNP. Here we have demonstrated that in F9 and ND7 cells lines, SmN unlike

a



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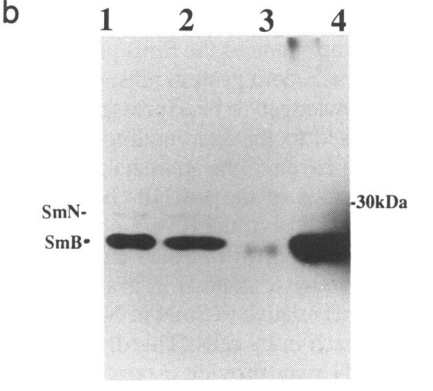

Figure 7. a. Immunoblots of snRNP polypeptides immunoprecipitated from 3T3 mouse fibroblast cells (lanes 1–3) and 3T3 cells stably transfected with the mouse SmN cDNA clone 201, artificially expressing high levels of SmN (3T3-MSN) (lanes 4–7). Lanes: 1, 3T3 4g3 anti-B'' (U2) immunoprecipitate; 2, 3T3 K8.43 anti-p67 (U1) immunoprecipitate; 3, 3T3 total cell extract; 4, 3T3-MSN 4g3 (U2); 5, 3T3-MSN K8.43 (U1); 6, 3T3-MSN KSm2 anti-SmD; 7, 3T3-MSN total cell extract. **b.** Immunoblot of snRNP polypeptides immunoprecipitated from 3T3 cells stably transfected with the mouse SmN cDNA clone 201, artificially expressing low levels of SmN (3T3-7SNa). Lanes: 1, 3T3-7SNa total extract; 2, 4g3 (U2) immunoprecipitate; 3, OX-12 non anti-Sm monoclonal; 4, K8.43 (U1) immunoprecipitate.

SmB, does not associate with the (U1) snRNP, but does however associate with the (U2) snRNP. Data from transfected 3T3-7SNa mouse fibroblast cells artificially expressing low levels of SmN also support this observation. The reported presence of SmN in (U1) snRNPs from PC12 cells (6) supports our data, because the actual level was considerably less than in immunoprecipitates of total snRNPS obtained using anti-Sm monoclonal antibody Y-12 (compared with A, B and C polypeptides). Hence association of the tissue specific SmN protein within the SnRNPs is more complex than that for the constitutively expressed SmB.

The association of SmN with the (U2) SnRNP is of interest as the (U2) SnRNP associates with the branch point region of the pre-mRNA close to the 3· splice acceptor. It may be postulated that the presence of SmN in the (U2) snRNP affects the choice of 3· splice acceptor, thus in turn determining which particular exons are included in the resulting mRNA. Previous studies have reported a correlation between the expression of SmN and the ability of such cell types to perform alternative splicing of the calcitonin/CGRP gene transcript with a different 3′ splice acceptor to produce CGRP mRNA (5,6,9,12–14).

However, some discrepancies do occur in this correlation (9,10) suggesting that SmN may be involved in different splicing decisions.

The F9 and ND7 cell lines used in this study are both capable of splicing the calcitonin/CGRP gene to CGRP mRNA either by natural expression of the calcitonin/CGRP gene (F9-(33)) or by transfection (ND7-(9)). It may be postulated that the association of SmN into specific snRNP particles (ie. (U2) snRNP in F9 and ND7 cells) may therefore be an important factor in influencing which particular splicing pathway is followed.

In contrast to the cell lines which express low to intermediate levels of SmN (F9, ND7 and 3T3-7SNa), SmN is common to both (U1) and (U2) snRNPs in 3T3-MSN cells expressing high levels of SmN and in rat brain where the expression of SmN (85%) is much higher relative to SmB (15%-Fig.6). This data suggests that the high level of total SmN expression in the brain causes it to replace the SmB core protein within the snRNPs. In view of this data, the status of the SmB protein in the context of it being described as a core protein must challenged. Indeed, as SmN is now acknowledged to be expressed predominantly by the neurons as opposed to the surrounding non-neuronal cells (9,10), it follows that there will be appreciable differences in the polypeptide compositions of the snRNPs between such cells.

Quantitative densitometry of the KSm5-probed Western blots of total protein samples (Fig.6) revealed an approximately six-fold excess of SmN relative to SmB in brain compared with a two-fold excess of SmB relative to SmN in ND7 cells, or roughly equal proportions of each in F9 cells. This difference in the levels of expression of SmN may provide a possible explanation for the difference observed in the composition of (U1) snRNPs between brain tissue and neuronal, embryonal carcinoma and the SmN transfected 3T3 cell lines. We suggest that in cells expressing low or intermediate levels of SmN (e.g. F9, ND7, 3T3-7SNa), the SmN is preferentially incorporated into (U2) snRNP whereas at higher levels of SmN expression (rat brain, 3T3-MSN), the (U2) snRNP becomes SmN-saturated and the excess SmN becomes incorporated into (U1) snRNPs. Earlier observations using the PC12 cell line (6) suggested that the level of SmN expressed in this cell line is also low, and just above the threshold level whereby all available (U2) snRNPs are saturated with SmN and hence SmN appears at very low levels in the (U1) SnRNP.

The replacement of SmB by SmN in brain (U1) and (U2) snRNPs implies that SmN is capable of performing similar splicing roles to that of SmB. The differences in primary structure between SmN and B may give the SmN snRNPs unique neuron-specific alternative splicing functions. The recent mapping of the human gene (SNRPN) encoding SmN to the Prader−Willi Syndrome critical region (16), and creation of a PWS-like phenotype in a murine model (19) in which no Snrpn message could be detected in the brain suggests that defects in mRNA processing (probably of neural-specific genes) due to this lack of SmN expression, may lead to PWS. The difference in snRNP association of SmN and SmB presented in this report suggests that SmN does have alternative roles in splicing to SmB. We suggest that the level of expression of SmN affects its localisation within the snRNPs which in turn affects the ability of these snRNPs to perform alternative mRNA splicing. This may resolve some of the discrepancies in the correlation between SmN expression and CGRP splice choice.

In situ hybridisation experiments with riboprobes reveal the neurons are predominantly the only brain cells expressing SmN (9,10) and that SmB is expressed at very low levels throughout the brain. Quantitative analysis of SmN relative to SmB expression in the whole brain is 85% verses 15% suggesting that the majority of Sm protein content of the brain resides in the neurons in the form of SmN. Hence other brain cells such as Schwann cells, astrocytes and glial cells (90% of total brain cells) may express very little in the way of total snRNP Sm polypeptides. The effect of this on the nature of RNA splicing performed in such non neuronal cells is an intriguing question.

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