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Expression of the SmB′ splicing protein in rodent cells capable of following an alternative RNA splicing pathway

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The expression of the SmB and SmB′ spliceosome proteins in a variety of cell types and tissues has been investigated. Although SmB is found in all cells studied, the SmB′ protein is found only in a small number of rodent cell types. The presence of this protein is correlated with the ability to utilize an alternative pathway of RNA splicing which is not available in most cell types. This is the first demonstration of tissue specific expression of a protein component of the spliceosome and suggests a role for SmB′ in the regulation of some cases of alternative RNA splicing.

RNA splicing alternative; Autoimmuno antigen; Tissue-specific expression

1. INTRODUCTION

Numerous examples of alternative RNA processing, producing different mRNAs from the same primary transcript in different cell types have been reported [1–4]. In contrast, the components of the splicing apparatus such as the Sm proteins and SnRNAs appear to be expressed in all tissues [5–7] paralleling the widespread requirement for processing of the primary transcript [8].

To investigate whether differences in these components occur between different tissues which might play a role in alternative splicing, we have studied the expression of two Sm proteins, B and B′ [9]. These two proteins are closely related, the slightly larger SmB′ protein (29 kDa compared to 28 kDa) containing an additional peptide not found in SmB. Both proteins are readily detectable in human cells [10,11], although studies on their expression in rat liver [12] and Friend cells [10] failed to detect SmB′ in these rodent cell types.

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Here we report that whilst SmB is detectable in all cells, SmB′ is only detectable in a small number of rodent cell types. The presence of SmB′ in these cells and of a specific isotype of SmB′ in similar human cells correlates with the ability of such cells to follow an alternative splicing pathway.

2. MATERIALS AND METHODS

2.1. *Western blotting*

Prior to electrophoresis, the protein content of cell samples was equalized by using a modified Lowry assay [13]. Equal amounts of protein from each sample were then electrophoresed on 12.5% polyacrylamide SDS gels, transferred to nitrocellulose and reacted with antibody as described [14]. Monoclonal antibody KSm5 [12], which detects the SmB and B′ proteins was used as the first layer and peroxidase conjugated rabbit anti-mouse immunoglobulin as the second layer. The fact that each sample contained an equivalent amount of protein was confirmed by scanning the protein bands in an unblotted portion of the gel stained with Coomassie Blue stain using a Joyce-Loebel chromoscan 3 densitometer.

2.2. *Two-dimensional gel electrophoresis*

Samples were electrophoresed on nonequilibrium pH gradient gels as described by O'Farrell et al. [15]. The gradient gel was then transferred to the slot of a 12.5% polyacrylamide gel and after electrophoresis, was treated as described above.

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3. RESULTS AND DISCUSSION

The KSm5 antibody used in these experiments [12] reacts with the closely related SmB and B′ proteins. Using this antibody we were able to detect only a single band with the mobility of SmB in a wide range of rodent cells (fig.1a). Although there was some variation in the amount of SmB present in the different cell lines, it was clearly detectable in all the lines studied including both suspension and attached cell lines, primary, immortalised and transformed cells, as well as transformed cell lines derived from all three germ layers. However, in the pluripotent mouse embryonal carcinoma (EC) cell line PCC3 [16] SmB′ was readily detectable, although the level of SmB was similar to that observed in most other cell types (fig.1a). A similar result was also obtained with two other murine EC cell lines, PCC4 and F9, suggesting that the presence of SmB′ is typical of mouse EC cell lines. In contrast overloading the amount of protein from differentiated cells so that the amounts of SmB in these cells exceeded that seen in the EC cells failed to reveal any SmB′ in the differentiated cells. As expected, both the SmB and SmB′ proteins were located in the nucleus of the EC cells and reacted with human SLE sera containing antibodies to Sm as well as with another monoclonal antibody directed against the SmB and B′ proteins (KSm4:12, not shown).

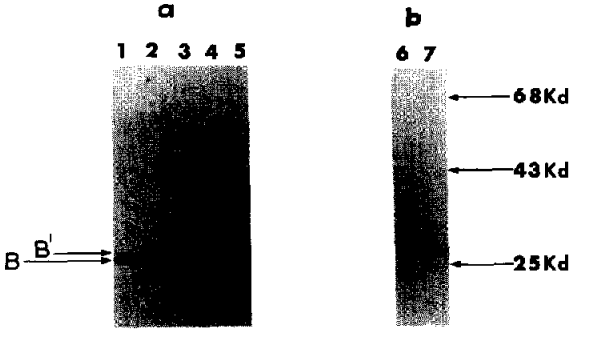

Fig.1. Western blot of protein samples from mouse (a) and human (b) cell lines with antibody KSm5. Track 1, PCC3; track 2, A20 (B lymphocytes); track 3, LS109.2 (pre-B cell); track 4, PYS (parietal endoderm); track 5, PSA5E (visceral endoderm); track 6, Tera 2; track 7, HeLa. Arrows indicate the position of molecular mass markers of the sizes indicated and of the SmB and B′ proteins.

To investigate the expression of SmB′ in human EC cells, we compared the proteins detected by KSm5 in HeLa cells with those observed in the human EC cell line Tera 2 [17]. As expected from previous studies [10,11], HeLa cells contained readily detectable levels of SmB′ protein which however, was less abundant than SmB. In contrast Tera 2 cells contained elevated levels of SmB′ resulting in a considerable excess of B′ compared to B protein (fig.1b). Hence, increased levels of SmB′ are a general characteristic of EC cells whatever their species of origin, although the basal level of this protein in differentiated cells varies dramatically between humans and rodents.

The expression of SmB′ in human and rodent cells was further studied by two-dimensional gel electrophoresis using a non-equilibrium pH gradient as the first dimension [15], followed by polyacrylamide gel electrophoresis and Western blotting with KSm5. In differentiated mouse cells typified by A20 lymphocytes (fig.2a) no B′ protein was detectable, the antibody recognising only a single basic spot with the molecular mass of SmB. The position of this spot correlated well with that observed by others who have used similar techniques to study the Sm proteins [18]. In PCC3 cells SmB′ was detectable (fig.2b) as a single spot at the same isoelectric point as SmB but at higher molecular mass. Hence in mouse cells, these experiments confirm those using one dimensional gels, SmB′ being detected only in EC cells.

In human cells the situation is more complex. As expected, in view of its detection in one-dimensional gel electrophoresis, SmB′ was observed in two-dimensional gels of HeLa cell proteins (fig.2c). The major SmB′ spot is, however, more basic than the major SmB spot, only a very small amount of SmB′ material being detectable (arrowed in fig.2c) at the same p*I* as the major SmB spot. In Tera 2 cells by contrast, considerably more B′ material was detectable at this point of the gel (arrowed in fig.2d), whilst the abundance of the more basic SmB′ spot was little different from that in HeLa cells. Hence, exactly as in mouse EC cells, an isotype of SmB′ with the same isoelectric point as SmB is over-expressed in human EC cells. Interestingly, a more basic form of SmB observed in HeLa cells (fig.2c) is absent in Tera 2 cells (fig.2d). The significance of this observation is at present uncertain, however.

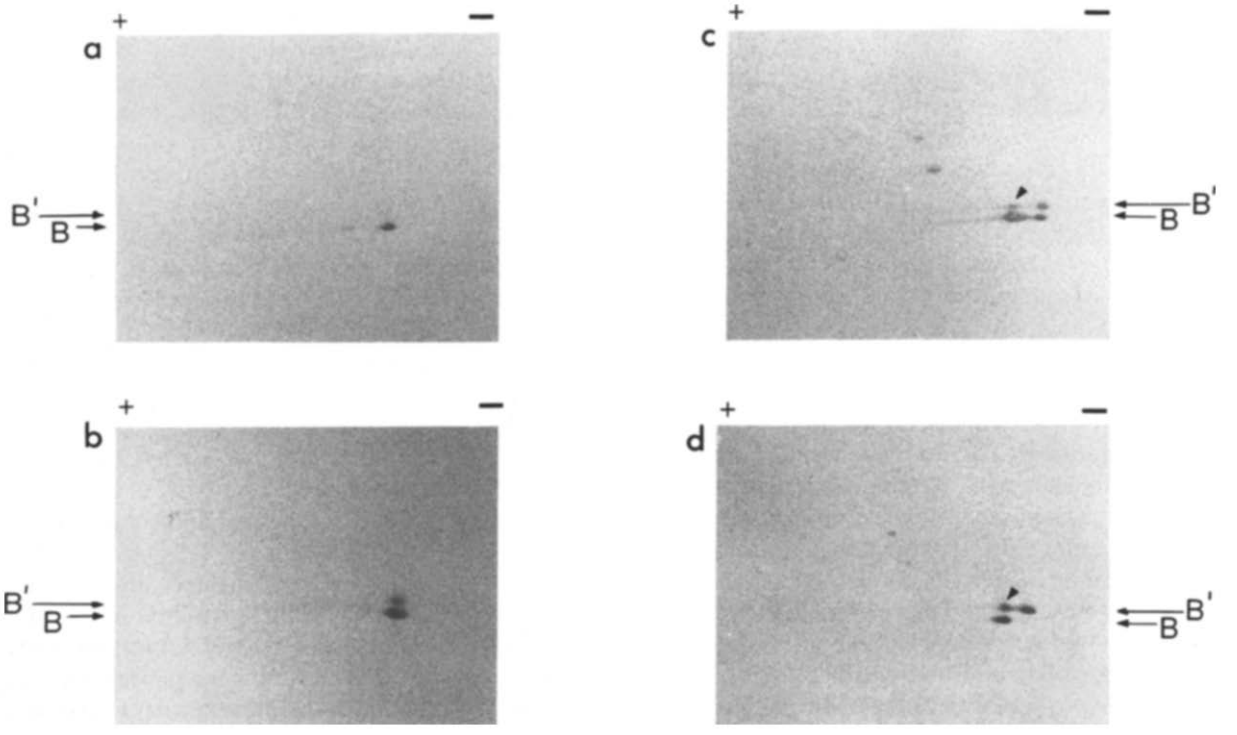

Fig.2. Two-dimensional Western blots with antibody KSm5. Protein samples were from A20 lymphocyte cells (a), PCC3 cells (b), HeLa cells (c) and Tera 2 cells (d). Arrows labelled B and B′ indicate the position of the SmB and B′ proteins in the second dimension gel as determined by co-electrophoresis of HeLa cell protein in a single slot of the second dimension gel followed by Western blotting. The + and − signs indicate the basic and acidic ends of the first dimension gel, respectively. The arrowed spot is discussed in the text.

The elevated levels of SmB′ in human and mouse EC cells detected in one-dimensional gels thus appear to be due at least in part to elevated expression of a more acidic SmB′ isotype. This occurs despite the fact that human cells have high levels of a more basic isotype of SmB′ which is undetectable in rodent cells.

The presence of SmB′ in EC cells suggested that it might also be detected in mouse embryos. Fig.3a shows that this was indeed the case, SmB′ being detectable in the total protein obtained from 13 day embryos. However, the expression of this protein in embryos was found to be tissue specific, the protein being undetectable in the foetal liver, but expressed at high level in foetal brain (fig.3a). Further experiments showed that such over-expression of SmB′ was also observed in adult brain (where SmB′ is detectable at considerable higher levels than SmB) compared to adult liver (fig.3a). Other adult tissues such as kidney and spleen showed a

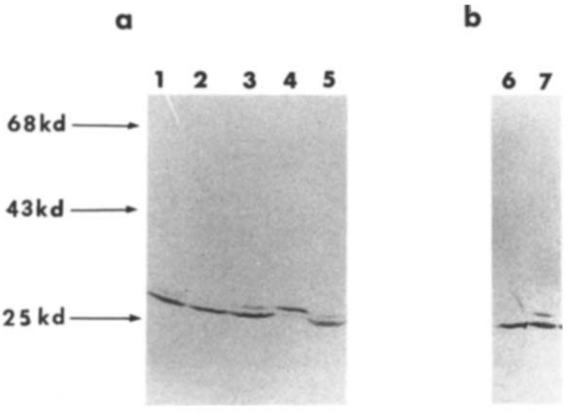

Fig.3. Western blot of rodent tissues (a) and cell lines (b) with antibody KSm5. Track 1, 13-day mouse embryo (strain CBA); track 2, 13-day foetal liver; track 3, 13-day foetal brain; track 4, adult mouse brain; track 5, adult mouse liver; track 6, A20 lymphocytes; track 7, PC12 adrenal medullary carcinoma. Arrows indicate the position and sizes of molecular mass markers.

similar pattern to that observed in the liver. This suggests that elevated expression of SmB′ represents a consistent tissue specific difference between brain and other organs regardless of the stage of development.

The detection of elevated SmB′ levels in cells as distinct as EC cells and foetal and adult brain raises questions as to the significance of this effect. One common feature of such cells is that both EC cells and some neurons in the brain express the alternatively spliced calcitonin/CGRP gene [19,20] and are capable of processing the primary transcript to mature CGRP mRNA [19,21].

Experiments in which a calcitonin/CGRP gene was constitutively expressed in a variety of cell types by transfection [19] and the construction of transgenic mice [21] suggested that this processing ability is found in only a small minority of cell types. Thus the vast majority of cells, including all the cells we have shown to lack SmB′, appear to be capable of processing the primary transcript to yield only the calcitonin mRNA. These experiments, together with mutational analysis of the calcitonin/CGRP gene, have led to the suggestion [19,21] that whilst the constitutively expressed splicing apparatus can readily produce calcitonin mRNA from the primary transcript, the production of CGRP mRNA required the presence of an additional splicing factor allowing cells containing it to follow an alternative splicing pathway. Our data suggest that SmB′ may be this splicing factor. In agreement with this idea SmB′ is detectable in three rat thyroid medullary carcinoma cell lines [22,23] which produce CGRP, further extending the correlation of SmB′ expression with the ability to splice for CGRP mRNA (fig.4).

If SmB′ is indeed required for specific RNA splicing, it should also be observed in rodent cell types such as the PC12 rat adrenal medullary carcinoma line [24] which is capable of processing the primary calcitonin/CGRP transcript to produce CGRP mRNA following transfection [19] although it does not naturally express the calcitonin/CGRP gene. Fig.3b shows that this prediction is correct. The SmB′ protein was clearly detectable in these cells but not in the A20 lymphocyte line which has been shown to produce only the calcitonin mRNA following transfection [19].

Hence expression of SmB′ appears to be associated with an alternative splicing pathway

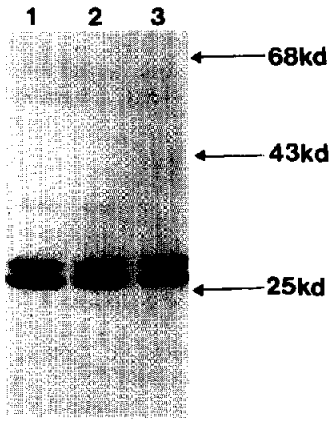

Fig.4. Western blot of rodent thyroid medullary carcinoma cell lines, with antibody KSm5. Track 1, rat MTC 6-23 cells; track 2, rat MTC 44-2 cells; track 3, rat MTC 6-23 C cell line. Arrows indicate the positions and sizes of molecular mass markers.

which in EC cells and some neurons allows the production of CGRP mRNA rather than calcitonin mRNA. The expression of SmB′ in other cells that do not normally express the calcitonin/CGRP gene presumably reflects other alternative splicing decisions in these cells for example that which produces substance P rather than substance K in some neurons [4]. The ability to follow this alternative splicing pathway may be dependent on the presence of the more acidic isotype of SmB′, which is present in both human and rodent EC cells. Alternatively this ability may simply depend on the elevation of total SmB′ levels above that typical of most cells in the species concerned, this possibility being suggested by the observation that variation in the concentration of splicing extracts can affect the choice of splice site in vitro [25].

The development of in vitro splicing systems which mimic the tissue specific regulation of alternative splicing observed in vivo will be necessary to distinguish these possibilities. Whatever the precise mechanisms, however, our findings indicate that cell type specific variation in at least one of the Sm components of the spliceosome does occur, and that such variation may play an important role in the regulation of at least one alternative RNA splicing pathway. Similarly, other examples of alternative splicing found in cell types lacking SmB′ (see for example [3]) may be regulated by as yet unidentified tissue specific variants of the Sm proteins.

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