Biochem. J. (1991) 277, 719–722 (Printed in Great Britain)

Differential expression of the mouse U1a and U1b SnRNA genes is not dependent on sequence differences in the octamer motif

Isabel LEA,*† Harry D. M. MOORE† and David S. LATCHMAN*

*Medical Molecular Biology Unit, Department of Biochemistry, University College and Middlesex School of Medicine, The Windeyer Building, Cleveland Street, London W1P 6DB, U.K., and †Institute of Zoology, London Zoo, Regents Park, London NW4, U.K.

The mouse U1b SnRNA gene is expressed in only a limited range of cell types, whereas the U1a SnRNA gene is expressed in all cells. These two genes differ in the sequence of the octamer motif, which plays a critical role in SnRNA gene regulation. We show that the U1b octamer binds the octamer-binding protein Oct-1 with higher affinity than does the U1a octamer in both U1b-expressing and -non-expressing cell lines and tissues. Moreover, the U1b octamer can direct a higher level of gene expression than the U1a octamer when linked to a heterologous promoter and introduced into a non-U1b-expressing cell line. Hence the tissue-specific expression of the U1b gene is not determined by the failure of its octamer motif to bind Oct-1 or the weak affinity of this binding.

INTRODUCTION

The octamer motif containing a conserved octamer core and an additional adjacent conserved A residue (consensus ATGCAAATNA) is found in the promoters of a wide variety of cellular genes, including those encoding histone H2B, the small nuclear RNAs and the immunoglobulin heavy and light chains (for review see [1]). In the genes which contain it, this motif plays a critical role in determining their specific expression patterns [2–4]. For example, in the case of the genes encoding the U series SnRNAs, which are expressed in all cell types, the octamer motif within these genes is capable of binding the octamer-binding protein Oct-1, which is also expressed in all cell types [5,6]. This binding is essential for the transcription of the SnRNA genes, with deletion of the octamer motif or its mutation abolishing the expression of the genes [7–9]. The interaction of the constitutively expressed Oct-1 protein with the octamer motif therefore results in the constitutive expression of the SnRNA genes.

In contrast with the constitutive expression of most SnRNAs, however, the mouse contains two distinct forms of the U1 SnRNA, one of which, U1a, is present in all cell types, whereas the other, U1b, is found in only a limited range of cells and tissues [10]. Thus, whereas U1b is found at similar levels to U1a in embryonic tissues, it is absent in most adult cell types, being detectable only in tissues such as testis, spleen and thymus which retain a stem cell population capable of further differentiation [10].

In view of the essential role played by U1 and the other SnRNAs in the splicing of mRNA precursors (for review see [11]), it is possible that a tissue-specific form of U1 might play a role in the regulation of alternative splicing events which differ in different tissues (for review see [12]), and it is therefore of importance to understand the processes regulating the differential expression of the U1a and U1b genes.

Interestingly, analysis of the promoter regions of the U1a and U1b genes has shown that their octamer motifs differ from one another by a single base pair (Fig. 1). In turn, both of these octamers differ from the consensus octamer sequence found in other SnRNA genes such as those encoding U2 and U4, the U1a octamer differing by two bases from the consensus and the U1b octamer differing by only a single base ([13], see Fig. 1).

In view of the importance of the octamer motif in SnRNA gene regulation, it is possible that these differences in the octamer motif are responsible for the different expression patterns of the U1a and U1b genes. For example, the U1b octamer might fail to bind the constitutively expressed Oct-1 protein and hence might be dependent upon an octamer-binding protein expressed only in embryonic or stem cells. Alternatively, the U1b octamer might bind Oct-1 but with much lower affinity than does the U1a octamer or the consensus sequence. Hence U1b would only be expressed in cells containing sufficient levels of Oct-1 to bind to all of its target promoters. In contrast, in other cell types where Oct-1 was limiting, U1b would be unable to compete with U1a and the other SnRNA genes for Oct-1 binding and hence would not be expressed (see [13] for a discussion of this idea).

To test these possibilities, we have studied the ability of the U1a and U1b octamer elements to bind Oct-1 as well as their ability to drive gene expression when linked to a heterologous promoter.

MATERIALS AND METHODS

Cells and tissues

C127 mouse fibroblasts [14] and C1300 mouse neuroblastoma cells [15] were grown respectively in Dulbecco's modified Eagle's medium or Royal Postgraduate Medical Institute 1640 medium, both of which were supplemented with 10% foetal calf serum. Mouse testis and liver were obtained from adult Balb/C mice.

Octamer consensus	A	T	G	C	A	A	A	T	N	A
U1a	A	T	G	T	A	G	A	T	G	A
U1b	A	T	G	C	A	G	A	T	A	A
U4	A	T	G	C	A	A	A	T	C	A

Fig. 1. Relationship of the octamer consensus sequence [1] and the octamer sequences in the U1a and U1b SnRNA genes and in the U4 SnRNA gene [13]

The eight-base octamer core sequence is overlined. In addition, the base two nucleotides downstream of the core is also conserved in most genes containing the octamer [1], and the two nucleotides adjacent to the octamer core in the various SnRNA genes are therefore shown for comparison.

Abbreviation used: CAT, chloramphenicol acetyltransferase.

Oligonucleotides

Complementary pairs of oligonucleotides containing the U1a or U1b octamer sequences were synthesized on an Applied Biosystems model 381A oligonucleotide synthesizer. All oligonucleotides were synthesized so that, when annealed, the double-stranded molecule would have a 5′ GATC single-stranded extension at either end to facilitate cloning. After annealing, the oligonucleotides were labelled by phosphorylation with [γ-^{32}P]ATP and T4 polynucleotide kinase.

DNA mobility shift assays

Nuclear extracts were prepared from approx. 5×10^7 cells as described by Dignam *et al.* [16]. For the binding assay, 10 fmol of [^{32}P]ATP-labelled oligonucleotide probe was mixed with 1 μl of nuclear extract in the presence of 20 mM-Hepes, 5 mM-$MgCl_2$, 50 mM-KCl, 0.5 mM-dithiothreitol, 4% Ficoll and 2 μg of poly(dIdC) per 20 μl reaction volume. Competitor DNA was added at appropriate molar excess at this stage, as required. The binding reaction was incubated on ice for 40 min prior to electrophoresis on a 4% polyacrylamide gel in $0.25 \times$ TBE ($1 \times$ TBE = 100 mM-Tris/HCl/100 mM-boric acid/2 mM-EDTA, pH 8.3) for 2–3 h at 150 V and 4 °C. DNA–protein complexes were visualized by autoradiography of the dried gel and quantified by scanning the autoradiographs on a Bio-Rad model 620 video densitometer.

Transfection

Annealed octamer oligonucleotides were cloned as 2-mer into the *Bam*HI site of pBL2 CAT vector [17], and recombinants were identified by screening of the resultant colonies with labelled oligonucleotide [18]. Recombinant plasmids were transfected as previously described [8] using 5 μg of DNA/2×10^6 cells on a 90 mm plate. Following transfection, cells were harvested and the protein content was determined by the method of Bradford [19]. The chloramphenicol acetyltransferase (CAT) activity of samples equalized for protein content was then determined by the method of Gorman [20].

RESULTS AND DISCUSSION

To investigate the affinity of the U1a and U1b octamer motifs for Oct-1, we prepared nuclear extracts from C127, cells which are mouse mammary fibroblasts that lack detectable U1b expression, and from C1300 cells, a mouse neuroblastoma which expresses high levels of U1b [10]. These extracts were then used in DNA mobility shift assays [21] with a labelled oligonucleotide (sequence ATGCAAATGAGAT) containing an octamer motif which binds Oct-1 with high affinity. As expected, a single DNA–protein complex formed with both cell extracts when this oligonucleotide was used as a probe, confirming that both cell types contained Oct-1 (Fig. 2).

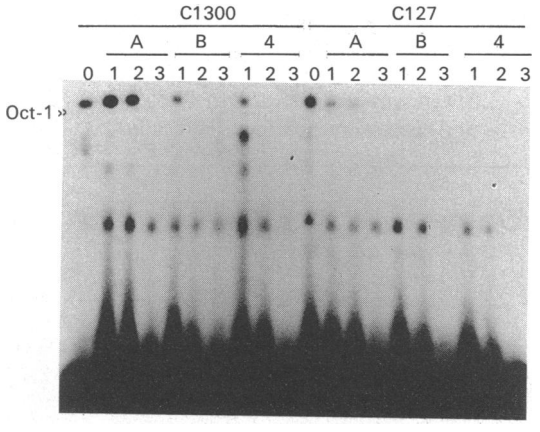

Fig. 2. DNA mobility shift assay using nuclear extracts prepared from C1300 cells or C127 cells and a labelled octamer oligonucleotide

The assays were carried out in the absence of competitor (track 0) or in the presence of a 1-fold (tracks 1), 10-fold (tracks 2) or 100-fold (tracks 3) excess of U1a (A), U1b (B) or U4 (4) octamer competitors. The arrow indicates the position of the shift produced by the constitutively expressed Oct-1 protein.

In order to determine the ability of the U1a and U1b octamers to compete for this protein, we carried out the binding reaction using the same labelled octamer oligonucleotide as before, but including various molar excesses of unlabelled U1a or U1b competitor oligonucleotide in the binding reaction. In these experiments (Fig. 2), the U1b octamer competed almost as well for binding as did an octamer oligonucleotide containing the consensus sequence found in the U4 SnRNA gene promoter, with effective competition being observed at a 10-fold molar excess of competitor. In contrast, the U1a octamer oligonucleotide competed significantly less well, with little competition even at a 100-fold excess of competitor. Hence the U1b octamer can compete as effectively for Oct-1 as the consensus octamer, in agreement with its differing from the consensus by only a single base, whereas the additional base difference in the U1a octamer significantly diminishes its affinity for Oct-1.

In order to test whether these differences in the affinity of the U1a and U1b octamers were confined to cell lines which had been cultured for long periods, we also prepared extracts from adult mouse liver, which expresses only U1a, and from adult mouse testis, which expresses both U1a and high levels of U1b [10]. In these experiments a similar pattern was observed, with the U1b octamer consistently exhibiting a higher binding affinity than the U1a octamer in both tissue types (results not shown).

Hence the U1b octamer is able to bind Oct-1 with higher affinity than the U1a octamer in U1b-expressing and -non-expressing cell lines and tissues. This suggested that the failure of U1b expression in most cell lines and tissues is not due to a failure to bind Oct-1 or a weak affinity for this factor. It remained possible from these experiments, however, that the constitutive expression of the U1a RNA was dependent upon its binding with high affinity a form of Oct-1 which was poorly bound by the consensus octamer motif and which would therefore not be detected when this motif was used as the probe in our previous competition experiments. Similarly, the U1b octamer might compete poorly or not at all for this form of Oct-1, explaining its tissue-specific expression pattern.

To test this possibility, we carried out further DNA mobility shift experiments using either labelled U1a or U1b octamers as the probe and excess unlabelled U1a or U1b as the competitor. In all of these experiments, whether carried out using extracts prepared from cultured cells (Fig. 3) or from tissues (results not shown), the U1b octamer consistently exhibited a higher affinity for octamer-binding protein than did the U1a octamer. This effect was observed regardless of whether the U1a or U1b octamer was used as the labelled probe and regardless of whether the cell line or tissue expressed U1b.

It is clear, therefore, that the tissue-specific expression of the U1b SnRNA is not dependent on the failure of its octamer motif to bind Oct-1 or on the low affinity of this binding. DNA mobility shift experiments can only assay the strength of binding, however, and not its functional consequences. We therefore wished to assess whether the U1b octamer was able to activate gene expression in a non-U1b-expressing cell line. Accordingly, 2-mer of the U1a and U1b oligonucleotides containing two tandem copies of the octamer motif were cloned upstream of the herpes simplex virus thymidine kinase promoter in the vector

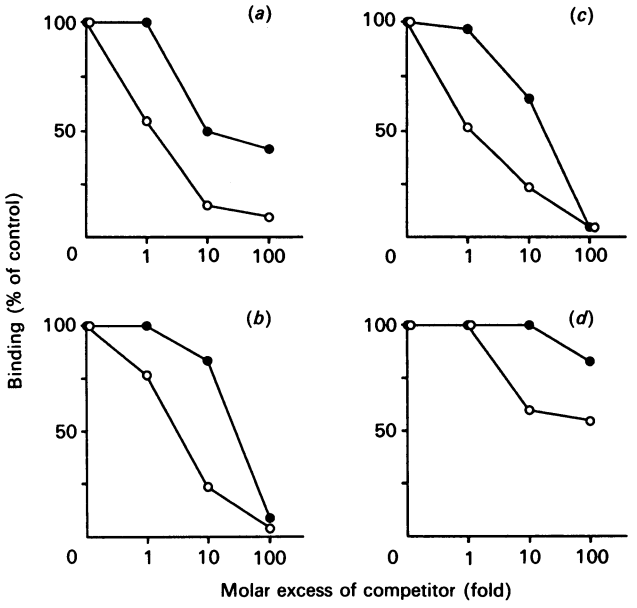

Fig. 3. DNA mobility shift assays showing competition with U1a and U1b octamers

Densitometric analysis is shown of the intensity of the Oct-1 band which forms using C1300 (*a*, *b*) or C127 (*c*, *d*) cell extracts on a labelled U1a octamer (*a*, *c*) or U1b octamer (*b*, *d*) in the absence or presence of the indicated excess of unlabelled U1a (●) or U1b (○) competitor. Values are expressed as percentages of the binding in the absence of competitor.

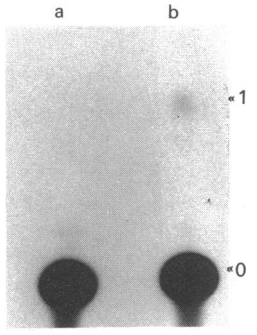

Fig. 4. Assay of CAT activity obtained by transfecting C127 cells with pBL_2 CAT vector containing a 2-mer of either the U1a (a) or the U1b (b) octamer oligonucleotide

The positions of the unacetylated (0) and monoacetylated (1) forms of chloramphenicol are indicated. Activity of the promoter driving the CAT gene results in the production of active CAT enzyme able to convert the unacetylated form of chloramphenicol into the monoacetylated form.

pBL2 CAT [17]. In this vector, the tk promoter drives expression of the readily assayable CAT gene [20], providing a simple means of testing the effect of any sequence on the activity of a heterologous promoter.

When these constructs were introduced into C127 cells, which do not express U1b, acetylation of chloramphenicol was detectable only in extracts of the cells transfected with the U1b construct. Hence in this experiment the U1b octamer directed a much higher level of gene activity than did the U1a octamer (Fig. 4). Hence the increased binding of Oct-1 by the U1b octamer compared with the U1a octamer has functional consequences, allowing it to direct a higher level of activity from a heterologous promoter even in a non-U1b-expressing cell line.

This indicates, therefore, that the failure of U1b expression in most cell types is not dependent upon the inability of its octamer motif to bind octamer-binding protein and activate gene expression in these cells. The mechanisms producing the tissue-specific expression of the U1b gene do not appear, therefore, to involve the octamer motif alone. It remains possible, however, that the octamer motif is unable to direct the expression of the U1b gene in non-expressing cells because binding of Oct-1 is prevented by an inhibitory protein present in these cells. If this is the case, however, this inhibitory protein must bind to a site adjacent to or only minimally overlapping the octamer, since we did not observe any such tissue-specific proteins binding to the octamer itself in our DNA mobility shift experiments.

Moreover, it is possible that the entire U1b promoter is not tissue-specific in its activity. Thus Moussa *et al.* [22] observed significant activity of a U1b gene with 400 bp of upstream sequence following transfection of mouse L cells, which do not normally express high levels of U1b. Hence the U1b promoter may actually be active in all cell types. If this is the case, the tissue-specific accumulation of the U1b RNA must be controlled either post-transcriptionally by differences in RNA stability in different cell types, or at the transcriptional level by differences in chromatin structure which control the accessibility of the U1b promoter to constitutive factors such as Oct-1. Further experiments will be necessary to resolve these possibilities.

The lack of involvement of the octamer motif in tissue-specific expression pattern does raise the question, however, of why the U1 SnRNA genes have octamer motifs which differ from the consensus. Thus the two octamer motifs found in the mouse U1a and U1b genes are also found in two rat U1 genes, although in this case, unlike U1a and U1b, both genes encode the same U1 RNA [23]. Similarly, the U1 gene in humans has an octamer motif identical with that in the mouse U1a gene and which therefore differs by 2bp from the consensus octamer found in most other octamer-containing genes [24]. We have previously shown that the nature of the octamer motif in the human U1 gene is responsible for its failure to be induced by the herpes simplex virus virion protein Vmw65, since it does not bind the complex of Oct-1 and Vmw65 that is necessary for such activation to occur [8]. It is possible, therefore, that the sequence differences between the U1 octamer and the consensus may play a role in some aspect of U1 gene regulation in both rodents and humans. The data presented here suggest, however, that they are unlikely to play any role in the differential expression of the mouse U1a and U1b genes.

I. L. was supported by an SERC/Case studentship. We thank the Arthritis and Rheumatism Council for purchasing the scanning densitometer used in these studies.

REFERENCES

1. Falkner, F. G., Moickat, R. & Zachau, H. G. (1986) Nucleic Acids Res. **13**, 7847–7863
2. Mason, J. O., Williams, G. T. & Neuberger, M. S. (1985) Cell **41**, 479–487
3. Parslow, T. G., Blair, D. L., Murphy, W. J. & Granner, D. K. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 2650–2654
4. Scheidereit, C., Heguy, A. & Roeder, R. G. (1987) Cell **51**, 783–793
5. Sive, H. L. & Roeder, R. G. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 6382–6386
6. Fletcher, C., Heutz, N. & Roeder, R. G. (1987) Cell **51**, 773–781
7. Mattaj, I. W., Lienhard, S., Jimcay, J. & De Robertis, E. M. (1985) Nature (London) **316**, 163–167
8. Kemp, L. M. & Latchman, D. S. (1988) EMBO J. **7**, 4239–4244
9. Dahlberg, J. E. & Lund, E. (1988) in Structure and Function of Major and Minor Small Ribonucleoprotein Particles (Birnsteil, M., ed.), pp. 38–70, Springer Verlag, Heidelberg

10. Lund, E., Kahan, B. & Dahlberg, J. E. (1985) Science **229**, 1271–1274
11. Maniatis, T. & Reed, R. (1987) Nature (London) **325**, 673–678
12. Latchman, D. S. (1990) New Biologist **2**, 297–303
13. Howard, E. F., Michael, S. K., Dahlberg, J. E. & Lund, E. (1986) Nucleic Acids Res. **14**, 9811–9825
14. Lowy, D. R., Rands, E. & Scolnick, E. M. (1978) J. Virol. **26**, 291–298
15. Augusti-Tocco, G. & Sato, G. (1969) Proc. Natl. Acad. Sci. U.S.A. **64**, 311–315
16. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. (1983) Nucleic Acids Res. **11**, 1475–1489
17. Luckow, B. & Schutz, G. (1987) Nucleic Acids Res. **15**, 5490
18. Grunstein, M. & Hugness, D. S. (1981) Proc. Natl. Acad. Sci. U.S.A. **72**, 3961–3964
19. Bradford, M. M. (1976) Anal. Biochem. **72**, 248–254
20. Gorman, C. M. (1985) in Gene Cloning (Glover, D. M., ed.), vol. 2, pp. 143–190, IRL Press, Oxford
21. Fried, M. & Crothers, D. M. (1981) Nucleic Acids Res. **9**, 6505–6525
22. Moussa, N. M., Lobo, S. M. & Marzluff, W. F. (1985) Gene **36**, 311–319
23. Watanabe, N. & Ohshima, Y. (1988) Eur. J. Biochem. **174**, 125–132
24. Lund, E. & Dahlberg, J. E. (1984) J. Biol. Chem. **259**, 2013–2021

Received 28 January 1991/27 February 1991; accepted 27 March 1991