Control of mouse U1a and U1b snRNA gene expression by differential transcription

Javier F. Cáceres*, Debbie McKenzie§, Rama Thimmapaya§, Elsebet Lund and James E. Dahlberg*
Department of Biomolecular Chemistry, University of Wisconsin—Madison, Madison, WI 53706, USA

Received May 13, 1992; Revised and Accepted July 24, 1992

ABSTRACT

The expression of mouse embryonic U1 snRNA (mU1b) genes is subject to stage- and tissue-specific control, being restricted to early embryos and adult tissues that contain a high proportion of stem cells capable of further differentiation. To determine the mechanism of this control we have sought to distinguish between differential RNA stability and regulation of U1 gene promoter activity in several cell types. We demonstrate here that mU1b RNA can accumulate to high levels in permanently transfected mouse 3T3 and C127 fibroblast cells which normally do not express the endogenous U1b genes, and apparently can do so without significantly interfering with cell growth. Expression of transfected chimeric U1 genes in such cells is much more efficient when their promoters are derived from a constitutively expressed mU1a gene rather than from an mU1b gene. In transgenic mice, introduced U1 transgenes with an mU1b 5’ flanking region are subject to normal tissue-specific control, indicating that U1b promoter activity is restricted to tissues that normally express U1b genes. Inactivation of the embryonic genes during normal differentiation is not associated with methylation of upstream CpG-rich sequences; however, in NIH 3T3 fibroblasts, the 5’ flanking regions of endogenous mU1b genes are completely methylated, indicating that DNA methylation serves to imprint the inactive state of the mU1b genes in cultured cells. Based on these results, we propose that the developmental control of U1b gene expression is due to differential activity of mU1a and mU1b promoters rather than to differential stability of U1a and U1b RNAs.

INTRODUCTION

U1 small nuclear RNA participates in the recognition of the 5' splice sites of pre-messenger RNAs during splicing (1–8). The accumulation of U1 RNAs is controlled in both mice and frogs (Xenopus), with electrophoretically distinguishable forms being characteristic of cells or tissues at different stages of development (9–12). Adult forms (called U1a RNAs) are present in all cell types whereas embryonic forms (U1b RNAs) are present only in cells that are capable of further differentiation such as germ line or embryonic stem cells. Strain-specific sequence variants of these forms exist, with two adult (mU1a1 and mU1a2) and six embryonic (mU1b1–b6) species having been described for mice (10,11). The seven nucleotide differences that distinguish all mU1b RNAs from mU1a RNAs, clustered between positions 60 and 77 in stem-loop n (10,11), appear to influence the efficiency of binding of a U1 RNA-specific protein, the A protein (13). Recently, developmentally controlled U1 snRNA variants have also been described in sea urchins (14), fruit flies (15) and plants (16).

In mouse cultured cells, the levels of mU1b RNAs apparently reflect the developmental lineage of the original cells. Thus, fully differentiated fibroblasts like C127 and 3T3 cells synthesize little, if any, mU1b RNAs (less than 2% of mU1a), whereas mouse L cells and embryonal carcinoma cells (EC cells) produce intermediate to high levels of these RNAs (30% and 55% of the total U1 RNAs, respectively) (10).

Although the different isoforms of U1 RNA might influence splice site utilization (17), they could also be functionally equivalent, as are the differentially expressed oocyte- and somatic-type 5S ribosomal RNAs of X. laevis (18). To date, there have been no reports of accumulation of high levels of embryonic forms of U1 RNA in cells that normally contain only the adult form. Thus, the consequences of accumulation of these RNAs in inappropriate cell types are unknown. Furthermore, the mechanism by which these RNAs accumulate in a differential manner in various cell types and tissues has not been determined. While a mechanism due to increased synthesis seems likely, one cannot rule out the possibility that stabilizing proteins are present only in embryonic tissues, and that U1b RNAs are preferentially degraded in 'non-expressing' cells.

Here, we show that expression of embryonic U1 RNA in mouse cells, both in culture and in intact animals is controlled...
transcriptionally, rather than by degradation. Furthermore, we show that accumulation of embryonic RNA is not detrimental to cells that normally have only the adult form.

MATERIALS AND METHODS

DNA templates

All U1 gene constructs (Figure 1) were cloned between the PstI and HindIII sites of a modified pAT153 vector (19) containing the kanamycin/G418 resistance gene of pCGBP9 (20) inserted between the BamHI and HindIII sites. The mouse mU1a1, mU1b2 and mU1b6 gene sequences were from clones pU1al-214, pU1b-136 and -453 (21), respectively, and the human U1 coding plus 3' flanking region sequences were from clone pHU1-1D (22). For generation of the chimeric genes, the various 5' flanking and U1 coding region sequences were connected via the Bell site present at position +27 of all the Ul coding regions (cf. Figure 1). Restriction enzyme digestions, ligations and other DNA manipulations were performed according to Sambrook et al. (23).

Growth and Transfection of mammalian cells

Mouse NIH 3T3, C127, F9 and LT-C18 cells (10) were maintained in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum. For transfection of NIH 3T3 cells, 10 or 20 µg of plasmid DNA and 40 µg of Lipofectin (Bethesda Research Laboratories) were used per 60 mm dish of cells. The DNA and the Lipofectin reagent were combined as suggested by the manufacturer. 24 hours later the medium was changed and after additional 24 hours of growth the transfected cells were split (1:3) and subsequently maintained in selective medium containing 0.4 mg of G418 ('Geneticin', Sigma) per ml of medium. After 14 to 18 days of selection, individual clones of G418-resistant cells were isolated and expanded into cell lines. For transfection of C127 cells, the calcium phosphate precipitation method was used (24); selection of G418 resistant clones was as above.

Preparation of nucleic acids

Total nucleic acids (DNA and RNA) of cultured cells and mouse tissues were isolated by the urea lysis method (10,25), using 4 ml of urea lysis buffer per 1-2 x 10⁶ cells. After two extractions with phenol/chloroform/isoamylalcohol (24:24:1), the nucleic acids were precipitated with ethanol, washed with 67% ethanol, dried and resuspended in 10 mM Tris pH 8, 1mM EDTA.

Analysis of RNA

For northern blot analyses, total RNAs were separated by electrophoresis either in partially denaturing gels containing 12% (30:0.8) polyacrylamide (for resolution of mouse U1a and U1b RNAs (10)) or in non-denaturing gels containing 15% (19:1) (26) or 12% (19:1) polyacrylamide (27) (for separation of human and mouse U1a RNAs). After electrophoresis and staining with ethidium bromide, the RNAs in the U1 RNA region of the gel were transferred to Gene Screen Plus (New England Biolabs) or Zeta Probe (BioRad) nylon membranes by electroblotting using an LKB Transphor unit. For hybridization, blots were incubated at 68°C in 0.9 M NaCl, 1% SDS, 10 mM Tris (pH 7.5), 1 mM EDTA containing a 32P-labeled U1-specific RNA probe as previously described (10).

Immunoprecipitation

32P-labeled snRNPs were precipitated from clarified cell-sonicates by incubation with human polyclonal anti-Sm antibodies (28) coupled to protein A-agarose. After proteinase K digestion, phenol extraction and ethanol precipitation, the immunoprecipitated RNAs were analyzed by polyacrylamide gel electrophoresis, as above, and individual RNAs were quantitated by Cerenkov counting of excised gel pieces. 32P-labeled precursor snRNAs containing mG-caps were isolated from total RNA by immuno-precipitation with rabbit polyclonal anti mG-cap antibodies (29).

Analysis of DNA

Restriction enzyme digestion, agarose gel electrophoresis and Southern blotting were according to standard procedures (23), using Gene Screen Plus (New England Biolabs) or Zeta Bind (Cuno) nylon membranes and alkaline transfer buffer (30). For Southern blots, hybridization conditions were as described for northern blots except the probes were 32P-labeled DNA prepared by random priming using a 'Prime a gene' kit (Promega) according to instructions. The structures of the mU1b6- and mU1b2-specific probes are indicated in Figure 6. The mU1al-specific probe (Fig. 2) corresponded to 5' flanking region sequences from position −220 to −800 and the plasmid-specific probe (Fig. 4B) was the BamHI-HindIII fragment containing the kanamycin resistance gene.

Transgenic mice

To generate transgenic mice, the 3.6 kb PstI fragment containing the mouse mU1b2 promoter and the human hU1 coding region (plus 3' end flanking sequences) was purified from the mU1b2/hU1 plasmid DNA (Fig. 1, bottom construct); in addition to the chimeric U1 gene, this fragment also contained 346 basepairs of pBR322 DNA sequences (corresponding to positions 29 to 375). This PstI fragment was introduced into fertilized mouse eggs (31,32) and transgenic mice were identified by Southern blot analyses of tail DNA using the pBR322 sequences as a transgene-specific probe. Homozygous transgenic mice were identified by quantitative slot blot hybridization. Tissues to be used for northern blot analyses of U1 RNAs were isolated from sexually mature mice as described previously (10).

RESULTS

Two general models could explain the low levels of U1b RNA in differentiated cells. On the one hand, the RNA could be unstable in such cells, perhaps due to the absence of a protecting protein. On the other hand, the U1b gene promoters might not be active in such cells. To differentiate between these models we have investigated both the stability of U1b RNA and the activity of U1b promoters in 3T3 and C127 fibroblast cells in which the level of U1b RNA is normally very low.

Stable expression of U1b RNA in 3T3 cells

To test the stability of U1b RNA in NIH 3T3 cells, we stably transfected the cells with a chimeric U1 gene that contained a mouse U1a1 promoter coupled to a mouse U1b6 coding region
(mU1a/U1b6, Fig. 1). Accumulated U1 RNAs were analyzed from these cells and from control cells which had been transfected by another chimeric U1 gene, mU1a/hU1, that would direct the synthesis of a human U1a-type RNA (hU1). mUlb6 RNA, hU1 RNA and endogenous mU1a RNA can be distinguished by electrophoresis in partially denaturing (10) or non-denaturing gels (26,27).

As illustrated in Figures 2A and B, very high levels of both U1b6 and hU1 RNAs could be achieved in such transfected cells (e.g., lanes 4). Thus, mU1b RNA is stable and can accumulate in differentiated mouse cells. We note that the amounts of accumulated mU1b or hU1 RNAs varied between different cell lines (for example, Fig. 2B, lanes 3–6), as would be expected if the transfected, exogenous genes were integrated in variable copy numbers and/or at different chromosomal locations. These data also show that the constitutive mU1a promoter is utilized efficiently in 3T3 cells.

The RNA products of the transfected genes were incorporated efficiently into U1 snRNPs (28). As illustrated in Figure 3, both mU1b6 RNA and hU1 RNA were precipitated to the same extent as mU1a RNAs using either polyclonal anti-Sm (lanes 2 and 4) or anti-RNP antibodies (not shown). Moreover, in E12 cells the relative levels of mU1a and mU1b RNAs that were precipitable as precursors (using anti-m7G cap antibodies; 29) correlated with the levels of mature RNAs in U1 snRNPs (data not shown).

Therefore, the control of accumulation of mU1b RNA in 3T3 cells apparently is not mediated through degradation of newly made RNAs.

Southern blot analyses of BamHI-digested DNAs isolated from the transfected cells (Figure 2C) showed that the number of copies of the exogenously introduced chimeric genes was significantly higher than that of the endogenous mU1a genes (e.g., lanes 2 and 3). However, since the endogenous mU1a genes detected by this 5' flanking region probe represent only about ten per cent of all of the mouse U1 genes (21,33–35), we estimate that the numbers of introduced and endogenous genes are comparable.
Figure 4. Differential activity of mU1a and mU1b promoters in transfected fibroblasts. (A) Differential expression of chimeric mUlb2/hUl (G cell lines) and mUla/hUl genes (F cell lines) in transfected C127 cells. Total cellular RNAs of untransfected C127 cells (lane 1), transfected G1 (lane 2), G4 (lane 3), G21 (lane 4) or F23 cells (lane 6) and human HeLa cells (lane 4) were separated as in Figure 2B and assayed for Ul RNAs by northern blot hybridization. (B) Comparison of gene copy numbers of the transfected, chimeric genes in G and F cell lines. Southern blot of BamHI-digested genomic DNAs of G (lanes 2 and 3) and F cells (lanes 5 and 6) (Fig. 2C) was hybridized with a plasmid-specific kanamycin resistance gene probe (Plas.). Linearized mUlb2/hUl (Lane 1) or mUla/hUl (lane 4) plasmid DNAs (arrowheads) were mixed with DNA of untransfected C127 cells. (C) (D) Stable transfaction of 3T3 cells with wildtype mUlb6 genes (B cell lines). (C) Anti-Sm precipitable 32P-labeled RNAs of untransfected 3T3 (lane1) and transfected B8 (lane 2) and B16 cells (lane 3) were analyzed in a partially denaturing gel as in Figure 3. (D) Copy number analysis of transfected mUlb6 genes in B cell lines. Southern blot of BamHI-digested genomic DNAs of 3T3 (lane 1), B8 (lane 2) and B16 cells (lane 3) were hybridized with an mUlb6-specific probe (Fig. 6). Linearized mUlb6 plasmid DNA (arrowhead) was mixed with 3T3 DNA (lane 1); endogenous (Endog.) mUlb6 genes are indicated by the bracket.

Table 1.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>U1 Gene</th>
<th>U1b RNA</th>
<th>hUl RNA</th>
<th>U2 RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>3T3</td>
<td>Transfected</td>
<td>1.5</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>E12</td>
<td>mUla/mU1b</td>
<td>37</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>F4</td>
<td>mUla/mU1b</td>
<td>46</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

Gene dosage compensation in stably transfected mouse 3T3 cell that carry multiple copies of chimeric Ul genes. The relative levels of individual snRNAs were determined by scintillation counting of excised gel pieces (Figure 3 and data not shown).

Hence, the amount of Ul RNA made per gene is about the same for the endogenous and exogenous templates.

Although the Ul RNAs from the exogenous genes represent approximately 40% of the total Ul RNAs of the transfected cells (cf. Fig. 3, lanes 2 and 4), the total amount of Ul RNA (i.e., the sum of endogenous mUla1 and mUla2 plus either mUlb6 or hUl RNAs) did not increase relative to the other major (endogenous) snRNAs; this is evident from the constant ratio of Ul to U2 (or U4 and U5) RNAs (see Table 1 and data not shown). Thus, we conclude that gene dosage compensation is operating in transfected 3T3 cells, as has been described previously for bovine papilloma virus-transformed mouse C127 cells carrying multiple copies of an hUl gene (27).

Differential Activities of mUla and mUlb promoters in transfected cell lines

To determine if mUlb promoters could function in cells that normally do not express mUlb genes, we stably transfected C127 cells with a chimeric gene that contains the 5' flanking region of an mUlb2 gene and the coding region of an hUl RNA gene (mUlb2/hUl, Fig. 1; G cell lines). As a control, C127 cells were also transfected with the chimera mUla/hUl (F cell lines). Northern blot analysis of Ul RNAs from such cells (Fig. 4A) demonstrated that the accumulation of hUl RNA was consistently lower when the cells received genes with the mUlb2 promoter (G cell lines, lanes 2 and 3) rather than the mUla promoter (F cell lines, lanes 5 and 6). Southern blot analysis of the RNAs of the transfected cells (Figure 4B), showed that similar levels of chimeric genes were present in all cell lines (compare lanes 2, 3, 5 and 6). Likewise, low levels of mUlb promoter activity were also observed in NIH 3T3 cells transfected with the mUlb2/hUl construct (data not shown).

The promoter of an mUlb6 gene, another variant of the mouse U1b genes, also had low activity in 3T3 cells. In two independent, stably transfected B cell lines, mUlb6 RNA accumulated for only 5-7% of the anti-Sm precipitable or total Ul RNA of the cells (Fig. 4C, lanes 2 and 3; data not shown), although Southern blot analysis showed that the copy numbers of the transfected mUlb6 genes were high relative to the endogenous genes (Fig. 4D, compare lane 1 with lanes 2 and 3). We conclude that, in contrast to mUla promoters, the mUlb promoters have little activity in differentiated cells.
Tissue-specific activity of an mUlb2 promoter in transgenic mice

The results in Figures 2–4 indicate that the differential control of mUlb gene expression in cultured cells is mediated by the 5’ flanking region of the genes, rather than by the structure of the RNA transcript. To test if this control reflects the situation in intact animals, we generated a line of transgenic mice carrying the chimeric mUlb2/hUl gene; hence, accumulation of the reporter RNA, hUl RNA, would indicate mUlb2 promoter activity.

Northern blot analysis of total RNA isolated from various tissues of homozygous and heterozygous mice (Fig. 5) shows that hUl RNA accumulates in adult testis, which normally do not accumulate mUlb RNAs (Lund et al., 1985), show no detectable hUl RNA (lanes Br, Ov and data not shown). Thus, the chimeric mUlb transgene was subject to normal tissue-specific controls, indicating that also in whole animals control is determined by sequences in the 5’ flanking region of the gene rather than by Ulb RNA instability.

Methylation of Endogenous mUlb genes

As an independent test of whether the mUlb6 genes are indeed transcriptionally inactive in cultured cells, we analyzed the degree of methylation of CpG sequences in the 5’ flanking regions of these genes. In vertebrates, most of the CpG sequences in genomic DNA are methylated as m^5CpG; non-methylated sequences are confined to the so-called ‘CpG islands’ present in the 5’ flanking sequences of genes (36,37). CpG islands associated with transcriptionally inactive genes are unmethylated in tissues of intact animals, but they are frequently methylated in tissue culture cells (38,39).

The developmentally expressed variants of the mUlb genes studied here (21) and other isolates of mUlb2 genes (40) have CpG islands in their immediate 5’ flanking regions, but the constitutively expressed mUlb genes lack CpG islands. We analyzed the state of methylation of the mUlb genes in genomic DNA of cultured cells and tissues, after first separating the genes from other, irrelevant sequences by digestion with PstI. The resulting PstI fragments were further digested with either MspI (which recognizes the sequence C-C-G-G regardless of methylation) or its isoschizomer HpaII (which does not digest C-m^5C-G-G) and the redigestion products were characterized by Southern blotting, using probes specific for the 5’ flanking regions of mUlb6 or mUlb2 genes.

As shown in Figure 6A, PstI-digestion generated a major 5.5 kb fragment that corresponds to the cloned mUlb6 gene (panel A and data not shown). Additional digestion with MspI reduced the size of this mUlb6-specific fragment by several kilobases (panel B). However, redigestion with HpaII left the 5.5 kb PstI fragments of DNA of 3T3 cells intact (panel C, lane 1), indicating that the MspI/HpaII sites of the mUlb6 genes were highly methylated in these cells. In contrast, not all of these HpaII sites were methylated in the DNA of L cells (lane 2), as demonstrated by the generation of some shorter fragments (cf. panel B). In the extreme case of embryonal carcinoma (EC) LT-C18 and F9 cells, the mUlb6 genes were unmethylated (panel C, lanes 3 and 4). The decreased methylation of mUlb6 genes in L cells and the lack of methylation in EC cells correlates with the fact that these cells synthesize moderate or large amounts of Ulb RNA, respectively (10). However, no methylation of CpG sequences is apparent in the 5’ flanking regions of mUlb genes of whole animal tissues like liver, brain and testis (panels B and C, lanes 5–7), regardless of the levels of accumulated Ulb RNA (10).
Figure 7. Lack of methylation of CpG islands in transfected mUlb6 genes. The methylation status of CpG sequences of transfected (B cell lines) and endogenous mUlb6 genes (brackets) was assayed by Southern blot hybridization using the mUlb6-specific probe (cf. Figure 6). Genomic DNAs of untransfected 3T3 cells (lanes 1) or transfected B4 (lanes 2), B8 (lanes 3) and B16 cells (lanes 4) were digested with either BamHI alone (panel A), BamHI+MspI (panel B) or BamHI+HpaII (panel C). Redigestion of the BamHI fragments with HpaII (or HinP1) trims the ends of the large endogenous mUlb6-specific fragments generating slightly faster migrating HpaII-resistant forms, as indicated by the brackets (compare panels A and C; data not shown). The amounts of B8 and B16 DNAs in panel C (lanes 3 and 4) were lower than those used in panels A and B. The dot (•) in panel C (lane 2) marks fragments derived from introduced mUlb6 genes that comigrate with fragments from endogenous genes in panel B.

These observations were extended to other potential methylation sites by redigestion of the PstI-generated fragments with HinP1, an enzyme that cuts DNA at GpCpGpC but not at GmpGpCpG sequences (Fig. 6A, panel D). Again, the mUlb6 genes of 3T3 and L cells showed a significant level of methylation of the HinP1 sites (lanes 1 and 2) whereas these sites were unmethylated in EC cells (lanes 3 and 4) and in adult tissues (lanes 5–7).

The members of another family of mUlb genes, the mUlb2 genes, were also methylated in cultured cells in which they were not expressed (Fig. 6B). Analysis of PstI-digested genomic DNAs was complicated slightly by polymorphisms in the Ulb2 gene family that resulted in size variations in three of the six mUlb2-specific PstI fragments (a/a', c/c' and f/f'; 35). Consequently, digests of DNAs of 3T3, L and F9 cells produced fragments a, c and f (panel A, lanes 1, 2 and 4) whereas those of LT-C18 cells and all tissues of LT mice yielded fragments a', c' and f' (lanes 3 and 5–7). Redigestion with MspI produced small fragments of mUlb2 genes that were not retained on the blot (panel B). Again, only the DNAs of 3T3 and L cells produced mUlb2 PstI fragments that were resistant to redigestion with HpaII or HinP1 (panels C and D, lanes 1 and 2).

Methylation of other mU1 genes

DNA methylation cannot account for the relatively low level of transcription of mUlb6 genes transfected into 3T3 cells (Fig. 4C). In BamHI-digested DNAs from such transfected cells (B cell lines) the exogenous genes were sensitive to digestion by either MspI or HpaII (Fig. 7, panels B and C, lanes 2–4). This difference in methylation between the endogenous (brackets; cf. Fig. 6A) and the introduced mUlb6 genes was confirmed by redigestion with HinP1 (data not shown).

The mUla gene studied here contains no CpG islands in the 5' upstream region (21). As expected, no methylation of CpG sequences was detected in that region of these constitutively expressed genes either in cultured cells or in the tissues (data not shown).

The observed patterns of methylation of endogenous mUlb genes are consistent with control of their expression being mediated at the level of transcription. This conclusion is in complete agreement with our findings (Figs. 4 and 5) that the 5' flanking regions of these genes are responsible for differential expression.

DISCUSSION

At least two classes of U1 small nuclear RNAs exist in mice, the constitutively expressed mUla RNAs, and the developmentally regulated mUlb RNAs (called the adult and embryonic forms, respectively). Here, we have investigated the mechanism of differential accumulation of mUlb RNA and examined whether control is exerted through stability of the RNA product or through inactivity of the promoter.

As model systems for these analyses we used primarily mouse tissue culture cells that showed low, intermediate or high levels of expression of their endogenous mUlb genes. By stably transfecting these cells with chimeric genes we were able to produce mUlb RNA in cells where it normally is not observed and to analyze the relative strength of mUla and mUlb promoters in such cells. Furthermore, we used the state of methylation of the endogenous mUlb genes of cultured cells as an indicator of whether these genes were transcriptionally active. We find that mUlb RNAs are stable in cells that normally do not accumulate them, whereas mUlb promoters are largely inactive. Thus, we conclude that the level of mUlb RNA in differentiated cells is controlled primarily through modulation of mUlb promoter activity.

It is unclear whether mUlb promoter inactivation occurs by the action of a specific inhibitory protein that represses gene activity or by the inability of the promoter to bind sufficient amounts of transcription factors. While mUla and mUlb promoters share elements that are characteristic of snRNA genes, such as the DSE and PSE (reviewed in references 41–43), the precise sequences of these elements are not identical; hence, they could differ in their abilities either to compete for a limiting transcription factor(s) or to function in the absence of such a factor. We have shown that the DSE acts as an enhancer that is specific for genes utilizing a PSE, such as U1 snRNA (44). This snRNA-specific activation is mediated by the Oct-1 transcription factor, which binds to octamer sequences within the DSE and interacts with other proteins, presumably at the PSE (45). Because of the promoter and factor specificity of the DSE, the significance of assays of mUlb octamer function that are based on mRNA transcription (46) remain unclear.

Sequences outside the shared elements differ significantly between mUla and mUlb promoters (21). For example, the 5' flanking region of the mUlb6 gene has three binding sites for the transcription factor Spl (47), whereas the promoter region
of the mU1a gene has no such sites (21). If binding of Sp1 to these sites is required for efficient transcription of U1b genes, then the decrease in the level of this factor during development (48) might lead to differential inactivation of these genes. Such a model does not rule out a possible role for a repressor-like factor that would specifically shut down mU1b transcription. We are currently testing these models using transiently transfected 3T3 cells, to determine whether a titratable inhibitor exists and whether the two classes of U1 genes compete equally in various types of cells (49).

Stably transfected genes with mU1b promoters are expressed only poorly in C127 or 3T3 fibroblast cells, which normally have almost no mU1b RNAs (10). It is unlikely that this is due to inopportune integration of the transfected genes into inactive chromatin, because the same result was obtained every time the experiment was repeated. The promoters themselves are functional in the right environment (or cell type), as evidenced by their activity when introduced into transgenic mice (Fig. 5) or when injected into X. laevis oocytes (data not shown). We did not test the activity of these genes in stably transfected L-cells, which express intermediate levels of mU1b RNAs, but Moussa et al. (50) did report transcription of exogenous mU1b 2 genes in such cells.

Methylation of CpG islands in promoter regions can be envisioned as a way of imprinting the inactivity of genes in cultured cells (38,31). When the CpG sequences of the endogenous mU1b genes of different cell lines were analyzed, a good correlation was apparent between low levels of mU1b RNAs and the extent of DNA methylation (Fig. 6). This result strongly supports our conclusion that mU1b genes are transcriptionally inactive in differentiated cells. The lack of methylation of these sequences in DNAs of differentiated tissues of adult animals does not contradict this conclusion since many genes that are transcriptionally inactive are unmethylated in whole animals (38,39).

In several cell lines that were stably transfected with DNA carrying the mU1a promoter and either hU1 or mU1b6 coding regions, up to half of the total accumulated U1 RNA were transcribed from the exogenous genes (Figs. 2 and 3). In spite of this new, additional source of active promoters, the total amount of U1 RNA per cell remained constant, relative to the levels of U2 or U4 RNAs (Table 1). Similar gene dosage compensation has been described before, in mouse cells transformed with recombinant bovine papilloma virus DNA containing a human U1 gene (27). It is unclear whether the intracellular level of U1 RNA is kept constant through a system that monitors total accumulated U1 RNA or through a mechanism that limits the total rate at which U1 synthesis can occur (for example by having a limited amount of a transcription factor). However, it is unlikely that gene dosage compensation is controlled by the same mechanism(s) that operate in the tissue- and stage-specific expression of mU1b genes during development.

The utility of having two independently controlled classes of mU1 RNA genes is unclear. In transfected 3T3 cells the mU1b6 RNA made from exogenous genes having mU1a promoters are incorporated into snRNPs and presumably can function in splicing of pre-mRNAs. Also, such stably transfected cells, in which up to 40% of the U1 RNA is the ‘inappropriate’ embryonic form, grow normally (J.C. and R.T., unpublished results). Thus, this control does not appear to be essential for cultured cells, but it could be important in whole animals at some stage of development. Therefore, it will be of interest to learn whether mice can develop normally with a high level of chimeric transgene(s) composed of the mU1a promoter and the coding region of an mU1b gene.

ACKNOWLEDGEMENTS

We thank Adrian Bird for stimulating discussions about DNA methylation, and members of the Dahlberg laboratory and Noruia Hernandez for critical comments. We thank Mel Simon for hospitality and enthusiastic support during our sabbatical leave (E.L. and J.E.D.) and Jessica Dousman for expert assistance with generation of transgenic mice. This work was supported by NIH grant GM 30220 to J.E.D. and E.L.

REFERENCES