Journal of Cellular Biochemistry 58:490–498 (1995)

U2-snRNP B″ Protein Gene Is an Early Growth-Inducible Gene

Jens Laitinen, Per Saris, Erkki Hölttä, and Ingvar Pettersson

Department of Pathology (J.L., E.H.) and Institute of Biotechnology (P.S.), University of Helsinki, FIN-00014, Helsinki, Finland; Department of Medical Cell Genetics, Medical Nobel Institute, Karolinska Institute, S-10401 Stockholm, Sweden (I.P.)

Abstract In this work we isolated mouse U2-snRNP–specific *b″* clones and analysed the expression of the mouse U2-snRNP–specific *b″* and U1-snRNP–specific *70K* genes in NIH-3T3 fibroblasts. Stimulation of growth-arrested NIH-3T3 cells with serum was found to evoke a rapid increase in the amount of cytoplasmic *b″* and *70K* mRNAs. These increases in mRNA did not require de novo protein synthesis. Moreover, the inhibition of protein synthesis by cycloheximide caused a superinduction in the amounts of the U1-snRNP–specific 70K transcripts. We also found that c-Ha-*ras*^{Val12} oncogene-transformed NIH-3T3 cells have higher levels of the *b″* and *70K* mRNAs than the normal 3T3 cells. These data imply that the *b″* and *70K* are early growth response genes, and their enhanced expression might be of significance in the processing of pre-mRNAs into mature mRNAs.

Key words: serum-stimulation, transformation, chromatin structure mRNA, RNA polymerase II, μg-small nuclear RNP, NIH-3T3 cells, *ras* oncogene

The small nuclear ribonucleoprotein particles (snRNPs) participate in the splicing of pre-mRNA as well as in the maturation of RNA polymerase II transcripts. The snRNPs are at least partially assembled in the cytoplasm and then migrate to the nucleus, where they, together with the pre-mRNA and hnRNP (heterogenous nuclear RNP) proteins, form a spliceosome [for reviews see Maniatis and Reed, 1987; Mattaj, 1988; Steitz et al., 1988; Parry et al., 1989]. The snRNPs contain one of the unique, uracil-rich, and nonpolyadenylated small nuclear RNAs (U1–U8 snRNA) associated with a subset of at least 14 distinct proteins, including 70K, A, A′, B, B′, B″, C, D, E, F, and G [Hinterberger et al., 1983; Pettersson et al., 1984; Reddy and Busch, 1988; Lührmann, 1988]. Seven proteins—B, B′, D, D′, E, F, and G—are common in all snRNPs [Lührmann, 1988]. The three proteins 70K, A, and C are unique for the U1-snRNPs, whereas the A′ and B″ are unique for the U2-snRNPs [Mimori et al., 1984; Pettersson et al., 1984].

Received November 28, 1994; accepted January 5, 1995.

Address reprint requests to Jens Laitinen, Department of Pathology, P.O. Box 21, Haartmaninkatu 3, University of Helsinki, FIN-00014, Helsinki, Finland.

Since the snRNPs are present at 10^5–10^6 copies/cell [Reddy and Busch, 1988; Lührmann, 1988], the snRNP protein genes must be efficiently transcribed in proliferating cells. However, their transcription is poorly understood. It has been shown that the transcripts of the sequenced snRNP proteins are polyadenylated [Wieben et al., 1985; Habets et al., 1987; Sillekens et al., 1987; Etzerodt et al., 1988; Dam et al., 1989]. Further, Philipsson and coworkers [Etzerodt et al., 1988] have documented that the *70K* transcripts accumulate early during the *Xenopus* oogenesis.

Here we report the expression pattern of the mouse U2-snRNP–specific *b″* and the U1-snRNP–specific *70K* genes in synchronized mouse 3T3 fibroblasts. Since rapid growth and cell transformation is known to be associated with an aberrant expression of several genes [Gutman and Wasylyk, 1991], including the snRNA [Reddy et al., 1981] and snRNP genes [Etzerodt et al., 1988], we addressed the question of whether the mouse U2-snRNP–specific *b″* and *70K* genes are rapidly activated upon serum stimulation of quiescent cells and whether there are any differences in the *b″* mRNA levels between the normal and transformed cells. We further present a characterization of the chromatin structure of the *b″* gene in the normal and

c-Ha-*ras*$^{Val\,12}$ oncogene-transformed NIH-3T3 cells. To our knowledge this is the first report describing the expression of snRNP genes in normal and *ras* oncogene-transformed cells upon growth stimulation.

EXPERIMENTAL PROCEDURES

λgt11 Cloning and Screening

The U2-snRNP–specific *b″* clones were obtained by screening a mouse lung carcinoma λgt11 expression cDNA library (Clontech, Palo Alto, CA) with an anti-RNP serum from a patient with an autoimmune disease. Identification of the recombinant phage clones, cloning, and sequencing were carried out as described [Nyman et al., 1989, 1990].

Cell Culture and Synchronization

The normal NIH-3T3 fibroblasts (N1) and c-Ha-*ras*$^{Val\,12}$ oncogene-transformed NIH-3T3 cells (E4) cells were grown and synchronized by serum starvation as described previously [Sistonen et al., 1987; Hölttä et al., 1988; Laitinen et al., 1990]. The DNA content of the cell nuclei stained with ethidium bromide was analyzed by flow cytometry (FACScan; Becton-Dickinson, Immunocytometry Systems, Mountain View, CA).

Isolation of Nuclei and Cytoplasmic RNA

Nuclei from 5×10^7 to 10^8 cells were isolated as described previously [Moreno et al., 1986; Laitinen et al., 1990]. In brief, the cells lysed in 10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 3 mM $MgCl_2$, 0.5% NP-40 were centrifuged at maximal speed in an Eppendorf centrifuge for 10 s, and the pelleted nuclei were resuspended in 400–1,000 μl of the lysis buffer without NP-40. The supernatant fraction was used for isolation of cytoplasmic RNA. The supernatant was treated with proteinase K (1 mg/ml) for 0.5–3 h at +37°C and then subjected to extractions with one volume of neutralized phenol-chloroform (1:1) and with chloroform. RNA in the aqueous phase was precipitated with 2.5 volumes of ice-cold ethanol with 0.3 M sodium acetate, pH 5.2, overnight at −20°C [Maniatis et al., 1984].

Micrococcal Nuclease Digestions

The MNase (Boehringer Mannheim, Mannheim, Germany) digestion was carried out as described elsewhere [Moreno et al., 1986; Laitinen et al., 1990].

Isolation of Genomic DNA

DNA was isolated using the protein salting-out method as described elsewhere [Laitinen and Hölttä, 1994; Laitinen et al., 1994] with the following modifications. After digestion with proteinase K, 5 M NaCl was added to a final concentration of 1.2 M, and the tubes were vortexed for 30 s. Thereafter, the precipitated proteins were removed by three successive centrifugations at 400*g*, 450*g*, and 500*g*, for 15 min at +4°C. The resulting supernatant was transferred into new tubes, and DNA was precipitated by adding two volumes of room-temperature ethanol. The purity of the DNA samples was checked by measuring the O.D. ratio 260/280 nm and was found to be between 1.7 and 2.

Preparation of Insert Probes for Southern and Northern Blot Analyses

For detecting mouse *b″* gene sequences, appropriate inserts were generated from the pGEM clones, pRNP11 (485 bp), pRNP31 (489 bp), or pJL5 (246 bp) by the polymerase chain reaction (GeneAmp; Perkin-Elmer, Norwalk, CT). Oligonucleotides hybridizing to the SP6 (5′ GATTTAGGTGACACTATAG 3′) and T7 (5′ TAATACGACTCACTATAGGG 3′) promoter sequences flanking the inserts in pGEM3 vectors were used as primers (Promega, Madison, WI), with 25 cycles of 1 min at 94°C, 1 min at 37°C, and 2 min at 72°C for amplification of the inserts [Saiki et al., 1988].

The insert probes specific to *70K, ornithine decarboxylase* (*odc*), c-*jun,* and *glyceraldehyde-3-phosphate dehydrogenase* (*gapdh*) genes were prepared from the pEX70K1 [Nyman et al., 1990], pODC16 [Hickok et al., 1986], pAH119 [Ryseck et al., 1988], and pRGAPDH-13 [Fort et al., 1985] plasmids. From pEX70K1 we isolated a 1,271 bp EcoRI DNA fragment, from pODC16 a 936 bp Hind III cDNA fragment, from pAH119 a 2,156 bp Eco RI–Pvu II DNA fragment, and from pRGAPDH-13 a 1.3 kb Pst I *gapdh* fragment.

The inserts were isolated and purified from 1% low melting point agarose (LMP-Agarose; BRL, Life Technologies Inc., Gaithersburg, MD) gels. The gel slice was melted at +65°C for 15 min in a microcentrifuge tube, extracted for 3 min with 1 volume of neutralized phenol, and freezed at −70°C. This was followed by centrifugation at 13,000*g* for 10 min at room temperature, and the insert in the aqueous phase was precipitated with ethanol.

Electrophoresis, Blotting, and Hybridization Analyses of Nucleic Acids

Aliquots of DNA (10 μg) or RNA (20 μg) were separated on 1.6–1.8% agarose or 1.6% agarose/formaldehyde gels, respectively [Maniatis et al., 1984]. The fractionated nucleic acids were transferred by capillary blotting to Hybond-N nylon filters (Amersham, Buckinghamshire, UK), dried, and baked at +80°C for 2 h [Laitinen et al., 1990].

^{32}P-dCTP–labelled probes were prepared by standard random priming reactions according to the directions of the manufacturer (DuPont or Amersham), and hybridizations were carried out as described earlier [Laitinen et al., 1990]. The levels of mRNA and the degree of chromatin digestion were quantitated by densitometric scanning (ScanJet Plus, Hewlett-Packard, Greeley, CO; Scan Analysis, Biosoft, Cambridge, UK) of the autoradiograms.

RESULTS

In search for clones of snRNP proteins, we screened a mouse lung carcinoma λgt11 expression library with an anti-RNP serum of a patient with an autoimmune disease [Nyman et al., 1990]. Antibodies eluted from the fusion proteins produced by two clones, pRNP11 and pRNP31 (EMBL accession numbers X63019 and X63020, respectively), reacted with two snRNP proteins, A and B″, in immunoblots of HeLa nuclear extracts (data not shown). The cDNA inserts were sequenced and found to contain overlapping sequences with only two mismatched bases (Fig. 1A). Unlike the pRNP31 which has a truncated 3′ end, the pRNP11 also has a potential polyadenylation signal, the AATAA sequence (bold characters in Fig. 1A). pRNP11 and pRNP31 contain one copy of the putative mRNA destabilization sequence ATTTA [Shaw and Kamen, 1986] at positions 325 and 403, respectively (Fig. 1A). Computer searches with the pRNP11/pRNP31 sequence revealed extensive homologies with the two human U-snRNP-associated proteins A [Sillekens et al., 1987] and B″ [Habets et al., 1987]. Figure 1B shows the deduced amino acid sequence of pRNP11/pRNP31 aligned with the human A and B″ sequences. The two human proteins have very similar amino- and carboxyterminal domains but contain unique sequences in the middle part [Sillekens et al., 1987]. The mouse protein encoded by pRNP11/pRNP31 is highly homologous both with the common carboxyterminal domain and with the B″ specific region. Thus the cDNA clones pRNP11 and pRNP31 encode the carboxyterminal half of the mouse B″ protein corresponding to residues 102–225 in the human B″ protein (Fig. 1B).

As the amount of U-snRNAs has been reported to be elevated in transformed Novikoff hepatoma cells as compared with the normal rat liver cells [Reddy et al., 1981], and it has been found that the transcripts of the U1-snRNP-specific *70K* gene accumulate early in oogenesis of the *Xenopus* [Etzerodt et al., 1988], we wished to study whether the mouse U2-snRNP-specific *b″* gene is a growth-inducible gene and whether its amount is increased in transformed cells. For studying the expression of the mouse *b″* gene we isolated a 246 bp subclone pJL5 for use as a probe (Fig. 1C). On Northern blots of total cytoplasmic RNA, it hybridizes to a single 1.0 kb RNA (Figs. 2 and 3) which is consistent with the reported size of the human *b″* transcript [Habets et al., 1987].

First, we examined the expression levels of the mouse *b″* mRNA during the growth stimulation by 10% FCS of serum-starved normal (N1 cells) and c-Ha-*ras*^{Val12} oncogene-transformed (E4 cells) NIH-3T3 fibroblasts [Sistonen et al., 1987]. Total cytoplasmic RNA was isolated from the cells and subjected to Northern blot analysis. Figure 2A shows the kinetics of induction of the U2-snRNP-specific *b″* mRNA in the E4 cells. The *b″* mRNA levels were low in the quiescent cells but increased rapidly after serum stimulation (Fig. 2A). Scanning of the autoradiograms with a densitometer showed that the *b″* tran-

Fig. 1. Sequences of the mouse *b″* cDNAs in comparison to the human *b″* and *a* genes. **A:** Comparison between the mouse *b″* cDNAs, pRNP31 and pRNP11. Mismatched bases in the aligned sequences are shown as gaps. The potential translational termination site TAA and the potential polyadenylation signal AATAA are indicated by bold capitals. **B:** The alignment of the predicted amino acid sequence of the mouse B″ protein (pRNP31) (upper sequence) with the human B″ (middle) and human A (lowest) proteins using the one-letter amino acid code. The two human protein sequences are from Habets et al. [1987] and Sillekens et al. [1987], respectively. **C:** Alignment of the mouse *b″* cDNA (pRNP11) (upper sequence) with the human *a* gene [Sillekens et al., 1987] (lower sequence). Underlined bases indicate the sequence of a 250 bp Pst I insert (pJL5) used for studying the *b″* gene expression. Gaps in the sequences are indicated as periods.

A

```
pRNP31 CGGAAAAAGG AAAAGAAGAA AGCTAAAACC ATGGAACAGG CTGCAGCTGC TGCAAACAAG AAGCCTGGTC   70

   71 AGGGAACACC AAATGCAGCT AATACCCAAG GCACTGCAGC GCCAAATCCT CAGGTCCCTG ATTATCCTCC  140
pRNP11        CG AAATGCAGCT AATACCCAAG GCACTGCAGC GCCAAATCCT CAGGTCCCTG ATTATCCTCC   62

  141 AAATTATATC CTATTCCTTA ATAACTTACC AGAGGAGACA AATGAGATGA TGTTATCCAT GCTGTTCAAC  210
   63 AAATTATATC CTATTTCTTA ATAACTTACC AGAGGAGACA AATGAGATGA TGTTATCCAT GCTGCTCAAC  132

  211 CAGTTCCCTG GATTCAAGGA AGTTCGCTTG GTACCGGGGA GACATGACAT TGCATTTGTA GAATTTGAGA  280
  133 CAGTTCCCTG GATTCAAGGA AGTTCGCTTG GTACCGGGGA GACATGACAT TGCATTTGTA GAATTTGAGA  202

  281 ATGATGGTCA GGCTGGAGCT GCCAGAGATG CTCTGCAGGG GTTTAAGATT ACACCGTCCC ATGCCATGAA  350
  203 ATGATGGTCA GGCTGGAGCT GCCAGAGATG CTCTGCAGGG GTTTAAGATT ACACCGTCCC ATGCCATGAA  272

  351 GATCACCTAT GCCAAGAAGT AACATGTGAT GCCAATGTGG AAGGACTTGG TTATTTATAG TGTTTGTTTT  420
  273 GATCACCTAT GCCAAGAAGT AACATGTGAT GCCAATGTGG AAGGACTTGG TTATTTATAG TGTTTGTTTT  342

  421 GATCATATTC GGTCAAGTCA TTTTAAATGG TTGGAAGTGA AGGTGAAGTT TTGGGAGAAG AGTTGTCGG   489
  343 GATCATATTC GGTCAAGTCA TTTTAAATGG TTGGAAGTGA AGGTGAAGTT TTGGGAGAAG AGTTGTCTAA  412

  413 TTTTTTTTAG TTTTGCTGAA CTATGAAATA CGGAGCCTTA ATTTTGTACA ATAAACTTTT ATTTGTATTC  482
  483 TCG                                                                          485
```

B

```
Mouse U2-B"   1  RKKEKKKAKT MEQAAAAANK KPGQGTPNAA NTQGTAAPNP QVPDYPPNYI  50
Human U2-B" 103  KKKEKKKAKT VEQTATTTNK KPGQGTPNSA NTQGNSTPNP QVPDYPPNYI 152
Human U1-A  206  .......... .......... .......... .......... .....PPNHI 210

Mouse U2-B"  51  LFLNNLPEET NEMMLSMLFN QFPGFKEVRL VPGRHDIAFV EFENDGQAGA 100
Human U2-B" 153  LFLNNLPEET NEMMLSMLFN QFPGFKEVRL VPGRHDIAFV EFENDGQAGA 202
Human U1-A  211  LFLTNLPEET NELMLSMLFN QFPGFKEVRL VPGRHDIAFV EFDNEVQAGA 260

Mouse U2-B" 101  ARDALQGFKI TPSHAMKITY AKK                               123
Human U2-B" 203  ARDALQGFKI TPSHAMKITY AKK                               226
Human U1-A  261  ARDALQGFKI TQNNAMKISF AKK                               283
```

C

```
pRNP11 CCAGAGGAGA CAAATGAGAT GATGTTATCC ATGCTGCTCA ACCAGTTCCC TGGATTCAAG GAAGTTCGCT  160
hu-a   CCAGAGGAGA CCAACGAGCT CATGCTGTCC ATGCTTTTCA ATCAGTTCCC TGGCTTCAAG GAGGTCCGTC  840

  161 TGGTACCGGG GAGACATGAC ATTGCATTTG TAGAATTTGA GAATGATGGT CAGGCTGGAG CTGCCAGAGA  230
  841 TGGTACCCGG GCGGCATGAC ATCGCCTTCG TGGAGTTTGA CAATGAGGTA CAGGCAGGGG CAGCTCGCGA  910

        Pst I
  231 TGCTCTGCAG GGGTTTAAGA TTACACCGTC CCATGCCATG AAGATCACCT ATGCCAAGAA GTAACATGTG  300
  911 TGCCCTGCAG GGCTTTAAGA TCACGCAGAA CAACGCCATG AAGATCTCCT TTGCCAAGAA GTAGCACCTT  980

  301 ATGCCAATGT GGAAGGACTT GGTTATTTAT AGTGTTTGTT TTGATCATAT TCGGTCAAGT CATTTTAAAT  370
  981 TTCCCCCCAT GCCTGCCCCT TCCCCTGTTC TGGGGCCACC CCTTTCCCCC TTGGCTCAGC CCCCTGAAGG 1050

  371 GGTTGGAAGT GAAGGTGAAG TTTTGGGAGA AGAGTTGTCT AATTTTTTTT AGTTTTGCTG AACTATGAAA  440
 1051 TAAGTCCCCC CTTGGGGGCC TTCTTGGAGC CGTGTGTGAG TGAGTGGTCG CCACACAGCA TTGTACCCAG 1120

  441 TACGGAGCCT TAATTTTGTA CAATAAACTT TTATTTGTAT TCTCG                          485
 1121 AGTCTGTCCC CAGACATTGC ACCTGGCGCT GTTAGGCCGG AATTAAAGTG GCTTTTTGAG GTTTGGTTTT 1190
```

Fig. 1

scripts were accumulated two- to threefold after the serum stimulation for 1–3 h (Fig. 2B). These results were based on three independent experiments. To see whether the rapid accumulation of the *b″* mRNA is a specific property of the U2-snRNP–specific *b″* gene, we also performed comparative Northern blot analyses with the U1-snRNP–specific *70K* gene. The data show that there is also a small increase in the *70K* transcripts after growth stimulation (Fig. 2, second panel). The individual matched background subtraction of the densitometric scans revealed that in the stimulated cells the *70K* transcripts were increased twofold. As a reference, we also analyzed the accumulation patterns of the *ornithine decarboxylase* (*odc*) transcripts, since *odc* is known to belong to the early response genes [Stimac and Morris, 1987; Jähner and Hunter, 1991]. As is evident from the Northern blot analyses, the magnitude of the increases in the *b″* and *70K* transcripts were roughly similar to that of the *odc* mRNA (Fig. 2, third panel). It was also found that in the quiescent cells the basal levels of the snRNP transcripts were dependent on the duration of serum starvation similarly to that earlier reported for the *odc* and c-*myc* mRNAs [e.g., see Thompson et al., 1985; Stimac and Morris, 1987; Sistonen et al., 1989]. Such increases were not seen in the expression patterns of *glyceraldehyde-3-phosphate dehydrogenase* (*gapdh*) gene, whose mRNA levels usually remain invariant upon growth stimulation (Fig. 2, fourth panel).

Next we wanted to determine whether ongoing protein synthesis is needed for the increase of the *b″* and *70K* transcripts. Figure 3 shows that the accumulation of the *b″* mRNA did not require protein synthesis. Treatment with cycloheximide even slightly increased the *b″* transcript levels in some experiments, but no consistent superinduction of the *b″* gene was detected (Fig. 3). The accumulation of the *70K* transcript was superinduced by cycloheximide like that of the c-*jun* transcripts (Fig. 3) [Lau and Nathans, 1987; Ryseck et al., 1988, Lamph et al., 1988]. The degree of *70K* superinduction is, however, less pronounced than that of the c-*jun* gene (Fig. 3, third panel).

As transformed cells often display an aberrant accumulation of several transcripts [Reddy et al., 1981; Hölttä et al., 1988; Sistonen et al., 1989] and show increased levels of RNA components of snRNPs [Reddy et al., 1981], it was of interest to determine whether the U2-snRNP–

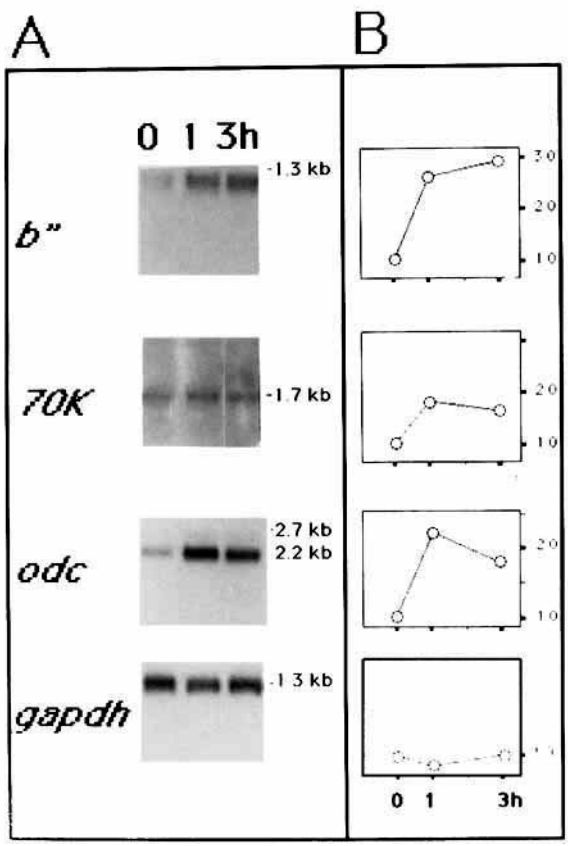

Fig. 2. Expression of mouse *b″*, *ornithine decarboxylase*, and *gapdh* genes in serum stimulated NIH-3T3 cells. E4 cells were synchronized by serum starvation for 16 h, whereafter 10% FCS was added to allow the cells to enter the growth cycle. **A:** Northern blot analyses of total cytoplasmic RNAs at different times after release from the growth arrest. The blots were sequentially hybridized to insert probes specific to *b″* (pJL5), *70K* (pEX70K1), *odc* (pODC16), and *gapdh* (pRGAPDH-13). *gapdh* was used as a control for loading. **B:** Densitometric scans of the relative levels of *b″*, *70K*, and *odc* mRNA (normalized to *gapdh* mRNAs) from data in A.

specific *b″* transcripts are accumulated in the c-Ha-*ras*^{Val12} oncogene-transformed cells. Similarly to that with the transcript levels of *odc* in the c-Ha-*ras*^{Val12} oncogene-transformed E4 cells [Hölttä et al., 1988], the levels of the *b″* and *70K* transcripts were clearly higher than those in the N1 cells (Fig. 4). This is also true for the *70K* mRNAs (data not shown). The *ras*-transformed E4 cells exhibited an about two- to threefold increase in the basal levels of cytoplasmic *b″* and *70K* mRNA when compared to the normal N1 cells. This difference was invariantly seen during growth stimulation by serum (1–3 h) both in asynchronous and synchronous cultures (data not shown).

Transcriptional activity of genes correlates with their sensitivity to micrococcal nuclease

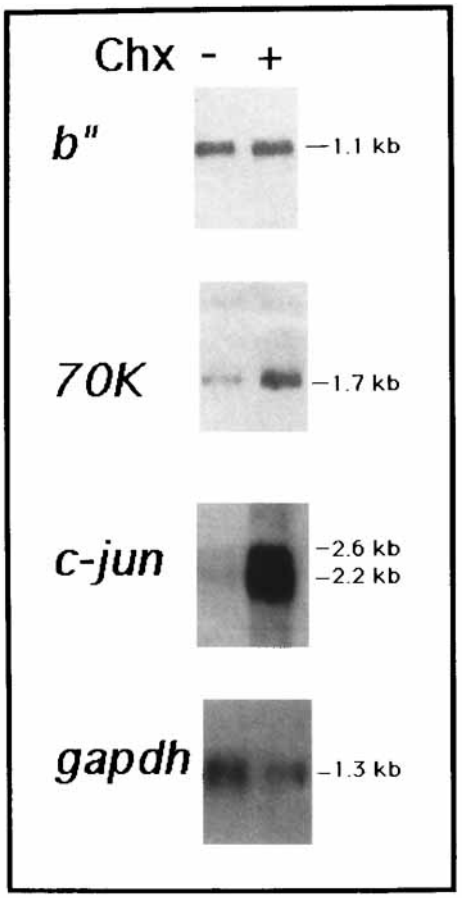

Fig. 3. Effect of cycloheximide on the *b″* gene expression in serum-stimulated 3T3 cells. E4 cells were synchronized by serum starvation and stimulated with 10% foetal calf serum for 3 h in the absence or presence of cycloheximide (10 μg/ml). The northern blots (see Fig. 2) were sequentially hybridized to insert probes specific to *b″* (pJL5), *70K* (pEX70K1) c-*jun* (pAH119), and *gapdh* (pRGAPDH-13). *gapdh* was used as a control for loading. The symbols − and + indicate the absence or presence of cycloheximide (Chx).

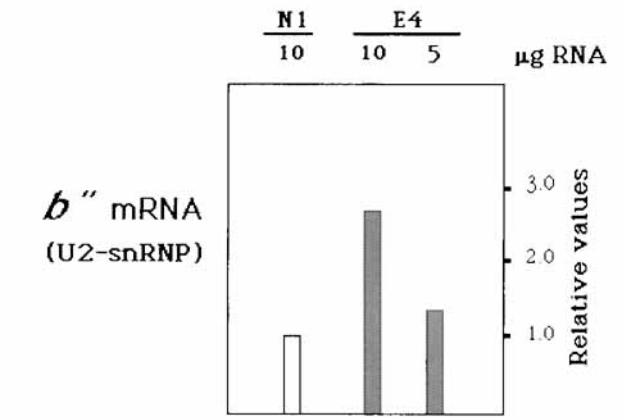

Fig. 4. Northern blot analysis of *b″* mRNA in quiescent normal and c-Ha-*ras*^{Val12} oncogene-transformed 3T3 cells. The normal (N1) and c-Ha-*ras*^{Val12} oncogene-transformed (E4) cells were synchronized and the levels of *b″* mRNA in the cells were analyzed as in Figs. 2 and 3. The bars indicate the relative abundance of *b″* mRNA in the normal (open bar) and *ras*-transformed cells (shaded bars) by densitometric scanning. The values are means from three independent experiments.

digestion [Smith et al., 1983; Yu and Smith, 1985; Moreno et al., 1986]. The growth-inducible accumulation of the *b″* mRNAs relative to the *gapdh* transcripts suggested that there may also be differences between the genes at the chromatin level. To study this, we isolated nuclei from the N1 and E4 cells, subjected them to MNase digestion, transferred the resulting DNA fragments by capillary blotting onto nylon filters, and probed them with the ^{32}P-dCTP–labelled insert probes specific to the *b″*, *odc*, and *gapdh* genes. It was found that the *b″* and *odc* genes are more accessible to the MNase digestions than the *gapdh* gene (Fig. 5A and data not shown). Densitometric scannings of the autoradiograms (Fig. 5B) clearly show that the *b″* chromatin displays a more decondensed nucleosomal organization in the transformed E4 cells than in the normal N1 cells (Fig. 5B).

DISCUSSION

Data base searches with the pRNP11 and pRNP31 nucleotide sequences revealed an extensive homology with the nucleotide sequence of the human U2-snRNP–specific *b″* gene [Sillekens et al., 1987; Habets et al., 1987, 1989]. Moreover, the predicted amino acid sequence of the mouse clones indicated a 91.8% similarity with the human B″ protein. Notably, the last 73 amino acids of the C-terminal region are identical between the mouse and human proteins. At this C-terminal region both the mouse and human B″ as well as the human A polypeptides contain the RNP consensus octamer (HDI-AFVEP), which is thought to be involved in U-snRNA binding [Query et al., 1989]. However, only the N-terminal region of the human B″ protein appears to be capable of directly binding to U2-snRNA [Scherly et al., 1990]. Thus, although no specific function has been assigned to the C-terminal region of the B″ protein yet, its evolutionary conservativeness supports the assumption that this region must contribute to some important structural/functional feature of the U2-snRNP. Interestingly, Gunderson et al. [1994] have recently found that the C-terminal part of A protein, which is very similar to that of B″ protein (Fig. 1) [Habets et al., 1987; Sillekens

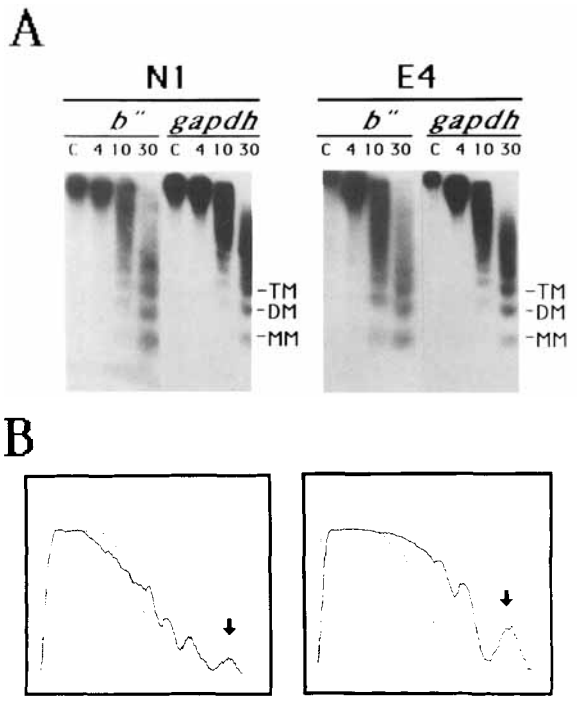

Fig. 5. Nucleosomal organization of the *b″* and *gapdh* genes. **A:** Micrococcal nuclease analysis of the nucleosomal organization of the *b″* and *gapdh* genes. The normal (**left panel**) and *ras* oncogene-transformed (**right panel**) cells were synchronized by serum starvation as in Fig. 2. Nuclei were digested with micrococcal nuclease (75 units/ml), extracted for DNA and electrophoresed on 1.6% agarose gels, and subjected to Southern blot analysis. The numbers on top of the gels indicate the digestion time with micrococcal nuclease in minutes. C indicates DNA from undigested nuclei. MM, DM, and TM stand for monomer, dimer, and trimer, respectively. **B:** Densitometric scans of the MNase digestion patterns representing 10 min digestions shown in A. The solid and dashed lines indicate the nucleosomal organization of *b″* and *gapdh* genes, respectively. Migration is from left to right. The arrows indicate the positions of DNA from the nucleosome monomer.

et al., 1987] is involved in the polyadenylation of its pre-mRNA via a direct interaction with poly(A) polymerase. Therefore, it is tempting to speculate that B″ protein would likewise be involved not only in splicing but also in the autoregulation of its own pre-mRNAs.

The data presented in this work show that stimulation of growth-arrested 3T3 cells with serum caused a rapid activation of the *b″* and *70K* genes. The rises in these snRNP transcripts were similar to the increase in the transcript levels (two- to threefold) of the early response *odc* gene following serum stimulation for 1–3 h. Significantly, the increase in the *b″* and *70K* mRNA was not dependent on de novo protein synthesis. In contrast to the *b″* transcripts, the rise in *70K* RNA was superinduced by cycloheximide treatment of the cells, suggesting that there are differences in controlling the expression of these genes.

Several studies have shown that transcriptionally active genes are preferentially cleaved by MNase [Smith et al., 1983; Weintraub, 1984; Yu and Smith, 1985; Moreno et al., 1986]. Further, we have earlier demonstrated that the nucleosomal organization of the immediate early genes *odc* and *c-myc* at their coding regions is more decondensed than that of the "house-keeping" *gapdh* gene [Laitinen et al., 1990]. Similarly, we found here that the nucleosomal organization of the *b″* gene is more loosened than that of the *gapdh* gene.

Only a few comparative studies exist on the quantities of snRNP components between the normal and transformed cells [see Reddy and Busch, 1988]. U-snRNAs have been found to display a tenfold elevation in Novikoff hepatoma cells as compared to the cells in normal liver. In this study we have explored the changes in the expression patterns of *b″* and *70K* genes upon morphological transformation of 3T3 cells by c-Ha-*ras*^{Val12} oncogene. It was found that the *ras*-transformed E4 cells have threefold higher levels of U2-snRNP–specific *b″* and *70K* transcripts than their normal counterparts.

We suggest that the increased expression of *70K* and *b″* genes could result in enhanced assembly of snRNP particles and pre-mRNA processing in serum synchronized cells and *ras* oncogene-transformed cells.

ACKNOWLEDGMENTS

We thank Dr. Nils R. Ringertz for the continuous support and research facilities, Dr. Moshe Yaniv for the plasmid pAH119, Dr. Olli A. Jänne for the pODC16 plasmid, Dr. Phil Fort for the pRGAPDH-13 plasmid, and Dr. Lea Sistonen and Dr. Kari Alitalo for the E4 and N1 cell lines. Dr. Ulf Nyman and Dr. Bensalah Lazrek are acknowledged for helpful advice and Evi Mellquist and Anna Maria Siivonen for technical assistance. We also thank Monica Schoultz for the FACS analyses and Björn Lindroos for the photography. This work was supported by the Nordic Council, University of Helsinki, the Finnish Cancer Organizations, the Sigrid Jusélius Foundation, the Magnus Ehrnrooth Stiftelsen, the Emil Aaltonen Foundation, the Finnish Academy of Sciences, and the Swedish Medical Research Council, grant B92-16X-09927-OIA (I.P.).

REFERENCES

Etzerodt M, Vignalli R, Gilberto G, Scherly D, Mattaj IW, Philipson L (1988): Structure and expression of a Xenopus gene encoding an sn RNP protein (U1 70K). EMBO J 7:4311–4321.

Fort P, Marty L, Piechaczyk M, Sabroury SE, Dani C, Janteur P, Blanchard JM (1985): Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. Nucleic Acids Res 13:1431–1442.

Gunderson SI, Beyer K, Martin G, Keller W, Boelens WC, Mattaj IW (1994): The human U1A snRNP protein regulates polyadenylation via a direct interaction with poly(A) polymerase. Cell 76:531–541.

Gutman A, Wasylyk B (1991): Nuclear targets for transcription regulation by oncogenes. Trends Genet 2:49–54.

Habets JW, Sillekens PTG, Hoet MH, Schalken JA, Roebroek AJM, Leunissen JAM, van de Ven WJM, van Wenrooij WJ (1987): Analysis of a cDNA clone expressing a human autoimmune antigen: Full-length sequence of the U2 small nuclear RNA-associated B″ antigen. Proc Natl Acad Sci USA 84:2421–2425.

Habets JW, Sillekens PTG, Hoet MH, McAllister G, Lerner MR, van Wenrooij WJ (1989): Small nuclear RNA-associated proteins are immunologically related as revealed by mapping of autoimmune reactive C-cell epitiopes. Proc Natl Acad Sci USA 86:4674–4678.

Hickok NJ, Seppänen PJ, Kontula KK, Jänne PA, Bardin CW, Jänne OA (1986): Two ornithine decarboxylase mRNA species in mouse kidney arise from size heterogeneity at their 3′ termini. Proc Natl Acad Sci USA 83:594–598.

Hinterberger M, Pettersson I, Steitz JA (1983): Isolation of small nuclear ribonucleoproteins containing U1, U2, U4, U5, and U6 RNAs. J Biol Chem 258:2604–2613.

Hölttä E, Sistonen L, Alitalo K (1988): The mechanisms of ornithine decarboxylase deregulation in c-Ha-*ras* oncogene-transformed NIH 3T3 cells. J Biol Chem 263:4500–4507.

Jähner D, Hunter T (1991): The stimulation of quiescent rat fibroblasts by v-*src* and v-*fps* oncogenic protein-tyrosine kinases leads to the induction of a subset of immediate early genes. Oncogene 6:1259–1268.

Laitinen J, Hölttä E (1994): Methylation status and chromatin structure of an early response gene (*ornithine decarboxylase*) in resting and stimulated NIH-3T3 fibroblasts. J Cell Biochem 55:155–167.

Laitinen J, Sistonen L, Alitalo K, Hölttä E (1990): c-Ha-*ras*^{Val12} oncogene-transformed NIH-3T3 fibroblasts display more decondensed nucleosomal organization than normal fibroblasts. J Cell Biol 111:9–17.

Laitinen J, Samarut J, Hölttä E (1994): A nontoxic and versatile protein salting-out method for isolation of DNA. Biotechniques 17:316–322.

Lamph WW, Wamsley P, Sassone-Corsi P, Verma I (1988): Induction of proto-oncogene JUN/AP-1 by serum and TPA. Nature 334:629–631.

Lau LF, Nathans D (1987): Expression of a set of growth-related immediate early genes in BALB/c 3T3 cells: Coordinate regulation with c-*fos* or c-*myc*. Proc Natl Acad Sci USA 84:1182–1186.

Lührmann R (1988): snRNP proteins. In Birnstiel ML (ed): "Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles." Berlin, Heidelberg, New York: Springer-Verlag, pp 71–99.

Maniatis T, Reed R (1987): The role of small nuclear ribonucleoprotein particles in pre-mRNA splicing. Nature 325: 673–678.

Maniatis T, Fritsch EF, Sambrook J (1984): "Molecular Cloning. A Laboratory Manual. New York: Cold Spring Harbor Laboratory.

Mattaj IW (1988): UsnRNP assembly and transport. In Birnstiel ML (ed): "Structure and function of major and minor small nuclear ribonucleoprotein particles." Berlin, Heidelberg, New York: Springer-Verlag, pp 100–114.

Mimori T, Hinterberger M, Pettersson I, Steitz JA (1984): Autoantibodies to the U2 small nuclear ribonucleoprotein in a patient with scleroderma-polymyotis overlap syndrome. J Biol Chem 259:560–565.

Moreno ML, Chrysogelos SA, Stein GS, Stein JL (1986): Reversible changes in the nucleosomal organization of a human *H4 histone* gene during the cell cycle. Biochemistry 25:5364–5370.

Nyman U, Ringertz NR, Pettersson I (1989): Demonstration of an amino terminal La epitope recognized by human anti-La sera. Immunol Lett 1:65–71.

Nyman U, Lundberg I, Hedfors E, Pettersson I (1990): Recombinant 70-kD protein used for determination of autoantigenic epitopes recognized by anti-RNP sera. Clin Exp Immunol 81:52–58.

Parry HD, Scherly D, Mattaj IW (1989): Snurpogenesis: The transcription and assembly of U snRNP components. Trends Biochem Sci 14:15–19.

Pettersson I, Hinterberger M, Mimori T, Gottlieb E, Steitz JA (1984): The structure of mammalian small nuclear ribonucleoproteins. Identification of multiple protein components reactive with anti-(U1)ribonucleoprotein and anti-Sm autoantibodies. J Biol Chem 259:5907–5914.

Query CS, Bentley RC, Keene JD (1989): A common RNA recognition motif identified within a defined U1 RNA binding domain of the 70K U1 snRNP protein. Cell 57:89–101.

Reddy R, Busch H (1988): Small nuclear RNAs: RNA sequences, structure, and modifications. In Birnstiel ML (ed): "Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles." Berlin, Heidelberg, New York: Springer-Verlag, pp 1–37.

Reddy R, Li W, Henning D, Choi YC, Nohga K, Busch H (1981): Characterization and subcellular localization of 7-8S RNAs of Novikoff hepatoma. J Biol Chem 256:8452–8457.

Ryseck R-P, Hirai SI, Yaniv M, Bravo R (1988): Transcriptional activation of c-*jun* during the G_0/G_1 transition in mouse fibroblasts. Nature 334:535–537.

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988): Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491.

Scherly D, Dathan NA, Boelens W, van Venrooj WJ, Mattaj IW (1990): The U2B″ RNP motif as a site of protein-protein interaction. EMBO J 9:3675–3681.

Shaw G, Kamen R (1986): A conserved AU sequence from the 3′ untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell 46:659–667.

Sillekens PTG, Habets WJ, Beijer RP, van Wenrooij WJ (1987): cDNA cloning of the human U1 snRNA-associated A protein: Extensive homology between U1 and U2 snRNP-specific proteins. EMBO J 12:3841–3848.

Sillekens PTG, Beijer RP, Habets WJ, van Wenrooij WJ (1988): Human U1 snRNP-specific C-protein: Complete cDNA and protein sequence and identification of a multigene family in mammals. Nucleic Acids Res 16:8307–8322.

Sistonen L, Keski-Oja J, Ulmanen I, Hölttä E Wikgren B-J, Alitalo K (1987): Dose effects of transfected c-Ha-*ras*^{Val12} oncogene in transformed cell clones. Exp Cell Res 168:518–530.

Sistonen L, Hölttä E, Mäkelä TP, Keski-Oja J, Alitalo K (1989): The cellular response to induction of the p21 c-Ha-*ras* oncoprotein includes stimulation of *jun* gene expression. EMBO J 8:815–822.

Smith DE, Fisher PA (1984): Identification, developmental regulation, and response to heat shock of two antigenically related forms of a major nuclear envelope protein in *Drosophila* embryos: Application of an improved method for affinity purification of antibodies using polypeptides immobilized on nitrocellulose blots. J Cell Biol 99:20–28.

Smith RD, Seale RL, Yu J (1983): Transcribed chromatin exhibits altered nucleosomal spacing Proc. Natl. Acad. Sci USA 80:5505–5509.

Steitz JA, Black DL, Gerke V, Parker KA, Krämer A (1988): Functions of the abundant snRNPs. In Birnstiel ML (ed): "Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles." Berlin, Heidelberg, New York: Springer-Verlag, pp 115–154.

Stimac E, Morris DR (1987): Messenger RNAs coding for enzymes of polyamine biosynthesis are induced during the G_0/G_1 transition but not during the traverse of the normal G_1 phase. J Cell Physiol 133:590–594.

Thompson CB, Challoner PB, Neiman PE, Groudine M (1985): Levels of c-*myc* oncogene mRNA are invariant throughout the cell cycle. Nature 314:363–366.

Van Dam A., Winkel I, Ziljstra-Baalbergen J, Smeenk R, Cuypers HT (1989): Cloned human snRNP proteins B and B″ differ only in their carboxyl-terminal part. EMBO J 8:3853–3860.

Weintraub H (1984): Histone-H1-dependent chromatin superstructures and the suppression of gene activity. Cell 38:17–27.

Wieben ED, Rohleder AM, Nenninger JM, Pederson T (1985): cDNA cloning of a human autoimmune nuclear ribonucleoprotein antigen. Proc Natl Acad Sci USA 82:7914–7918.

Yu J, Smith RD (1985): Sequential alterations in globin gene chromatin structure during erythroleukemia cell differentiation. J Biol Chem 260:3035–3040.