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Mouse ubiquitous B2 repeat in polysomal and cytoplasmic poly(A)+RNAs: uniderectional orientation and 3'-end localization

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ABSTRACT

A cDNA library in pBR322 was prepared with cytoplasmic poly(A)$^+$RNA from mouse liver cells. From 1 to 1.5% of clones hybridized to either B1 or B2 ubiquitous repetitive sequences. Several clones hybridizing to a B2 repeat were partially sequenced. The full-length B2 sequence was found at the 3'-end of abundant 20S poly(A)$^+$RNA (designated as B2$^+$mRNA$_x$) within the non-coding part of it. B2$^+$mRNA$_x$ is concentrated in mouse liver polysomes and absent from cytoplasm of Ehrlich carcinoma cells. The B2 sequence seems to be located at the 3'-end of some other mRNAs as well. To determine the orientation of the B2 sequence in different RNAs, its two strands were labeled, electrophoretically separated, and used for hybridization with Northern blotts containing nuclear, cytoplasmic and polysomal RNAs. In nuclear RNA, the B2 sequence is present in both orientations; in polysomal and cytoplasmic poly(A)$^+$RNAs, only one ("canonical") strand of it can be detected. Low molecular weight poly(A)$^+$B2$^+$RNA /1/ also contains the same strand of the B2 element. The conclusion has been drawn that only one its strand can survive the processing. This strand contains promoter-like sequences and AATAAA blocks. The latter can be used in some cases by the cell as mRNA polyadenylation signals.

INTRODUCTION

Mouse genome contains two dominating short repetitive sequences designated as B1 and B2 /2/. These two sequences are very abundant (ca. 10^5 copies of each) and efficiently transcribed. About 2% of total hnRNA is represented by B1 and B2 transcripts. In particular, almost all double-stranded sequences of B-type (100-200 bp long hairpin-like sequences) are transcribed from B1 and B2 /2/. Like other repetitive sequences, most of B1 and B2 transcripts are degraded within the nucleus in the course of processing /1/.

However, since dsRNA-B partly hybridizes to polysomal

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poly(A)$^+$RNA, short repeats may be included in the mRNA structure /3-5/. Later, cloned B1 and B2 sequences were shown to hybridize to polysomal poly(A)$^+$RNA immobilized on Northern filters /1/. However, hybridization may be explained by the existence of partial homology between B-type repeats and mRNA. We decided therefore to clone cDNA sequences complementary to mRNA, to select those hybridizing with B1 and B2 repeats, and to determine their structure and organization.

In this paper, we describe the results of such selection and sequencing of cDNA clones derived from polysomal poly(A)$^+$ RNA abundant in mouse liver which contains the B2 element and was designated as B2$^+$ mRNA$_x$. The full-length B2 sequence was found at its 3'-end. Moreover, all cytoplasmic RNAs of mouse liver and Ehrlich carcinoma cells were found to have their B2 sequence oriented in the same way, which makes it possible to use AATAAA blocks as signals for polyadenylation. The oppositely oriented B2 sequences present in nuclear RNA are eliminated during processing. This observation explains why cytoplasmic RNA does not contain dsRNA-B /6/ in spite of the presence of B1 and B2 sequences.

MATERIALS AND METHODS

Preparation of cDNA library. cDNA was synthesized in a 200-µl volume containing 50 mM tris-HCl, pH 8.1, 50 mM KCl, 5 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM dATP, TTP, dGTP and dCTP, 50-100 µCi of [^{32}P]dCTP (350 Ci/mmole, The Radioactive Centre, Amersham), 10 µg of poly(A)$^+$RNA, 3 µg of oligo$(dT)_{12-18}$ 0.4 mg of RNAase inhibitor from human placenta /7/, and 20 units of reverse transcriptase. Synthesis of the second DNA strand was performed with the aid of reverse transcriptase or DNA-polymerase as described earlier /8/. Double-stranded DNA was treated with nuclease S1 /8/. Synthesis of oligo(dC) at the 3'-ends of cDNA and of oligo(dG) at the 3'-ends of plasmid pBR322 DNA linearized with restriction endonuclease PstI was performed in 20-50 µl volumes containing 200 mM potassium cacodylate, 30 mM tris-HCl, pH 7.6, 0.2 mM dithiothreitol, 1 mM $CoCl_2$, 0.1 mM dCTP (or dGTP), 1 µg of DNA and

10-20 units of terminal nucleotidyl transferase for 15-30 min at 37°C. The length of homopolymeric tracts was found to vary from 10 to 20 nucleotides. DNA was deproteinized with chloroform and purified by Sephadex G-50 chromatography.

For transformation, 10 ng of cDNA was mixed with 50 ng of vector DNA in 150 µl. NaCl, tris-HCl, pH 7.5, and EDTA were added to 200 mM, 50 mM and 1 mM, respectively, and the sample was incubated for 3 min at 65°C, for 3 hours at 42°C, and then cooled slowly (during 4 hours) to 20°C. The resultant chimeric plasmid DNA was used to transform E.coli HB101 to tetracycline resistance. The yield of recombinant colonies was $6x10^4$ per µg of cDNA, more than about 90% of them being of the Amp^S, Tet^r phenotype. Bacterial colonies containing recombinant plasmids were grown and fixed on nitrocellulose filters essentially as described by Grunstein and Hogness /9/. Duplicate filters were hybridized with ^{32}P-labeled cDNA synthesized from mouse liver cytoplasmic poly$(A)^+$RNA or ^{125}I-labeled double-stranded RNA-B /2/. In some cases, the hybridization was also performed with purified fragments of clone 54.

DNA analysis. The DNA of clones was prepared as described in /2/. After restriction with PstI, the DNA was fractionated electrophoretically and transferred to nitrocellulose filters according to Southern /10/.

The DNA was sequenced using the method of Maxam and Gilbert /11/ in the modified protocol of Dobrynin et al. /12/. The 3'-end labeling was carried out according to the instructions supplied by the manufacturer (New England Nuclear) utilizing /α-^{32}P/ cordycepin triphosphate and terminal nucleotidyl transferase. In some experiments, [^{32}P]dCTP and the Klenow enzyme were used for 3'-end labeling /11/.

DNA strand separation. DNA strands were separated according to /11/. The labeled DNA fragment was denatured by heating to 85-90° for 10 min in 60% (w/v) DMSO, 0.05% xylene cyanol and 0.05% bromphenol blue, cooled quickly in a dry ice/acetone bath and separated in a gel containing 4.5% (w/v) acrylamide, 0.15% N,N'-methylenebisacrylamide. Electrophoresis

was carried out in a cold room at 7 V/cm overnight.

RNA preparation. Nuclear, cytoplasmic and polysomal RNAs from mouse liver or Ehrlich carcinoma cells were prepared as described in /1/. Double-stranded RNA-B was isolated and *in vitro* labeled with ^{125}I as described in /2/. 3 M sodium acetate precipitation of RNA was used to remove DNA traces.

Northern blot analysis of RNA. Polyadenylated RNA was denatured by heating to 60°C for 3 min in 6 M urea/formamide and separated in denaturing 1.5% agarose gels containing 7 M urea and 25 mM sodium citrate, pH 3.5 /1/. RNA was transferred directly to DBM paper and hybridized to ^{32}P-labeled DNA fragments as described earlier /1/.

/α-^{32}P/dCTP (2000-3000 Ci/mmole or 350 Ci/mmole) and /α-^{32}P/ cordycepin triphosphate (5000 Ci/mmole) were purchased from the Radiochemical Centre (Amersham, UK). Non-radioactive deoxynucleotides and oligo(dT)$_{12-18}$ were from Boehringer Mannheim. Poly(U)-Sepharose 4B was from Pharmacia Fine Chemicals. AMV reverse transcriptase was a gift from Dr. V.M. Kavsan (Institute of Molecular Biology and Genetics, USSR Academy of Sciences, Kiev); restriction enzymes were gifts from Dr. A.A.Janulaitis (Institute of Applied Enzymology, Vilnus). The Klenow enzyme was a gift from Dr. V.G.Korobko (Institute of Bioorganic Chemistry, Moscow); terminal nucleotidyl transferase was a gift from Dr. A.A.Bocharov (this Institute).

RESULTS

Selection of cDNA clones hybridizing to repetitive sequences of B-type

Cytoplasmic polyadenylated RNA prepared from mouse liver was used to construct a large library of approximately 60,000 independent recombinants in pBR322. The first and second cDNA strands were synthesized in the same tube without phenol extraction or ethanol precipitation between the steps. cDNA molecules were inserted into the PstI site of pBR322 using oligo(dC)-oligo(dG) extensions of 10-20 nucleotides yielding approximately (6-7)x10^{4} transformants per 1 µg of cDNA.

Replicas from plates containing about 1000 colonies per plate were used for colony hybridization with either ^{32}P-labeled mouse liver cDNA or ^{125}I-labeled double-stranded RNA-B prepared from hnRNA of Ehrlich carcinoma cells /2/ (Fig.1).

From 30 to 40% of colonies bound total cDNA. The presence of negative colonies may be caused by a low abundance of corresponding mRNAs. On the other hand, only a few of colonies (ca. 1-1.5%) bound dsRNA of B-type. These were purified and grown. DNA was isolated and subjected to restriction analysis. As a first approach to the construction of restriction maps of the inserts, pBR322 recombinant plasmids have been treated with the restriction enzymes EcoRI or BamHI which are known to cleave the original pBR322 plasmid only once. The results of these digestions indicate that the recombinant plasmids are larger than the wild type pBR322 containing inserts of ~400 bp (clones 23, 35) and ~1000 bp (clones 41, 54, 91) (data not shown). To identify the inserts of these five clones, hybridizing with dsRNA-B, their DNAs were digested with restriction endonuclease PstI, electrophoresed on agarose gel, transferred to a nitrocellulose filter and hybridized to ^{125}I-labeled dsRNA-B or ^{32}P-labeled DNAs containing either B1 or B2 sequences (Fig. 2).

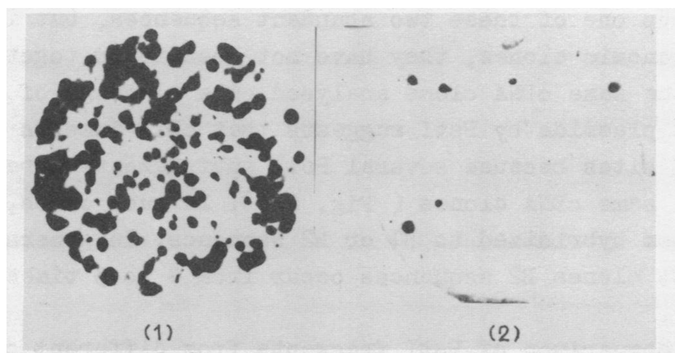

Fig. 1. Random spreads of mouse liver cDNA recombinant clones hybridized with [^{32}P] cDNA transcribed from cytoplasmic poly(A)$^{+}$RNA (1) and [^{125}I] dsRNA-B (2).

2x10^{6} cpm of [^{32}P] cDNA or [^{125}I] dsRNA-B was hybridized to duplicate filters as described in Materials and Methods.

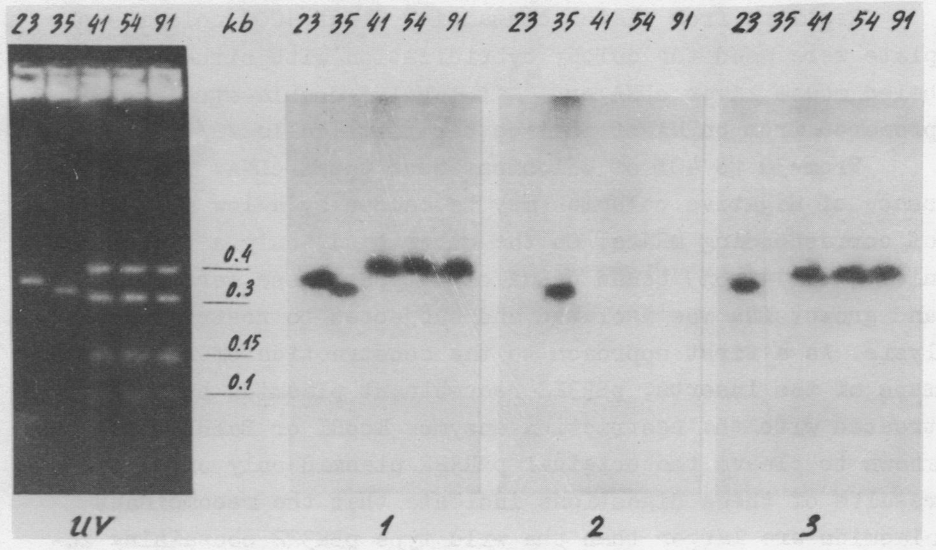

Fig. 2. Identification of B1- and B2-containing cDNA inserts in the clones hybridizing with dsRNA-B.
Plasmid DNAs digested with PstI and analysed by the Southern method were used for hybridization to $[^{125}I]$ dsRNA-B (1), $[^{32}P]$ B1 genomic DNA sequence (2), and $[^{32}P]$ B2 genomic DNA sequence (3).
UV - 3.2% agarose gel stained with ethidium bromide.

In all of the cases, the insertions hybridized to either B1 or B2. Thus, the binding of dsRNA-B to a cDNA clone usually requires one of these two abundant sequences, but in contrast to genomic clones, they have not been found together in one and the same cDNA clone analysed. The cleavage of the recombinant plasmids by PstI suggests that cDNA inserts contain PstI sites because several PstI subfragments were detected in the same cDNA clones (Fig. 2). In such cases, only one of them hybridized to B1 or B2 sequence. In general, among cDNA clones B2 sequences occur from 4 to 5 times as often as B1.

The comparison of PstI fragments from different clones containing the B2 sequence suggested that many of them originated from the same mRNA, although they contained different portions of the latter. The conclusion was further confirmed by cross-hybridization between PstI subfragments of different

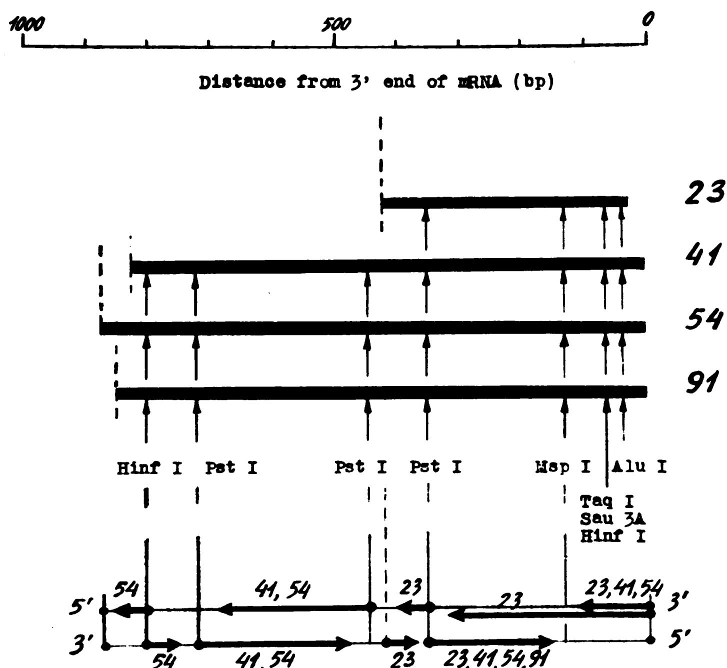

Fig. 3. Restriction maps of the cDNA inserts of four recombinant plasmids derived from $B2^{+}mRNA_{x}$.
The regions covered by sequencing are indicated with arrows. The numbers above the arrows refer to the different clones analysed.

clones non-containing B2 as well as by DNA sequencing (see below). We have designated this B2-containing mRNA as $B2^{+}mRNA_{x}$. Among fifteen B2-containing clones analysed, eight contained $B2^{+}mRNA_{x}$ sequences. It means that this $B2^{+}mRNA_{x}$ is the major B2-containing mRNA abundant in mouse liver.

Full-length B2 sequence is present in $B2^{+}mRNA_{x}$.

Fig. 3 shows the physical maps of clones used for sequencing. One can see that these are four independent clones as the insertions have different length. In all of them, the B2-containing PstI subfragment is located at the end of an insertion. The marked fragments have been sequenced using the Maxam and Gilbert procedure /11/. The longest noninterrupted region that was sequenced is the whole insert of clone 23.

B2-containing PstI subfragments (~ 400 bp in length) were

found to be identical in the overlapping parts of four clones. Direct sequencing of PstI subfragments (280 bp) located far upstream the B2 element in clones 54 and 41 also revealed identical sequences (not shown here). Thus, all of them have been transcribed from the same $B2^{+}mRNA_{x}$. The lengths of oligo (dC) tails of the inserts were found to be different. Besides, the AT-rich zone and oligo(dA) typical of B2 /13/ were absent from the insert of clone 23. Oligo(dA) was also lost in the B2 of clones 41 and 54. In clones 41, 54 and 91, the B2 element is followed with a stretch of predominantly adenosine residues containing AATAAA blocks and, in the case of clone 91, with $(dA)_{7}$. The presence of a "poly(A) addition signal" at one end of the inserts suggests that these clones may contain 3'-noncoding part of mRNA and that the B2 element is located at the 3'-end of the cloned mRNA. Further analysis of these clones using separated cDNA strands for Northern hybridization (see below) has confirmed this interpretation.

Fig. 4 shows a schematic representation of the organization and partial sequence of a cDNA insert derived from $B2^{+}$ $mRNA_{x}$. For comparison, the consensus B2 sequence determined from genomic clones /13/ is also shown.

The following conclusions can be drawn from the sequence analysis. (i) The whole B2 unit is present in $B2^{+}mRNA_{x}$. It is well matched to genomic B2. The deviation from the genomic consensus sequence is not higher than the deviation of individual genomic sequences from the latter /13/. (ii) The B2 sequence is located at the 3'-end of $B2^{+}mRNA_{x}$, just before a poly(A) stretch. It follows from the fact that, in all cases, the B2-containing PstI fragment is terminated with an oligo (dC) tail and that, in one case, a poly(A) stretch exists. In other cases, the very end of the cDNA was lost during cloning. (iii) The 5'-end part of a sequenced $B2^{+}mRNA_{x}$ segment contains an open reading frame which can encode a polypeptide consisting of 39 amino acids. It lacks an initiator codon because the size of mRNA is much greater than that of the sequenced part (see below). (iv) The B2 sequence is located in the nontranslatable part of mRNA, 54 nucleotides downstream the stop codon TGA of the open reading frame. Considering that the ge-

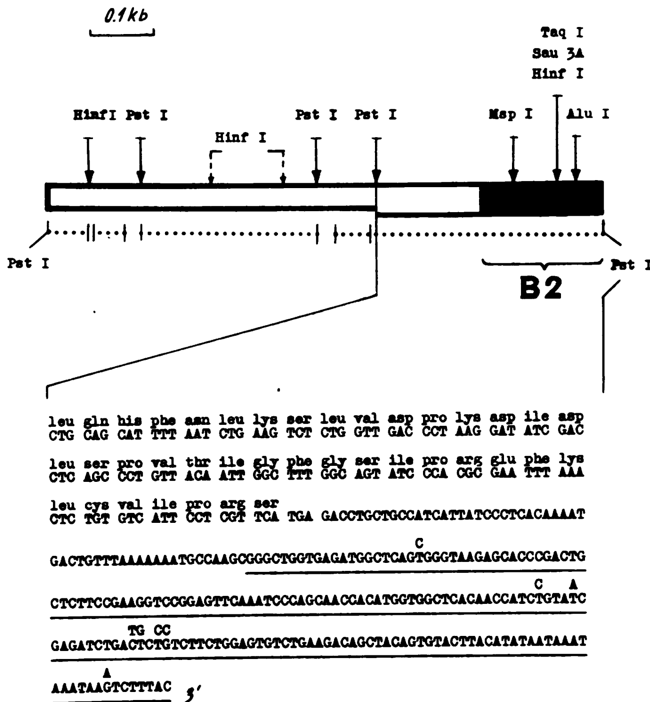

Fig. 4. Schematic representation of the organization and partial sequence of the cDNA inserts derived from B2[+]mRNA$_x$.

... shows the region with a determined nucleotide sequence;

—— shows the B2 sequence.

The letters above the B2 sequence indicate the deviation of the consensus B2 repeat.

nomic B2 sequences are terminated with oligo(dA) of variable length /13/, one can calculate that the whole non-translatable region of B2[+]mRNA$_x$ is 245-250 nucleotides long.

B2[+]mRNA$_x$ is an mRNA abundant in mouse liver

To analyse the nature of B2[+]mRNA$_x$, we hybridized the Northern filters containing electrophoretically separated poly(A)[+] RNAs from different sources with the subfragments of clone 54 containing and lacking the B2 sequence (Fig. 5).

In agreement with the previous data /1/, the B2-containing subfragment binds to the heterogeneous material of mouse liver

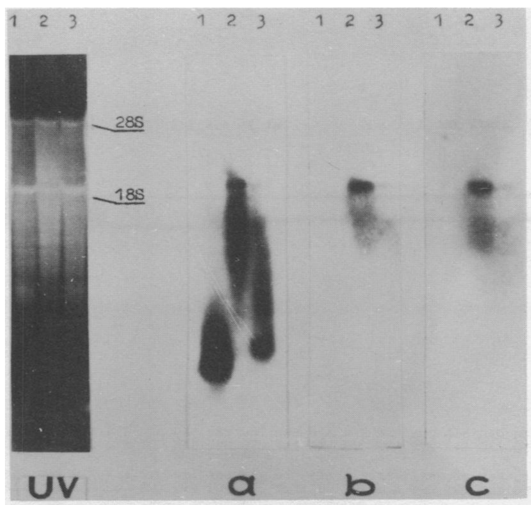

Fig. 5. Northern blot hybridization of mouse cell RNAs to cDNA PstI-fragments of clone 54.

(a) Hybridization to the $B2^{+}$ fragment (373 bp);
(b) Hybridization to the $B2^{-}$ fragment (280 bp);
(c) Hybridization to the $B2^{-}$ fragment (150 bp).

1 - poly(A)$^{+}$RNA of the Ehrlich carcinoma cytoplasm; 2 - poly(A)$^{+}$RNA of the liver polysomes; 3 - poly(A)$^{+}$RNA of the liver cytoplasm.

polysomal and cytoplasmic poly(A)$^{+}$RNA. In addition, polysomal RNA contains a discrete band ~2 kb long binding the B2 sequence. In cytoplasmic poly(A)$^{+}$RNA from Ehrlich carcinoma cells, the hybridization of B2 sequences to high molecular weight species was very low. On the other hand, the fragments of clone 54 lacking the B2 sequence and corresponding to two different parts of $B2^{+}mRNA_{x}$ hybridized almost exclusively to the 2 kb discrete band. (A small hybridization smear is probably due to a partial degradation of ~2 kb mRNA). They hybridized neither to poly(A)$^{+}$RNA of liver cells nor to total poly(A)$^{+}$RNA of Ehrlich carcinoma cells. Thus, the cloned $B2^{+}mRNA_{x}$ indeed represents the most abundant B2-containing 20S mRNA which is specific for mouse liver.

One can also see a prominent band on stained gels of polysomal poly(A)$^{+}$RNA, which coincides with the position of 20S mRNA. Its content is compatible with the percentage

of clones (~0.5%) containing $B2^{+}mRNA_{x}$, as follows from the colony hybridization experiments.

Thus, 20S $B2^{+}mRNA_{x}$ belongs to the class of mRNAs actively synthesized and enriched in polysomes in mouse liver cells.

<u>All B2 sequences in cytoplasmic poly(A)$^{+}$RNA are oriented in the same direction corresponding to the "canonical" one</u>

To analyse the orientation of B2 sequences in different RNA fractions, we developed hybridization experiments with the separated strands of the B2 repeat. The B2-containing PstI subfragment from clone 54 with the determined primary structure (see above) was labeled at its 3'-ends using [^{32}P] cordycepin triphosphate and terminal transferase; then, the two strands were separated by gel electrophoresis in 4.5% polyacrylamide (Fig. 6). This fragment contains oligo(dC) at the 3'-end of one strand (designated as C) and oligo(dG) at the 5'-end of another strand (designated as G). Because of large asymmetry, the strands are very well separated (Fig. 6a). According to sequencing data, the C-strand has orientation of the B2 shown in Fig. 4. AluI digestion of the fragment should eliminate a small piece containing labeled 3'-oligo(dC), thus making possible to identify every strand (see Fig. 6 b and c). The both equally labeled strands were eluted and separately hybridized to the Northern filters containing different RNA fractions (Fig. 7).

One can see that nuclear RNAs from mouse liver and Ehrlich carcinoma cells hybridized equally well to the both strands of B2. On the other hand, all fractions of cytoplasmic and polysomal poly(A)$^{+}$RNA bound only one strand of the B2 sequence. No hybridization with the second strand could be detected even in an over-exposed autoradiograph. It is true of $B2^{+}mRNA_{x}$, of the heterogeneous poly(A)$^{+}$B2^{+}RNA fraction, and of the discrete low molecular weight polyadenylated B2-containing RNA.

The sequencing has revealed that the B2 strand present in cytoplasmic and polysomal RNAs corresponds to the "canonical" one (see Fig. 4) which contains elements pertinent to

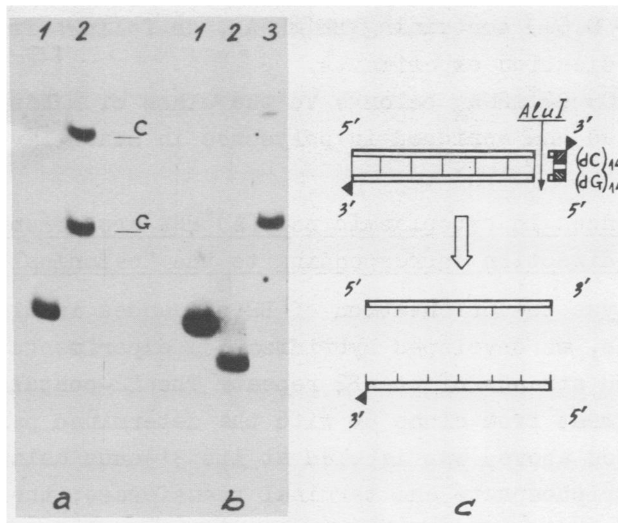

Fig. 6. DNA strand separation of the B2^{+} fragment of cDNA clone 54.

The excised fragment was 3'-labeled (see the text) and analysed by electrophoresis and autoradiography.

(a) Strand separation of the original fragment.
1 - the native fragment; 2 - the denatured fragment.
C - strand containing 3'-oligo(dC);
G - strand containing 5'-oligo(dG).

(b) Identification of G- and C-strand by AluI digestion followed by strand separation.
1 - the native fragment; 2 - the native fragment digested with AluI; 3 - the denatured fragment after AluI digestion.

(c) The scheme illustrating the identification of G- and C-strands.

the RNA polymerase III promoter and AATAAA blocks /13/. These experiments also provide direct evidence for localization of the B2 sequence at the 3'-end of B2^{+}mRNA$_{x}$.

DISCUSSION

Short interspersed repeats of B-type in some mRNAs

The above results show that B1 and B2 sequences are present in 1-1.5% of the total population of mouse cytoplasmic poly(A)$^{+}$RNAs. At least some of these RNAs may be considered as mRNAs. First, they are detectable within well purified mouse liver polysomes. Second, at least one of them (20S B2^{+} mRNA$_{x}$) was shown to be one of the major components among

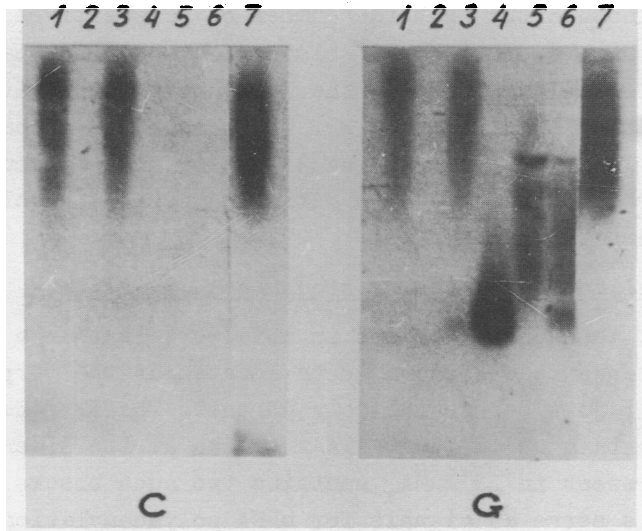

Fig. 7. Northern blot hybridization of mouse cell RNAs to separated DNA strands of the B2$^+$ fragment of cDNA clone 54.

C - hybridization to the strand containing 3'-oligo(dC);
G - hybridization to the strand containing 5'-oligo(dG);

1 - nuclear poly(A)$^-$ and 3 -poly(A)$^+$RNAs from Ehrlich carcinoma cells; 2 - cytoplasmic poly(A)$^-$, and 4 - poly(A)$^+$ RNAs of Ehrlich carcinoma cells; 5 - polysomal, and 6 -cytoplasmic poly(A)$^+$RNA from liver cells; 7 - nuclear liver poly(A)$^+$RNA.

polysomal poly(A)$^+$RNAs. Third, again in the case of B2$^+$mRNA$_x$, a long sequence uninterrupted by termination codons was found in the sequenced segment of this mRNA.

Recently, a B2 sequence was also detected in mouse mRNA for the protein of the major histocompability complex (H2), i.e. in an individual well characterized mRNA /14/.

The results of our early hybridization experiments have suggested that repetitive sequences exist within some non-repetitive mRNAs /15/, although most authors arrived at quite opposite conclusions at that time /16/.

Later, we found that dsRNA-B hybridized to some extent with polysomal poly(A)$^+$RNA /3-6/. Similar observations were done by Naora et al. /17/. In this paper, RNAs hybridizing to B1 or B2 sequences have been cloned and analysed.

It is noteworthy that the number of B2 positives is about 4 times as high as that of B1$^+$ clones. On the other hand, the hybridization signal of B1 on the Northern filters with mRNA is similar to that of B2. The possible explanation is that B2 is located closer to the 3'-end of mRNA molecules than B1 and therefore they are more often transcribed by oligo(dT) primed reverse transcriptase.

3'-end localization of the B2 sequence in mRNAs

One of the central points in this work is that a full-length B2 sequence was shown to be located at the very 3'-end of B2$^+$mRNA$_x$, just before the poly(A) tail. The B2 sequence was shown to contain up to four AATAAA blocks at its 3'-end /13/. Its copy present in B2$^+$mRNA$_x$ contains two such blocks (Fig.4). These blocks serve as signals for mRNA polyadenylation, being located approximately 15 nucleotides upstream the poly(A) tract, and probably they fulfill such a function for B2$^+$mRNA$_x$.

Although B2$^+$mRNA$_x$ is a dominating B2-containing mRNA in mouse liver polysomes, some B2 repeats belong to other RNA sequences. B2$^+$mRNA$_x$ is not present in the cytoplasm of Ehrlich carcinoma cells and it does not prevail in MOPC 21 cells /1/.

B2-containing cDNA clones different from B2$^+$mRNA$_x$ have not yet been characterized in detail. However, our preliminary results show that, in all cases studied, the B2 element is located at the terminal cDNA insert near the oligo(dC) tail. In the mRNA for the histocompability complex, the B2 sequence also occurs at the 3'-end /14/. Consequently, such a localization is typical of the B2 sequence in mRNA.

Interestingly enough is that a short interspersed repetitive sequence ("suffix") was detected at the 3'-end in several mRNAs of D. melanogaster /18/. Recently other authors have reported that repetitive elements exist in unique mRNA /19, 20/. Sutcliffe et al. /21/ have selected cDNA clones made from rat brain poly(A)$^+$RNA that carry a common repetitive 82-nucleotide sequence (designated as ID sequence). Just as B2 sequences, ID sequences terminate with oligo(dA) and hybridize to low molecular weight RNA. They are homologous to the

repetitive sequence found in the second intron of the rat growth hormone gene /22/ and partly homologous to the B2 sequence described in this paper.

RNA processing eliminates most of B2 sequences, in particular, all the sequences oriented in a "non-canonical" direction

Another important observation made in this work is that only one orientation of the B2 sequence can be detected in all cytoplasmic RNAs while nuclear RNA contains both B2 strands equally represented. This explains the presence of dsRNA-B in nuclear RNA but not in cytoplasmic RNA although the latter can hybridize to melted dsRNA /3-6/.

Many B2 sequences are present in hnRNA as constituents of RNA polymerase II transcripts located mostly within introns. For example, introns of the rat growth hormone gene and of the mouse albumin gene contain B2 in the "canonical" orientation while intron of the mouse α-fetoprotein gene contains it in the opposite orientation /22, 23/. On the other hand, exons contain only "canonically" oriented B2 sequences (the data of this work) which may fulfill the function of polyadenylation, and only these B2 elements survive the processing.

Certain data indicate that some of B2-containing transcripts are synthesized by RNA polymerase III /1/. Obviously in that case, the synthesis would yield only a canonical strand of the B2 sequence possessing RNA polymerase III promoter and terminator sequences as well as polyadenylation signal. In fact, a small poly(A)$^+$RNA containing the B2 sequence, which is the best candidate for being transcribed by RNA polymerase III, has the orientation mentioned above.

The function of small poly(A)$^+$B2-containing RNA is completely obscure. One may speculate, for example, that this RNA is involved in processing. Its poly(A) track might readily form duplexes with the oligo(U) segment of hnRNA belonging to B2 complements (having the opposite orientation). The next step would be the formation of a complete B2 duplex followed by excision of non-canonical B2 sequences with specific nucleases.

It was shown recently by electron microscopy of mRNA sub-

jected to self-annealing that both complements from short repetitive elements can be found in mRNA /19/. The approach used in our work, being probably the most direct one has shown clearly that at least the repetitive sequences of the B2 family exist in only one orientation in mature poly(A)$^+$ RNA of mouse liver and Ehrlich carcinoma cells. The same approach is now being used to study the transcription characteristics of B1 and Alu sequences as well as B2 in other tissues.

On the biological significance of B-type repeats

The repeats of B-type seem to represent a special class of transposable elements /13, 24/. Their transcription by RNA polymerase III followed by reverse transcription of a short RNA product was postulated. Such short RNA molecules consisting of a B2 sequence and a poly(A) tail were recently discovered in tumor cells /1/.

Transposable elements are usually considered as "selfish DNA" and their survival in the genome is attributed to independent mechanisms for their multiplication and transposition rather than to a natural selection process. Due to the existence of such mechanisms, transposable elements can withstand the pressure of selection /25/.

However, several properties of such elements allow one to suspect that the above idea is over-simplified. Sometimes, repeats of the B-type are included in mRNA. Both B1 and B2 contain regions of homology to several functional elements of the genome. For example, B2 carries a signal of polyadenylation and the data presented here show that this signal is a functional one. Hence, although most of the occasional insertions of B2 into a gene may either inactivate it (insertion into an exon) or have no effect (insertion into an intron), some of them may create signals that positively change gene functioning. Such events should be fixed by natural selection.

The creation of polyadenylation sites is not the only possible way for B-repeats to influence genome functioning. Both B1 and B2 segments were found to be homologous to consensus exon-intron junctions whereas two segments in B1 sequences

were shown to be homologous to papova virus replication origins /13/; these findings suggest that B-type repeats are involved in DNA replication and hnRNA processing. Furthermore, B-type repeats may be involved in the recognition process during either recombination or conversion of genes.

It is likely that the transposable repetitive sequences are not merely parasitic sequences. They may be considered as a kind of symbionts which use the genome machinery for amplification but, at the same time, supply the genome with certain signal sequences that influence genome functioning.

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