Major transcripts containing B1 and B2 repetitive sequences in cytoplasmic poly(A)$^+$RNA from mouse tissues

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The cytoplasmic poly(A)$^+$RNAs containing ubiquitous B1 and B2 repeats of the mouse genome in normal tissues and tumors have been studied. Only one strand of the repeats is represented in cytoplasmic RNA in all the cases. Some tumor cells were found to be enriched in 1.4 kb B1$^+$mRNA, 1.6 kb B2$^+$mRNA and small (0.2–04 kb) B1$^+$ and B2$^+$ poly(A)$^+$RNAs. On the other hand, mouse liver and kidney contained high amounts of 2 kb B2$^+$mRNA. Its content increased noticeably in the regenerating liver, but in hepatoma it dropped to a zero level. Thus, the switching on (or off) of B1- and B2-containing mRNAs occurred non-coordinately. At the same time, the activation of the synthesis of small B2$^+$RNA and small B1$^+$RNA was simultaneous.

B1 repetitive element *B2 repetitive element* *Transcription* *Normal mouse tissue* *Mouse tumor tissue*

1. INTRODUCTION

The mouse genome contains two major families of short interspersed repeats designated as B1 and B2 sequences [1]. These sequences are efficiently transcribed comprising a significant fraction of hnRNA. In the course of processing, the major part of B1 and B2 sequences are degraded. A small part survives and appears in cytoplasmic poly(A)$^+$RNA. It has recently been discovered that only one strand of B2 is detectable in cytoplasm of mouse liver and Ehrlich carcinoma cells [2].

Here, we describe the partial sequence of one of the mouse liver cytoplasmic poly(A)$^+$RNAs containing B1. The full-length B1 is shown to be located at the 3′-end of this RNA. The systematic study of major cytoplasmic transcripts containing B1 and B2 in different mouse tissues has also been undertaken. Only one strand of B1 and B2 repeats is represented in cytoplasmic RNA in all the cases. A novel B1$^+$RNA abundant in different tumors has been detected. The transcription of small B1$^+$ and B2$^+$RNAs is enhanced in most tumor cells compared to normal ones.

2. MATERIALS AND METHODS

For hybridization, we used the DNAs of clones pRTM35 and pRTM54 containing B1 and B2, respectively. The isolation of plasmids and DNA insertions was described earlier [2].

Polyadenylated nuclear and cytoplasmic RNAs were isolated as described previously [3,4]. Aliquots (5 μg) of RNA were fractionated by electrophoresis in a 1.5% agarose gel in 7 M urea in citrate buffer (pH 3.5) or in a 1% agarose gel containing 6% formaldehyde. The RNA was transferred to a DBM or nitrocellulose filter and hybridized with ^{32}P-labeled DNA strands as described previously [2].

The 3′-end labeling of PstI-DNA fragments was carried out according to the instructions supplied by the manufacturer (New England Nuclear) utilizing [α-^{32}P]cordicepin triphosphate or [α-^{32}P]-dideoxyadenosine triphosphate and terminal nucleotidyl transferase. DNA strands were separated as described earlier [2].

The sequences were determined by the chemical degradation procedure of Maxam and Gilbert [5].

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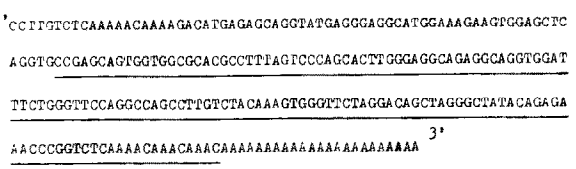
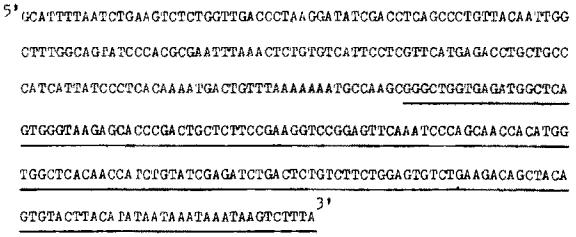
3. RESULTS

It has been shown that a full-length B2 repetitive sequence is present at the 3′-end of 2 kb mRNA$_x$ abundant in liver cells and some other polysomal poly(A)$^+$RNAs [2]. The question arises as to the organization of the B1 repetitive sequence in mature poly(A)$^+$RNAs; From the cDNA library prepared with cytoplasmic poly(A)$^+$RNA of mouse liver, we selected several clones hybridizing with the B1 sequence. Fig.1a shows a nucleotide sequence of the insertion from one such clone (pRTM35). It contains a full-length copy of the B1 repeat. Prior to B1, another sequence is located. B1 includes a conserved part, ~130 nucleotides long, and a short AC-rich motif $(A_3C)_3$. Right after the latter, the oligo(A) sequence terminating the insertion is located. It consists of 23 A residues. Thus, in the mRNA that served as a template for the cloned cDNA, the B1 sequence seemed to be located at the 3′-end before the poly(A) track added post-transcriptionally, the situation being similar to that of B2$^+$mRNA$_x$ (see fig.1b). However, the possibility cannot be excluded that $(A)_{23}$ is a fraction of genomic oligo(dA)/oligo(dT) sequence and therefore the location of B1 in mRNA differs from that postulated above. In contrast to B2, the B1 sequence does not contain polyadenylation signal AATAAA, which may be considered as a guide for detecting post-transcriptional poly(A). Three other cDNA clones containing full-length B1 elements were also isolated but since the A-rich region was lost from them, probably during the cloning procedure, the orientation of B1 in corresponding RNA transcripts remained unclear (not shown). In any case, these data demonstrate that the hybridization of the B1 sequence with cytoplasmic and polysomal poly(A)$^+$RNA described previously [3] depends on the presence of full-length B1 elements in these RNAs.



a



b

Fig.1. Nucleotide sequences of cDNA inserts of pRTM35 (a) and pRTM54 (b). Only plus (+) strands corresponding to those present in poly(A)$^+$RNA are shown in the 5′ to 3′ direction. The B1 (in pRTM35) and B2 (in pRTM54) repetitive elements are underlined. The sequence organization of pRTM54 was previously described [2] and given here for a better illustration of further experiments.

DNA inserts of the pRTM35 (containing B1) and the pRTM54 (containing B2) clones were further used for hybridization analysis. The main experiment was conducted to analyze the distribution of B1 and B2 sequences among mRNAs in mouse tissues - 6 normal cells and 8 tumors - and to detect the B1 and B2 orientation in these RNAs (fig.2). As probes, separated strands of B1 and B2 sequences were taken. The strand containing the signal sequences (like the RNA polymerase III promoter and terminator, polyadenylation signals, etc.) was designated as 'plus (+) strand' or 'canonic strand' (see fig.1), and the other (complementary) strand as 'minus (−) strand', or 'opposite strand'. These probes were hybridized to the Northern filters containing cytoplasmic poly(A)$^+$ RNA from the above-mentioned mouse tissues.

Both strands of B1 and B2 hybridized fairly well to nuclear RNA of Ehrlich carcinoma cells (fig.2). The same was observed for hn RNA from mouse liver and other mouse tissues (not shown). However, the cytoplasmic poly(A)$^+$RNA bound exclusively to the − strand, i.e., it contained only the + strand of B1 and B2 sequences. Some faint signal was visible after hybridization with the + strand, but no bands were seen distinctly. Very probably this smeared material results from the nuclear RNA leakage.

In cytoplasm of most cell types tested, both B2 and B1 + strands were present in molecules of a messenger size and in the small molecular mass RNA fraction. Small poly(A)$^+$RNA containing B2 (more abundant) and B1 sequences are described

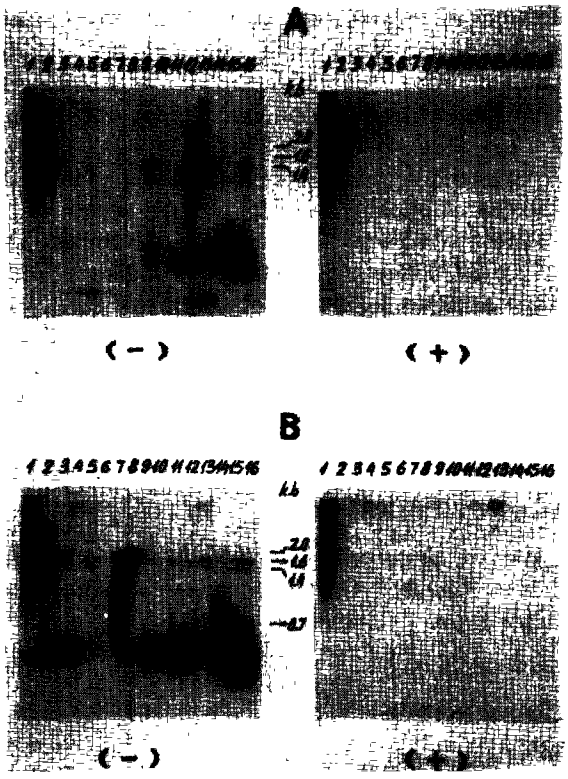


Fig.2. Northern blot hybridization of mouse cell RNAs to separated DNA strands (minus and plus) of the $B1^+$ fragment of pRTM35 (A) and $B2^+$ fragment of pRTM54 (B). 1, Nuclear poly(A)$^+$RNA from Ehrlich carcinoma cells; 2–16, cytoplasmic poly(A)$^+$RNAs from testicles (2), kidney (3), brain (4), lung (5), heart (6), liver (7), regenerating liver (48 h after partial hepatectomy) (8), hepatoma 22 (9), mammary gland carcinoma (Ca 755) (10), Lewis lung carcinoma (11), cervical carcinoma (CCU-5) (12), colon adenocarcinoma (Acatol-1) (13), lymphoma (L1210) (14), Ehrlich ascites carcinoma (15) and MOPC plasmacytoma (16). All tumor cell lines were a gift of Dr E.S. Revasova (USSR Cancer Research Center, Moscow). RNA was transferred to a DBM filter and hybridized with ^{32}P-labeled DNA. Thereafter radioautography was performed with an intensifying screen at −70°C. The labeled probe was removed by treating the filter with 250 ml of a solution containing 0.1×SSC, 0.1% SDS for 15 min at 90°C. After incubation, the filter was air-dried at room temperature and used for rehybridization.

elsewhere [3,4]. At least, the small $B2^+$RNA is synthesized with the aid of RNA polymerase III [4]. One can see from fig.2b that even the nuclear small $B2^+$RNA is represented exclusively by a + strand, in contrast to high molecular mass RNA. The signals present in the B2 repeat seem to be involved in small $B2^+$RNA biosynthesis.

The content of small $B2^+$RNA varies strongly from one tissue to another, being rather high in brain and testicles and negligibly low in lung and heart. Seven of 8 tumors analysed contained a high amount of small $B2^+$RNA. Its content in the cells of 6 tumors was higher than in such normal tissues as brain. Under the conditions used, small $B1^+$ RNA was not recovered in normal tissues tested while in the tumors it was well detected. In general, the content of $B2^+$RNA was much higher than that of $B1^+$RNA. An independent transcription of B2 and B1 repeats by RNA polymerase III can be coordinately regulated.

Messenger-size B1- and B2-containing RNAs are represented by a few major bands and some smeared material. Liver and kidney contain the 2 kb $B2^+$mRNA$_x$ described previously [2]. Other normal tissues tested and all tumors are practically void of $B2^+$mRNA$_x$. Thus, the expression of corresponding gene is tissue specific. Interestingly, in regenerating liver the content of $B2^+$mRNA$_x$ increases manyfold while in hepatoma cells it completely disappears (fig.2b, lanes 7–9).

Many tumors have a 1.6 kb B2-containing poly(A)$^+$RNA which seems to correspond to the mRNA encoding a Qa/Tla class I major histocompatibility complex (MHC) antigen described by Brickwell et al. [6]. In two cases (adenocarcinoma and plasmacytoma, lanes 13 and 16), a high level of this RNA was observed. Unlike Brickell et al. [6], we detected this RNA in several normal tissues (testicles, kidney, lung), its content being practically equal for some normal and tumor cells. Finally, in some tumor cells one could clearly see 1.4 kb and 0.7 kb B2-containing poly(A)$^+$RNAs.

At the same time, only one major band 1.4 kb long was observed among B1-containing poly(A)$^+$ RNA (fig.2A). The latter was found in tumor cells only. It was especially abundant in colon carcinoma, plasmacytoma and hepatoma (fig.2A). This poly(A)$^+$RNA was absent from cytoplasm of all normal tissues tested.

The major types of RNAs found in cytoplasmic poly(A)$^+$RNA preparations were also detectable in poly(A)$^+$RNA from purified polysomes where their content did not change as compared to the cytoplasmic fraction (some examples are shown in fig.3).

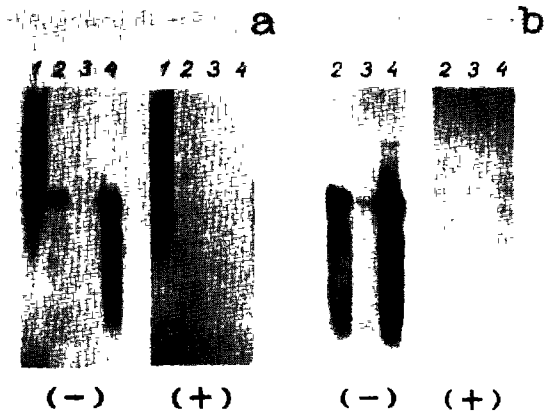


Fig.3. Northern blot hybridization of polyadenylated nuclear RNA from Ehrlich carcinoma cells (1) and polysomal poly(A)$^+$RNAs from mouse kidney (2), brain (3) and liver (4) cells to separated DNA strands (minus and plus) of the B2$^+$ fragment of pRTM54. RNA was transferred to nitrocellulose filters and hybridized with ^{32}P-labeled DNA. The filters were exposed for 0.5 (a) or 5 (b) days.

4. DISCUSSION

The data presented allow the following conclusions to be made. (i) Only one (+) strand of both B1 and B2 repeats survived the processing in all the tissues studied and could be detected in putative mRNAs. (ii) The synthesis of different mRNAs containing B2 sequences was not coordinate. Their formation changed independently from each other and from small B2$^+$RNA. Also, no correlation could be seen between the synthesis of 1.4 kb B1$^+$RNA and small B1$^+$RNA. (iii) The synthesis of 1.6 kb B2$^+$ poly(A)$^+$mRNA and specifically of 1.4 kb B1$^+$ poly(A)$^+$RNA was activated in some tumors compared to normal tissues. (iv) In general, the tumors are characterized by increased synthesis of small B1$^+$ and B2$^+$RNAs. The activation of both goes in parallel.

One may speculate that the formation of small B1$^+$ and B2$^+$RNAs is the first step in retroposition of the B-type sequences to the new sites of the genome. Thus, the activation of the B2 and B1 transcription by RNA polymerase III may induce the activation of retroposition.

An intriguing issue to be studied is the nature of mRNAs containing B-type repeats whose transcription is activated in a variety of tumors. The 1.6 kb B2$^+$RNA seems to correspond to a MHC antigen mRNA [6,7] while the nature of the 1.4 kb B1$^+$RNA is unknown. The 1.6 kb B2$^+$RNA is still present in several normal tissues while the 1.4 kb B1$^+$RNA is not. Thus, the latter rather than the former may be more specific for tumors.

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