Nucleic Acids Research

Sequences hybridizing to mRNA, oligo(dT) and dsRNA from pre-mRNA are contiguous in the cloned mouse DNA fragments

O.N.Tokarskaya, N.A.Tchurikov, P.L.Ivanov, D.A.Kramerov and A.P.Ryskov

Institute of Molecular Biology, USSR Academy of Sciences, Moscow B-334, Vavilov street 32, USSR

ABSTRACT

Fragments from the DNA of mouse embryos produced by restriction endonuclease HindIII were cloned in pBR322 plasmid and examined for the ability to hybridize _in situ_ with [^{32}P] labeled cDNA synthesized from the polysomal poly(A)$^+$mRNA template. Several of the selected clones were examined for the presence of specific sequences inside the cloned mouse DNA fragments by the blotting procedure of Southern /1/. The data obtained indicate that the majority of the cloned mouse DNA fragments contained sequences hybridizing with cDNA, oligo(dT) and double-stranded regions from pre-mRNA. The results of hybridization experiments and double digestion with HindIII+HaeIII endonucleases provide evidence that these sequences could be contiguous in the given restriction DNA fragments.

INTRODUCTION

Complementary sequences and double-stranded structures of different types were found in the nuclear precursor of mRNA (DNA-like RNA, dRNA, heterogeneous nuclear RNA, hnRNA) /2-5/. According to our classification /6/, dsRNA-A represents long (300–800 or more base pairs in length) double-stranded structures which cannot snap back after pre-mRNA melting. They arise mainly during pre-mRNA isolation as a result of the interaction of long complementary sequences. DsRNA-B and dsRNA-C (100–200 and 20–30 base pairs in length, respectively) can snap back after melting of pre-mRNA and thus represent true hairpin -like structures in pre-mRNA. The function of these sequences in pre-mRNA and eukaryotic genomes is yet unknown. It has been suggested that dsRNA-B may serve as signal sequences for the processing of pre-mRNA /7-8/. Different complementary sequences may direct the folding of pre-mRNA molecules as part of

425

the processing events necessary for mRNA formation. On the
other hand, they can be specific and functionally significant
only for certain, for example, repetitive structural genes.

Further progress in our understanding of the structural
organization and the functional role of these sequences may be
based on the analysis of cloned DNA fragments which contain
structural genes and sequences complementary to dsRNA. The
isolation of clones containing sequences complementary to hams-
ter /9/ and mouse /10/ dsRNA from pre-mRNA has been recently
described. Molecular hybridization analysis of the DNAs of such
clones suggests that they contain two most abundant types of
dsRNA sequences accounting for 50-75% of the total dsRNA-B /10/.

In the present work, we have selected several clones hy-
bridizing with mouse polysomal mRNA (or cDNA) and found out
thatthe majority of the cloned mouse DNA fragments contained
sequences hybridizing to cDNA transcribed from polysomal mRNA,
oligo(dT) and dsRNA from pre-mRNA. The results of molecular
hybridization experiments and double digestion with restriction
endonucleases provide evidence that these sequences are conti-
guous in two individual restriction DNA fragments.

MATERIALS AND METHODS

Bacterial plasmids and strains. A small (2.6×10^6 daltons)
plasmid pBR322 (a gift of Dr. H.Boyer) conferring resistance to
ampicillin and tetracyclin /11/ was used as a vector. E.coli,
strain HB101, was used for growing plasmid pBR322. E.coli,
strain χ1776 (a gift of Dr. R.Curtiss), was used as a host for
the recombinant plasmid DNA transformation and cloning in a P3
physical containment.

Preparation of DNA. Mouse DNA was prepared from the Ehr-
lich ascite cells by the procedure of Gross-Bellard et al./12/.
For cloning, DNA was obtained from mouse embryos /12/. Plasmid
pBR322 DNA was isolated according to Bolivar et al. /11/. Re-
combinant plasmids were grown according to Curtiss et al./13/
with further purification using the ethidium bromide CsCl den-
sity gradient centrifugation /11/. DNAs of recombinant clones
containing sequences homologous to dsRNA-B1 (B1⁺clone) and

dsRNA-B2 (B2$^+$clone) were prepared as described /10/.

Preparation of recombinant plasmids. 20 µg of HindIII digested mouse embryonic DNA and 0.05 µg of HindIII digested pBR322 plasmid DNA were ligated in 200 µl of a solution containing 100 mM NaCl, 50 mM Tris-HCl buffer (pH 7.5), 10 mM MgCl$_2$, 10 mM β-mercaptoethanol, 0.08 mM ATP, 50 µg/ml of bovine serum albumin and DNA ligase (a gift of Dr. G.Dolganov). After a 1 hr incubation, the reaction mixture was diluted 10-fold with the ligase buffer, more ligase was added, and the reaction was continued for 16 hrs.

Isolation of polysomal poly(A)$^+$mRNA was performed as described /6/.

Isolation of pre-mRNA and its double-stranded regions (dsRNA). Ehrlich ascites carcinoma cells (2×10^8 cells) were incubated in 25 ml of the Eagle medium containing 5 mCi of [^{3}H] uridine for 1 hr. Pre-mRNA was isolated by the hot phenol fractionation procedure /14/ or by the Scherrer and Darnell method /15/ (in the latter case, the cells were labeled in the medium containing 0.05 µg/ml of actinomycin D). DsRNA was isolated from pre-mRNA (>18S) by digestion with a mixture of pancreatic and T1 RNAases and gel filtration through Sephadex G-75 as previously described /4/.

Preparation of cDNA. DNA complementary to mouse polysomal poly(A)$^+$mRNA was synthesized in a reaction mixture containing 50 mM Tris-HCl (pH 8.1), 5 mM MgCl$_2$, 10 mM dithiothreitol, 50 mM KCl, 100 µg/ml of actinomycin D (Calbiochem), 0.5 mM each of dATP, dGTP, and dTTP, 50–100 µCi [^{32}P]dCTP (350 Ci/ mmol) (Amersham), mRNA (50 µg/ml), oligo(dT)$_{12-18}$ (4 µg/ml) (Collaborative Research), RNA-dependent DNA polymerase from avian myeloblastosis virus (240 units/ml) (provided by Drs. D. and J.W.Beard). The solution was quickly vortexed and incubated for 1 hr at 37°C. The reaction mixture was then made 10 mM EDTA and 0.1 M NaOH and heated to 65°C for 30 min. After readjusting the pH of the solution to 7.5, the sample was placed on a Sephadex G-50 column equilibrated with 0.1 M NaCl – 0.01 M Tris-HCl (pH 7.5). The excluded fraction was collected and precipitated with ethanol.

Identification of recombinant clones. Colonies of trans-
formed bacteria were screened for the presence of cDNA sequen-
ces by colony hybridization according to the procedure of Grun-
stein and Hogness /16/. 10×10^6 cpm of the $[^{32}P]$ labeled cDNA
were hybridized to each of 8 cm nitrocellulose filters in
5xSSC for 24 hrs at $65°C$. After hybridization, the filters were
extensively washed with 2xSSC at $65°C$, 2xSSC-7 M urea - 0.1%
sodium dodecyl sulfate at $42°C$, and 2xSSC at room temperature.
Autoradiography of the dried filters was performed with an in-
tensifying screen at $-70°C$ for 1 to 5 days. Colonies which
gave positive autoradiography response were picked from the
replica plates, grown individually in suspension cultures, and
plasmid DNA was isolated as described /11/.

Restriction endonuclease digestion and gel electrophor-
esis. DNA was digested with restriction endonucleases HindIII
and HaeIII (provided by Dr. V.Nosikov) in 10 mM Tris-HCl, pH
7.6, 10 mM $MgCl_2$, 1 mM dithiothreitol, 50 mM NaCl at $37°C$. The
number of enzyme units used and the time of digestion varied
with different batches of enzymes.

Native or digested plasmid DNAs were fractionated by
electrophoresis in slab gels of 1 or 3% agarose. The gels were
stained with 10 µg/ml of ethidium bromide to visualize the DNA
bands.

Transfer of DNA to nitrocellulose filters and hybridi-
zation. The DNA in the agarose slab gels was denatured in 1 M
NaCl-0.5 M NaOH for 30 min. After neutralization of the solu-
tion with 1.5 M NaCl - 0.5 M Tris-HCl, pH 7.0, the DNA was
transferred by blotting the gel onto nitrocellulose filters
overnight according to the procedure of Southern /1/ in the
presence of 6xSSC. Thereafter, the nitrocellulose filters were
rinsed in 2xSSC, air-dried, baked at $80°C$ for 5 hrs in a vacu-
um oven, soaked in a 2-fold concentrated Denhardt's solution
/17/, and hybridized to $[^{32}P]$ labeled cDNA, $[^{32}P]$ labeled nick-
-translated DNA or $[^{125}I]$ labeled dsRNA in a mixture containing
2xSSC, 0.1% sodium dodecyl sulfate, 2x Denhardt's solution,
100 µg/ml of denatured E.coli DNA, and 100 µg/ml of poly(U)
for 16 hrs at $65°C$. Washing and autoradiography of the filters
were carried out as described above.

Molecular hybridization assays. Non-radioactive plasmid
DNAs immobilized on the filters were hybridized to $[^{32}P]$label-
ed cDNA or $[^{3}H]$labeled mRNA in a solution containing 0.4 M
NaCl, 0.01 M Tris-HCl (pH 7.5), 0.001 M EDTA, 0.2% sodium do-
decyl sulfate and 100 µg/ml of poly(U) for 15 hrs at 65°C.
After annealing, the filters were washed 3 times for 2 hrs
with 7 M urea in 2xSSC - 0.1% sodium dodecyl sulfate at 42°C
and 3 times for 1 hr with 2xSSC at 65°C. In some experiments,
to remove the cDNA fraction enriched in sequences complementa-
ry to dsRNA-B1 and dsRNA-B2, the total cDNA was repeatedly
hybridized to the DNAs of B1^{+} and B2^{+} clones immobilized on
nitrocellulose filters. Non-hybridized cDNA (B1-, B2-depleted
cDNA) was then used for hybridization to the DNA of the studi-
ed recombinant clones.

In vitro labeling. DsRNA was labeled with ^{125}I according
to the procedure of Prensky et al. /18/. The reaction was per-
formed at 80°C for 6 min. Before hybridization, $[^{125}I]$labeled
RNA was passed through nitrocellulose filters (0.45 µm). The
total mouse DNA and the DNAs of B1^{+} and B2^{+} clones were label-
ed with ^{32}P by nick translation to a specific activity of
$5x10^{7}$ cpm/µg according to /19/.

Isolation of DNA fragments from agarose gel. This was
done by electrophoretic elution /20/. The eluted samples were
concentrated 10-fold and NaCl was added to a concentration of
0.5 M. Then the DNA solution was passed through Dowex 50Wx8 to
remove ethidium bromide and DNA fragments were precipitated
with ethanol.

RESULTS

1. Analysis of cloned mouse DNA fragments

Mouse DNA digested with restriction endonuclease HindIII
was ligated to HindIII-digested pBR322. Under the conditions
used, up to 90% of the transformants that harboured hybrid
plasmids were obtained. These recombinant clones were tested
for hybridization of their DNA to $[^{32}P]$labeled cDNA by the
technique of colony hybridization /16/. 100-120 clones of 500
tested gave strong positive hybridization. Several of them

were chosen at random for further study.

The purified DNAs from the selected plasmids were digest-
ed with HindIII restriction endonuclease and fractionated by
electrophoresis in a 1% agarose gel. The results shown in Fig.1
demonstrate the presence of more than one insert in the majo-
rity of clones. This probably depends on the fact that under-
restricted DNA was used for the ligation procedure. However,
the secondary ligation of different mouse DNA fragments could
not be excluded and therefore all different HindIII mouse DNA
fragments even present in the same clone were considered as
independent genomic fragments. Two clones, viz. 5 and 92, con-
tain a single insert. The behaviour of clone №31 is rather un-
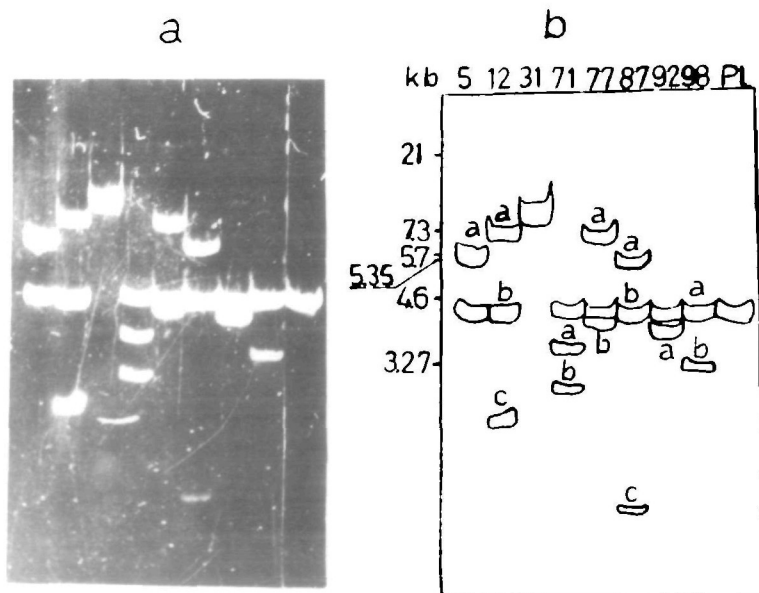usual, perhaps due to elimination of the HindIII restriction

Fig. 1. Electrophoresis of HindIII-cleaved mouse DNA
fragments cloned in pBR322 plasmid.

Fragments separated in 1% agarose gel are visualized
by staining with ethidium bromide followed by ultravio-
let irradiation (input per track, 0.5 μg DNA).
a - UV visualization
b - graphic scheme; Pl - plasmid pBR322; λ DNA di-
gested with EcoRI restriction endonuclease was
used as size markers.

site during the cloning procedure. Although the hybridization
properties of this clone are similar to the others, it was
discarded in subsequent experiments. The majority of inserts
ranged in size from 2 to 8 kilobases (kb). All of the indivi-
dual restriction fragments were further analysed by the gel
blotting technique using for the hybridization $[^{32}P]$labeled
cDNA and oligo(dT) synthesized by reverse transcriptase, $[^{125}I]$
labeled dsRNA from pre-mRNA, and $[^{32}P]$labeled nick-translated
total mouse DNA and DNA of clones containing B1 and B2 sequen-
ces. Experimental procedures for the isolation of the latters
and their properties were described in the previous work /10/.
These clones contain two most abundant sequences transcribed
into dsRNA-B, designated as dsRNA-B1 and dsRNA-B2, and account-
ing for up to 75% of the total dsRNA-B. They belong to a class
of highly repetitive DNA sequences scattered throughout the
whole mouse genome and only a part of them (about 1/4) is or-
ganized in palindromic structures.

The results of the hybridization experiments are shown in
Fig. 2 and summarized in Table 1. Almost all fragments of the
clones hybridize to cDNA. The only exception is fragment 98b.
The same fragments which hybridize to cDNA also bind dsRNA.
No exception was found with our fragments. Only in the case of
fragment 87a, hybridization to dsRNA was much weaker. For
those fragments which do not comigrate with plasmid DNA, it
was also possible to determine the nature of dsRNA binding
sequences by hybridization with labeled DNA of plasmids con-
taining B1 and B2 sequences (Fig. 2c,d). Two fragments, 87a
and 87c, hybridized with neither B1 nor B2 sequences. All the
others bound either B1 or B2, or both of them. The results are
in agreement with the previous data showing B1 and B2 sequen-
ces to be responsible for the formation of the most abundant
classes of dsRNA-B /10/.

Ten of the fourteen fragments hybridizing to cDNA also
bind oligo(dT) (Fig.2e). Thus, these two types of sequences
are also very often (but not always) located in the close
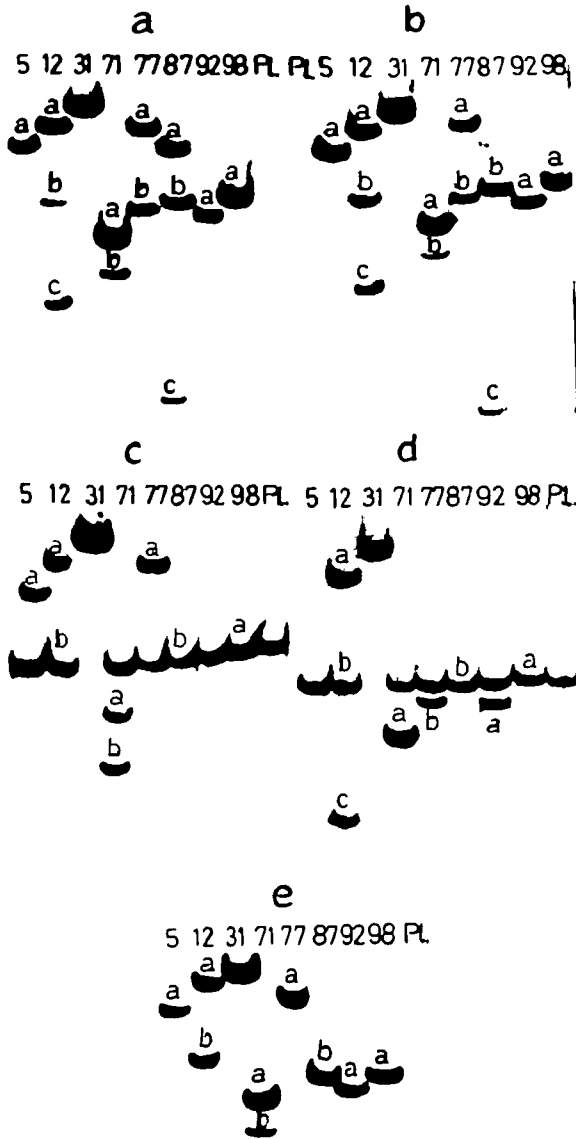neighbourhood in the mouse genome.

Fig. 2. Autoradiography of HindIII DNA fragments after blott-hybridization to: a) [32P] cDNA; b) [125I] dsRNA; c) [32P] DNA of B1+ clone; d) [32P] DNA of B2+ clone; e) [32P] oligo(dT).

Hybridization to oligo(dT) was performed in a 2xSSC solution at 28° overnight; after hybridization, the filter was washed with the 2x SSC solution at room temperature, dried, and exposed to X-ray film for 3 hrs.

Table 1. Properties of mouse DNA fragments cloned in plasmid pBR322

Clone No.	Fragment	Fragment size (in base pairs)	Hybridization to				
			$[^{32}P]$ cDNA	total $[^{125}I]$ dsRNA	$[^{32}P]$ DNA of B1$^+$ clone	$[^{32}P]$ DNA of B2$^+$ clone	$[^{32}P]$ oligo(dT)
5	a	6400	+	+	+	−	+
12	a	8000	+	+	+	+	+
	b	4300	+	+	−	−	+
	c	2730	+	+	+	+	−
71	a	3600	+	+	+	+	+
	b	3000	+	+	+	−	+
77	a	7000	+	+	+	−	+
	b	4200	+	+	−	+	−
87	a	6400	+	−	−	−	−
	b	4300	+	+	−	−	+
	c	2000	+	+	−	−	−
92	a	4000	+	+	−	+	+
98	a	4300	+	+	−	−	+
	b	3400	−	−	−	−	−

total $[^{125}I]$dsRNA – dsRNA isolated from pre-mRNA by RNAase digestion /3/ and in vitro labeled with ^{125}I;

$[^{32}P]$DNA of B1$^+$ and B2$^+$ clones – nick-translated mouse DNA fragments from recombinant clones containing sequences complementary to dsRNA-B1 and dsRNA-B2 /10/;

$[^{32}P]$oligo(dT) – DNA homopolymer synthesized from poly(A) sequences by RNA-dependent DNA polymerase from avian myeloblastosis virus.

2. The contiguity of sequences hybridizing to cDNA and dsRNA

The most intriguing feature is the hybridization of indi-
vidual fragments with both cDNA and dsRNA. It may be explained
either (i) by the contiguity of genes and sequences coding for
dsRNA or (ii) by the existence of sequences complementary to
dsRNA in cDNA preparations. The second explanation seemed to
be plausible as we found that clones containing B1 or B2 se-
quences bound from 1 to 2% of polysomal mRNA or cDNA in the
hybridization reaction (unpublished data).

To choose among the two possibilities, the following ex-
periments were performed. First, the DNA of clones was hybri-
dized with cDNA from which B1 and B2 sequences have been re-
moved. For this, the total cDNA was repeatedly (2-3 times)
hybridized to the DNAs of B1$^+$ and B2$^+$ clones immobilized on
nitrocellulose filters. The non-hybridized fraction of cDNA
(B1-, B2-depleted cDNA), the total cDNA and mRNA were then
used for molecular hybridization experiments with the DNAs of
different clones. The results of these experiments are listed
in Table 2. It was found that the percentage of hybridization

Table 2. Hybridization characteristics of recombinant clones

Clone No.	Hybridization, %		
	Polysomal [3H]mRNA[x]	[32P] cDNA[xx]	B1-, B2-depleted [32P] cDNA[xxx]
5	0.015	0.026	0.0025
12	0.025	0.08	0.0069
71	0.04	0.15	0.12
77	0.017	0.097	0.0056
87	0.016	0.03	0.0038
92	0.009	0.025	0.0085
98	0.011	0.017	0.012

Hybridization of cloned DNAs immobilized on nitrocellulose filters was carried out as described in Methods. 10×10^6 cpm of $[^{32}P]$cDNA and 5×10^6 cpm of $[^3H]$mRNA were taken in the experiments. Control filters containing pBR322 DNA bound 100-150 cpm of $[^{32}P]$cDNA and 30-40 cpm of $[^3H]$mRNA. These backgrounds were subtracted from the experimental figures.

[x] Hybrids were treated with RNAase; [xx] $[^{32}P]$cDNA - DNA complementary to mouse liver polysomal poly(A)$^+$mRNA synthesized by RNA-dependent DNA polymerase from avian myeloblastosis virus; [xxx] B1-, B2-depleted $[^{32}P]$cDNA - total $[^{32}P]$ cDNA nonhybridized to DNA of B1$^+$ and B2$^+$ clones after two cycles of hybridization.

was reduced significantly in the case when B1-, B2-depleted cDNA was used as compared with the total cDNA and mRNA. It is not surprising because the fraction of repetitive cDNA sequences was removed from the reaction. However, hybridization was yet detected for all clones. For several clones (NN 71, 92, 98), the percentage of hybridization was high enough. The results of these experiments suggest the presence of structural gene sequences in the tested DNAs. However, this suggestion needs some further direct support as we still cannot exclude the possibility that some other dsRNA sequences remaining in cDNA after removal of B1 and B2 participate in the hybridization reaction. Therefore, in the second series of experiments we tried to separate physically sequences hybridizing to cDNA and to dsRNA. Fragments 98a and 71a were chosen for the analysis. For this, fragment 98a (together with comigrated plasmid DNA) was eluted from 1% agarose gel, purified, and additionally digested with restrictase HaeIII. DNA of the clone N 71 was

also digested with restrictases HindIII+HaeIII. The subfrag-
ments obtained were analysed by the blotting technique follow-
ed by hybridization to total cDNA, B1-, B2-depleted cDNA and
total dsRNA. The results are shown in Fig. 3 (A and B, tracks
3-5). The number and location of the subfragments derived from
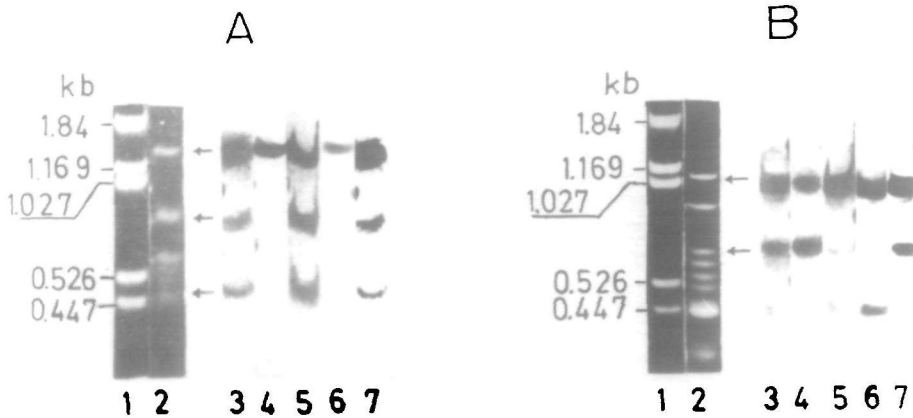the inserted sequences and plasmid vector DNA are depicted in

Fig. 3. Analysis of DNA subfragments obtained after HaeIII
        digestion of fragment 98a (A) and fragment 71a (B).

   1. SV40 DNA digested with HindIII used as site markers.
      Fragments in 3% agarose gel were visualized by
      ethidium bromide staining.
   2. DNA subfragments in 3% agarose gel were visualized
      by ethidium bromide staining.
      (A) Fragment 98a and comigrated plasmid DNA were
          digested with HaeIII.
      (B) Total DNA of clone 71 was digested with HindIII+
          +HaeIII. The hybridizing subfragments derived
          from 98a and 71a are shown with arrows.
   3-7. The corresponding radioautograms after Southern
      hybridization with:
      $[^{32}P]$ cDNA, (1-5)x10^6 cpm input per blott (3);
      $[^{125}I]$ dsRNA, (5-7)x10^5 cpm input per blott (4);

      $[^{32}P]$ B1-, B2-depleted cDNA, (1-2)x10^6 cpm input
          per blott (5), control hybridization to DNA
          of B1$^+$ and B2$^+$ clones was not detected (data
          not shown);
      $[^{32}P]$ oligo(dT), (0.5-1)x10^6 cpm input per blott (6);
      $[^{32}P]$ total mouse DNA, (1-2)x10^6 cpm input per
          blott (7); hybridization to unique DNA repre-
          sented by fragment 98b was not observed un-
          der the conditions used (data not shown).

Fig. 3 (A and B, tracks 2). The hybridizing subfragments are
shown with arrows. Three subfragments derived from 98a were
hybridized to total cDNA (Fig. 3A, track 3) but only one of
them (the upper one) could hybridize to total dsRNA (Fig. 3A,
track 4). The two lower subfragments could hybridize only to
cDNA. A similar digestion of fragment 71a yielded two sub-
fragments from which the upper one hybridized more efficient-
ly to cDNA and the lower one to dsRNA (Fig. 3B, tracks 3 and
4). Consistent with these data are our results from experi-
ments in which the blotted HaeIII subfragments of 98a and of
71a were hybridized to B1-B2-depleted cDNA (Fig.3, A and B,
tracks 5). In this case, no hybridization was observed to cont-
rol DNA (B1$^+$, B2$^+$ clones; data not shown); this means that
only the hybridization to cDNA is detected.

It is evident that three subfragments derived from 98a
and one subfragment (the upper) derived from 71a contain cDNA
or presumptive structural gene sequences. Sequences homologous
to dsRNA are located in one (the lower) subfragment derived
from 71a and in one (the upper) subfragment derived from 98a.
Thus, these results demonstrate the sequences hybridizing to
cDNA and dsRNA to be contiguous in the single restriction
fragments 98a and 71a. Possibly, by using suitable restriction
endonucleases, one can demonstrate a similar situation also
for other fragments studied.

3. Further studies on cDNA binding fragments

Among the fragments studied, one should reveal those
which hybridize exclusively to cDNA, i.e. contain presumptive
structural genes. These are the subfragments of fragments 98a
and 71a excised by restriction with endonuclease HaeIII and
possibly fragment 87a excised from clone N 87 by digestion
with endonuclease HindIII (Fig. 2). Of greatest interest is
fragment 71a which binds more than 0.1% of total cDNA even in
the case of the B1-, B2-depleted cDNA, i.e. it hybridizes to
the abundant class of cDNA or mRNA.

Further hybridization experiments were performed with
these subfragments to know the location of oligo(dT) hybridi-
zing sequences. It is clearly seen that the two upper subfrag-
ments derived from 98a and 71a can bind oligo(dT) (Fig. 3, A

and B, tracks 6). A small subfragment derived from 71a and practically not hybridizing to cDNA and dsRNA also hybridized with oligo(dT) (Fig. 3B, track 6). No hybridization was detected with other subfragments including the two subfragments derived from 98a and containing cDNA sequences.

The subfragments were also hybridized with total mouse DNA labeled with ^{32}P by nick translation. Under the given experimental conditions, hybridization to unique DNA (fragment 98b) was not observed (data not shown) and only repetitive sequences could be detected. As it can be seen from Fig. 3 (A and B, tracks 7) all the HaeIII subfragments of 98a and of 71a hybridize to total $[^{32}P]$ DNA, although less effectively than fragments which contain B1 and B2 sequences. It means that sequences complementary to cDNA which are within fragments 98a and 71a are repetitive in mouse genome but not to such an extent as dsRNA hybridizing sequences.

DISCUSSION

The main result of the paper is the demonstration of the contiguity between sequences hybridizing to cDNA and to dsRNA (in particular to dsRNA-B1 and dsRNA-B2) in the individual fragments of mouse genome. Different interpretations of this observation are possible.

It has been shown previously that ds sequences in pre-mRNA (dsRNA-B) are characterized by the following typical features: they are transcribed from repetitive regions of the genome, have low complexity, identical or similar in different pre-mRNA molecules and can hybridize to polysomal mRNA to some extent /6-8, 21/. Two types of sequences (B1 and B2) responsible for the synthesis of about 2/3 of all dsRNA-B were isolated later /10/. It has been suggested that dsRNA-B may serve as signal sequences for the processing which are located at the borderline between mRNA and non-informative regions of pre-mRNA /7,8/.

Consistent with this hypothesis are the above mentioned findings showing that the individual cloned fragments can hybridize to both cDNA and dsRNA. However, the situation seems to be more complex. We have found that a small but significant

fraction of cDNA transcribed even from purified polysomal mRNA
hybridizes with B1 or B2 sequences. B1 and B2 sequences are
ubiquitous in the genome and this can explain why very many
DNA fragments give a positive test upon hybridization to cDNA.
The nature of mRNA (cDNA) molecules containing sequences com-
plementary to dsRNA is not clear. They comprise only a few
percent of the total chains.

Nevertheless, at least in two cases, we could demonstrate
that, besides sequences hybridizing to dsRNA, the DNA frag-
ments also contained sequences binding cDNA exclusively and
that these two types of sequences were localized close one to
another. The question arises what is the nature of sequences
binding this "real" cDNA. One possibility is that most of the
genes are neighboured by repetitive sequences transcribed in
dsRNA and used as signal sequences. This idea is corroborated
by the results demonstrating that, even after a complete re-
moval of B1 and B2 sequences from cDNA, it still hybridizes
to cloned DNA fragments (Table 2). If such an interpretation
is correct, the B1 or B2 sequence can be used as a guide for
the search of a structural gene in a close neighbourhood.

The second possibility is that only a specific set of
transcribed genetic elements is flanked by B1, B2 or other
repetitive sequences responsible for dsRNA formation. For
example, these may be multiple genes similar to mobile dis-
persed genes of Drosophila melanogaster /22,23/. The latters
are known to be framed with direct repeats /24/.

One should consider in this respect that, in both cases
analyzed in detail, the sequence hybridizing exclusively to
cDNA was represented by a repetitive sequence of the mouse ge-
nome. Further analysis of these questions should lead to their
solving and to a better understanding of the mammalian genome
organization.

An additional result of this investigation is the demonst
ration of contiguity of oligo(dA·dT) clusters and sequences
hybridizing to cDNA. The cases of coincidences are not so re-
gular as for dsRNA-hybridizing sequences but their percentage
is higher than could be expected from the random occurrence
of oligo(dA·dT) clusters in the mouse genome. Moreover, we

could note a high degree of coincidence between highly repe-
titive sequences of B1 and B2 types and oligo(dA·dT) clusters
within individual cloned fragments. The sequencing of three
cloned B1 sequences discovered the existence of dA-rich clus-
ters in close neighbourhood to them (Kramerov, Kraev et al.,
manuscript in preparation). This is in accordance with the
data obtained by Flavell et al. /25/. These authors have
shown by fractionating the total rabbit DNA on poly(U)-Sepha-
dex that 50% of fragments 400 kb long which contain an
oligo(dA·dT) cluster comprise highly repetitive sequences as
well.

The significance of these data also depends on the solv-
ing of the above mentioned question about the nature of gene-
tic elements studied here.

ACKNOWLEDGEMENTS

REFERENCES

1   Southern, E.M. (1975) J.Mol.Biol. 98,503-517.
2   Jelinek, W., Darnell, J.E. (1972) Proc. Natl. Acad. Sci.
     USA 69,2537-2541.
3   Ryskov, A.P., Farashyan, V.R., and Georgiev, G.P. (1972)
     Biochim. Biophys. Acta 262, 568-572.
4   Ryskov, A.P., Saunders, G.F., Farashyan, V.R., and Geor-
     giev, G.P. (1973) Biochim. Biophys. Acta 312, 152-164.
5   Ryskov, A.P., Yenikolopov, G.N., and Limborska, S.A.
     (1974) FEBS Letters 47, 98-101.
6   Kramerov, D.A., Ryskov, A.P., and Georgiev, G.P. (1977)
     Biochim.Biophys.Acta 475, 461-475.
7   Ryskov, A.P., Tokarskaya,O.V., Georgiev, G.P., Coutelle,
     Ch., and Thiele, B. (1976) Nucleic Acids Res. 3, 1487-1498.
8   Ryskov, A.P., Kramerov, D.A., and Georgiev, G.P. (1976)
     Biochim.Biophys.Acta 447, 214-229.
9   Jelinek, W.R. (1978) Proc.Natl.Acad.Sci.USA 75,2679-2683.
10   Kramerov, D.A., Grigoryan, A.A., Ryskov, A.P., and Geor-
     giev, G.P. (1979) Nucleic Acids Res. 6, 697-713.
11   Bolivare, F., Rodriguez, R.L., Greene, P.J., Betlach, M.C.,
     Heynecher, H.L., Boyer, H.W., Grosa, J.M., and Falkov, S.
     (1977) Gene 2, 95-113.
12   Gross-Bellard, M., Oudet, P., Chambon, P. (1973) Europ.
     J. Biochem. 36, 32-38.

13 Curtiss, R.III, Inoue, M., Pereira, D., Hsy, J.C., Alexan-
    der, L., and Rock, L. (1977) Miami Winter Symp. 13, 99–114.
14 Georgiev, G.P., Ryskov, A.P., Coutelle, C., Mantieva, V.L.
    and Avakyan, E.R. (1972) Biochim. Biophys.Acta 259,259–283.
15 Scherrer, K. and Darnell, J.E. (1962) Biochem.Biophys.Res.
    Communs. 7, 486–490.
16 Grunstein, M., Hogness, D.C. (1975) Proc.Natl.Acad.Sci.
    USA 72, 3961–3965.
17 Denhardt, D.T. (1966) Biochem.Biophys.Res.Communs. 23,
    641–646.
18 Prensky, W.,Steddensen, D.M., Hughes, W.L. (1973) Proc.
    Natl.Acad.Sci. USA 70, 1860–1864.
19 Rigby, P.W.J., Dieckmann, M., Rhodes, C., and Berg, P.
    (1977) J.Mol.Biol. 113, 237–251.
20 Wienand, U., Schwarz, Z., Feix, G. (1979) FEBS Letters
    98, 319–323.
21 Ryskov, A.P., Limborska, S.A., and Georgiev, G.P. (1973)
    Mol.Biol.Reports 1, 215–219.
22 Ilyin, Y.V., Tchurikov, N.A., Ananiev, E.V., Ryskov, A.P.,
    Yenikolopov, G.N., Limborska, S.A., Maleeva, N.A., Gvoz-
    dev, V.A., and Georgiev, G.P. (1978) Cold Spring Harbor
    Symp. Quant. Biol. 42, 959–969.
23 Strobel, E., Dunsmuir, P., and Rubin, G.M. (1979) Cell
    17, 429–439.
24 Finnegan, D.J., Rubin, G.M., Young, M.W., and Hogness,
    D.S. (1978) Cold Spring Harbor Symp. Quant, Biol. 42,
    1053–1063.
25 Flavell, R.A., Van der Berg, F.M., and Grosveld, G.C.
    (1977) J.Mol.Biol. 115, 715–734.