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Long double-stranded sequences (dsRNA-B) of nuclear pre-mRNA consist of a few highly abundant classes of sequences: evidence from DNA cloning experiments

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ABSTRACT

DNA preparations from about hundred randomly selected clones containing mouse DNA fragments were screened for the existence of sequences complementary to long double-stranded regions of pre-mRNA able to snap back after melting (dsRNA-B). Many clones containing such sequences were found. The cloned sequences can be subdivided into three groups: (1) those complementary to about a half (at least to 30-40%) of the total dsRNA, designated as sequences B1; (2) those complementary to a part of sequence B1; and (3) sequences complementary to about a quarter (at least to 15%) of the total dsRNA referred to as sequence B2. The size of DNA sequence complementary to dsRNA is about 400 base pairs.

Melting experiments with hybrids show that the members of B1 family are very similar if not identical, while the divergence among B2 sequences is higher, but still the number of substitutions does not exceed 9% of bases. Thus, the major part of dsRNA-B consists of a small number of highly abundant sequences as was suggested earlier on the basis of renaturation kinetics /1-3/. Sequences B1 and B2 are represented by many copies in the mouse genome and in pre-mRNA, and many of them probably do not form hairpin-like structures.

INTRODUCTION

Double-stranded (ds) sequences of different length were found in the nuclear precursor of mRNA, pre-mRNA (DNA-like RNA, dRNA, heterogeneous nuclear RNA, hnRNA) /4-6/. According to our classification /4/, dsRNA-A represents the long (300-800 or more base pairs in length) ds regions which are not able to snap back after pre-mRNA melting. DsRNA-B and C (100-200 and 20-30 base pairs in length, respectively) are able to snap back and thus represent true hairpin-like structures. It has been suggested that dsRNA-B may serve as a signal sequencing for processing located at the borderline between mRNA

and non-informative regions of pre-mRNA /1,2,7/. They, for example, may be involved in splicing /8/.

For further analysis of the structure and functions of double-stranded regions in pre-mRNA, it was very important to have these sequences in a homogeneous state. This problem can be solved by cloning the corresponding DNA fragments. The present paper described the isolation of clones containing fragments of mouse DNA complementary to dsRNA-B prepared from pre--mRNA. The studies on hybridization of isolated DNA fragments with ds RNA confirmed our previous observation as to the low complexity of dsRNA-B /1-3/. Most of it was found in two very abundant classes, hybridizing to the DNA of isolated clones. The concentration of two particular dsRNA-B sequences in pre--mRNA, total DNA, and the palindrome fraction of DNA was also determined.

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 /9/ was used as a vector. E.coli, strain HB101, was used for growing plasmid PBR322. EK2 host E.coli strain χ1776 (a gift of Dr. R.Curtiss) was used as a host for the recombinant plasmid DNA transformation and cloning.

Preparation of DNA. Mouse DNA was prepared from the Ehrlich ascite cells by the procedure of Gross-Bellard et al. /11/. For cloning the DNA was obtained from mouse embryos/11/.

Plasmid PBR322 DNA was isolated according to Bolivar et al. /9/. Recombinant plasmids were grown according to Curtiss et al. /10/ with further purification using the ethidium bromide CsCl density gradient centrifugation /9/. In the preliminary experiments on selection of clones containing sequences hybridizing with dsRNA the CsCl equilibrium centrifugation was omitted.

Construction of recombinant plasmids and cloning. EcoRI endonuclease /12/, HindIII endonuclease /13/, T4 phage induced DNA ligase /14/ were prepared in this laboratory by

Dr. G.N.Yenikolopov.

Mouse and plasmid DNAs (125 μg/ml) were first treated with HindIII endonuclease in a buffer containing: 7 mM Tris-HCl, pH 7.9, 7 mM $MgCl_2$, 1 mM DTT. The concentration of NaCl was then brought to 50 mM and EcoRI endonuclease was added. The small fragment of plasmid DNA formed by cleavage /9/ was removed by chromatography on Biogel A15m.

The HindIII-EcoRI recombinant plasmid DNA (0.2 μg/ml) with non-self-complementary cohesive ends was ligated with HindIII-EcoRI digested mouse DNA (30 μg/ml). The ligation was performed at 12°C in a buffer containing 50 mM Tris-HCl, pH 7.9, 0.08 mM ATP, 1 mM DTT and 60 μg/ml of bovine serum albumine. After a 1 hr incubation, the reaction mixture was diluted 10-fold with the ligase buffer, more ligase was added, and the mixture was incubated for another 16 hr. The ligated DNA was diluted 5-fold with 0.1 M Tris-HCl, pH 7.5. The transformation was performed with *E.coli* strain χ1776 according to Curtiss *et al*. /10, 15/ with slight modification. The work was conducted in P3 conditions.

Isolation of pre-mRNA and its double-stranded regions. Ehrlich ascites carcinoma cells ($2x10^8$ cells) were incubated in 25 ml of the Eagle medium containing 15 mCi of [^{3}H]uridine for 1 hr. Pre-mRNA ($2x10^6$ cpm/μg) was isolated by the hot phenol fractionation procedure /16,17/ or by the Scherrer and Darnell method /18/ (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), digested with a mixture of pancreatic and TI RNAases, by gel filtration through Sephadex-G-75 /4.6/. DsRNA-A and dsRNA-B were separated by electrophoresis as described earlier /4/.

In some experiments denatured dsRNA was labeled with ^{125}I according to Prensky *et al*. /19/. The reaction was performed at 80°C for 6 min. Before hybridization, [^{125}I]RNA was passed through Millipore filters (0.45 μm).

Isolation of palindromic DNA. The main band of mouse DNA was prepared by centrifugation in a CsCl density gradient in the V 65 rotor (55000 rpm, 11 hr, 15°C). After dialysis, the DNA (80 μg) was denatured in 0.3 M NaOH (12 ml), the alkali

was neutralized with HCl, and this solution was poured into 1l of a buffer (2 mM Tris-HCl,pH 7.5, 0.1 M NaCl), heated to 65°C. After a 3 min incubation ($C_0t=10^{-5}$), the solution was rapidly cooled in a mixture of dry ice and acetone, and then concentrated to 15 ml by ultrafiltration in the Amicon cell (filter PM30) at 4°C. The DNA was treated with SI nuclease /20/ in 0.1 M NaCl, 30 mM $NaCH_3COO$, pH 4.6, 1 mM $ZnSO_4$, 5% glycerol, for 30 min at 20°C and then for 40 min at 45°C. After deproteinization with chloroform and purification on hydroxyapatite, the palindromic DNA was dialized, concentrated, and labeled using the nick-translation reaction /21/. The palindromic fraction isolated in this way contained 5% of the total DNA. It should contain inverted repeats separated by both short and long joining loops /22/.

Hybridization experiments. Plasmid DNA was incubated in 0.5 M NaOH at 37°C for 1 hr and then 20 volumes of 6xSSC containing 5% formaldehyde and 1 volume of 0.5 M K_2HPO_4 were added. CH_2O was used to prevent renaturation of palindromes. The DNA solution was passed through a Millipore filter (0.45 µm). The filters were dried in vacuo (2 hr at 80°C) and incubated in 2xSSC containing 0.5% CH_2O (2 hr at 65°C).

In selection experiments, 20-30 filters were incubated together in 0.7 ml of a solution containing denatured [^{3}H]-dsRNA ($1.5x10^5$ cpm), 5xSSC, 0.5 ug/ml poly(U) and 0.2% SDS for 15 hr at 65°C. After annealing, the filters were washed 3 times with 7 M urea in 2xSSC at 41°C, treated with RNAase (10 ug/ml, 40 min), dried, and counted. The hybridization with [^{125}I] dsRNA and [^{32}P] palindromic DNA was performed in the same way.

In saturation experiments, each filter (10 µg DNA) was wetted with a small but exact volume of hybridization mixture containing denatured ^{3}H dsRNA (2500 cpm) and 0.8 mg/ml poly(U), 2xSSC, 0.5% SDS, and put separately one from another in a flat-bottom vessel containing mineral oil. Annealing was performed for 2-3 hr at 65°C. Then filters were washed, treated with RNAase, and the radioactivity was counted.

A similar procedure was used for hybridization of clone DNAs either with total mouse DNA, or with palindromic DNA, or

with mouse DNA-insertion of clone MM35 which was labeled with [^{32}P]dNTP of the nick-translation reaction /21/. Before hybridization, labeled DNAs were fragmented by boiling for 30 min in 0.3 M NaOH. (After that, the maximal DNA size distribution was about 800-1000 nucleotides according to electrophoretic data). In these experiments, the hybridization mixture contained labeled DNA, 2xSSC, 0.8 mg/ml poly(U), 50 μg/ml denatured E.coli DNA and 3x Denhard solution /23/. In the case of total mouse DNA, the filters were treated with SI nuclease after hybridization and washing. The sum of the radioactivity which remained on the filter and of the acid insoluble radioactivity released by SI nuclease was considered as a hybridized material. Such a procedure was used in order to exclude overestimation of the hybridization depending on the presence of single-stranded tails in the hybrids.

In rehybridization experiments, dsRNA bound to a filter after primary hybridization was eluted by boiling for 4 min in 2 ml of an aqueous tRNA solution (100 μg/ml). RNA was reprecipitated with ethanol, treated with DNAse I (50 μg/ml, 1.5 h, 20°C), deproteinized with chloroform and, after reprecipitation with ethanol, used for rehybridization, as described above.

Before hybridization, [^{3}H]pre-mRNA (>45S) was treated with 0.1 M NaOH for 20 min at 20°C. Such a fragmentation of RNA (the maximum of distribution was about 5S) prevented most of the pre-mRNA long double-stranded regions from snapping back.

Determination of Tm of hybrids. [P^{32}] cRNA used in the melting experiments was synthesized using the core RNA-polymerase of E.coli /24/, a kind gift of Dr. R.Beabealashvili. Mouse DNA fragments excised from clones MM35, 14 were used as a template. The hybridization of cRNA with clone DNAs was performed as described above for the hybridization of dsRNA-B. In the cRNA-B1 and cRNA-B2 preparation experiments (see Results), the filters after annealing were treated mildly with RNAase A (0.2 μg/ml of RNAase, 0°C, 10 min) and then the filters were washed extensively with 2xSSC, 0.5% SDS solution.

For Tm determination, the filters with hybrids were

washed and then heated to an appropriate temperature in 2xSSC, 0.2% SDS solution for 6 min. After rapid cooling, the solution was applied on a GF/B filter for radioactivity measurement. Then the cycle was repeated at a higher temperature, and so on.

THE RESULTS

Isolation of clones containing DNA fragments complementary to dsRNA. In the first series of experiments, DNA was prepared from 110 randomly selected clones of PRB322 plasmid containing insertions of mouse DNA with EcoRI and HindIII restriction sites at the ends. DNAs from each five clones were combined, and the combined samples were immobilized on nitrocellulose filters in the presence of 5% formaldehyde (to avoid the snapback of palindromic regions in denatured DNA) and used for hybridization. All groups but one bound some amount of [^{3}H] dsRNA, (dsRNA-B), [^{125}I] dsRNA (total long dsRNA) and [^{32}P] palindromic DNA. Six combinations hybridizing with the highest proportion of dsRNA were selected and the hybridization was repeated with DNAs of thirty individual clones present in these groups. DNA of 15 clones among the 30 analyzed ones was found to contain sequences which bound melted dsRNA.

Such a scheme did not allow one to determine the exact percentage of clones containing sequences complementary to dsRNA; it is clear nevertheless that it cannot be lower than 20%. The upper limit is 50%.

Fifteen selected clones were grown, DNA was isolated and used in several hybridization experiments.

It should be pointed out that, in both selection experiments, the binding of dsRNA-B usually parallels that of two other samples tested for hybridization. This finding suggests that dsRNA-B is represented by the most abundant sequences among the total dsRNAs and total DNA palindromes.

DsRNA-B contains highly abundant sequences. To determine the percentage of dsRNA-B sequences complementary to DNA of any given clone, the hybridization was performed with a

large excess of immobilized DNA and at a very low R_0t values to exclude dsRNA renaturation. DsRNA-B for these experiments was purified electrophoretically. The results are presented in Fig. 1. All clones could be divided into four groups according to their hybridization properties. DNA of the clones belonging to the first group binds 39–45% of dsRNA-B, that from the second one - about 30%, from the third - about 23%, and from the fourth - 11-16% of dsRNA-B. Such a high binding of dsRNA clearly shows that the clones contain sequences widely distributed among dsRNA-B.

DsRNA-B practically did not hybridize with DNA of clone №1. On the other hand, the total dsRNA was bound by this DNA to the same extent as by the DNA of the clones belonging to group IV (data not shown). One may suggest that this clone contains a sequence complementary to one of dsRNA-A sequences. It is interesting that the DNA from a number of clones hybridizing with dsRNA-B (№№ 3, 31, 61) at the same time does not bind purified dsRNA-A (Fig. 1). This result proves the earlier found differences between sequences

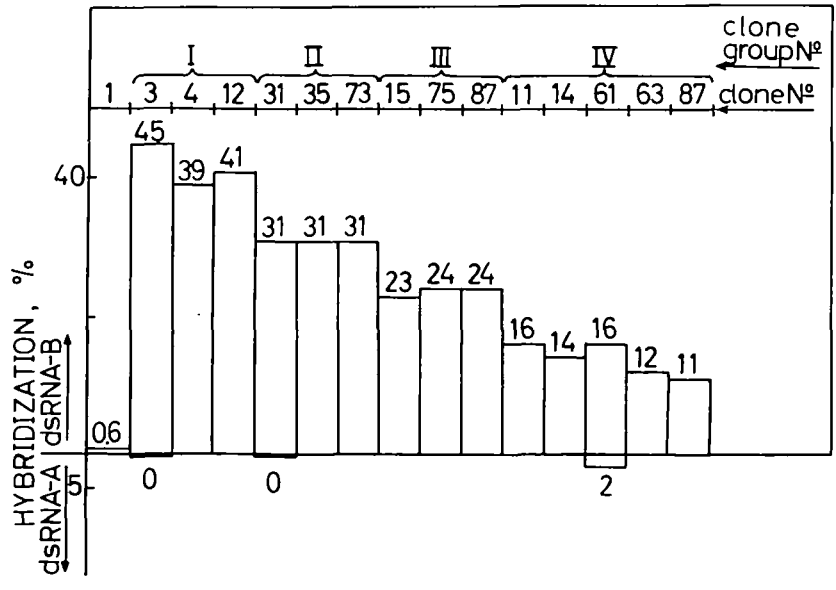

Fig. 1. The binding of total dsRNA-B or dsRNA-A to the excess of DNA from several clones.

present in dsRNA-A and dsRNA-B /3/. Their cross-contamination seems to be very low.

Existence of two highly abundant classes of dsRNA-B. The question arises: how similar are the sequences present in different clones whose DNA hybridizes to dsRNA-B? If the percentages of dsRNA binding by a number of clones are added up, the sum would exceed 100%. Therefore one may suggest that many of clones contain the same sequence complementary to dsRNA-B. To check this possibility, we hybridized dsRNA-B to DNA of clone №35 (group II) or №14 (group IV), eluted the bound material from the filter, treated it with DNAase, melted, and used repeatedly for hybridization with DNA of the same or other clones (table 1). In this series of experiments, dsRNA-B was not purified by gel electrophoresis. Therefore, the samples contained also dsRNA-A (about 30% of the total material). For this reason, the figures of original hybridization

Table 1

Hybridization of [^{3}H] labeled dsRNA-B sequences preselected by binding to DNA of clones № 35 and № 14 with DNA prepared from different clones

№ of experiment	DNA on filter (clone №)	Hybridization, % total dsRNA	dsRNA complementary to DNA of clone № 35	dsRNA complementary to DNA of clone № 14
1	4	24	50	
	14	7	0	
	15	14	32	
	31	22	60	
	35	22	47	
	75	19	35	
	87	17	37	
2	3	30	50	48
	14	11	0	58
	35	21	70	0
	61	17	0	46
	63	10	0	35
	89	15	31	0

For each experiment, 27000 cpm of total [^{3}H] dsRNA or 500 cpm of preselected [^{3}H] dsRNA was taken.

were 1.5 times lower than in the experiment presented in Table 1.

One can see that from 50 to 70% of the RNA was bound upon rehybridization of the eluted RNA with the same DNA; this figure can be considered as an index of the hybridization efficiency in our conditions. The DNAs prepared from clones №№ 4 and 31 bound practically the same amount of preselected dsRNA-B as the DNA from clone №35. Thus, all the three contain the same sequence complementary to dsRNA-B, and we designated it as sequence B1. Considering the 60% efficiency of hybridization reaction, one can estimate that the content of sequence B1 in purified dsRNA-B (see Table 1) is about 50%.

DNA of four other clones (№№ 15, 75, 87, 89) belonging to group III combined with 31-37% of preselected dsRNA-B1. This figure correlates well with a lower binding of the total dsRNA-B by DNAs of these clones. Therefore, the DNA of these clones contains only a part of sequence B1.

DNA prepared from three other clones (№№ 14, 61, 63) belonging to group IV did not hybridize with dsRNA-B1 at all. On the other hand, the DNAs of this group of clones hybridized quite well with dsRNA preselected on the DNA from clone №14. The binding capacity for the DNA of clones №№ 14 and 61 is the same and it is somewhat lower in the case of the DNA from clone №63. Thus, these clones also contain the common sequence (or part of it in the case of clone №63) which is however completely different from sequence B1. It will be referred to as sequence B2.

Considering the 60% efficiency of hybridization, one may estimate the content of sequence B2 in dsRNA-B being equal to 20-25%. Thus, sequences B1 and B2 total about three quarters of the whole dsRNA-B.

As could be expected, the preselected dsRNA-B2 did not hybridize with the DNA of clones containing sequence B1 (№№ 35 and 89). On the other hand, the DNA from clone №3 most efficiently binding to the total dsRNA hybridized to both B1 and B2 sequences (Table 1 and 2). Thus, the clones of group I contain two main sequences complementary to dsRNA-B. Conse-

quently, there is a good correlation between the groups of clones given in Table 1 and the presence of the particular sequences in them. Clones of group I contain both sequences B1 and B2, clones of group II - sequences B1, clones of group III - part of B1, and those of group IV - sequence B2. There are some exceptions: for example, clone №89 put in the group IV contains a small part of sequence B1 but not B2.

Some properties of sequences B1 and B2. It is important to know the size of sequences hybridizing to dsRNA-B, in particular, to find the number of dsRNA sequences present in cloned DNA fragments. As was shown above, some clones seem to contain more than one sequence complementary to dsRNA. Probably this is not a common rule, as the size of cloned DNA fragments is rather low: 2-3 kb on the average and 4.3 kb only in one case. Clones №№ 11 and 31 contain DNA less than 1 kb in length. However, to answer the above question one is bound to know the length of sequences hybridizing with dsRNA. For this purpose, we isolated electrophoretically a fragment of mouse DNA (2.5 kb) from B1-containing clone №35, labeled it by nick translation, and hybridized it with DNA from clone №35 and three other clones (№№ 12, 15, and 31) containing sequence B1 (Table 2). Comparison of the figures obtained shows that about 16% of the DNA fragment from clone №35 is homologeous to the DNA of any other clone. As the

Table 2

Hybridization of mouse [^{32}P] DNA fragment of clone № 35 with DNAs prepared from different clones

DNA on filter (clone №)	Hybridization of [^{32}P] DNA (background subtracted)		Homology, %
	cpm bound	% of cpm bound	
35	2340	85.1	100
12	372	13.5	15
15	320	12.0	14
31	398	14.5	17
PBR322 (background)	58	2.1	-

size of mouse DNA from clone №35 is equal to 2.5 kb, the length of the homology region should be about 400 base pairs. This figure correlates quite well with the size of the dsRNA region in pre-mRNA which comprises about 200 base pairs or 400 bases in a linear structure.

At this point, it is not possible to exclude a possibility of the existence of two different and separated palindromes having 200 base pairs in length in sequence B1 which are present in all three clones (the lower limit for dsRNA-B is 100 base pairs or 200 base pairs in a linear structure). However, this seems to be rather unlikely. The result of the described experiment also demonstrates that flanking sequences for fragment B1 differ in different clones.

Another question is how closely different sequences belonging to the same class, B1 or B2 are related. To answer this question, we measured the melting temperature curves of hybrids formed by homologeous and heterologeous DNA and RNA. First, the inserted mouse DNA fragments containing sequences B1 and B2 were cut out from the plasmid DNA of clones №35 (B1) and №14 (B2) by the EcoRI and HindIII endonuclease treatment and purified electrophoretically. These DNAs were transcribed with the aid of E.coli RNA polymerase to prepare [^{32}P] cRNAs. Then cRNA transcribed from the DNA of clone №35 was hybridized with the immobilized DNA of clone №31 which also contained sequence B1. After mild RNAase A treatment of hybrids, about 10% of the input labeled cRNA was recovered on a nitrocellulose filter. As only sequence B1 is common for mouse DNA of these two clones, the hybridized cRNA corresponds to the transcript from sequence B1. In the same way, sequence B2 was purified by hybridization of the cRNA transcribed from clone №14 to the DNA of clone №61.

The hybridized cRNAs designated as cRNA-B1 (№35) and cRNA-B2 (№14) were eluted and rehybridized with DNAs prepared from a number of different clones containing sequences B1 and B2, respectively. Thereafter, the melting temperature for all hybrids was measured (Fig. 2A and B). One can see that the Tm for a hybrid formed by cRNA-B1 (№35) with the DNA of clone №35 is only by 1-2°C higher than the Tm of its hybrid

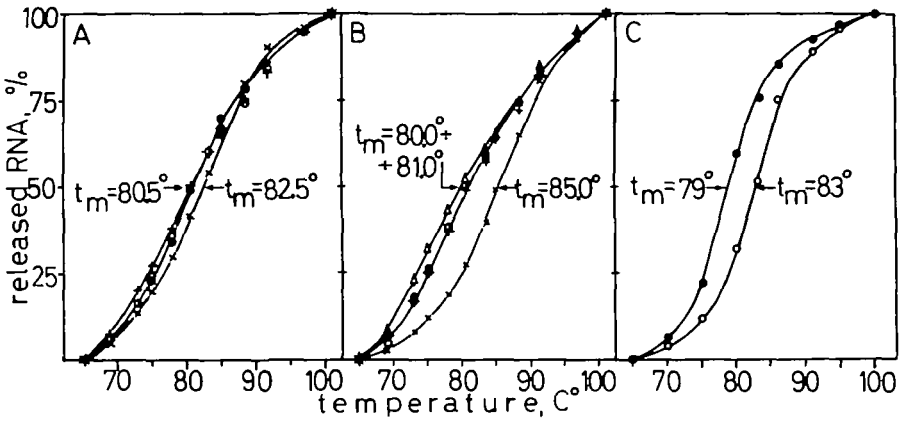

Fig. 2. Melting curves of the hybrids between:

A. cRNA-B1 and DNAs of the clones № 35 (—x—), № 3 (—o—), № 31 (—●—), and № 87 (—+—);

B. cRNA-B2 and DNAs of the clones № 14 (—x—), № 3 (—+—), № 11 (—Δ—), № 61 (—●—) and № 63 (—o—);

C. dsRNA and DNAs of the clones № 35 (—o—), and № 14 (—●—).

Melting of the hybrids was performed by increasing of temperature during the incubation of filters in 2xSSC.

with the DNA of other clones containing sequence B1. Thus, there may be small variations in sequences B1 present in different sites of the genome. The number of substitutions does not exceed 3% of all bases.

At the same time, the Tm of a hybrid between cRNA-B2 (№14) and the DNA of clone №14 was by 4–5°C higher than the Tm of hybrids obtained with the DNA of other clones containing sequence B2, thus indicating a higher divergence of sequence B2. The substitutions between two different sequences B2 may involve 6–8% of bases.

In another experiment, the melting of hybrids between the DNA of clones №№ 35 and 14 and the total dsRNA-B was obtained (Fig. 2c). One can expect that since dsRNA-B is transcribed from different regions of the genome, the mismatching in such hybrids would be of the same order as in the case of cross-hybridization experiments described above. Actually the Tm

values were found to be equal to 83°C (sequence B1) and 79°C (sequence B2). The first figure is equal to that obtained for hybrids between the DNA of clone № 35 and cRNA-B1 (№ 35). The second one is by 6°C lower than the Tm of hybrids between the DNA of clone № 14 and cRNA-B2 (№ 14). Again a conclusion can be drawn that in family B1 only a very low divergence, if any, takes place while in family B2 the variation may involve up to 9% of the total number of bases.

The representation of sequences B1 and B2 in total DNA, palindromic DNA and pre-mRNA. Frequent occurence of sequences complementary to dsRNA-B among random DNA fragments suggests that their high content in the mouse genome equals from $2x10^5$ to $3x10^5$ copies per genome. Considering the fact that they are mostly represented by a few types of very abundant sequences, one may conclude that the number of these individual sequences should be equal to or higher than several tens of thousands of copies. To check this, we hybridized the sheared mouse DNA with the immobilized DNA prepared from clones (Table 3). The DNA from clones containing sequence B1 or a part of it binds 0.4-0.5% of the total mouse DNA while the DNA from clone containing both B1 and B2 hybridizes with about 1% of the latter.

Assuming that sequence B1 itself binds 0.5% of the total DNA and its size is equal to 200-400 base pairs, one can calculate the total number of sequences B1 per genome to be equal to $(4+8) \times 10^4$. This estimate coincides with the above mentioned value.

In other experiments, the DNA of clones was hybridized with sheared palindromic DNA. Both B1 and B2 containing DNA fragments bind 2.5-3% of the latter. Thus, palindromes are 4-6 times enriched in sequence B1 comparing with the total DNA. In our experiments, about 5% of the total DNA was recovered in the fraction of palindromes. Thus, about 0.13% of the total DNA is represented by sequence B1 organized as an inverted repeat. This corresponds to about a quarter of all sequences B1. The remaining three quarters of sequences B1 seem to be scattered throughout the genome as simple repeats not forming

Table 3

Hybridization of total mouse DNA, palindromic DNA, and pre-mRNA cloned DNA immobilized on filter

DNA on filter (clone №)	Type of sequence present	Total DNA[x]	Palindromic DNA[x]	Pre-mRNA[x]
			Hybridization, %	
12	B1&B2	1.0	4.7	1.6
31	B1		3.1	
35	B1	0.52	2.5	1.5
87	B1			1.2
89	part of B1	0.38	2.7	
14	B2		2.4	0.63
63	B2			0.76
11	B2, or its part			0.43
PBR322 DNA (background)		0.04	0.4	0.02

[x] Background was subtracted in all cases. The total radioactivity input was equal to 3.2×10^5 (total DNA), 6×10^4 (palindromic DNA) and 8.7×10^4 (pre-mRNA) counts per min.

palindromes. Of course, these calculations are very approximate as the efficiency of hybridization may vary in different cases.

Finally, the DNA of clones was hybridized with nuclear pre-mRNA (hnRNA). About 1.5% of the latter hybridized with DNAs containing sequences B1 and about 0.6 with those containing B2. Thus, pre-mRNA is 2-3 times enriched in these sequence comparing to total DNA, i.e. sequences B1 and B2 in DNA are preferentially transcribed. On the other hand, their content is much higher than that of dsRNA-B (~ 0.5%) indicating that not all sequences B1 and B2 form double-stranded structures in pre-mRNA.

DISCUSSION

The main conclusion from the above results is that the major part if not all dsRNA-B is represented by a few classes

of highly reiterated homogeneous sequences. Two of them, designated as B1 and B2, the most abundant, were cloned as parts of different mouse DNA fragments.

It is interesting that all sequences belonging to family B1 are very similar if not identical, as follows from melting experiments. The upper limit for mismatching is 3% of bases but in fact it may be even lower. Thus, either sequence B1 appeared rather recently in the course of evolution, or the involving of this sequence is limited. Family B2 of sequences is more heterogeneous.

The low complexity of dsRNA-B compatible with the existence of 1-3 different sequences has been shown previously by analysing the renaturation kinetics of melted dsRNA-B /1-3/ although the quantitation of RNA renaturation data is always rather tentative. The fingerprint analysis of dsRNA digests showed the existence of several abundant oligonucleotides /25/. However, only the cloning data have provided a direct proof for the statement.

The existence of clones containing incomplete sequences B1 and B2 is probably due to their divergence leading to the appearance of corresponding restriction sites within these sequences.

Both sequences B1 and B2 belong to a class of highly repetitive DNA sequences scattered throughout the whole mouse genome. As was mentioned above, only part of them ($\sim 1/4$) is organized in palindromic or hairpin-like structures.

In agreement with our previous data /26/, we have found that palindromes are enriched in sequences B1 and B2 as compared with the total DNA. However, the content of sequences B1 and B2 in palindromes is lower than in dsRNA-B, thus indicating that palindromes are much more heterogeneous than dsRNA-B. The question about the nature of different types of palindromes and their relation to dsRNA awaits further studies.

Among our clones there are a few whose DNA does not hybridize with dsRNA-B but with the total dsRNA. They may contain sequences corresponding to dsRNA-A and their further study may reveal the organization and significance of this

dsRNA as well.

When the experimental part of this work was finished, the paper by Jelinek /27/ appeared in which the author described the isolation of clones containing sequences complementary to dsRNA from the humster genome and made a conclusion about their wide distribution in the genome and a rather high divergency.

The cloning of the two most abundant sequences transcribed into dsRNA-B which is described in this paper opens many new lines of experiments. First, the sequencing becomes possible now. Second, their relation to mRNA /28,29/ may be studied. In particular, we have isolated and cloned recently a number of mouse DNA fragments hybridizing with mouse mRNA, i.e. fragments carrying structural genes. Most of them contain at the same time sequences B1 and/or B2 as well. Further study of their relation to the gene sequence may lead to a better understanding of the dsRNA function.

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