CHARACTERISTICS OF CLONED ALPHA-SATELLITE SEQUENCES FROM EXTRACHROMOSDMAL DNA OF HeLa CELL CULTURES

T. B. Krokhina, T. G. Tsvetkova, and I. V. Gar'kavtsev

UDC 577.21

KEY WORDS: cycloheximide; extrachromosomal alphoid DNA.

Small polydispersed circular DNA (spcDNA) is the name given to a class of extrachromosomal molecules found in many eukaryotic cells, and formed mainly by highly repeating and moderately highly repeating sequences [5].

The writers previously obtained fractions of spcDNA from HeLa cells treated with different doses of cycloheximide (CHI) This antiobiotic was found to increase excision of highly repeating DNA sequences: Alu 1 by 2.5 times, the alpha-satellite by 3-5 times, the classical satellite by 5-7 times [3]. The problem of the existence of a possible specificity of expulsion of definite groups of sequences, including those within the classes of repeats examined, was not cleared up. Alpha-satellite DNA is most convenient for such investigations, for it has been shown to have chromosomal specificity, and this family of sequences is present in the juxtacentromeric regions of all chromosomes.

The aim of this investigation was to clone and characterize alphoid extrachromosomal DNA from cells treated with CHI: to establish the primary sequence of the isolated fragments, to assess their variability as regards polymorphism of lengths of restriction fragments (PLRF), and to detect possible chromosomal specificity.

EXPERIMENTAL METHOD

The method of Hirt [4] was used to isolate spcDNA from HeLa cells treated with CHI (30 μg/ml, 20 h). Additional purification was carried out by centrifugation twice in a CsCl density gradient. As the vector for obtaining recombinant plasmids, we used pTZ19R and pTZ18R, hydrolyzed by Sau3A. After transformation clones with an insertion were selected by the color reaction on McConkey's agar. Recombinants carrying alphoid DNA were detected by hybridization with a labeled pHR19 probe [2]. Hybridization in situ was carried out by the standard method, and sequencing by Sanger's method [6].

EXPERIMENTAL RESULTS

To select spcDNA, discarded at the translation block, after the last round of centrifugation aliquots of the spcDNA fractions from the control cells and cells treated with CHI were hybridized with the labeled probe BluR8 (Fig. 1). Hybridization in fractions 1, 2, and 3 is found only for cells treated with the inhibitor. The spcDNA of these fractions is probably discarded under the influence of CHI, for in the control no hybridization took place in fractions with the same coefficient of refraction. We formed a clone bank of this group of sequences. From 400 clones obtained, only two had homology with alphoid DNA – probe pHR19 [2]: pK1 and pK2. The length of the cloned fragments was approximately: for pK1 – 390 bp, for pK2 — 184 bp. The primary sequence of these clones is shown in Fig 2.

All-Union Medical Genetic Research Center, Academy of Medical Sciences of the USSR Moscow (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 1, pp. 72-74, January, 1992. Original article submitted June 5, 1991.

0007-4888/92/0001-0105$12.50

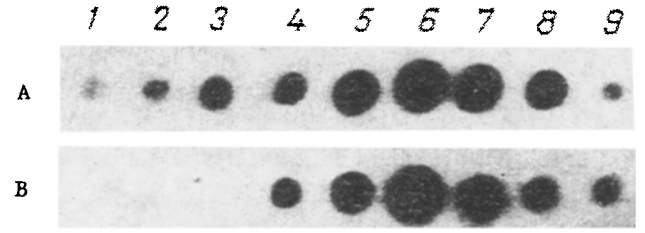

Fig. 1. Results of dot hybridization of aliquots of spcDNA obtained by centrifugation in CsCl density gradient, with ^{32}P-labeled clone BluR8. A) spcDNA fractions obtained from HeLa cells treated with CHI (30 μg/ml, 20 h); b) fractions from control cells. Numbers indicate serial numbers of fractions in order of diminishing coefficients of refraction (from left to right).

Fig. 2. Primary sequency of nucleotides of alpha-satellite DNA of clones pK1 and pK2. a) pK1.

5'

```
TTTACAGAGA GCAGACTTGA AACACTCTTT TTGTGGAATT TGCAAGTGGA  50
GATTTCAGCC GCTTTGAGGT CAATGGTAGA AAGGAACTAT CTTCGTATAA 100
GACTAGACAG ATGATTCTCA GAAATCTTTG TGATGTGTGC GTTCAACTCA 150
CAGAGTTACT TTTCTTTTCA TAGAGCAGTT AGGACATCTG TTGTAAGTCT 200
GTCAGTGGAT ATTCAGACTC TTGAGCTTCG TTGAACGGAT TCTTCATATG 250
TCAGTAGAAA CTCTGTTGTA TGTCTGCAAT GATATCAGAC CTCTTGAGCC 300
TCGTTGAGTC GGGATTTCTT CATATTATGC  GACAGAAT AATTCTCAGT 350
AACTTCCTTG TGTTGTGTGT ATTCAACTCA  AGTTGAAC
```

3'

b) pK2

5'

```
TGAAGTCCTG CTGGCTGTTG CACTTGGACA AGCCACGCAG GAGGGAGTGG  50
TACCGCTGAG CAAGCCTCTG CTCTAAGTGA GCTTTCAGGT CTCTGATTTG 100
CTGGGCATGT GATGAATCGA TCTTAAAGAC GAGCATTGAA CTCATCTTTC 150
GTAGATCTAC GAAGTCGAGC ATTCAGCGTA TGAC
```

3'

To study the organization of the genome of these sequences, PLRF was determined. On hybridization of DNA isolated from the blood of six individuals and hydrolyzed by the set of restriction endonucleases available to us, as molecular probes we used alpha-satellite DNA of clones pK1 and pK2. The following restriction endonucleases were used in the work: EcoRI, PstI, BamHI, HindIII, MspI, TagI, XnaI, KpnI, SalI, Sau3A, BspRI.

The results showed that In relation to all restriction endonucleases examined, neither clone possessed PLRF, and only a change in the copy number of particular fragments was observed. The exception was a case in which an additional band appeared for restriction endonuclease KpnI during hybridization with clone pK4 These data are evidence that the cloned sequences do not possess high variability in the genome but are quite conservative.

Hybridization in situ established the chromosomal localization of these sequences. They have a similar distribution among chromosomes. After statistical analysis of the number of grains of silver above the chromosomes of 10 metaphase plates it was shown that hybridization is observed mainly on chromosomes 1, 5, and 19 (Fig. 3), However, no marked chromosomal specificity was found even with washing under harsh conditions. Nevertheless, the character of distribution among chromosomes indicates that both recombinant clones contain alphoids belonging to group 1 [1]. Comparison with

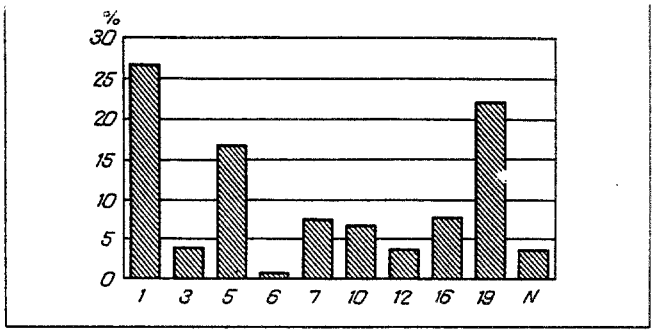

Fig. 3. Histogram of distribution of number of grains of silver above chromosomes during hybridization in situ with probe ^{3}H-pK1. Horizontal axis – individual chromosomes; N) all remaining chromosomes. Vertical axis – percentage of grains of silver above an individual chromosome as a ratio of total number, taken as 100%.

the bank of these sequences of alpha-satellite DNA (All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR) confirmed this conclusion.

In the present investigation, sequences of alpha-satellite DNA from the fraction of extrachromosomal DNA of HeLa cells, treated with cycloheximide, not described previously, were thus cloned. The clones obtained do not possess chromosomal specificity and are conservative, since PLRF was found only for one restriction endonuclease (KpnI) of the 11 studied. This may evidently be an argument in support of the view that the discarding of highly repeating sequences during inhibition of translation is a stochastic statistical process.

LITERATURE CITED

1. I. A. Aleksandrov, Yu. V. Yurov, S. P. Mitkevich, and V. M. Gindilis, Dokl. Akad. Nauk SSSR, **288**, No 1, 242 (1986).
2. I. V. Gar'kavtsev, T. G. Tsvetkova, and N. A. Lyapunova, Mol. Genet. Mikrobiol. Virusol., No. 5, 11 (1989).
3. T. V. Krokhina, G. V. Raevskaya, D. I. Chernyi, and I. V. Gar'kavtsev. Byull. Éksp. Biol. Med., No 5 (1991).
4. B. Hirt, J. Molec Biol., **26**, 365 (1967).
5. R. S. Jones and S. S. Potter, Nucleic Acid Res., **3**, 1027 (1985).
6. F. Sanger, S. Nicklen, and A. R. Coulson, Proc. Nat. Acad. Sci USA, **74**, 5463 (1977).