INHIBITION OF TRANSLATION BY CYCLOHEXIMIDE INCREASES THE NUMBER OF HIGHLY REPETITIVE SEQUENCES IN EXTRACHROMOSOMAL CIRCULAR DNA

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Changes arising in cells of higher eukaryotes in response to extremal factors are currently being intensively studied. Under these conditions synthesis of heat shock proteins has been shown to begin in the cell, certain receptors increase in number, adaptive synthesis of certain enzymes and reparative DNA synthesis commence, and so on. However, the most interesting topic is the study of the effect of physical and chemical factors on the genome, for genome changes not only have adaptive value, but they are also material for evolutionary processes. Yet there have been few studies of the response of the genome to stress. Exposure to chemical and physical factors in some cases leads to amplification or deletion of particular DNA sequences [8] and to bursts of transpositions of MDH of the Drosophila genome [7].

In the investigation described below cycloheximide, an antibiotic inhibiting protein synthesis in eukaryotic cells, was used as agent creating extremal conditions for the cells. There is evidence that cycloheximide (CHI), in high doses, increases excision of small polydispersed circular DNAs (spcDNAs) in transplantable cells [11, 12]. Since a considerable part of the human genome consists of tandemly organized and dispersed DNA repeats, it is to be expected that mainly these sequences will be found among the spcDNAs formed under the influence of CHI.

The aim of the investigation was to study the qualitative composition of the spcDNA of intact cells and of cells treated with different doses of CHI, and to compare the content of different classes of repeats in spcDNA under normal conditions and during treatment with CHI.

EXPERIMENTAL METHOD

Experiments were carried out on a culture of HeLa (wild-type strain) cells obtained from the cell bank of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR. The cells were grown on rollers in medium 199 with the addition of 10% bovine serum. Supercoiled circular DNA was obtained by Hirt's method [10] and separated from contaminating linear fragments by centrifugation twice in a CsCl density gradient. After fractionation aliquots were hybridized with the ^{32}P-labeled sequence AluI (clone Blur8) by the standard method [3].

EXPERIMENTAL RESULTS

Cultures of HeLa cells treated and not treated with CHI were used. The antibiotic was added for 16 h in two concentrations: 1 μg/ml — a concentration combining maximal inhibition of protein synthesis with minimal cytotoxic effect, as shown by the reversibility of the changes taking place in the cells, and 50 μg/ml — the cytotoxic dose.

Electron microscopy showed that the length of the isolated spcDNAs varied from 0.15 to 0.6 μ, roughly equivalent to 500-2000 base pairs. Length was estimated relative to mitochondrial DNA (5.5 μ; 17.5 kbp [4]; Fig. 1).

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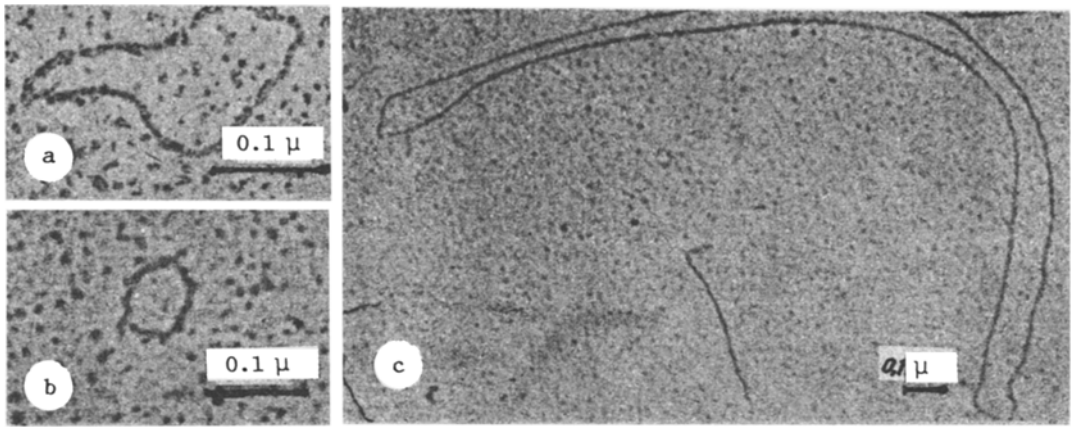

Fig. 1. Electron micrographs of circular DNA molecules. a, b) spcDNA from HeLa cells treated with CHI (50 μg/ml, 16 h). a) 2.1 kbp; b) 0.67 kbp. 140,000×; c) mitochondrial DNA, 17.5 kbp, 50,000×.

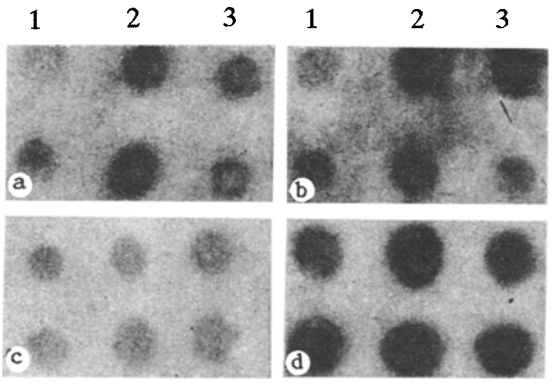

Fig. 2. Results of dot hybridization of spcDNA fractions from control cells (1), from cells treated with 1 μg/ml CHI (2), 50 μg/ml CHI (3), with probes: classical satellite (a), alpha-satellite (b), AluI (c), and rRNA gene (d).

To determine the qualitative composition of the spcDNA fractions thus obtained hybridization was carried out with the available probes: the classical satellite — clone pPT301 [6], an alpha-satellite — clone HRS185 [2], rRNA genes — clone pB [13], histone genes — clone p604 (kindly provided by M. Birnstiel), the telomeric DNA sequence — clone pTI, and AluI — clone Blur8. Fractions of circular DNA molecules, in both control and experimental series, were found to contain all these sequences (Fig. 2).

To enable the relative number of the above-mentioned sequences to be compared in the control and experimental samples, the material applied to the filters was normalized with respect to concentration of total DNA isolated from all the test samples and also from extrachromosomal DNA.

Table 1 gives an approximate estimate of changes in the relative content of the test sequences in spcDNAs isolated from the control cells and from cells treated with different doses of CHI. For this purpose the radioactivity of individual spots obtained by dot hybridization was determined on a scintillation counter. Table 1 gives mean values of the relative change of the count obtained in three independent experiments. The control was taken as 1 independently for each sequence examined. The tendency of the changes in the parameters was similar for the two doses of CHI, indicating that scatter of these sequences takes place not only under very strict conditions (a high dose of CHI), but also with a concentration giving reversible changes (1 μg/ml). The exception is the classical satellite sequence which, with a dose of 1 μg/ml of CHI, is represented in the spcDNA fraction by a larger number of copies than with a dose of 50 μg/ml. A similar tendency is observed for the alpha-satellite, but it is much weaker. The increase in the number of highly repetitive, nontranscribable

TABLE 1. Comparison of Content of Repeats in spcDNA Fractions Obtained from Intact Cells and Cells Treated with Various Doses of CHI

Sequence	Untreated cells	1 μg/ml CHI	50 μg/ml CHI
Classical satellite	1	7,05±1,20	4,50±1,40
Alpha-satellite	1	4,80±1,77	3,75±0,29
AluI	1	2,40±0,15	2,70±0,12
Histone genes	1	0,95±0,07	0,90±0,14
rRNA genes	1	1,22±0,18	1,00±0,03
Telomeric DNA	1	1,09±0,16	1,18±0,20

sequences undergoing excision as a result of the translation block averaged 5-7 times for the classical satellite, 3-5 times for the alpha-satellite, and 2.5 times for AluI. The number of rRNA and histone genes and amount of telomeric DNA "evicted" under these circumstances remains at the control level.

The human genome thus responds to an extremal influence (in this case, to inhibition of protein synthesis) by increased excision of nontranscribable highly repetitive sequences, but the virtual absence of changes in excision of moderate transcribable repeats (genes of rRNA and histones).

A high frequency of recombination events is characteristic of structurally simpler tandemly organized repeats, collected into groups. Our findings suggest that inhibition of intracellular protein synthesis leads to a deficiency of certain short-living protein(s), involved in stabilizing the frequency of genetic recombination. These conditions collectively lead to a marked increase in excision of short, cluster-forming, highly repetitive DNA sequences, which evidently reflects processes taking place in the cell genome in an extremal situation.

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