DETECTION OF POSTREPARATIVE DNA LESIONS AND IRREGULARITY OF THEIR DISTRIBUTION IN FRACTIONS OF METAPHASE CHROMOSOMES DIFFERING IN SIZE

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A fundamental problem in molecular biology and theoretical medicine is that of relations between organization of chromatin in the cell nucleus and gene expression. There is reason to suppose that chromatin may exist in a number of discrete states, transitions between which correspond to the epigenetic reprograming of the genome [5]. In our view one way to analyze these states is to study the principles of distribution of lesions in polynucleotide strands of DNA in chromosomes of different sizes, depending on the structure of the nuclear chromatin. The correctness of this approach is explained by the fact that a parameter of these states, such as the ratio between the numbers of euchromatin and heterochromatin regions in the chromosomes, is also a function of their geometric size.

Data on the distribution of breaks in DNA of metaphase chromasomes of different sizes, after damage induced by a chemcial mutagen in a cell in the S period, are reported for the first time in this paper.

EXPERIMENTAL METHODS

A culture of Djungarian hamster fibroblasts of strain DM-15 (4/21 GPRT$^-$) with known karyotype [2] was generously provided by E. S. Kakpakova (All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR). The cells were cultured for 24 h in the presence of ^{3}H- or ^{14}C-thymidine, then washed with medium 199 with addition of calf serum (10%) and streptomycin sulfate (100 U/ml). Next, in the middle of the S period appropriate concentrations of N-nitroso-N-methylurea (NMU) were added for 1 h. The mutagen was removed by washing with nutrient medium and, at the end of the C_2 period, colcemid (0.05 μg/ml) was used for 2 h. The interphase cells were separated from mitotic cells by shaking in cold Hanks' solution. Metaphase chromosomes were isolated from the latter and fractionated in a 5-15% sucrose gradient at 1 g [7, 15]. By pooling the corresponding fractions, three groups of metaphase chromosomes were obtained: small (SMC), medium (MMC), and large (LMC). Chromosomes from each group were lysed on the surface of a 5-20% alkaline sucrose gradient in test tubes for ultracentrifugation in the SW55Ti rotor of an L8-70 centrifuge (Beckman, USA). The lytic solution consisted of 0.6 M NaOH, 0.9 M NaCl, 0.1 M EDTA, and 4% sodium laurylsarcosinate. A sucrose gradient was prepared on the basis of 0.9 M NaCl, 0.3 M NaOH, 0.01 M EDTA. Lysis was carried out for 2 h at 20°C in darkness, and the sample was centrifuged at 200,000 g (20°C, 90 min). The gradient was separated into 30 fractions and the molecular weight of the single-stranded DNA fragments determined [9, 11], with an empirical correction for dependence of the sedimentation constant on the spinning of the rotor.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that the function $\ln \frac{\% C_i}{M_i \cdot \Delta M_i} = f(M_i)$, where % Ci denotes radioactivity of each fraction as a percentage of total activity of the sedimentation peak, Mi the

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0007-4888/86/0009-1187$12.50

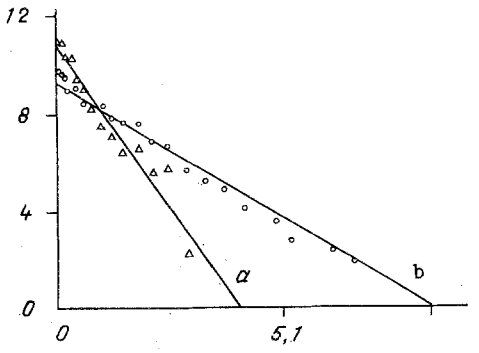

Fig. 1

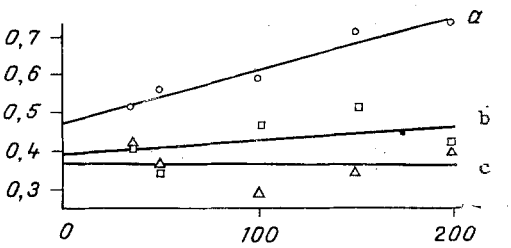

Fig. 2

Fig. 1. Sedimentation peak profile in alkaline sucrose gradient (in the presence of NMU, 150 μg/ml) expressed as a linear function: $\ln \frac{\% C_i}{M_i \cdot \Delta M_i} = f(M_i)$. Abscissa, ln of functions; ordinate, value of M (in daltons $\times 10^6$). a) For fraction of small chromosomes, b) for fraction of large chromosomes. Here and in Table 2: graph plotted by computer (Hewlett-Packard).

Fig. 2. Accumulation of single breaks in DNA from different groups of chromosomes, depending on dose of mutagen. Abscissa, NMU concentration (in μg/ml); ordinate, ratio $\frac{I}{M} \cdot 10^{-7}$. a) For fraction of small chromosomes, b) for fraction of medium chromosomes, c) for fraction of large chromosomes.

difference between molecular weights of the lower and upper boundaries of the i-th fraction, is approximated by a straight line, indicating a random distribution of molecular weights. Accordingly, using the tangent of the angle of slope it is possible to calcualte the size of the DNA fragments [11]. In DNA of SMC, by contrast with LMC, the number of single breaks (SB) of DNA is dose-dependent (Fig. 2). This phenomenon can be explained by an increase in the quantity of heterochromatin per unit of molecular weight of DNA in SMC comapred with LMC. For example, in man the dimensions of regions of C-chromatin in chromosomes 1, 9, 16, and Y are (minimal estimates) 1.06, 0.93, 0.68, and 0.71 μ respectively. The DNA content in these chromosomes is 249×10^6, 137×10^6, 93×10^6, and 53×10^6 base pairs respectively [14]. In that case, the normalized length of C-chromatin per base pair will be 4.3×10^{-9}, 6.8×10^{-9}, 7.5×10^{-9}, and 13×10^{-9} μ. The important fact is that structural changes in metaphase chromosomes do not involve C-regions [13]. Thus the data given above can be explained by an increase in the number of SB in heterochromatin regions, the relative number of which is greater in small chromosomes. We also know that euchromatin is significantly more vulnerable to the direct action of several agents: DNase I [12], carcinogenic chemical compunds [10], and ionizing radiation [6]. At the same time, it has been shown [1] that chromosomal aberrations are located more frequently in heterochromatin regions or at junctions between hetero- and euchromatin. This contradiction may be connected with the fact that the relative number of lesions immediately after exposure to genetically harmful substances is substantially greater in euchromatin, but repair processes are impaired in heterochromatin regions. This last fact is in agreement with suggestions put forward by a number of workers [1, 3], but as yet it has not been confirmed experimentally. Our own data confirm these hypotheses directly. At the same time, the interpretation given of the experiments is complicated by data obtained on the quantity of labeled DNA in SMC and LMC. In the karyotype of the Djungarian hamster the ratio between the numbers of large and small chromosomes is 1:1. If the DNA content in these groups of chromosomes is roughly proportional to their length, we have DNA LMC:DNA SMA = 6.5. However, the quantity of DNA (as ^{3}H-thymidine) entering the gradient from LMC and SMC is in the ratio of 2.5 and 4.5, for doses of NMU of 50 and 150 μg/ml respectively. This indicates a defiiency of large chromosomes, isolated after treatment of the cell with small doses of NMU. These differences may be due to at least two circumstances: 1) incomplete outflow of large chromosomes during lysis of the cells, which in no way affects the interpretation of the results given above; 2) partial degradation of large chromosomes and transfer of their fragments into SMC. Fragmentation of only 10% of large chromosomes down to the size of small could lead to the ratio of 2.5. In that case the increased number of SB in the fraction of small chromosomes could be explained by the effect mentioned above. However, the number of

SB in DNA of SMC clearly depends on the dose of mutagen, and at the same time, with an increase in the latter, the ratio of the amounts of DNA entering the gradient from LMC and SMC approaches their ratio in the karyotype. Hence it can be concluded that accumulation of SB in SMC is not connected with any significant fragmentation of large chromosomes. Meanwhile the possibility cannot be completely ruled out that with an increase in the concentration of mutagen in SMC a certain number of DNA fragments from MMC and LMC accumulates. In that case the results would indicate that the method of analysis used enables these fragments to be concentrated, i.e., the resolving power of the method of determination of DNA lesions is significantly enhanced. As we know [4], after exposure to the action of chemical and physical mutagens, some dividing cells die after a few mitoses, although molecular analysis indicates 100% successful completion of repair. The possibility cannot be ruled out that some cells with lesions revealed by the method described above may be ancestors of modified clones with all the consequences which this entails.

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