FUNCTIONING OF NUCLEOLUS-ORGANIZING REGIONS OF CHROMOSOMES IN HUMAN BLOOD LYMPHOCYTE CULTURES

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The development of a selective method of staining nucleolus-organizing regions (NOR) of metaphase chromosomes and the nucleolus of the interphase nucleus with silver nitrate has led to the active study of functioning of these cell structures by means of cytological methods. It has been shown that staining NOR with silver nitrate (Ag-staining), reflecting the functioning of ribosomal genes, is a stable, inherited, and characteristic feature of somatic cells of a given individual [1, 2, 5, 6, 12]. However, some workers have noted the existence of intercellular variability of Ag-staining of NOR within the same tissue, namely in blood lymphocyte cultures stimulated to divide by phytohemagglutinin (PHA) [1, 2, 4, 5]. Variability of this kind may be either the result of the presence of lymphocyte populations differing in the functioning of their ribosomal genes, or nothing more than a reflection of the gradual formation of NOR activity as the cell leaves the Y_o phase under the influence of PHA.

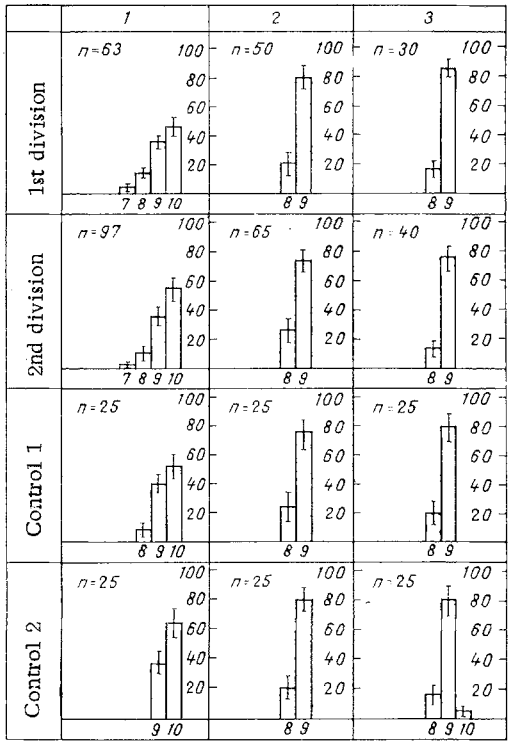

Fig. 1. Distribution of cells by total number of Ag^+-NOR in 1st and 2nd divisions. 1, 2, 3) Different individuals. For each histogram: horizontal axis — total number of Ag^+-NOR found in metaphase plates; vertical axis — percent of metaphase plates with a particular number of Ag^+-NOR. n) Number of cells studied.

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TABLE 1. Characteristics of Individual Chromosomes Varying in Ag-Staining of Their NOR

Individual	Varying chromosomes	Number of Ag-negative chromosomes	
		absolute	%
1	22	20	40
	13	2	4
	21	1	2
2	15	11	22
3	14	9	18

Legend. Total number of varying chromosomes analyzed for each individual was 50.

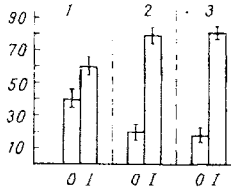

Fig. 2. Intensity of Ag-staining of varying chromosomes. 1, 2, 3) Individuals. Horizontal axis — characteristics of staining, in points (0, 1); vertical axis — percent of varying chromosomes with particular intensity of staining.

The aim of this investigation was to study the time course of formation of NOR activity by Ag-staining metaphase chromosomes in the first and second cell divisions of lymphocytes after stimulation by PHA in order to test the validity of the second hypothesis.

EXPERIMENTAL METHOD

Cultures of blood lymphocytes from three individuals, conventionally designated Nos. 1, 2, and 3, were used. Fixation was carried out 72 h after the beginning of culture in the presence of PHA-P (0.01 ml, from Difco, USA). Films were prepared by the standard method. To distinguish between metaphases of the first and second divisions bromodeoxyuridine (BUdR) was added to the cultures in a dose of 10 μg/ml from the beginning of cultivation. The films were stained both to detect BUdR [7] and also by the AgI method [4]. From 50 to 160 metaphase plates (MP) were analyzed for each case. Control films were stained simultaneously by the G method to identify the 10 acrocentric chromosomes carrying the NOR, and by the AgI method to evaluate the state of the NOR. Chromosomes in such films either contained (control 1) or did not contain (control 2) BUdR. In each of these groups 25 MP were analyzed. Ag-staining of NOR was characterized by the number of stained NOR and the intensity of staining, which was expressed in points (from 0 to 3). The associative capacity of the NOR was studied by determining the number of cells containing associations, the number of associations per cell (the whole sample of cells taken for analysis was counted), the number of associations per cell with associations, and the number of chromosomes per association. The criterion of association of acrocentric chromosomes was the presence of Ag-stained substrate between their short arms.

EXPERIMENTAL RESULTS

The data in Fig. 1 show that in films from all three individuals the lymphocytes were heterogeneous for the number of Ag-stained NOR (Ag^+-NOR), which was expressed as a change in staining of one or two NOR in the minority of the group of cells tested. The relative percentages of "major" and "minor" classes with respect to number of Ag^+-NOR did not differ in metaphases of the 1st and 2nd cell divisions in the experimental cultures and remained the same in the two control groups.

TABLE 2. Characteristics of Associative Capacity of Chromosomal NOR in 1st and 2nd Division Lymphocytes

Individual	Cell division	Number of cells studied	Number of cells containing associations, %	Number of associations per cell in whole sample	Number of associations per cell with associations	Number of associating chromosomes per association
1	1 st	63	77,8 ± 5,23	1,05 ± 0,13	1,34 ± 0,17	2,24 ± 0,18
	2 nd	97	49,5 ± 6,07	0,58 ± 0,08	1,16 ± 0,16	2,16 ± 0,20
			$P < 0,05$	$P < 0,05$	$P > 0,05$	$P > 0,05$
2	1 st	50	74,0 ± 6,20	1,10 ± 0,15	1,48 ± 0,20	2,34 ± 0,20
	2 nd	65	53,8 ± 6,19	0,65 ± 0,09	1,20 ± 0,19	2,01 ± 0,22
			$P < 0,05$	$P < 0,05$	$P > 0,05$	$P > 0,05$
3	1 st	30	66,7 ± 8,60	0,90 ± 0,17	1,35 ± 0,26	2,20 ± 0,29
	2 nd	40	35,0 ± 7,54	0,45 ± 0,11	1,29 ± 0,30	2,20 ± 0,35
			$P < 0,05$	$P < 0,05$	$P > 0,05$	$P > 0,05$
Generalized values	1 st	143	74,1 ± 6,43	1,03 ± 0,15	1,39 ± 0,20	2,28 ± 0,21
	2 nd	202	48,0 ± 6,42	0,57 ± 0,09	1,19 ± 0,19	2,14 ± 0,24
			$P < 0,05$	$P < 0,05$	$P > 0,05$	$P > 0,05$

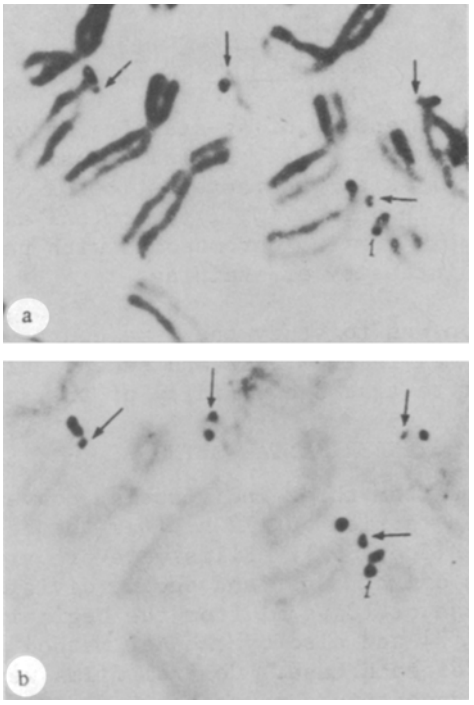

Fig. 3. Changes in size of grains of silver in NOR of 2nd cell division chromosomes during Ag-staining. a) Fragment of 2nd division metaphase plate with marked asymmetry in size of silver grains; b) the same after additional staining with silver nitrate. 1) NOR in which asymmetry of size of silver grains is not observed.

To judge which individual chromosomes varied in their Ag-staining, preparations of chromosomes after combined Ag and G staining were analyzed. Intercellular variability in the number of Ag^+-NOR, as Table 1 shows, was due to variation in staining of any single chromosome in each individual (absence of staining of chromosomes 13 and 21 in individual 1 occurred in only one or two cells). In all cases this chromosome was palely stained (Fig. 2) (averaged results of the study of the two control groups are given in Table 1 and Fig. 2, because the data for these groups were identical). It can be concluded from the results that within the limits of sensitivity of the method used there was no significant difference in functioning of NOR between cells of the 1st and 2nd cell divisions and that the phenomenon of intercellular variability of Ag staining of NOR of human metaphase chromosomes is evidently not connected with the order of cell division of lymphocytes after stimulation by PHA.

The results of investigation of the associative capacity of NOR of the acrocentric chromosomes in 1st and 2nd division cells are given in Table 2. A statistically significant decrease in the number of MP containing associations was found in cells of the 2nd division, and this resulted in a significant decrease in the number of associations per cell when the whole cell sample was analyzed. Depending on the number of associations per cell containing associations, and also on the number of associating chromosomes per association, no difference was found between cells of the 1st and 2nd divisions. Consequently, the main difference between cells of the 1st and 2nd divisions as regards associative power of their acrocentric chromosomes was a decrease in the number of MP containing associations in the 2nd cell division. This phenomenon, which was observed previously [3, 8], was explained by marked shortening of interphase preceding the 2nd cell division, compared with its duration before the 1st division. Wachtler et al. [11] suggest that associations of acrocentric chromosomes in mitosis are the result of a change in nucleolar function of the lymphocytes after stimulation by PHA. The reduction in their number was interpreted by these workers as separation of associations arising previously. In our opinion this problem requires further study.

Since analysis of chromosomes in MP of the 2nd division could distinguish between sister chromatids taking up BUdR, and at the same time, could evaluate the state of the NOR on the basis of its Ag staining, it was possible to investigate yet another problem which has been discussed in the literature, namely the smaller size of the grains of silver in the palely stained chromatid than in the darkly stained chromatid [9, 10]. This phenomenon is explained by inhibition of the transcribing activity of the NOR whose DNA has incorporated BUdR into both strands of the molecule, and consequently stains more palely. We stained such chromosomes additionally by the Ag method and found that in this case the difference in size of the grains of silver either was reduced or disappeared virtually completely (Fig. 3). Since asymmetry of Ag staining was found not in all MP of the same film and not in all NOR of a given MP, and since it was reduced or abolished after additional staining with silver nitrate it can be concluded that this asymmetry is due to reduced ability of the regions of the nucleolar organizer, whose DNA has incorporated BUdR twice, to stain with Ag. There are no grounds for asserting that this phenomenon depends on inhibition of transcription of BUdR-incorporating regions of the chromosomes and not on a change in the properties of the transcript or its after-product transcribed from BUdR-containing DNA sequences.

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