COMPARATIVE STUDY OF NUCLEOLUS ORGANIZING REGIONS OF METAPHASE CHROMOSOMES IN HUMAN BLOOD LYMPHOCYTES AND SKIN FIBROBLASTS

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It is now possible by means of silver staining of metaphase chromosomes to study activity of ribosomal genes located in what are called the nucleolus-organizing regions (NOR) of chromosomes. It has been shown on human chromosomes that the number of stained NOR in the metaphase plates (MP), and the intensity of their staining are inherited characteristics of the individual [1, 6, 7, 10]. The question arises whether this characteristic has its own particular features for cells of different tissues, allowing for possible differences between them in the character and intensity of their protein synthesis. For example, a considerable decrease in the number of functioning NOR has been observed in meiocytes in the pachytene of oogenesis [5], and absence of functioning NOR has been found in blood cells and bone marrow cells, unstimulated by PHA, from patients with acute and chronic myeloid leukemia [2]. Nevertheless, the study of differences between tissues with NOR function in somatic cells is represented only by single publications dealing with the study of blood lymphocytes and skin fibroblasts, and the results of these investigations are contradictory in character [4, 8]. The aim of the present investigation was to study function of NOR of metaphase chromosomes of blood lymphocytes stimulated to divide by PHA, and of skin fibroblasts of the same individuals, by the method of silver staining.

EXPERIMENTAL METHOD

Standard preparations of metaphase chromosomes from blood lymphocytes cultured in the presence of PHA and of skin fibroblasts cultured on slides, after 13-15 passages, and obtained from four persons identified conventionally as A, B, C, and D, were studied. The preparations were subjected to combined silver and G Staining, so that it was possible to evaluate the state of the NOR and to identify the chromosomes carrying them simultaneously. After silver staining, carried out by the modified Ag-1-H method [3], the preparations were incubated in 0.2 N CaCl for 6-7 min at 65°C and stained with a 0.2% solution of Giemsa's stain in phosphate buffer (pH 6.8) for 6-7 min. NOR activity was analyzed by determining the number of NOR stained with silver per MP and the intensity of staining of individual NOR, which was expressed in points (from 0 to 3). The associative ability of the NOR also was determined on the basis of 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 containing associations, and the number of associating chromosomes per association. The criterion of associations of NOR of acrocentric chromosomes was the presence of substrate stained with silver between them.

EXPERIMENTAL RESULTS

The data given in Fig. 1 show that in every case heterogeneity for the total quantity of Ag + NOR was present in MP of blood lymphocytes and skin fibroblasts: in individuals A and C there were two subpopulations of cells with 9 and 10 Ag + NOR, whereas in individuals B and D there were three in each case (8-10 Ag + NOR). The main class of cells for Ag + NOR content was the same for lymphocytes and fibroblasts in each case. The relative percentages of major and minor classes with respect to this parameter was the same for individuals C and D. For individual A the minor class in MP of fibroblasts was 16%, compared with 40% in lymphocytes. For individual B the appearance of a group of MP with 10 stained NOR, which was not observed in the lymphocytes, was observed in the fibroblasts.

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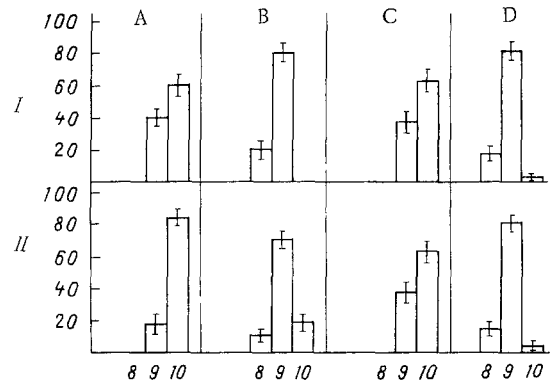

Fig. 1. Distribution of cells by total number of Ag + NOR in lymphocytes and fibroblasts of four individuals (A-D). Here and in Fig. 2: I) lymphocytes, II) fibroblasts. For each histogram: horizontal axis — number of Ag + NOR per MP, vertical axis — percentage of cells. In all cases 50 MP were studied for each type of cell.

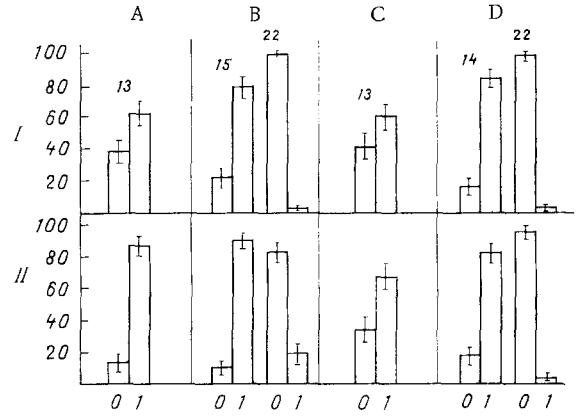

Fig. 2. Intensity of staining of varying chromosomes in lymphocytes and fibroblasts of four individuals (A-D). 13, 14, 15, 22) Chromosomes changing their silver staining. For each histogram: horizontal axis — degree of staining, in points: vertical axis — percentage of cells with given intensity of staining.

As Table 1 shows, intercellular variability for number of Ag + NOR was due both in the blood lymphocytes and skin fibroblasts to changes in staining of one or two chromosomes characteristic of each individual, and identical for cells of both types. Changes in silver staining of NOR of the other chromosomes were observed in single cells, amounting to not more that 2-4% of the total number of cells analyzed for each case (Table 1). Only in case B was staining of NOR of the 22nd chromosome, which was practically inactive in MP of the blood lymphocytes, observed in a culture of skin fibroblasts (Table 1). In all cases the varying chromosome was palely stained (Fig. 2) and grains of silver were present in NOR. The intensity of staining of the remaining NOR of the acrocentric chromosomes was identical both in blood lymphocytes and in skin fibroblasts for each individual.

The types of silver staining of NOR of acrocentric chromosomes of cultures of skin fibroblasts and blood lymphocytes in the same individuals thus do not differ from one another, in agreement with the observations of Mikelsaar and Schwarzacher [8]. The phenomemon of intercellular variability for Ag + NOR content is similar both qualitatively and quantitatively in the types of cells compared.

TABLE 1. Characteristics of Individual Chromosomes Varying in Silver Staining

Individual	Type of cells	Varying chromosomes	Number of chromosomes changing their silver staining	
			abs.	%
A	Lymphocytes	13	19	38
		22	1	2
	Fibroblasts	13	7	14
		22	1	2
B	Lymphocytes	15	11	22
		22	1	2
	Fibroblasts	15	5	10
		22	10	20
C	Lymphocytes	13	20	40
		15	1	2
	Fibroblasts	13	17	34
		14	1	2
		15	1	2
D	Lymphocytes	14	9	18
		22	1	2
	Fibroblasts	14	8	16
		22	2	4

TABLE 2. Characteristics of Associative Capacity of NOR of Chromosomes in Lymphocytes and Fibroblasts

Individual	Type of cells	Total number of cells	Number of cells with associations, %	Number of associations		Number of associating chromosomes per association
				per cell	per cell with associations	
A	Lymphocytes	50	68±7,5	0,80±0,11	1,23±0,16	2,1±0,21
	Fibroblasts	50	22±5,8	0,24±0,06	1,10±0,19	2,0±0,34
P			<0,05	<0,05	>0,05	>0,05
B	Lymphocytes	115	63±4,5	0,84±0,08	1,34±0,16	2,2±0,22
	Fibroblasts	50	22±5,8	0,24±0,07	1,10±0,30	2,0±0,41
P			<0,05	<0,05	>0,05	>0,05
C	Lymphocytes	50	80±5,6	1,10±0,10	1,40±0,18	2,3±0,20
	Fibroblasts	50	34±7,0	0,34±0,08	1,00±0,24	2,0±0,34
P			<0,05	<0,05	>0,05	>0,05
D	Lymphocytes	70	49±6,0	0,64±0,08	1,30±0,22	2,2±0,33
	Fibroblasts	50	12±4,6	0,12±0,05	1,00±0,17	2,0±0,58
P			<0,05	<0,05	>0,05	>0,05

The results of comparison of the associative power of NOR of acrocentric chromosomes in MP of blood lymphocytes and skin fibroblasts are given in Table 2. Compared with the blood lymphocytes, in MP of skin fibroblasts of all four individuals a statistically significant decrease was observed in the number of MP containing associations and, as a result, a decrease in the number of associations per cell, calculated for the whole sample of cells studied. Cells containing associations did not differ in number or in number of associated chromosomes per association in the tissues compared. Consequently, the main difference between the associative capacity of NOR of the metaphase chromosomes of blood lymphocytes and skin fibroblasts was a decrease in the number of cells containing associations in cultures of skin fibroblasts. The differences found are not the result of differences in the technique of obtaining preparations of metaphase chromosomes. The technique of preparing metaphase chromosomes of blood lymphocytes and skin fibroblasts differed in the incubation time of the preparations in hypotonic solution: this was 8 min for blood lymphocytes and 35 min for skin fibroblasts. An additional investigation of metaphase chromosomes of cultures of blood lymphocytes showed that during incubation for 40 min in 0.6% hypotonic KCl solution the number of cells containing associations (with a complete set of chromosomes) and other criteria of the associative capacity of the NOR remained the same as after incubation for 8 min. Differences revealed in the associative capacity of NOR of acrocentric chromosomes of cultures of blood lymphocytes

and skin fibroblasts, on the one hand, and the equal number of Ag + NOR and the equal intensity of their staining in these tissues, on the other hand, may be evidence of the relative nature of the proportionality described by some workers [9] between the intensity of silver staining of NOR and their associative capacity. It can be postulated that the active state of NOR, being inherited, is a characteristic and specific feature for each individual, essential for realization of the proliferative powers of the cell, and with respect to this feature blood lymphocytes do not differ from skin fibroblasts when cultured *in vitro*.

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PRESERVATION OF PARENTAL FEATURES IN MAN—CHINESE HAMSTER CELL HYBRID

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By studying different features in hybrid and parental cells it is possible to assess the contribution of genetic and environmental factors to the phenotype of hybrid cells.

In the investigation described below the effect of long-term culture of hybrid cells in a nonselective medium on colony forming ability in selective media containing the same components as were used to select the hybrid cells during hybridization was studied.

EXPERIMENTAL METHOD

Hybrid cells of subclones MOI-8-1 and MOI-8-3 were used. The cells were obtained by fusion of normal human embryonic muscle cells (IMG 812) with transplantable chinese hamster cells (MO-1), deficient for hypoxanthinephosphoribosyl transferase and resistant to 1 mM ouabain; chinese hamster cells (O-1) resistant to 1 mM ouabain also were used.

The method of obtaining the hybrid cells was described previously [1]. The cells were cultured in Carrel flasks or on Eagle's medium with the addition of 20% bovine serum without antibiotics (henceforward this medium will be called normal medium) or on HATG medium, containing hypoxanthine (10^{-4} M), aminopterin (4×10^{-7} M), thymidine (1.6×10^{-7} M), and glycine (3×10^{-6} M). The cells were subcultured every 4-5 days. In the experiments the cells were

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