GENETIC DETERMINATION OF NUCLEOLAR ORGANIZER ACTIVITY IN HUMAN CHROMOSOMES

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A method of selective staining of nucleolar organizer regions (NOR) of human and animal chromosomes with silver nitrate has recently been developed and it has been shown that this staining is determined by two factors: by the presence of ribosomal genes in the chromosome and by their transcription activity [6, 7, 9]. In human somatic cells NOR carriers are five pairs of acrocentric chromosomes. It has been shown that individuals may differ in the number of stained chromosomes and the degree of staining; different chromosomes, moreover, behave relatively indepentently of one another [1, 4]. The question arises whether this individual variation in NOR is genotypically determined or whether it is susceptible to environmental influences also. It has been concluded from segregation analysis of a number of families that the character of Ag-staining of a given chromosome is inherited by children from their parents [2, 5]. On the other hand, these and other investigations revealed intercellular variability of the pattern of Ag-staining in the same individual. Changes in NOR activity in somatic cell hybrids also have been discovered [6].

The question of stability of the functional state of ribosomal genes in somatic cells and the degree to which it is inherited in generations of individuals thus remains open. An investigation on twins provides an approach to the solution of this problem which has not hitherto been used.

The paper gives the first results of a study of the genetic determination of NOR of human chromosomes in twins.

EXPERIMENTAL METHOD

Blood lymphocyte cultures from 16 pairs of twins of both sexes were used: eight mono- (MT) and eight dizygotic (DT) pairs of twins. The twins, aged from 20 to 40 years, were chosen from the register of twins of the Institute of Medical Genetics, Academy of Medical Sciences of the USSR. The zygosity of the twins was determined with respect to genetic markers such as ABO, Rh, MN, P, Le, and haptoglobin antigens of erythrocytes, acid phosphatase

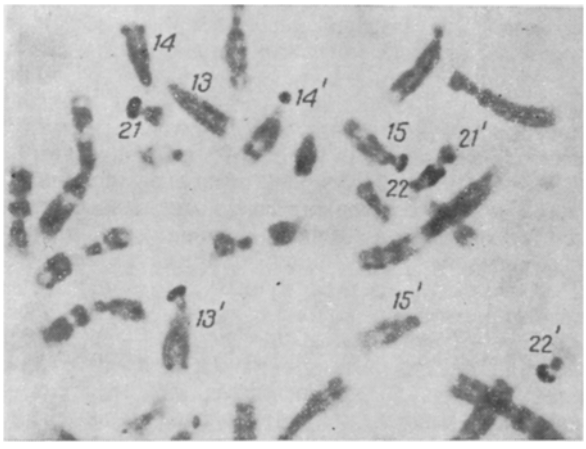

Fig. 1. Fragment of metaphase plate with complete set of acrocentric chromosomes. Successive staining by Ag- and G-methods (3000 ×), seven of ten acrocentrics are Ag-stained. Degree of staining of NOR of separate chromosomes: chromosomes 13, 14, 15' — 0 points; chromosome 15 — 1 point; chromosome 21 — 3 points; remaining acrocentrics — 2 points.

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TABLE 1. Mean NOR Activity in MT and DT

Chromosome No.	Percent of chromosomes with NOR activity		Degree of NOR activity, conventional units	
	MT	DT	MT	DT
13	69,5±10,3	65,0±8,7	1,33±0,20	1,51±0,21
14	72,6±8,1	75,6±7,1	1,62±0,18	1,75±0,16
15	76,9±5,9	74,5±6,2	1,51±0,16	1,35±0,02
21	99,6±0,9	93,6±3,2	2,24±0,11	2,07±0,12
22	74,4±11,4	77,8±11,3	1,63±0,24	1,53±0,26
All acrocentrics	78,5±5,6	80,2±2,8	1,67±0,09	1,64±0,05

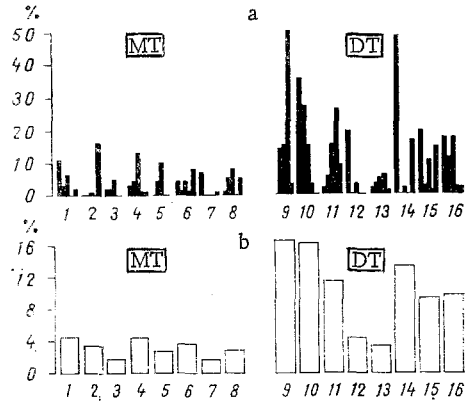

Fig. 2. Differences in percentage detection of NOR in individual chromosomes (a) and mean values for set of acrocentrics (b). Horizontal axis: 1-8) Nos. of MT pairs; 9-16) Nos. of DT pairs. Vertical axis: differences between twins of each pair (in %). a) Separate rectangles for each pair of twins from left to right correspond to chromosomes 13, 14, 15, 21, and 22.

and phosphoglucomutase activity of erythrocytes, and ability to taste phenylthiocarbamide. The probability of the type of zygosity among the pairs of twins chosen for analysis was not below 0.9. Lymphocyte cultures were obtained by the standard method; chromosomal preparations were air-dried. The method of Ag-staining [1], in the writers' modification, was as follows. The preparations were kept for two weeks at room temperature, after which two drops of a 50% solution of silver nitrate were applied, coverslips were placed on them, and they were incubated in a wet chamber at 37°C for 18-48 h. The preparations were then taken through distilled water and 70°, 96°, and 100° alcohol, and air-dried. The chromosomes were identified by G-staining, for which purpose the Ag-stained preparations were treated with 0.2 N cesium chloride solution at 58-65°C and then stained for 6-12 min with Giemsa's stain, made up in phosphate buffer at pH 6.8. From 25 to 50 metaphase plates with a complete set of identifiable acrocentric chromosomes were analyzed from each individual. NOR activity of the chromosome set was judged by the number of Ag-stained chromosomes and the intensity of silver impregnation in each of the five pairs of chromosomes.

EXPERIMENTAL RESULTS

Nucleolar organizers located in human acrocentric chromosomes 13, 14, 15, 21, and 22 can be stained with silver in each or only one homolog of each pair or they remain completely unstained. The degree of staining of NOR also may vary within wide limits (Fig. 1). The frequency of active NOR in a given individual or in the group of twins as a whole was expressed as the number of Ag-positive chromosomes as a percentage of the total number of chromosomes of the given pair analyzed. It was calculated separately for each of the five pairs of chromosomes. The index of NOR activity was the mean content of Ag-material in each pair of acrocentrics, expressed in conventional units. To calculate this index, the intensity of staining of NOR was classified visually on a four-point scale: 0) no staining, 1) weak, 2) average, 3) intense staining. The final index of activity was calculated as the sum of the products of the fraction of chromosomes with a given degree of staining and the appropriate number of points.

The results of quantitative analysis based on both indices are shown in Table 1 and Fig. 2.

The results give information on the comparative frequency to which different acrocentric chromosomes participate in nucleolar organization. On average about eight of ten acrocentrics stain in each cell; among different individuals this figure varied from six to ten. It was

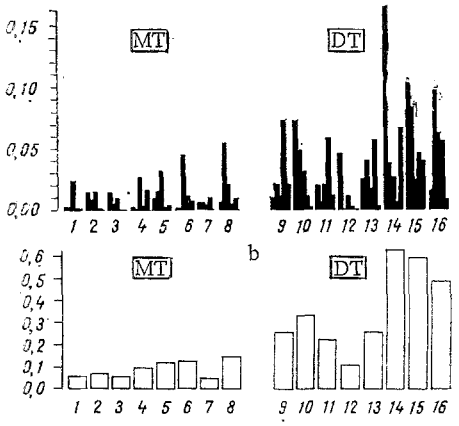

Fig. 3. In-pair differences of NOR activity in individual chromosomes (a) and mean values for set of acrocentrics (b). Vertical axis: differences in NOR activity between twins of each pair (in conventional units). Remainder of legend as to Fig. 2.

TABLE 2. Interindividual and In-Pair Dispersions of Differences between Indices of NOR Activity in Acrocentric Chromosomes of MT and DT

Dispersion	Percent of chromosomes with active NOR		Degree of activity on NOR, conventional units	
	MT	DT	MT	DT
Interindividual*	247	63	$658 \cdot 10^{-4}$	$228 \cdot 10^{-4}$
In-pair†	0,57	7,2	$26 \cdot 10^{-4}$	$332 \cdot 10^{-4}$

*Scatter of mean values in pairs relative to mean for group of twins.
†Scatter of values of half-differences between individual twins of pairs in each group.

the same in the MT and DT groups. Chromosomes 13, 14, 15, and 22 did not differ statistically significantly in frequency of staining. Chromosome 21 was practically always stained: Its staining detection index was 93-99%, significantly higher than the mean. Similar results were obtained for the degree of staining of NOR: chromosomes 13, 14, 15, and 22 on average were equally stained, whereas the relative differences from the mean were greater for chromosomes 21 than the differences for frequency of staining. The material thus showed that chromosome 21 plays a much more active part than other chromosomes in the formation of the nucleolus.

Comparison of the mean percentage of chromosomes with active NOR and the degree of activity for groups of MT and DT, and also of interindividual dispersions of these indices in the two groups showed that the groups chosen for analysis were sufficiently similar. The probability of difference between the mean values and dispersion of the degree of staining was under 0.05: For dispersion of frequencies of active NOR this probability was approximately 0.05. Conversely, the difference between the twins of each pair of MT was less as regards all characteristics than that between twins of the DT pairs (Table 1). Dispersions within the pair were more than 12 times greater for DT than for MT (probability of coincidence under 0.01). A clear idea of the distribution of differences for both indices of nucleolar organizer activity in individual chromosomes of MT and DT is given by the histograms in Fig. 2. For most pairs of DT these differences were appreciably greater than the corresponding diffirences for MT although the frequency of active chromosomes in pairs 12 and 13 and the degree of activity in pair 12 were within the same limits as for MT.

If attempts are made to represent the differences in NOR activity thus revealed as alternatives (present — absent), limited by a certain conventional threshold value, the degree of similarity of the features studied in MT and DT can be determined. For instance, comparison of histograms of frequency of active NOR in individual MT and DT chromosomes, with a threshold value of 15%, shows that concordance in MT was 0.975 and in DT 0.625. With respect

to degree of activity (level below 0.5) the corresponding values of these indices are 0.975 and 0.725.

The results of this first investigation on twins are in full agreement with existing data of segregation analysis [2, 5] and they indicate a high degree of inheritability of the functional state of ribosomal genes for each carrier chromosome, so far as this state can be judged by a cytogenetic test: Ag-staining of nucleoproteins in the nucleolar organizer region. From this point of view the feature studied does not differ from structural characteristics of polymorphic regions of chromosomes with respect to G- and Q-staining, according to which a high degree of concordance is found among monozygotic twins [3, 8].

However, it is too early to conclude that the use of Ag-staining of the nucleolar-organizing chromosomes can be used as a reliable criterion of the zygosity of twins. A special analysis of the contribution of genotypic and environmental factors in the low level of variability that can be detected in individual twins of monozygotic pairs with respect to particular chromosomes must be undertaken on a much wider sample of twins. A special study of the nature of the frequently observed intercellular variability of Ag-staining of NOR within the same individual is required.

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