RELATIONSHIP BETWEEN INTENSITY OF TRANSCRIPTION AND CONTENT OF rRNA GENES IN INDIVIDUAL NUCLEOLUS-ORGANIZING REGIONS OF HUMAN CHROMOSOMES

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Numerous investigations have shown that nucleolus-organizing regions (NOR) are the site of rRNA genes and in man they are located in the vicinity of the secondary constriction of the short arms of five pairs of acrocentric chromosomes (pairs 13, 14, 15, 21, and 22).

It has been shown that staining NOR of acrocentric chromosomes with silver nitrate (Ag staining) reflects the transcription activity of ribosomal genes and is a stable and inheritable feature of the particular chromosome of the set and a particular individual [3, 4, 11, 12]. In such cases definite differences exist in the intensity of Ag staining between chromosomes and between homologs of one pair [3-5, 7, 10, 14]. This variability may be based on two causes: 1) variability of the content of rRNA genes in the NOR of individual chromosomes; 2) changes in transcription activity of ribosomal genes. Comparison of the number of copies of rRNA genes on chromosomes of the same individual, determined by the nucleic acid hybridization method on preparations of metaphase chromosomes, and comparison of the transcription activity of the NOR, revealed by Ag staining, may provide an experimental approach to the discovery of the causes of this variability.

One attempt at an investigation of this kind has already been made by workers in the West [13]. However, a shortcoming of this investigation is that it was conducted on unidentified chromosomes, and in situ hybridization was carried out on preparations which had previously been stained with silver and, as the authors themselves point out [9], this was bound to interfere with hybridization labeling.

The aim of the present investigation was to determine the relative content of rRNA genes by in situ hybridization in all ten individual NOR and to compare these data with the degree of their functional activity and with the morphological features of the satellite strands.

EXPERIMENTAL METHOD

Experiments were carried out on preparations of metaphase chromosomes, prepared by the standard method, from peripheral blood lymphocytes of two karyotypically normal individuals, stimulated to proliferate. To identify the chromosomes, in all cases modified R-staining was used [2]. In this method, 6 h before fixation, 5-bromodeoxyuridine (BUdR) was added to the culture. The chromosome preparations were stained with the fluorochrome Hoechst 33258, irradiated with UV rays, and then stained by the Giemsa stain with phosphate buffer (pH 6.8).

To determine the length of the satellite strands, the preparations were stained initially with azure and eosin. The length of the satellite strands of the acrocentric chromosomes was estimated in points: 0) no satellite strands present, 1 point) length of the satellite strand less than that of the short arm of chromosome 17; 2 points) length of the satellite strand equal to that of the short arm of chromosome 17; 3 points) length of the satellite strand greater than that of the short arm of chromosome 17 [6]. The preparations

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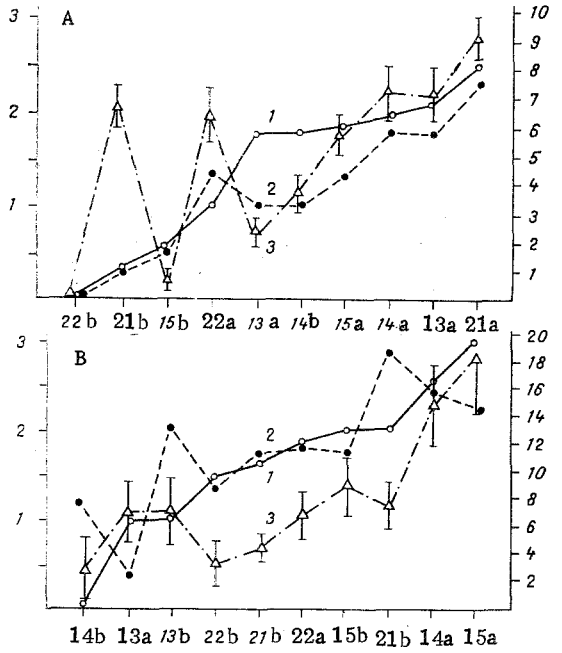

Fig. 1. Relationship between intensity of Ag staining (1), length of satellite strand (2) and intensity of hybridization labeling with the ^{3}H-DNA probe 28S rRNA (3) of the NOR of chromosomes of two individuals (A and B). Abscissa: homologs (A and B) of acrocentric chromosomes, arranged in order of increasing intensity of Ag staining; ordinate: on left) point ratings of Ag staining and length of satellite strands, on right) point ratings of hybridization labeling.

were then stained again by the R-method, as described above, and the chromosomes were identified. Ten metaphase plates were studied for each individual.

Some preparations were stained by a combination of the Ag and R methods [2, 12]. In this case the preparations were stained with silver nitrate by the method in [12] immediately after staining with the fluorochrome, and they were then irradiated with UV light. Functional activity (transcription of rRNA) was assessed by the intensity of Ag staining of NOR, expressed in points (from 0 to 3 points). For each individual chromosome an average score of points for 20 metaphase plates was obtained.

In situ hybridization was carried out on intact preparations of metaphase chromosomes of the same individuals by the method in [8]. A fragment of the gene of 28S rRNA, labeled by the nick translation method to a specific radioactivity of $(1\text{-}5)\cdot10^7$ cpm/μg, cloned from the rat library, was used as the probe for in situ hybridization. After development of the autoradiographs, they were stained by a modified R method (BUdR + Hoechst + Giemsa stain). The preparations were coated with type UK-19 photographic emulsion with a greater than normal gelatin content (Photographic Chemical Research Institute Project, Moscow). The preparations were exposed for 3-6 weeks at 4°C. The intensity of hybridization labeling was determined by counting the number of grains above the individual NOR. To obtain more complete characteristics of hybridization labeling, the size of the grains in the label also was estimated in points: 1 point) grain smaller than half the short arm of chromosome 17; 2 points) the grain equal to half the short arm of chromosome 17; 3 points) the grain larger than half the short arm of chromosome 17. The total relative intensity of labeling was obtained as the sum of the product of the number of grains and the corresponding score for the size of the silver grains, in points. For each individual the mean intensity of labeling after in situ hybridization was obtained for individual chromosomes by analysis of 21 and 15 metaphase plates respectively.

EXPERIMENTAL RESULTS

For each of the two individuals studied correlation curves were plotted for the length of the satellite strands, the intensity of Ag staining, and the intensity of labeling in con-

TABLE 1. Comparison of Length of Satellite Strand, Intensity of Ag Staining, and Intensity of in Situ Hybridization Labeling of Chromosomes of Pair 15 (individual B), One Homolog of Which has an Enlarged Short Arm

No. of cell	Length of satellite strand		Intensity of Ag staining		Intensity of in situ hybridizatn.	
	15p+	15	15p+	15	15p+	15
1	3	1	3	2	12	5
2	2	2	3	2	22	8
3	3	2	3	3	16	12
4	1	1	2	2	28	6
5	2	1	3	2	12	6
6	3	2	3	2	10	4
7	2	2	3	2	10	6
8	2	2	3	2	18	12
9	2	2	3	2	16	10
10	3	2	3	1	16	12
Mean	2,3	1,7	2,9	2,0	16,0	8,0

TABLE 2. Analysis of Correlation between Intensity of Ag Staining (x), Length of Satellite Strand (y), and Intensity of Hybridization Labeling of NOR of Two Phenotypically Normal Individuals (A and B) by ^{3}H-DNA Probe 28S rRNA (z)

Individual	Coefficient of correlation		Coef. of multiple determination, %
	paired	multiple	
A	$r_{x,y} = +0{,}924$	$r_{x \cdot yz} = 0{,}932$	87
	$r_{x,z} = +0{,}635$	$r_{y \cdot xz} = 0{,}955$	91
	$r_{y,z} = +0{,}772$	$r_{z \cdot xy} = 0{,}799$	64
B	$r_{x,y} = +0{,}647$	$r_{x \cdot yz} = 0{,}844$	71
	$r_{x,z} = +0{,}791$	$r_{y \cdot xz} = 0{,}648$	50
	$r_{y,z} = +0{,}493$	$r_{z \cdot xy} = 0{,}791$	62

nection with in situ hybridization (Fig. 1). Individual chromosomes were arranged on the graphs in the order of increasing intensity of Ag staining. Each pair of acrocentric chromosomes was conventionally divided into homologs (a and b), homologs from each plate with a more marked feature being placed in one group and those with a less marked in the other. The validity of this approach for "identifying" homologs in the pair was based on the result of our comparison of three features (length of the satellite strand, intensity of silver staining, and intensity of hybridization labeling) in pairs of homologs in cases when one homolog could be unambiguously identified by reference to a morphologic distinguishing feature. Table 1 gives the results of analysis of each of these three features of chromosome 15 of individual B, in whom one homolog of this pair was characterized by enlargement of the short arm (15p+), easily distinguishable in every metaphase plate. It will be clear from Table 1 that the marker chromosome has values for all three features that are either equal to those of the homolog or higher. Homologs of other pairs of chromosomes evidently also differ with respect to these features and, consequently, conventional separation of the homologs (a and b) by the method we adopted can be regarded as justified.

Analysis of the graphs in Fig. 1 shows that all 10 acrocentric chromosomes differ with respect to each of the three features. To determine the degree of correlation between the parameters studied, coefficients of paired and multiple correlation and also coefficients of multiple determination (r^2), expressing the dependence of one parameter on the other two, in percent, were calculated (Table 2). On the whole, it can be concluded from the shape of the curves in Fig. 1 and the results of correlation analysis (Table 2) that in both cases there was sufficiently high positive correlation between the three features studied: length

of the satellite strand, intensity of Ag staining, and intensity of hybridization labeling. Against this background, the high intensity of hybridization labeling (6.8 ± 0.8 points) associated with weak Ag staining (0.3 point) and a short satellite strand (0.3 point) of chromosome 21a of individual A, stands out in sharp contrast (Fig. 1). It can evidently be postulated that a considerable proportion of the copies of the ribosomal genes in the NOR of this chromosome are in a repressed state, possibly as a result of their methylation. A similar case was analyzed previously in our laboratory [1]. In an individual with an enlarged short arm of 1 homolog of chromosome 13 ($13p^+$) a disproportionately weak intensity of silver staining was discovered, evidence of functional inactivity of the majority of the ribosomal genes of this NOR. By the combined use of restriction endonucleases, including those sensitive to methylation in the recognition site, the degree of methylation of part of the DNA of the nontranscribable spacers (NTS) of the ribosomal genes was discovered in the DNA of this individual. Comparison of these observations with a cytogenetic analysis suggested that methylated NTS are located in the NOR of chromosome $13p^+$.

It can be concluded from these results that interchromosomal variability of the rDNA content exists among 10 acrocentric chromosomes in man, and that the variability of transcription activity of the NOR is based both on the variability of the rDNA content in the individual NOR, or even its complete absence, and on the presence of rDNA in an inactive state.

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