SYNTHESIS OF RIBOSOMAL RNA IN LYMPHOCYTES WITH TRISOMY FOR CHROMOSOME 21

E. F. Davidenkova,* E. I. Shvarts,
A. B. Chukhlovin, and O. A. Rozenberg

UDC 616.899.65-05:616.153.
32-008.939.633.2-074

The conditions of isolation and cultivation of peripheral blood lymphocytes for 24 h are described. A method of isolating ribosomes from minimal numbers of lymphocytes (separated from 10-20 ml peripheral blood) by means of magnesium precipitation followed by low-speed centrifugation is given. When the specific radioactivity in polysomes of normal lymphocytes and lymphocytes trisomic for chromosome 21, stimulated by phytohemagglutinin, was compared no difference was found between euploid and trisomic cells with respect to this index. A decrease in the percentage of mitogen-reactive lymphocytes is postulated in the population trisomic for chromosome 21.

An urgent task in medical genetics is the study of the mechanisms of realization of genetic information in cells with an aneuploid genome. In Down's syndrome the chromosomal imbalance is due to trisomy for chromosome 21, which is a component of the nucleolar organizer and contains cistrons of ribosomal DNA [2, 3]. No results obtained by studying the efficiency of synthesis of ribosomal RNA (rRNA) in human aneuploid cells with an additional nucleolus-organizing chromosome are to be found in the literature. As a result of transcription of ribosomal cistrons a high-molecular-weight precursor with sedimentation constant of 45S is formed in the first place and, under the influence of nucleases, it is converted into 28S and 18S rRNA. The latter migrate into the cytoplasm where they participate in the formation of large and small subunits of ribosomes. Consequently, determination of the specific radioactivity of rRNA isolated from polysomes is an integral indicator of the efficiency of rRNA synthesis.

The object of this investigation was to study the specific radioactivity of rRNA isolated from polysomes of phytohemagglutinin (PHA)-stimulated lymphocytes of patients with Down's syndrome.

EXPERIMENTAL METHOD

Heparinized venous blood in a dose of 10-15 ml was used. The diagnosis of Down's syndrome was made on the basis of the results of clinical and karyological investigation. Lymphocytes were isolated by Pegrum's method [6]. The cells, numbering $10 \cdot 10^6$-$15 \cdot 10^6$, were incubated in medium No. 199 containing 10% fetal calf serum and also 10 mg penicillin and 5 mg kanamycin to 100 ml. The density of the cell suspension was $2 \cdot 10^6$/ml. Phytohemagglutinin (Burroughs Wellcome, England) was added in a dose of 30 μg to 1 ml incubation medium. Incubation continued for 21 h. The cells were incubated for the last 4 h with ^{3}H-uridine (specific radioactivity 17 Ci/mmole) in a concentration of 8 μCi/ml. Polysomes were isolated by Palmiter's method [4]. The residue of ribosomes was dissolved in 0.15 ml of a buffer containing 2% Na dodecylsulfate, 0.2 M NaCl, 0.04 M EDTA, pH 5.8 [9]. Heparin (1.5 mg/ml) also was added to the buffer. Before electrophoresis in polyacrylamide gel (PAG) glycerol was added (25% by volume) to the samples. Electrophoresis was carried out by the method of Peacock and Dingman [5], by adding dodecylsulfate to the electrode buffer in a final concentration of 2%. The gels were scanned with an attachment to the SF-4A spectrophotometer, cut into 2-mm sections, and, after hydrolysis in 30% H_2O_2, the radioactivity of the samples was determined in dioxan scintillator on the

*Corresponding Member of the Academy of Medical Sciences of the USSR.

Academic Group of Corresponding Member of the Academy of Medical Sciences of the USSR E. F. Davidenkova, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 10, pp. 1263-1264, October, 1976. Original article submitted January 12, 1976.

TABLE 1. Specific Radioactivity (in counts/min/μg 28S rRNA) of rRNA in Ribosomes of PHA-stimulated Lymphocytes from Normal Subjects and Patients with Down's Syndrome

Experiment No.	Specific radioactivity	
	Down's syndrome	normal subjects
1	10 051	11 312
2	7 005	5 159
3	3 107	5 588
4	17 560	6 312
5	6 100	5 550
6	7 900	5 282
7	11 190	11 863
M	7 900	5 588
P	>0,05	

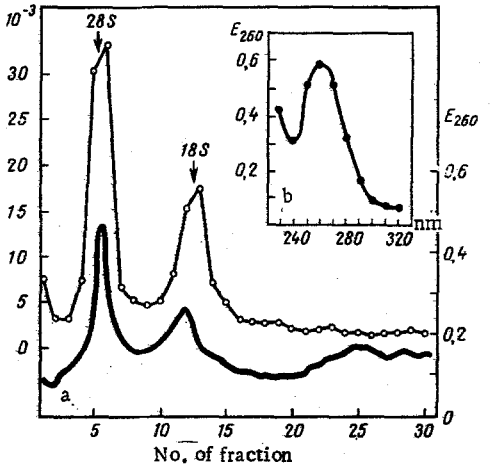

Fig. 1. Analysis of rRNA by electrophoresis in PAG. RNA from $10.0 \cdot 10^6$ lymphocytes extracted by phenol method and fractionated in 2.5% PAG: a) abscissa, no. of fractions; ordinate, right: optical density at 260 nm (E_{260}), left: specific radioactivity (in counts/min); b) characteristics of UV-absorbing material of ribosomes from which rRNA was isolated. Abscissa, wavelength (in nm); ordinate, optical density (E_{260}).

Mark II (Packard, USA) counter. The content of rRNA in individual peaks (28S and 18S) and the specific radioactivity were calculated by Zardi's method [9].

EXPERIMENTAL RESULTS AND DISCUSSION

By a combination of methods of short-term cultivation of lymphocytes, isolation of polysomes, and subsequent analysis by electrophoresis, the investigation could be carried out on a minimal amount of biological material; this is very important when human pathology is being studied (Fig. 1).

Values of the specific radioactivity of rRNA in PHA-stimulated lymphocytes from patients and subjects of the control group are given in Table 1. No statistically significant differences were found between the specific radioactivities of rRNA in the two groups compared.

Analysis of the results is best begun with an examination of previous studies of the structural organization of chromosome 21 in the human genome. By methods of molecular and somatic hybridization the genetic determinants of indophenol oxidase antiviral protein and rRNA on chromosome 21 have been mapped, i.e., reading of both messenger and rRNA takes place from it [2, 3, 8]. The nucleolar organizer in the human genome

consists of chromosomes of groups D and G, ten chromosomes in all. On hybridization of 28S and 18S RNA with DNA from patients with Down's syndrome an increase in the number of ribosomal cistrons by 10% was found [2].

The writers previously found a sharp decrease in the incorporation of ^{3}H-uridine into total RNA of PHA-stimulated lymphocytes of patients with Down's syndrome after incubation of the cells with the mitogen for 24 and 48 h. Synthesis of RNA in these experiments was assessed from the rate of incorporation of radioactive precursor into $1 \cdot 10^6$ cells [1].

This decrease can be presumed to be based upon a disturbance of the function of the nucleolar organizer. This hypothesis is also supported by the data of Rigas [7], who found a decrease in DNA synthesis in PHA-stimulated patients' lymphocytes. For the cell to enter the phase of DNA replication activation of rRNA synthesis in the G-phase is known to be necessary. However, in the investigation described above no difference was found in the efficiency of rRNA synthesis in lymphocytes trisomic for chromosome 21 compared with the control values. In this connection the decrease in incorporation of ^{3}H-uridine into total RNA found previously was probably due to a decrease in the percentage of cells responding to the mitogen.

LITERATURE CITED

1. E. F. Davidenkova, A. B. Chukhlovin, and E. I. Shvarts, Genetika, No. 4, 147 (1974).
2. K. Bross and W. Krone, Humangenetik, 18, 71 (1973).
3. A. S. Henderson, D. Warburton, and K. C. Atwood, Proc. Nat. Acad. Sci. USA, 69, 3394 (1972).
4. R. D. Palmiter, Biochemistry (Washington), 13, 3606 (1974).
5. A. C. Peacock and C. W. Dingman, Biochemistry (Washington), 6, 1818 (1967),
6. G. D. Pegrum, D. G. B. Perera, and E. Thompson, Immunology, 23, 13 (1972).
7. D. A. Rigas, P. Elsasser, and F. Hecht, Internat. Arch. Allergy, 39, 587 (1970).
8. F. H. Ruddle, Nature, 242, 165 (1973).
9. L. Zardi and R. Baserga, Exp. Molec. Path., 20, 69 (1974).

DEPENDENCE OF CYTOGENETIC ACTION OF TEPA ON ITS CONCENTRATION IN HUMAN LYMPHOCYTE CULTURES

T. G. Selezneva and A. N. Chebotarev

UDC 612.6.05.014.46

The cytogenetic action of TEPA [tris(2-methyl-1-aziridinyl)-phosphine oxide; mol. wt. 173.154] on cultures of human lymphocytes was investigated. With an increase in concentration of the mutagen from 0.125 to 16.0 μg/ml the cytogenetic effect increased: the proportion of aberrant metaphases rose from 6.0 to 61.0% and the total number of breaks from 7.96 to 116.3. A method of finding the smallest effective concentration of a test substance compared with a control is suggested, and for TEPA its value is 0.120 μg/ml. The fraction of chromatid breaks remained constant at 51.72% when different TEPA concentrations were used. The distribution of chromosome breaks among the cells is satisfactorily described by a geometric distribution.

KEY WORDS: cultures of human lymphocytes; chromosomal aberrations.

An important factor in the testing of chemical compounds for mutagenic activity is the determination of their smallest effective concentration compared with a control. In the recommended techniques [1] the need

Laboratory of Mutagenesis, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician N. P. Dubinin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 10, pp. 1265-1267, October, 1976. Original article submitted February 18, 1976.