of the chromosomes remained from each parental line. These workers note that all active NOR of chromosomes in the parental lines also remained active in the hybrids; reactivation of the NOR in one chromosome also was observed [10].

Unlike the authors cited, we investigated "human × hamster" hybrid cells; the human cells, moreover, were normal diploid human cells of embryonic origin. So far as relations between human and hamster chromosomes in hybrid clone MOM-8 are concerned our results agree with those of Wang et al. [9]. In our hybrids, elimination mainly of human chromosomes occurred. The simultaneous NOR activity and the interspecific associations which we found indicate that these "human × Chinese hamster" hybrids constitute a third type of hybrids with this phenomenon. The appearance of associative capacity between NOR of Chinese hamster chromosomes and depression of NOR of Chinese hamster chromosome III in hybrids of independent origin are evidence of complex changes in the regulation of NOR activity in the hybrid cells. It is an interesting fact, although one difficult to demonstrate, that NOR of human chromosomes in hybrid clones are more often stained brown, which is more characteristic of NOR of the Chinese hamster, or they stain a deep red color, and subsequent treatment with silver nitrate does not change the color of the stain.

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ACTIVITY OF NUCLEOLUS-ORGANIZING REGIONS OF CHINESE HAMSTER CHROMOSOMES IN CLONES OF DIFFERENT PLOIDY

G. D. Iashvili, V. G. Chernikov, and E. M. Pichugina

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Activity of nucleolus-organizing regions (NOR) in mammalian chromosomes, revealed by differential staining with silver nitrate [3-6], is subject to variation, for which hereditary factors are to some extent responsible [8-10]. Egolina et al. [1] showed that the number of Ag-staining NOR and the degree of affinity for the stain are genetically determined and they found intercellular individual variability in the number of Ag-stained NOR, which did not depend on technical procedures. However, contradictory data have been obtained on NOR activity in heteroploid cell cultures or in tumor and leukemic cells. Hubell and Hsu [7], for instance, described heteroploid human tumor cells with 18 acrocentric chromosomes; the number of NOR, moreover, was no greater than that in diploid cells. Meanwhile, Miller et al. [11, 12] report a tetraploid line, isolated from a mouse embryo, in which a proportional increase in NOR activity was observed. Mamaev et al. [2] investigated NOR of chromosomes of blood and bone marrow cells in acute leukemia and chronic myeloid leukemia, and also ovarian tumor cells by the silver staining method. They did not observe selective staining of NOR or inactivation

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

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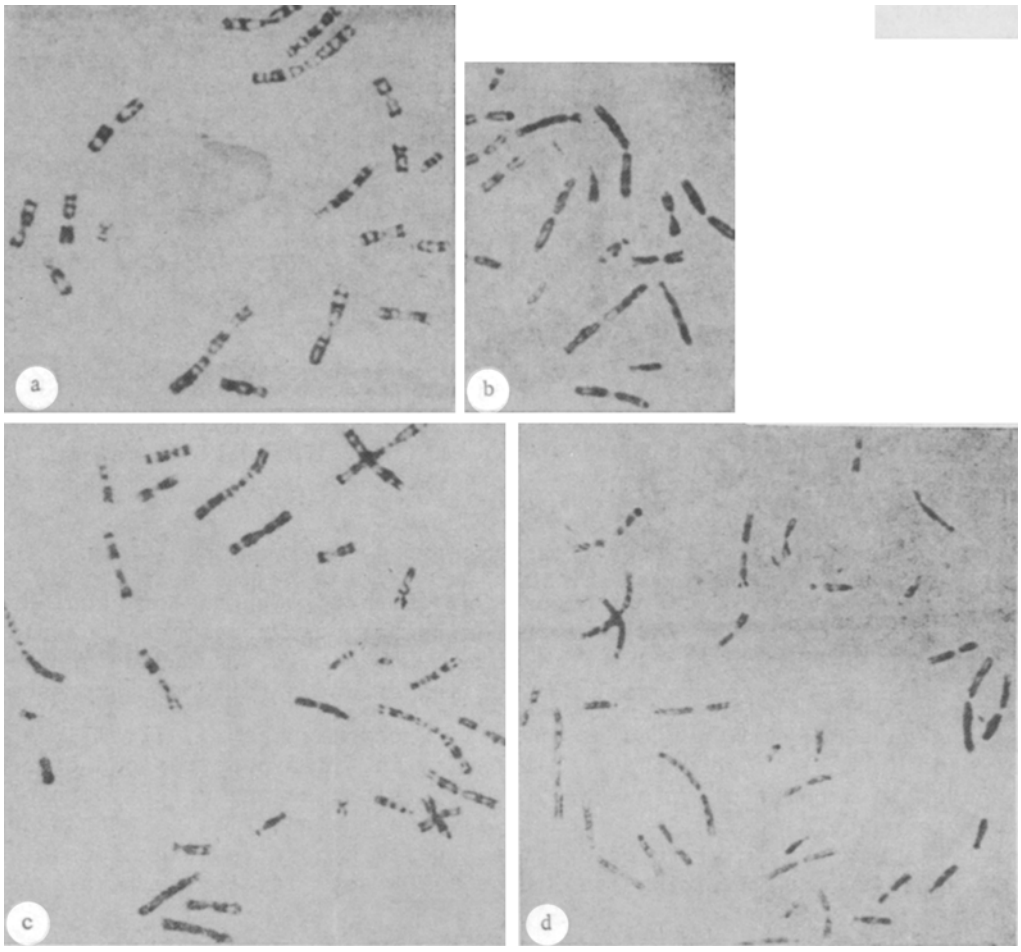

Fig. 1. Metaphase plates of M-15-2 (hypodiploid) and MO-2-3 (hypotetraploid) Chinese hamster cells: a) clone M-15-2, G stain; b) clone M-15-2, Ag stain; c) clone MO-2-3, G stain; d) clone MO-2-3, Ag stain. Magnification 770×.

of additional NOR in the tumor cell. These researchers conclude that in leukemias and tumors with a diploid number of chromosomes the number of stained NOR may be the same as in normal cells of the same individual, but with an increase in the number of chromosomes in the cells, the activity of ribosomal genes in the extra chromosomes may be preserved, and this leads to an increase in the total number of positively stained NOR.

The problem under discussion consists essentially of the study of the question whether the NOR activity of the corresponding chromosomes is autonomous and uncontrolled, i.e., depends purely on the genetically determined activity of each nucleolus-organizing chromosome, or whether these chromosomes, together with others, form a system in which the separate elements obey the principles of regulation and compensation depending on the physiology of the cell. Some light has been shed on this problem by analysis of hybrids or by comparing cell clones originating from the same ancestral clone and differing in ploidy. We investigated activity of NOR in Chinese hamster cells differing in ploidy.

EXPERIMENTAL METHOD

Clones M-15-2 and M, with modal chromosome numbers of 18 (hypodiploid) and 36 (hypotetraploid), respectively, of common origin (from 237_1 cells), and resistant to 6-mercaptopurine, were used. To isolate clones with increased ploidy, the selective agent ouabain (1 mM) was used. Resistance to ouabain is known to be due to a dominant mutation. For that reason, the probability of finding clones with increased ploidy is greater among cells surviving exposure to ouabain. From M-15-2 cells, clones MO-2-3, MO-2-4, and MO-2-5, resistant to ouabain, were isolated. Clone MO-1 was isolated from M cells. All ouabain resistant clones were hypotetraploid with a modal chromosome number of 36. The cells were cultured on Eagle's medium with

TABLE 1. NOR Activity in Hypodiploid and Hypotetraploid Clones*

Clone	Modal number of chromosomes	Mean number of active NOR per cell				
		chromosome I	chromosome II	chromosome III	chromosome IV	total for four chromosomes †
M-15-2	18	1,00	1,27	1,00	0,09	3,36±0,99
MO-2-3	36	1,80	2,40	1,92	0,28	6,40±1,33
MO-2-4	36	1,72	2,44	1,96	0,32	6,28±1,73
MO-2-5	36	1,76	2,40	1,92	0,28	6,36±1,56
M	36	1,76	2,44	1,96	0,28	6,44±1,58
MO-1	36	1,80	2,44	1,92	0,32	6,40±1,68

*In each clone 25 metaphase plates were studied.
†Values of the 95% confidence interval for the mean also are given.

20% bovine serum. Chromosome preparations were obtained by the standard air-drying method. Chromosomes were stained by the G-method using trypsin [13]. For silver staining the method of Goodpasture and Bloom [4] was used. NOR activity was analyzed only in metaphases with 18 and 36 chromosomes.

EXPERIMENTAL RESULTS

No changes in karyotype were found in clone MO-1 compared with the original M cells. In clones MO-2-3, MO-2-4, and MO-2-5 an increase in the number of chromosomes took place on account of doubling of all chromosomes of the original M-15-2 cells, including nucleolus-organizing chromosomes, without any preferential duplication of any particular chromosomes (Fig. 1).

Silver staining revealed four nucleolus-organizing chromosomes, I, II, III, and IV, in the clones. The data in Table 1 show that NOR activity in all hypotetraploid clones was almost twice as high as in hypodiploid clone M-15-2. In this case the effect both of the mutation of resistance to ouabain itself and of short-term selection of cells with ouabain for manifestation of NOR activity can be ruled out, because in hypotetraploid clone M, not treated with ouabain, NOR activity was indistinguishable from NOR activity in the remaining hypotetraploid clones.

The results described above indicate that a change in ploidy in clonal derivatives leads to a proportional increase in NOR activity which is stable. This supports autonomy of manifestation of NOR activity and absence of features of regulation or compensation, in agreement with data obtained on tumor and leukemic cells [2].

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