# SIMULTANEOUS ACTIVITY OF NUCLEOLUS-ORGANIZING REGIONS OF HUMAN AND CHINESE HAMSTER CHROMOSOMES IN HYBRID SOMATIC CELLS

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Eliceiri and Green [3] first showed in hybrid "human × mouse" somatic cells that the 28S rRNA locus is transcribed only on mouse chromosomes. It was later shown that rRNA only of that species whose chromosomes are not eliminated is produced in interspecific somatic hybrids. This observation was confirmed in a study [5] in which rodent chromosomes were eliminated and, despite the presence of the nucleolus-organizing chromosomes of the rodents, activity of nucleolus-organizing regions (NOR) was observed only on human chromosomes. Meanwhile, simultaneous transcription of the 28S rRNA of both species was found in "Syrian hamster × mouse" hybrid cells [2, 8], but later, activity of NOR of the chromosomes of both species was discovered [6] in one cell. These same researchers showed association between chromosomes of different species. These observations proved that one hybrid cell can produce 28S rRNA of different species. It was also shown later by the silver impregnation method [10] that simultaneous activity of NOR of the chromosomes and associations between chromosomes of mouse and Chinese hamster occur in hybrid cells.

Simultaneous activity of NOR of the parental chromosomes and the appearance of associations between them in hybrid rodent cells have thus now been demonstrated.

The object of this investigation was to study simultaneous activity of NOR and associations between human and Chinese hamster chromosomes in hybrid cells in which normal human embryonic fibroblasts or muscle cells were used as the parental line.

## EXPERIMENTAL METHOD

Transplantable hypotetraploid (2n = 36) Chinese hamster cells (clone M), deficient for hypoxanthine phosphoribosyltransferase, transplantable hypotetraploid (2n = 36) Chinese hamster cells (clone MO-1), deficient for hypoxanthine phosphoribosyltransferase and resistant to 1 mM ouabain; normal human embryonic fibroblasts (strain F20, 46, XX); normal human embryonic muscle cells (strain IMG 812; 46, XY); hybrid cells (clone MF-2, 15th passage), obtained by fusion of M cells with F20 cells; hybrid cells (clone MOM-8, 15th passage) obtained by fusion of MO-1 cells with IMG 812 cells, and two subclones of hybrid cells, MOM-8-1 (75th passage) and MOM-8-3 (83rd passage), obtained from clone MOM-8, were used in the experiments. The method of obtaining hybrid cells was described previously [1]. The cells were cultured in Carrel flasks on Eagle's medium with the addition of 20% bovine serum without antibiotics. Chromosome preparations were obtained by the standard air-drying method. Chromosomes were stained by the G method, using trypsin [7]. The method in [4] was used for silver staining.

## EXPERIMENTAL RESULTS

M and MO-1 Chinese hamster cells have four nucleolus-organizing chromosomes designated I, II, III, and IV. Chromosomes I, II, and III are metacentric, in which the NOR is located in the telomere region of the long arm. Nucleolus-organizing chromosome IV is large and acrocentric, with the NOR in the distal end of the long arm. With respect to location of NOR, chromosomes of the Chinese hamster thus are in clear contrast with human chromosomes, in which these regions are located in the short arms of 10 acrocentric chromosomes.

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TABLE 1. Number of Human and Chinese Hamster Chromosomes in a Series of Hybrid Cells of Clone MOM-8 at the 15th Passage

| Serial No. of metaphase plate | Human (46 chromosomes) | Chinese hamster (36 chromosomes) | Total (82 chromosomes) | |
|---|---|---|---|---|
| | | | identified chromosomes | unidentified chromosomes and fragments |
| 1 | 5 (10,9) | 25 (69,4) | 30 (36,6) | 0 (0) |
| 2 | 7 (15,2) | 33 (91,7) | 45 (54,9) | 5 (6,1) |
| 3 | 4 (8,7) | 21 (58,3) | 36 (43,9) | 11 (13,4) |
| 4 | 4 (8,7) | 33 (91,7) | 37 (45,1) | 0 (0) |
| 5 | 9 (19,6) | 40 (111,1) | 52 (63,4) | 3 (3,6) |
| 6 | 3 (6,5) | 29 (55,6) | 23 (28,0) | 0 (0) |
| 7 | 6 (13,0) | 31 (86,1) | 37 (45,1) | 0 (0) |
| 8 | 9 (19,6) | 32 (89,9) | 42 (51,2) | 1 (1,2) |
| 9 | 7 (15,2) | 32 (89,9) | 39 (47,6) | 0 (0) |
| 10 | 6 (13,0) | 41 (113,9) | 49 (59,7) | 2 (2,4) |
| Extreme values | 3—9 (6,6—19,6) | 20—41 (55,6—113,9) | 23—49 (28,0—59,7) | 0—11 (0—13,4) |
| Mean value | 6,0 (13,0) | 30,8 (85,6) | 39,0 (47,6) | 2,2 (2,7) |

Legend. Number of chromosomes (in % of original parental set) given in parentheses.

TABLE 2. Percentage of Cells with Associations in Parental and Hybrid Cells

| Type of associations | Strain of human cells | | Clone of Chinese hamster cells | | Hybrid clone | | | |
|---|---|---|---|---|---|---|---|---|
| | IMG-812 (50) | F-20 (50) | MO-1 (50) | M (50) | MOM-8 (90) | MOM-8-1 (90) | MOM-8-3 (90) | MF-2 (50) |
| Hamster — hamster | — | — | 0 | 0 | 11,1 | 32,2 | 33,3 | 38,0 |
| Hamster — human | — | — | — | — | 4,4 | 8,9 | 3,3 | 8,0 |
| Human — human | 28,0 | 32,0 | — | — | 8,9 | 0 | 0 | 8,0 |
| Total | 28,0 | 32,0 | 0 | 0 | 21,1 | 35,5 | 33,3 | 48,0 |

Legend. Number of metaphases studied shown in parentheses.

TABLE 3. Activity of NOR of Chromosomes in Parental and Hybrid Cells

| Clone and strain | Number of metaphases studied | Mean number of NOR per cell | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Chinese hamster chromosomes | | | | human chromosomes | | total activity |
| | | I | II | III | IV | D | G | |
| MO-1 | 25 | 1,8 | 2,4 | 1,9 | 0,3 | — | — | 6,4 |
| IMG 812 | 50 | — | — | — | — | 4,8 | 3,5 | 8,3 |
| MOM-8 | 90 | 0,8 | 0,8 | 0 | 0,04 | 0,2 | 0,03 | 1,9 |
| MOM-8-1 | 90 | 0,9 | 0,8 | 0 | 0,6 | 0,1 | 0 | 2,4 |
| MOM-8-3 | 90 | 1,0 | 0,9 | 0 | 0 | 0,1 | 0 | 2,0 |
| M | 25 | 1,8 | 2,4 | 2,0 | 0,3 | — | — | 6,5 |
| F-20 | 50 | — | — | — | — | 4,9 | 3,6 | 8,5 |
| MF-2 | 50 | 1,2 | 1,2 | 0 | 0,1 | 0,1 | 0,1 | 2,7 |

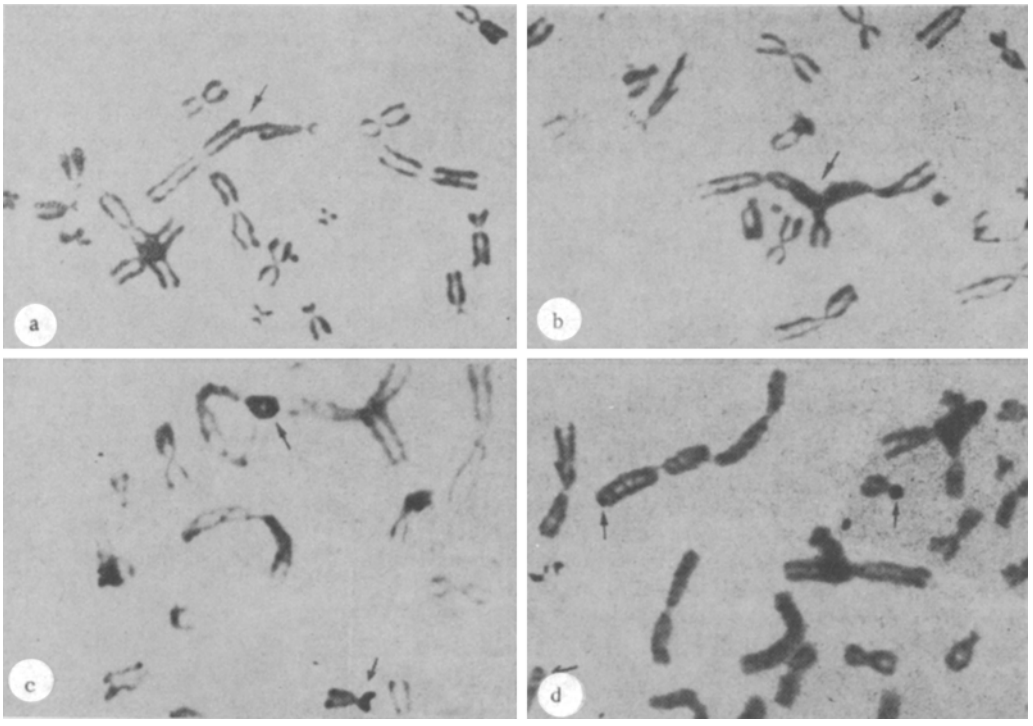

Fig. 1. Fragments of metaphase plates of hybrid cells. a) Arrow indicates association between NOR of Chinese hamster chromosomes I and IV; b) arrow indicates association between two NOR of Chinese hamster chromosomes I and I and NOR of human chromosome D; c, d) long arrows indicate NOR of Chinese hamster chromosomes, short arrows NOR of human D chromosomes.

The chromosome composition of hybrid clone MOM-8 is given in Table 1. Cells of this clone, by the 15th passage, contained 13% of chromosomes from the original number of the human parental chromosome set and 85.6% of Chinese hamster chromosomes. The mean number of identified and unidentified chromosomes in the hybrid cells was 41.2%.

It will be clear from Table 2 that Chinese hamster chromosomes do not form associations in parental clones MO-1 and M, whereas in human IMG 812 and F20 cells the number of cells with associations is considerable (28-32%). Both intraspecific and interspecific associations are observed in the hybrid clones (Fig. 1a, b), with the exception of hybrids MOM-8-1 and MOM-8-3, which had almost completely lost the acrocentric human chromosomes at times corresponding to the 75th and 83rd passages, so that no human–human associations were observed. Associations of the hamster–hamster type were most numerous in the hybrid cells.

Simultaneous activity of NOR of human and Chinese hamster chromosomes was observed in all hybrid clones (Fig. 1c, d). In clone MOM-8 the fraction of cells with simultaneous activity of NOR was 18.9%, in clone MOM-8-1 it was 12.2%, in clone MOM-8-3 7.8%, and in clone MF-2 it was 20%. In this case also the decrease in the fraction of cells with simultaneous NOR activity in clones MOM-8-1 and MOM-8-3 was connected with greater elimination of the human chromosomes than in clones MOM-8 and MF-2.

Table 3 gives results showing a decrease in NOR activity of human and Chinese hamster chromosomes in the hybrid cells compared with parental cells. Complete suppression of NOR of Chinese hamster chromosome III was noted. Total NOR activity of the chromosomes per cell was much lower in the hybrids than in the parental lines; the difference, moreover, was statistically significant ($P < 0.01$) in hybrids of independent origin (1.9 in clone MOM-8 and 2.7 in clone MF-2), whereas there was virtually no difference between the parental lines of these two hybrid clones.

Transcription of rRNA of both species [2, 8] and NOR activity of the chromosomes of both parents [6, 10] were found previously. It must be noted that in the investigations cited the parental lines were transplantable cells. These hybrids, so far as chromosome elimination is concerned, were regarded by the researchers concerned as codominant [6, 10], for more than 50%

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.

LITERATURE CITED


1. K. N. Grinberg, G. D. Iashvili, E. M. Pichugina, et al., Byull. Éksp. Biol. Med., No. 11, 85 (1982).
2. G. L. Eliceiri, J. Cell Biol., 53, 177 (1972).
3. G. L. Eliceiri and H. J. Green, J. Mol. Biol., 80, 253 (1969).
4. C. Goodpasture and S. E. Bloom, Chromosoma, 53, 37 (1975).
5. D. A. Miller, V. G. Dev, R. Tantravahi, et al., Cytogenet. Cell Genet., 21, 33 (1978).
6. O. J. Miller, V. G. Dev, R. Tantravahi, et al., Exp. Cell Res., 115, 457 (1978).
7. M. Soabright, Lancet, 2, 971 (1971).
8. D. Toniolo and G. Basilico, Nature, 248, 411 (1974).
9. H. S. Wang, V. Niewczas, H. R. de S. Nazareth, et al., Cytogenet. Cell Genet., 24, 233 (1979).
10. L. G. Weide, V. G. Dev, and C. S. Rupert, Exp. Cell Res., 123, 424 (1979).


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


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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


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