Positioning of the NOR-bearing chromosomes in relation to nucleoli in daughter cells after mitosis

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Summary

It is known that chromosomes occupy non-random positions in the cell nucleus. But it is not clear to what extent their nuclear positions, together with their neighbourhood, are conserved in daughter cells. To address specific aspects of this problem, we used the model of the chromosomes carrying ribosomal genes, that are organized in clusters termed Nucleolus Organizer Regions (NORs). We compared the association of chosen NOR-bearing chromosomes (NOR-chromosomes) with nucleoli, as well as the numbers of nucleoli, in the pairs of daughter cells, and established how frequently the daughter cells had equal numbers of the homologs of certain NOR-chromosomes associated with individual nucleoli. The daughter cells typically had different numbers of nucleoli. At the same time, using immuno-FISH with probes for chromosomes 14 and 15 in HeLa cells, we found that the cell pairs with identical combinations appeared significantly more frequently than predicted by the random model. Thus, although the total number of chromosomes associated with nucleoli is variable, our data indicate that the position of the NOR-bearing chromosomes in relation to nucleoli is partly inherited through mitosis.

Key words: chromosome positioning, nucleoli, NORs, daughter cells

Introduction

Chromosomes are not randomly arranged in the vertebrate cell nucleus (Cremer and Cremer 2001, Cremer and Cremer 2006, Foster and Bridger 2005, Parada and Misteli 2002, Pederson 2004). But it is not clear to what extent their nuclear positions, together with their neighbourhoods, are conserved in daughter cells (Bickmore and Chubb 2003). Using similar experimental approaches, the results of recent studies argued that the chromosomes were arranged similarly in maternal and daughter cells (Essers *et al.* 2005, Gerlich *et al.* 2003), or that positions of chromosomes in daughter nuclei were conserved only partly and in most cases largely differed from the positions seen in mother cell nuclei (Walter *et al.* 2003).

To address specific aspects of this problem, we used the model of the chromosomes carrying ribosomal genes. These genes are organized in clusters termed Nucleolus Organizer Regions (NORs) (McClintock 1934, Bush and Smetana 1970). Nucleoli disintegrate during mitosis, and at the beginning of the next G1 phase NORs from more than one chromosome cluster and participate in the formation of a given nucleolus (Raška, 2003, Raška *et al.* 2004). In the middle of G1 phase the position of chromosomes and the number of nucleoli in the nucleus are already stable and do not change significantly until the end of the interphase (Walter *et al.* 2003, Cremer and Cremer 2006, Foster and Bridger 2005, Parada and Misteli 2002). We therefore selected for our study pairs of daughter cells in this period of the cell cycle, and compared the association of chosen NOR-bearing chromosomes (NOR-chromosomes) with nucleoli in the pairs of daughter cells from the human derived HeLa cell line. We thus did not

investigate the maternal cell with regard to the daughter cells, but focused on the similarity between the two daughter cells.

The aim of our study was to establish how frequently the daughter cells had equal numbers of the homologs of certain NOR-chromosomes associated with individual nucleoli. Since the inheritance of the chromosome positioning in relation to nucleoli depends on the number of nucleoli per nucleus, we also compared the numbers of nucleoli in the two daughter cells. It should be mentioned that the approach used here did not allow us discriminate between the individual homologs of the chromosomes associated with each nucleolus.

Methods

HeLa cells were cultivated in flasks at 37°C in Dulbecco modified Eagle's medium (DMEM, Sigma, USA) containing 10% fetal calf serum, 1% glutamine, 0.1% gentamycin, and 0.85g/l $NaHCO_3$ in atmosphere supplemented with 5% CO_2. The preparations of the couples of postmitotic cells were obtained by shaking and seeding mitotic cells on the glass coverslips. In such procedure we could get sufficient numbers of clearly distinguished pairs of the postmitotic daughter cells. In vivo time-lapse observations encompassing a period from mitosis to mid G1 showed that the cells of different pairs did not mix during this period (data not shown).

Commercial Cy3- and FITC- labeled whole chromosome painting probes for human chromosomes 13, 14, 15, 21 and 22, supplied ready to use in hybridization mixture (Appligene Oncor, USA). Primary monoclonal antibody against mouse fibrillarin (clone 17C12), kindly donated by Kenneth M. Pollard (Scripps Research Institute, La Jolla, CA), was used for immunovisualization of nucleoli. Secondary anti-mouse antibodies (Jackson ImmunoResearch Laboratories) were conjugated with Cy3 or FITC.

The combined detection of fibrillarin and *in situ* hybridization (immuno-FISH) was performed after Pliss *et al.* (2005). After fibrillarin imunolabeling the cells were postfixed with methanol/acetic acid (3:1) overnight at -20°C, then the regular FISH procedure followed (Pliss *et al*. 2005), except the post hybridization washing. Namely, the cells were washed in 50% formamide in 2xSSC, pH 7, for 15 min at 43°C, in 0.1% Tween-20 /2xSSC for 8 min at 43°C; in 0.1% Igepal (ICN Biomedicals, Inc) / 4xSSC for 3 x 4 min at 37°C, in PBS 3 x 3 min at RT (Harničarová *et al*. 2006). Coverslips were mounted in Mowiol supplemented with DABCO and viewed using Olympus AX70 Provis equipped with the Photometrics CCD camera.

Results

Nuclei of HeLa cells contained usually 2-5 nucleoli, with average number 4.03 ± 0.12 (Kalmárová *et al*. 2007). The number of nucleoli were most frequently different in the daughter cells (Fig. 1). Specifically, in 77% cases, the daughter cells contained different numbers of nucleoli. We additionally compared our data with a random model. In this model the appearance of the pairs of daughter cells with *i* and *j* nucleoli was calculated as product of the experimentally found frequencies of the cells with *i* and *j* nucleoli. Comparing the incidence of the nucleoli in 100 pairs of daughter cells, we found a close correspondence with the random model (Fig. 1).

Next we visualized chromosomes 14 and 15, performing hybridization with Cy3- and FITC-labeled probes, in combination with immuno labeling of nucleoli using antibody against fibrillarin. The HeLa cells typically possess four homologs of chromosome 15 and three homologs of chromosome 14. Different numbers of these chromosomes can be associated with each nucleolus (Kalmárová *et al.* 2007). Accordingly, different cells may have different combinations of the nucleolar association. In case of the chromosome 15, all four homologs are nucleoli-associated (Kalmárová *et al.* 2007, Smirnov *et al.* 2006). For instance, five

combinations are possible in cells with four nucleoli (Fig. 2). In one extreme situation, all four chromosomes are associated with one nucleolus. In the other extreme sitiuation, there is one chromosome associated with each of the nucleoli (Fig. 3, A-C). In case of the chromosome 14, not all homologs are associated with nucleoli (Fig. 3, D-F) (Kalmárová *et al*. 2007, Smirnov *et al.* 2006), which increases the number of possible combinations to seven (Fig. 2). Comparing these combinations in the daughter cells, we surprisingly found that in 50% of cell pairs, for both chromosome 14 and chromosome 15, the combinations were identical (Table). To evaluate these data, we used a random pairing model in which appearance of the pairs of daughter cells with combinations *i* and *j* was calculated as product of the experimentally found frequencies of the cells with the combinations *i* and *j*. The pairs with identical combinations appeared with significantly higher frequency in the experiment (50%) than in the random model (32% for chromosome 15 and 25% for chromosome 14) (Table).

Chromosomes	**Frequency of symmetrical distribution**		
	Experiment	**Random model**	**n**
15	0.50	0.32	160
14	0.50	0.25	100

Table. Similarity of the position of the NOR-bearing chromosomes with respect to nucleoli in the daughter cells. In 50% of the cell pairs the combinations are identical for both chromosome 14 and chromosome 15, which significantly exceeds the values predicted by the random model.

Additionally, in the case of chromosome 14 we observed a significant symmetry in the distribution of the non-associated chromosomes after mitosis: in 62% cases the daughter cells had equal number of such chromosomes, while the random model predicted only 44%.

Discussion

In this study we observed that the daughter cells typically had different numbers of nucleoli (Fig. 1). Such an asymmetry, observed also by other authors (see e.g. Leung *et al.* 2004), is not entirely compatible with the claim that global chromosome positions are basically heritable through mitosis (Gerlich *et al*. 2003). Interestingly, in spite of this asymmetry, we found that chromosomes 14 and 15 showed a similar pattern of nucleolar associations more frequently than predicted by the random pairing model (Fig. 3, Table). Taken together, our results support the view of Walter *et al*. (2003), according to which there is only a limited similarity in chromosome positioning between the daughter cells.

Thus, although the total number of chromosomes associated with nucleoli is variable, our data indicate that the distribution of the NOR-bearing chromosomes among the nucleoli is partly conserved through mitosis.

Acknowledgments

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Legends

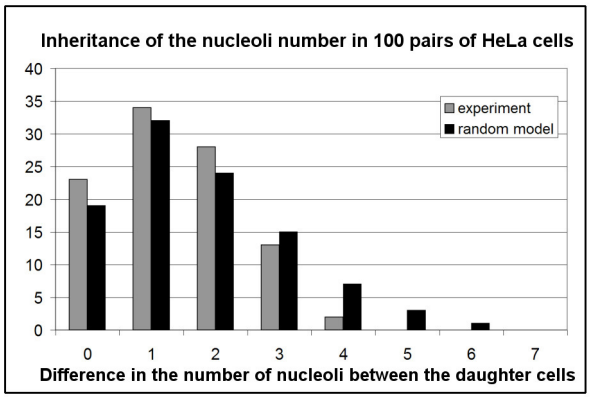

Fig. 1. Differences in the nucleoli number between the daughter HeLa cells.

The number of nucleoli in the daughter cells most frequently differed by one (grey bars). Only in 23% of cell pairs, the number of nucleoli was identical. Observed differences in the number of nucleoli between the daughter cells closely corresponded to those in the random model (black bars).

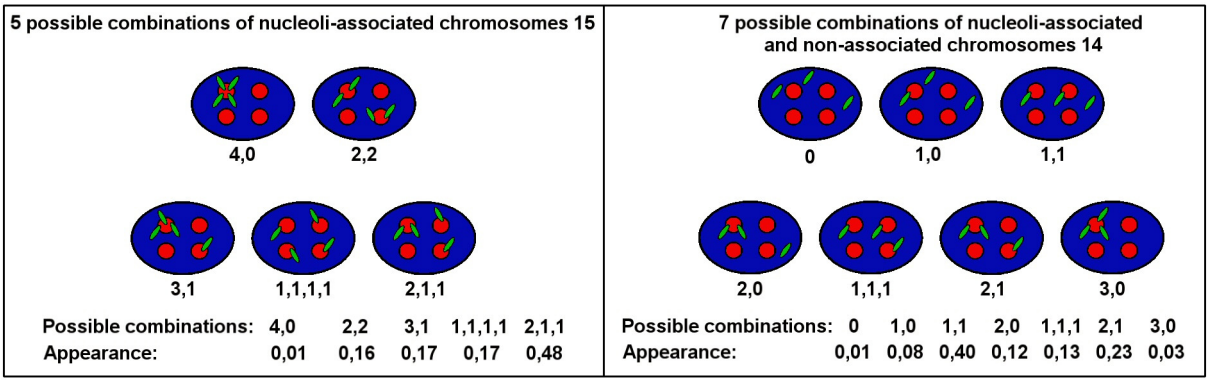

Fig. 2 Scheme depicting relations between nucleoli (red) and chromosomes 14 and 15 (green) in the cell nucleus (blue): all possible combinations of the nucleolar associations for the case of cells with four nucleoli are shown. All chromosomes 15 are associated with nucleoli, but some chromosomes 14 are not nucleoli-associated.

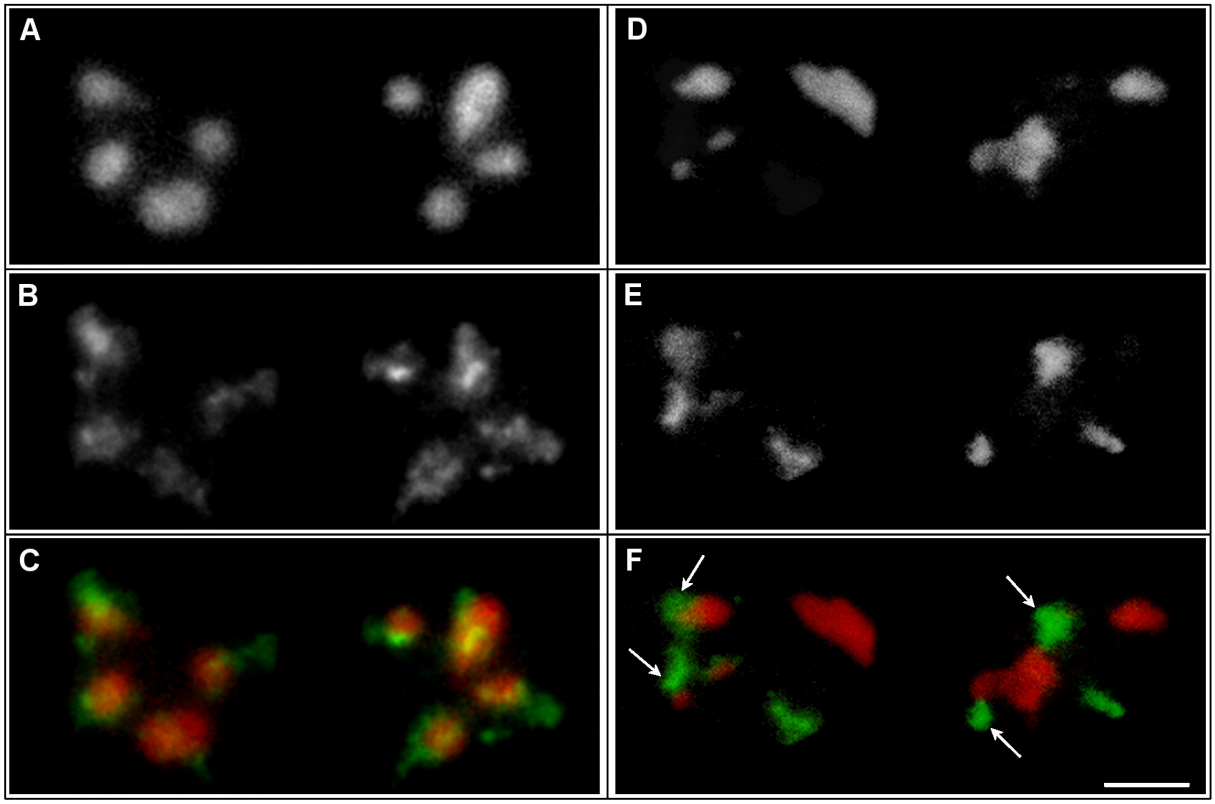

Fig. 3 Combinations of positions of chromosomes 14 and 15 in relation to nucleoli as compared in daughter HeLa cells. FISH signal with the specific probes for chromosomes 14 and 15 (B, E; green in C, F) in a couple of interphase daughter cells. Immunocytochemistry with fibrillarin was used to visualize nucleoli (A, D; red in C, F). In the chosen example of chromosome 15 (A, B, C), all four homologs are associated with nucleoli, deeply penetrating into them, which is typical for the chromosome 15 (Kalmárová *et al.* 2007). This case corresponds to the combination (1, 1, 1, 1) in Fig. 2. In the chosen example of chromosome 14 (D, E, F), two chromosome homologs are nucleoli associated (arrows in F), and one is separated from the nucleoli. This case corresponds to the combination (1, 1) in Fig. 2. Bar: 10 μm.