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Relationship between large-scale chromatin organization and nucleolus in human cells

PhD thesis-short report

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Contents:

Abbreviations ______ *4*

1. General introduction ______ *5*

1.1. Chromosome territories and their positioning in the interphase nucleus ______ 5

1.2. Positioning of chromosomes as compared in the daughter cells after mitosis ______ 6

1.3. The nucleolus ______ 7

1.4. Nucleolus Organizer Regions (NORs) ______ 8

2. Specific aims of the thesis ______ *10*

3. Materials and Methods ______ *11*

3.1. Cell culture ______ 11

3.2. DNA probes and antibodies ______ 11

3.3. Immunofluorescence ______ 12

3.4. Immuno-FISH and FISH ______ 12

3.5. Microscopes used ______ 12

3.6. Statistical evaluation ______ 12

4. Results and discussion ______ *13*

4.1. NORs and their transcription competence during the cell cycle. ______ 13

4.2. Positioning of NORs and NOR-bearing chromosomes in relation to nucleoli 14

4.3. Positioning of the NOR-bearing chromosomes in relation to nucleoli in daughter cells after mitosis ______ 15

5. Conclusions ______ *16*

6. Summary ______ *17*

7. References ______ *19*

8. Publications and Presentations of Markéta Kalmárová (Fialová) ______ *25*

Abbreviations

DABCO	1,4-diazabicyclo[2.2.2]octane
DAPI	4',6-diamidino-2-phenylindole
DMEM	Dulbecco modified Eagle's medium
EM	electron microscopy
FISH	fluorescence *in situ* hybridization
GFP	green fluorescent protein
HeLa	human cells, derived from adenocarcinoma cervix cells
LEP	diploid human embryonic lung fibroblast cells
LM	light microscopy
NORs	Nucleolus Organizer Regions
PBS	Phosphate Buffered Saline
PCC	premature chromosome condensation
Pol I	RNA polymerase I
rDNA	ribosomal genes
RT	room temperature
SSC	standard sodium citrate
UBF	Upstream Binding Factor

1. General introduction

Discovered by Brown in 1831, cell nucleus continues to be an object of great fascination for scientists today, because chromosomes are deposited, and the first steps gene expression take place, in this organelle.

1.1. Chromosome territories and their positioning in the interphase nucleus

Chromosomes exist as distinct condensed structures during mitosis. But their organization in the interphase nucleus represents a special problem. The first experimental evidence for existence of interphase chromosomes in the form of the chromosome territories was provided in 1970s by Cremer and colleagues by inducing focal DNA damage with a laser UV microbeam (Cremer et al., 1974; Lamond and Sleeman, 2003; Cremer and Cremer, 2006). Further understanding of these territories was achieved using isotopic and non-isotopic *in situ* hybridization with genomic DNA probes (Manuelidis, 1985). Following the development of fluorescence *in situ* hybridization (FISH) with chromosome-specific DNA libraries, the territorial organization of chromosomes became accepted scientific dogma (Cremer et al., 1988; Lichter et al., 1988). More recently, introduction of green fluorescent protein (GFP) (Robinett et al., 1996; Belmont and Straight, 1998) and incorporation of fluorescently tagged nucleotides into DNA, have facilitated the visualization of genome dynamics in living cells. It was confirmed that each chromosome does indeed occupy its own territory (Foster and Bridger, 2005). Based on statistical evaluation of the chromosome territories positioning, numerous studies have shown that, in vertebrate cells, the chromosome territories are non-randomly arranged in the nuclear space (Cremer and Cremer, 2001; Parada and Misteli, 2002; Cremer and Cremer, 2006). It has been found that gene-rich chromosomes tend to be located towards the nuclear interior, whereas gene-poor chromosomes tend to be located towards the periphery (Bridger and Bickmore, 1998; Parada and Misteli, 2002; Gilbert et al., 2005). Chromosomes also may be positioned in a size-dependent manner, with the larger chromosomes located more peripherally (Sun et al., 2000; Bolzer et al., 2005). However, there are some cases where the position of chromosomes varies whereas the gene density of chromatin has not changed. Changes in the spatial organization of chromosomes and genes have been observed in several diseases, including cancer, epilepsy and laminopathies,

a group of diseases caused by mutation in the nuclear protein lamin A/C. It is believed that particular chromosome positioning places genes into special neighborhood favorable for their expression or silencing (Parada and Misteli, 2002). But the situation is complicated by recent data indicating that chromosome territories partially intermingle in human cell nuclei (Branco and Pombo, 2006). Moreover, some genes can be found in loops extended far beyond the area defined as chromosome territory (Baxter et al., 2002; Mahy et al., 2002; Chubb and Bickmore, 2003; Kioussis, 2005; Wegel and Shaw, 2005).

Thus, the rationales for the large-scale order of chromosome positioning, and its relation to gene activity, remain to a large extent unclear.

1.2. Positioning of chromosomes as compared in the daughter cells after mitosis

Non-random territory organization implies the need for a mechanism to sucessfully convey chromosome positional information to daughter nuclei (cellular memory) (Williams and Fisher, 2003). Inheritance of interphase chromosomal order was considered by Boveri in 1909, on the basis of his studies of nematode chromosomes (e.g. Bickmore and Chubb, 2003). Boveri proposed that chromosome arrangements are similar in the two daughter nuclei. Early studies on some plant species supported this view (Heitz, 1932, after Walter et al., 2003). According to the more recent studies, the chromosome positioning is largely maintained in some species (Abranches et al. 1998; Santos et al., 2002) whereas the results obtained on mammalian cells were controversial. Thus, FISH experiments suggested a strong correlation of the whole chromosomes and centromeres positioning within each pair of cells derived from the same mother cell (Sun and Yokota, 1999). In other studies (Gerlich et al., 2003; Walter et al., 2003; Essers et al., 2005) fluorescence live cell imaging techniques were used to investigate whether chromosome position in mammalian cells can be maintained from a mother nucleus through the events of mitosis, and into the two daughter nuclei. Some authors claimed that the chromosome arrangements were similar in daughter cells (Gerlich et al., 2003 Essers et al., 2005), while others observed that positions of chromosomes in daughter nuclei differed significantly from the positions seen in mother cell nucleus (Walter et al., 2003). Although differences in experimental procedure could be responsible for these divergent results (Bickmore and Chubb, 2003), further studies of the chromosome positioning in the daughter cells are needed to elucidate this problem.

1.3. The nucleolus

Discovered in XVIII century, nucleolus is the most prominent of all nuclear subcompartments. The early observations of the nucleolus as a subcellular structure remained largely descriptive until the early 1960s. It was then established that nucleolus contains ribosomal genes that are transcribed, and ribosomal subunits are generated in this organelle (Busch and Smetana, 1970; Hadjiolov 1985; Raška et al., 1990; Scheer and Hock, 1999; Olson et al., 2000; Fatica and Tollervey, 2002).

Three major substructures can be distinguished in the nucleoli at the ultrastructural level: the fibrillar center, the dense fibrillar component, and the granular component. The fibrillar center (FC) contains ribosomal DNA (rDNA) and at least some of the essential components of Pol I transcription machinery. Dense fibrillar component (DFC) usually forms a rim around the FC and sometimes protrudes into the FC. DFC contains a high concentration of ribonucleoproteins, and is, therefore, electron-dense (Raška, 2003). Transcription of rDNA occurs apparently in this region including the boundary between FC and DFC (Koberna et al., 2002). Also the first steps in the pre-rRNA processing take place in this nucleolar subcompartment (e.g., Shaw and Jordan, 1995). The third nucleolar subcompartment is granular component (GC), where the later steps of pre-rRNA processing and formation of pre-ribosomal take place. In addition to these substructures, chromatin structures and nucleolar interstices can be seen within nucleoli. Importantly, nucleoli are surrounded with a mass of heterochromatin. This mass is believed to be composed to a large extent of DNA sequences from the NOR-bearing chromosomes (Stahl, 1976; Kaplan et al., 1993), the corresponding ribosomal genes apparently being encompassed within the nucleolus itself. Some chromosomes, besides NOR-bearing chromosomes, are also known to be regularly associated with nucleolus (Stahl, 1976; Ochs and Press, 1992; Kaplan et al., 1993; Sullivan et al., 2001).

In metabolically active mammalian and plant cells as well as in yeast, the nucleolus contains tens to hundreds of active ribosomal genes that are usually characterized by high levels of transcription accounting for about a half of the total cellular RNA production (Raška et al., 2006). This is understandable since the proteins perform most of the everyday work in the cell, and the production of ribosomes, that are protein “manufactures”, is essential for cell metabolism.

The functional analysis of nucleolar structures with respect to the metabolic processes has been significantly expanded. The new findings include ultrastructural identification of nascent molecules of ribosomal RNA as well as active ribosomal genes (Koberna et al., 2002). Microscopic approaches enabled to identify, although so far only in yeast, some key steps in maturation of pre-ribosomal RNA (Osheim et al., 2004).

The most spectacular progress in our knowledge of nucleolus was provided by functional proteomic analysis. Less than 200 different proteins were identified in human nucleolus at the very beginning of 21st century. With implementation of the nucleolar proteomics, this number increased to 350 in the year 2002, and in 2005 to almost 700. Current studies of the isolated nucleoli from various types of human cells suggest presence of more than 2000 proteins, although not all these nucleolar proteins were identified in each of the examined cell types (Lam et al., 2007).

Functional proteomic analysis of nucleoli not only helped us to answer a number of “ribosomal” questions, but also confirmed and expanded our knowledge of the non-ribosomal nucleolar functions. It seems that nucleolus plays a role in the control of cell cycle, senescence and regulation of telomerase activity, oncogene activities and tumor suppression, cell stress reactions, metabolism of mRNA molecules, maturation of the “extranucleolar” RNAs and control of viral infections. These functions are not well understood (Olson et al., 2002; Raška et al., 2006, Boisvert et al., 2007).

1.4. Nucleolus Organizer Regions (NORs)

Ribosomal genes coding 5.8S, 18S and 28S rRNA form arrays of tandem repeats at specific chromosomal sites termed nucleolus organizer regions (NORs). In humans, NORs are located on the short arms of the acrocentric chromosomes 13, 14, 15, 21 and 22 (Henderson et al., 1972; Long and David, 1980; Puvion-Dutilleul et al., 1991). Some NORs can be identified as secondary constrictions on metaphase chromosomes, or visualized by immunocytochemistry or silver staining, due to the abudance of specific associated proteins (Goodpasture and Bloom, 1975; Weisenberger and Scheer, 1995; Roussel et al., 1996; Gebrane-Younes et al., 1997). It is generally accepted that such NORs, termed "transcriptionally competent" or "competent" (Dousset et al., 2000; Savino et al., 2001), are transcriptionally active during previous interphase (Weisenberger and Scheer, 1995; Roussel

et al., 1996; Gebrane-Younes et al., 1997). The proteins responsible for this unique chromatin structure are components of the RNA polymerase (pol I) transcription machinery, including Upstream Binding Factor (UBF), which remain associated with NOR during mitosis when pol I transcription is repressed (Weisenberger and Scheer, 1995; Jordan et al., 1996; Roussel et al., 1996). UBF binding confers to NORs a unique architecture (e.g. McStay, 2006). The reactivation of pol I transcription starts in late anaphase. Primary nucleoli reform around the active NORs at the end of mitosis (Ochs et al., 1985; Benavente et al., 1987; Jimenez-Garcia et al., 1994) and fuse into final nucleoli in early G1. It should be also noted that short arms of human acrocentric chromosomes contain, apart from ribosomal genes, also some repetitive sequences (Kaplan et al., 1993; Sullivan et al., 2001).

Transcription activity of rDNA can be regulated at the level of whole NORs, which is particularly seen in the hybrid cells as so called "nucleolar dominance" (McStay, 2006, Stefanovsky and Moss, 2006; Lewis et al., 2007; Preuss and Picaard, 2007).

Relationships among the NORs, nucleoli and chromosome territories apparently play important role in the functioning of nucleus, and all these relationships are not sufficiently understood. Also, a synthetic view on the regulation of the expression of ribosomal genes has not been yet elaborated.

2. Specific aims of the thesis

In this thesis, I wished to expand our knowledge of the large-scale order of the NOR-chromosome positioning with respect to nucleoli, while assessing the competence of their NORs, as well as transcription activity of ribosomal genes. Accordingly, we first characterized the chosen model cell lines with respect to their NORs and competent NORs. The obtained data were used in subsequent studies of nucleolar associations of the NOR-bearing chromosomes, and localization of the transcriptionally competent and non-competent NORs. In the pairs of daughter cells, we also compared nucleolar associations of chosen NOR-bearing homologues with nucleoli, as well as numbers of nucleoli.

The specific aims of the thesis are:

- To establish the regularities in distribution of competent (active) as well as non-competent (inactive) NORs among the NOR-chromosomes in two human-derived cell lines, transformed HeLa and primary LEP cells.
- To establish the fate of competent NORs during the cell cycle in the two cell lines.
- To analyze nucleolar association of the NOR-bearing chromosomes in HeLa and LEP cells, with respect to the established occurrence of competent/non-competent NORs in different NOR-bearing chromosomes.
- To investigate nuclear/nucleolar location of competent and non-competent NORs in the two cell lines.
- In the pairs of daughter HeLa cells, to compare positioning of the NOR-bearing chromosomes in relation to nucleoli.
- To establish whether the same number of nucleoli is seen in the pairs of daughter HeLa cells after mitosis.

3. Materials and Methods

3.1. Cell culture

Human HeLa cells, derived from adenocarcinoma cervix cells, that have stable karyotype without considerable variations (Macville et al., 1999; Smirnov et al., 2006), and diploid human embryo lung fibroblasts (LEP; Sevapharme) were cultivated in flasks or on coverslips 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.

3.2. DNA probes and antibodies

We used 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), and pA and pB rDNA probes, prepared from a pA and pB plasmid constructs (Erickson et al., 1981), kindly donated by James Sylvester (Nemours Children's Clinic Research, Orlando, FL). The pA probe contains the 3` end of 18S rDNA, the 5.8S rDNA, both internal transcribed spacers, and most of the 28S rDNA. The pB probe contains the promoter, the external transcribed spacer, and the 5` end of the 18S subfragment. The probes were labeled by biotin using nick-translation kit BIONICK Labeling System (GIBCO-BRL, Invitrogen) according to the manufacturer`s instructions. The rDNA probes were stored in hybridization mixture containing 25 ng of probe, 0.5 mg/ml sonicated salmon sperm DNA, 50% deionized formamide, 2x SSC and 10% dextran sulfate at -20°C. Both rDNA probes exhibited the same pattern of FISH-labeling. Therefore, only the results obtained with pB probe were used for statistical analysis.

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. Autoantibodies against human UBF and NOR 90 antigen were kindly provided by U. Scheer, Biocenter of the University of Wurzburg. Biotinylated rDNA probe was detected after FISH with monoclonal rabbit anti-biotin antibodies (Enzo, Roche). Secondary anti-mouse and anti-rabbit antibodies (Jackson ImmunoResearch Laboratories) were conjugated with Cy3 or FITC.

3.3. Immunofluorescence

Cells growing on coverslips were washed in phosphate-buffered saline (PBS), fixed in methanol at -20°C for 30 min and air-dried. Following three washes in PBS, the cells were incubated with anti-fibrillarin antibody, washed in PBS and incubated with secondary antibodies conjugated with either FITC or Cy3.

3.4. Immuno-FISH and FISH

The combined detection of fibrillarin and *in situ* hybridization (immuno-FISH) was performed after Pliss et al., 2005. After fibrillarin imunolabeling, as described above, the cells were postfixed with methanol/acetic acid (3:1) overnight at -20°C. The chromosomal spreads on coverslips were rinsed in 2xSSC. 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). After FISH, biotinylated rDNA probes were detected using respective primary and secondary antibodies.

The results of all single labeling (fibrillarin immuno-labeling and FISH), double labeling (fibrillarin immuno-labeling combined with FISH and double-FISH) and triple labeling experiments (fibrillarin immuno-labeling and double FISH) were compatible.

3.5. Microscopes used

Coverslips were mounted in Mowiol supplemented with DABCO and viewed using Olympus AX70 Provis equipped with the Photometrics CCD camera or Leica TCS NT confocal microscope.

3.6. Statistical evaluation

All statistical evaluations were obtained by analysis of 100 HeLa and LEP cells.

4. Results and discussion

4.1. NORs and their transcription competence during the cell cycle.

Several studies demonstrated that at least some subunits of RNA polymerase I along with its main transcription factors can be detected on the competent NORs even in metaphase (Babu and Verma, 1985; Weisenberger and Scheer, 1995; Jordan et al., 1996; Roussel et al., 1996; Gebrane-Younes et al., 1997; Sirri et al., 1999). However, the hypothesis claiming identity of active and UBF-loaded NORs has not been directly proven, since individual NORs could not be observed in the interphase cell nucleus (Busch and Smetana, 1970). To address this issue, we studied regularities in distribution of the transcriptional competence among different chromosomes on mitotic spreads. Next, the distribution of transcriptional competence was compared with distribution of transcription signals on NORs in telophase cells, and also in metaphase cells after stimulation of rDNA transcription with roscovitine. Finally, employing premature chromosome condensation (PCC) with calyculin A, we analysed the pattern of NOR competence during the interphase.

In HeLa cells, around 13 NORs were regularly revealed on mitotic spreads of HeLa cells. Thus the rDNA clusters show a stable pattern of distribution among the chromosomes of HeLa cells. Around 9 competent NORs regularly appeared on specific mitotic chromosomes in HeLa cells. The results obtained on mitotic chromosomal spreads show a high regularity in number and intensity of the UBF/silver signals on specific chromosomes in HeLa cells. There was a significant discrepancy between the pattern of rDNA distribution and the pattern of NOR competence: even when rDNA and UBF/silver signals coincided on the same chromosome, their intensities do not correlate.

The competent NORs were regularly distributed among the chromosomes of the diploid LEP cells. Transcription activity of the NORs correlated with their transcription competence in metaphase after roscovitine stimulation and in telophase. These results strengthen the view that transcriptional competence of NORs identified in mitosis through UBF immunofluorescence serves as a reliable indicator of their transcription activity in interphase.

Using premature chromosome condensation with calyculin A we showed that the same number of competent NORs can be observed in interphase and in mitosis. We infer that a uniform pattern of transcription competence persists throughout the whole cell cycle. The results obtained in this work expand our knowledge on the fate of NORs in the cell cycle, and strongly argue that the presence of UBF on NORs (or their silver stainability) in mitosis serves as an indicator of their transcription activity in the previous interphase.

4.2. Positioning of NORs and NOR-bearing chromosomes in relation to nucleoli

Nucleoli reform after mitosis around transcriptionally active, and therefore necessarily competent, NORs (Ochs et al., 1985; Benavente et al., 1987; Jimenez-Garcia et al., 1994), and the integrity of nucleoli depends on expression of ribosomal genes (Melese and Xue, 1995; Scheer and Hock, 1999; Dousset et al, 2000). However, the position of the non-competent NORs, that exhibit a condensed chromatin structure (O'Sullivan et al., 2002), and chromosomes carrying the non-competent NORs, with respect to nucleoli remains unclear. In the present study, we analyzed nuclear positions of competent and non-competent NORs, as well as chromosomes bearing NORs, with respect to nucleoli in HeLa and LEP interphase cells.

Nucleolar association of the interphase NOR-bearing chromosomes correlated with transcriptional competence of their NORs in HeLa and LEP cells. We infer that the tendency of rDNA-bearing chromosome homologues to associate with nucleoli correlates with the number of transcriptionally competent NORs in these homologues.

Comparing the number of extranucleolar rDNA foci with the number of non-competent NORs, we found that the majority of the transcriptionally non-competent NORs in HeLa and LEP cells were situated within the nucleoli. We conclude that most of the transcriptionally non-competent NORs are situated in the nucleoli, and some NOR-bearing chromosomes are positioned in such manner that their NORs are located in the nucleolus, while the bulk of the chromosome territory is distanced from the nucleolus.

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

Although it is widely accepted that arrangement of chromosomes in the cell nucleus is non-random, it is not clear to what extent positions of chromosomes, together with their neighbourhood, are conserved in daughter cells after mitosis. To address specific aspects of this problem, we used the model of the chromosomes carrying NORs. At the beginning of G1 phase NORs from more than one chromosome cluster and participate in the formation of nucleoli (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 (Parada and Misteli, 2002; Walter et al., 2003; Foster and Bridger, 2005; Cremer and Cremer, 2006). We therefore selected for our study pairs of daughter cells in this period of the cell cycle, and compared the association of chosen 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 homologues 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.

In this work we found that the number of nucleoli were most frequently (in around 80% cases) different in the daughter cells. Comparing combinations of nucleolar associations for the chromosomes 14 and 15 we surprisingly discovered that in 50% of the daughter cell pairs, the combinations were identical.

Taken together, our data indicate that the distribution of the NOR-bearing chromosomes among the nucleoli is partly conserved through mitosis.

5. Conclusions

Concerning the behaviour of NORs during the cell cycle, several conclusions were reached:

- HeLa cells show an abnormal but stable pattern of rDNA distribution, however, not all acrocentric chromosomes carry ribosomal genes. In LEP cells all 10 acrocentric chromosomes are rDNA positive.
- Around 70 or 80% of the NORs, in HeLa and LEP cells respectively, are transcriptionally competent. In both HeLa and LEP cells, the transcription competence is non-randomly distributed among the NOR-bearing chromosomes.
- Stimulated transcription activity of the NORs exactly follows the pattern of transcription competence, which persists over the whole cell cycle, except probably for a short period in S phase.
- Employing premature chromosomal condensation to assess persistence of the transcription competence on the different NORs during the cell cycle, the pattern of transcription competence observed on mitotic chromosomal spreads persists throughout the interphase, except for a short period in S phase.

Study analysing nuclear positions of NORs, and NOR-chromosomes with respect to nucleoli, showed that in HeLa and LEP interphase cells:

- There is a positive correlation between the close nucleolar associations of the NOR-bearing chromosomes and the transcription competence of their NORs.
- Most of the non-competent NORs are located in nucleoli.
- Some intranucleolar NORs are situated on elongated chromatin protrusions connecting nucleoli with respective chromosome territories that are distanced from nucleoli.

The study correlating position of NOR-bearing chromosomes and nucleoli in the two daughter cells showed that:

- The two daughter cells typically have different numbers of nucleoli.
- The distribution of the NOR-bearing chromosomes among the nucleoli is partly conserved in daughter cells.

6. Summary

In human cells ribosomal genes are organized as clusters called Nucleolus Organizer Regions (NORs) that are situated on the short arms of acrocentric chromosomes. It was found that essential components of the RNA polymerase I transcription machinery, including Upstream Binding Factor (UBF), can be detected on some NORs, termed "competent" NORs, during mitosis. The competent NORs are believed to be transcriptionally active during interphase. But since individual NORs cannot be observed in the cell nucleus, their interphase status remained unclear. To address this problem, we detected the competent NORs by two commonly used methods, UBF immunofluorescence and silver staining, and combined them with FISH for visualization of rDNA and/or specific chromosomes. We found that the numbers of competent NORs on specific chromosomes were largely conserved in the subsequent cell cycles, with certain NOR-bearing homologues displaying a very stable pattern of competence. Importantly, those and only those NORs, which were loaded with UBF, incorporated bromo-uridine in metaphase after stimulation with roscovitine and in telophase, suggesting that competent and only competent NORs contain ribosomal genes transcriptionally active during interphase. Applying premature chromosome condensation with calyculin A, we visualized individual NORs in interphase cells, and found the same pattern of competence as observed in the mitotic chromosomes.

It is widely accepted that chromosomes occupy more or less fixed positions in mammalian interphase nucleus. However, relation between large-scale order of chromosome positioning and gene activity remained unclear. We approached this problem by studying the model of the human ribosomal genes. Employing FISH and immunocytochemistry, we found that, in HeLa and LEP cells, the large-scale positioning of the NOR-bearing chromosomes (NOR-chromosomes) with regard to nucleoli is linked to the transcription activity of rDNA. Namely, the tendency of rDNA-bearing chromosomes to associate with nucleoli correlates with the number of transcriptionally competent NORs in the respective chromosome homologues. Regarding the position of NORs, we found that not only competent but also most of the non-competent NORs are included in the nucleoli. Some intranucleolar NORs (supposedly non-competent) are situated on elongated chromatin protrusions connecting nucleoli with respective chromosome territories spatially distanced from nucleoli. The cause of such an arrangement of the apparently non-competent NORs remains to be elucidated.

It is not clear to what extent nuclear positions of chromosomes, together with their neighbourhood, are conserved in daughter cells. We studied this problem by comparing the association of chosen 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 homologues of certain NOR-chromosomes associated with individual nucleoli. The daughter cells typically had different numbers of nucleoli. As nucleoli play a crucial role in the arrangement of chromosomes in the cell nucleus, our data show that the position of chromosomes cannot be precisely maintained through mitosis. 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 maintained through mitosis.

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ELSO Drážďany 21.9.-24.9.2003: Transcriptionally competent NORs in HeLa cells (poster).

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EMBO/FEBS konference (Nuclear structure and Dynamics) v La Grande Motte Francie 26.9. 2005, Nucleolar association of the NOR-bearing chromosomes (poster)

The American Society for Cell Biology (ASCB) 45th Annual Meeting, at the Moscone Center in San Francisco, CA 14.12. 2005, Nucleolar association of the NOR-bearing chromosomes (poster).

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