

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.