Оригинальные работы

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FLUORESCENCE INTENSITY PROFILES OF *IN SITU* HYBRIDIZATION SIGNALS DEPICT GENOME ARCHITECTURE WITHIN HUMAN INTERPHASE NUCLEI

An approach towards construction of two-dimensional (2D) and three-dimensional (3D) profiles of interphase chromatin architecture by quantification of fluorescence in situ hybridization (FISH) signal intensity is proposed. The technique was applied for analysis of signal intensity and distribution within interphase nuclei of somatic cells in different human tissues. Whole genomic DNA, fraction of repeated DNA sequences (Cot1) and cloned satellite DNA were used as probes for FISH. The 2D and 3D fluorescence intensity profiles were able to depict FISH signal associations and somatic chromosome pairing. Furthermore, it allowed the detection of replicating signal patterns, the assessment of hybridization efficiency, and comparative analysis of DNA content variation of specific heterochromatic chromosomal regions. The 3D fluorescence intensity profiles allowed the analysis of intensity gradient within the signal volume. An approach was found applicable for determination of assembly of different types of DNA sequences, including classical satellite and alphoid DNA, gene-rich (G-negative bands) and gene-poor (G-positive bands) chromosomal regions as well as for assessment of chromatin architecture and targeted DNA sequence distribution within interphase nuclei. We conclude the approach to be a powerful additional tool for analysis of interphase genome architecture and chromosome behavior in the nucleus of human somatic cells.

Introduction. The investigation of high-order chromatin arrangement is a promising direction in cell biology and genetics due to the immense impact of nuclear organization on functional genome activity in somatic cells. Substantial progress made throughout last decades has given us the look at how positioning of chromosomes in the nucleus is linked to genome functions. We know now that chromosomes are compartmentalized into specific volumes, arrangement of chromatin is likely to be conserved throughout mitotic division, transcriptional activity is determined by positioning of a gene within the chromosomal volume, chromatin arrangement differs between cell types [1–3]. Despite of these achievements, a number of gaps in our incomplete knowledge concerning nuclear organization in interphase nuclei of different human somatic cells evidence for the need of technology improvement in chromatin arrangement studies. An approach to quantify fluorescence *in situ* hybridization (FISH) signals proposed earlier was shown to be useful for applied molecular cytogenetics [4, 5]. This communication extends the potential of quantitative FISH (QFISH) technique for chromatin arrangement studies. Additionally, we propose for the first time the construction of three-dimensional (3D) intensity profiles allowing the analysis of intensity distribution within signal area supposed to be useful for determination of DNA sequence positioning and variation in interphase.

Materials and methods. Peripheral blood lymphocytes were obtained from individuals with normal male and female karyotype. Chromosomal preparations of blood lymphocytes were made according to standard protocols with fewer modifications [6, 7]. Interphase nuclei of chorionic villi were obtained from specimens of spontaneous abortions, which were identified to possess diploid chromosome complement [8]. The fresh-frozen brain tissue samples were acquired from the Brain Bank of the National Research Center of Mental Health of Russian Academy of Medical Sciences (NRCMH RAMS). The processing of the adult brain tissue for FISH was performed according to a step-by-step protocol described earlier [9]. To prepare the suspension of volumetric interphase nuclei an acetic acid free processing procedure was applied. In total, 20 samples of blood lymphocytes, 20 samples of chorionic villi, and 3 samples of the adult brain were studied. FISH was performed according to the previously described protocols [6, 10–12]. DNA probes used were those

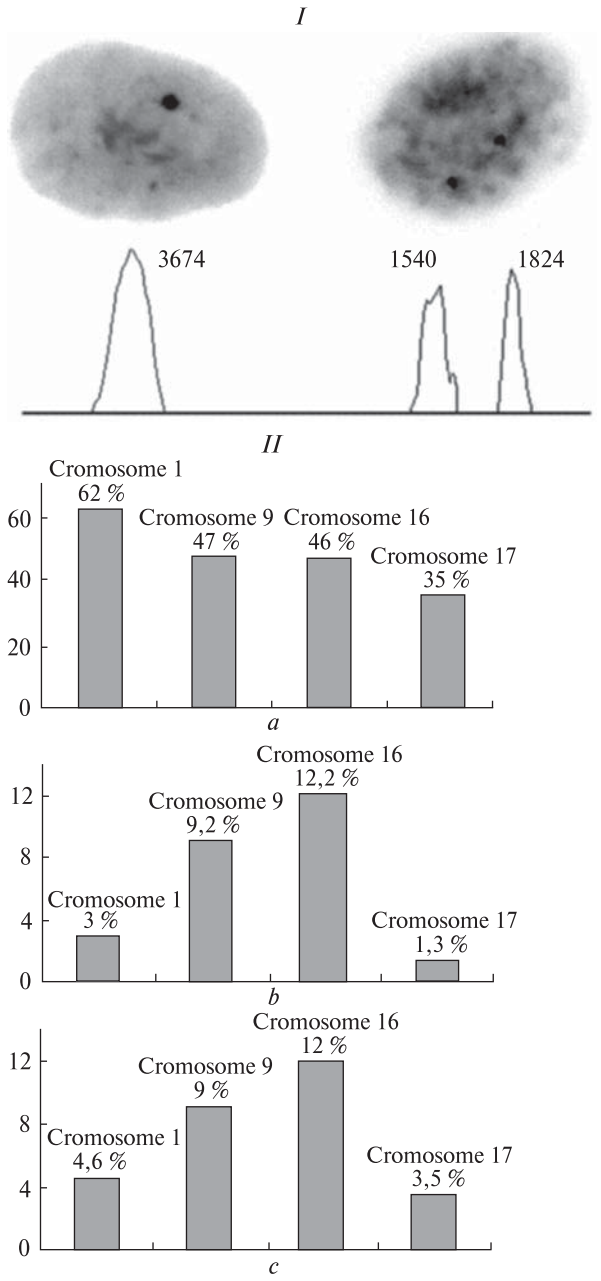

Fig. 1. QFISH with construction of 2D intensity profiles on human interphase nuclei: *I* – QFISH on interphase nuclei of chorionic villi showing that left nucleus have a single signal (relative intensity 3364 pixels) in contast to right one with two signals (relative intensities 1540 and 1842 pixels). Comparing the intensities of these signals, we come to the conclusion that left nucleus have a paired signal; *II* – the frequency of paired signals revealed by chromosome enumeration probes for chromosomes 1, 9, 16, and 17 in the adult brain (*a*), chorionic villi (*b*) and blood lymphocytes (*c*)

painting classical satellite DNA of chromosomes 1, 9, and 16; alphoid satellite DNA of chromosomes 17, and X; Cot1-DNA probe, cloned classical satellite DNA sequences, all the centromeric DNA, short interspersed repetitive elements, produces fluorescent R-banding or paints gene-rich euchromatic regions of the genome depending on temporal hybridization conditions; total-human-DNA-probe suppressed by Cot1 DNA during hybridization (paint euchromatic regions of the genome). All the probes, taking part of the original collection of laboratory of cytogenetics of NRCMH RAMS, were described previously [6, 7, 13, 14]. QFISH was performed as described previously [4, 5, 15]. The construction of intensity profiles was made by digital capturing of microscopic image by the monochrome CCD camera, LG-3 grayscale scientific PCI frame grabber, and Scion Image Beta 4.0.2 software acquired from www. scioncorp.com. Both 2D (two dimensional) and 3D profiles of FISH signal intensities were obtained via the use of corresponding software options and the macros supplied by the manufacturer. Volumetric brain derived interphase nuclei were analyzed through the corresponding stacking options of the software. For 2D profile analyses, 50 nuclei were evaluated per sample per probe (DNA probes for chromosomes 1, 9, 16, 17, X and Y) and 30 nuclei were evaluated per sample per probe in case of 3D profile analyses.

Results and discussion. To test the versatility of the approach dividing and postmitotic cells were analyzed. The technique was elaborated through studying interphase nuclei of chorionic villi, cultured peripheral blood lymphocytes, and cells of the adult brain. First, 2D profiles have demonstrated interphase paired signals for autosomes, which have been present in all the tissues analyzed. This appeared as single signal with doubled relative intensity in a nucleus. The occurrence of paired signals significantly differed between tissues being, however, slightly different from sample to sample of the same tissue (Fig. 1). The higher incidence was in human brain cell suspensions (average 48 %), while chorionic villi and blood lymphocytes demonstrated significantly lower incidences of paired signals, average– 6.4 and 7.3 %, respectively. As to the X chromosome, paired signals were occasionally detected (1–5 nucleus per sample). Nuclei exhibiting single signal with relative intensity similar to each single signal of disomic nuclei

were considered as monosomic and were excluded. Both chorionic villus and blood lymphocyte were characterized by occurrence of replicated signals. The rate of their incidence exceedingly varied from sample to sample being observed in all samples analyzed. Thus, the range of occurrence of replicated signals was between 4 and 46 % in blood lymphocytes and was 10–51 % in chorionic villi. The human brain occasionally demonstrated replicated signals. Replicated signals appeared as a doubled signal with two peaks of identical relative intensity having a connecting fluorescent track (data not shown). Replicated signals were usually coupled with a single signal in the case of chromosome X or occurred simultaneously in the case of autosomes. However, deviated patterns of signal appearance were observed in a small proportion of cells (less than 2 %).

Additional interesting observation made during the testing of the approach was referred to the possibility of assessment of both hybridization efficiency and DNA sequence size variation in a chromosomal region. To monitor hybridization efficiency, dependence between signal relative intensity and time was obtained. The 100 % of hybridization efficiency was considered the time point when relative intensity of hybridization signals stopped to change. Using different DNA probes, we succeeded to define this point as nearly 960 minutes or 16 hours (Fig. 2). This feature of the approach was not only useful for selecting optimal hybridization conditions, but also provided for selecting conditions for the analysis of DNA seuquences distributions (using Cot1-DNA- and total-human-DNA-probe) in 3D intensity profiles assay. Through comparing the ratio of relative signal intensities of homologous chromosomes in metaphase spreads and interphase nuclei, we found almost complete equality between these values in all the samples analyzed (data not shown). Therefore, the assay proposed can define relative size of DNA sequence in a specified chromosomal region in interphase. Comparing the intensity of profiles obtained by sequential quantification of FISH signals through the stacks of volumetric nuclei, we found that the area of intensity profiles (the value of relative intensity) changed insignificantly, whereas the shape of the curve tended to change. This means that analysis of intensity profiles of FISH signals allows to analyze DNA size and behavior independently

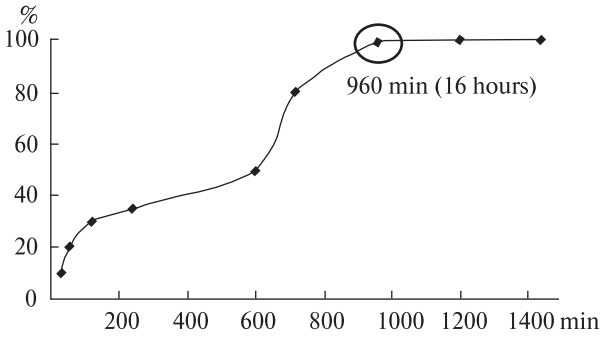

Fig. 2. QFISH identification of optimal hybridization conditions. Relative intensities were measured at different time points. The 100 %-efficiency was considered the point when relative intensities ceased to change: 960 minutes or 16 hours

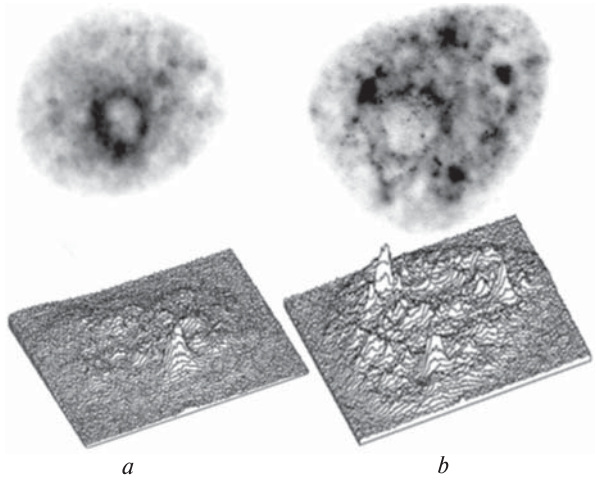

Fig. 3. QFISH with construction of 3D intensity profiles on interphase nuclei of the adult human brain with Cot1-DNA probe at temporal conditions when all large satellite DNAs are painted: *a*– assembly of satellite DNA appearing as single peak; *b*– assemblies of satellite DNA appearing as multiple peaks (mainly 3 peaks)

from processes happening with nucleus during preparation of cell suspensions.

During analyses with DNA probes painting different repetetive DNAs of cellular genome, we have noticed that the distribution of intensity within area is non-random and tends to vary within nuclear area. To address this type of DNA behavior, QFISH constructing 3D intensity profiles was applied. The latter has shown that satellite DNAs tend to gather forming large assemblies, which corresponded to single, double and multiple 3D fluorescent intensity peakes (Fig. 3, *a*). Using this tentative classification, we were able to show tis-

sue-specific patterns of repetetive DNA assemblies. Thus, large assemblies with single 3D fluorescent intensity peak per nucleus were more frequent in adult brain cells (mean 34 %), whereas in chorionic villi and blood cells satellite DNA assemblies were relatively uncommon (mean 5 and 3 %, respectively). Large assemblies appearing as double 3D fluorescent intensity peak per nucleus were less common as to single-peak-assemblies in the adult brain being, however, more frequent as to other tissues; mean frequencies of such assemblies were 17 % (the adult brain), 7 % (chorionic villi), and 4 % (blood lymphocytes). Satellite DNA assemblies characterized by multiple 3D fluorescent intensity peaks per nucleus (more than 2 relatively large 3D intensity profiles) were rare in the adult brain (mean frequency 3 %), but were more common in chorionic villi (mean frequency 9 %), and even more common in blood lymphocytes (mean frequency 33 %). The assemblies were almost exclusively referred to satellite DNA (both classical and alpha satellite), whereas gene-rich euchromatic regions and gene-poor euchromatic regions tended to be distributed regularly within nuclear area. Although gene-rich euchromatic regions more commonly were distrubted along nuclear periphery in contrast to gene-poor euchromatic regions that more commonly tended to position closely to nuclear center, no definitive conclusion could be drawn, inasmuch as consistent pattern of this DNA behavior was not observed due to exreme arrangement variation of these DNA types.

The behavior of DNA within nuclear volume is highly variable and appears to be crucial for functional genome activity [1–3]. However, an integrated view of direct relationship between chromosome or specific DNA type positioning in the nucleus and critical intracellular processes (i.e. transcription or translation) is far from being created. In major part this is due to technical limitations, which are inteneded to be overcomed by enhancing and modifying molecular cytogenetic techniques [4]. The approach proposed seems to be an interesting additional tool for studying chromatin behavior in interphase. Moreover, it granted to make important observations of DNA positioning in interphase nuclei.

We found that genome organization varies between tissues, as the incidence of paired chromosome regions and large type-specific DNA assemblies was different in each tissue analyzed. This agrees with a previous study of murine genome orgnization in different tissues that concluded the existence of tissue-specific patterns of chromatin arrangement in interphase [16]. Futhermore, we have observed that non-transribed DNA sequences (satellite DNAs) possess rather complex behavior allowing to suggest a functional role of their positioning in interphase. The organization of different DNA types on chromosomes (chromosome banding relationship to base composition in a chromosomal region) is featured by specific gene content: G-negative/R-positive bands are gene-rich regions, G-positive/R-negative bands – gene-poor regions, chromosomal region consisting of constitutive heterochromatin (C-bands) – non-transcribed sequences apparently lacking genes (satellite DNA) [17]. This classification was previously used for analyses of genome organization in lymphocytes and cancer cell lines. As a result, there was found that chromatin arrangement closely correlates with banding patterns of human chromosomes: gene-poor regions and C-band-regions are preferentially localized at nuclear periphery and around the nucleolus, whereas gene-rich regions are more frequently positions inside the nuclear volume rarely tethering nuclear membrane and the nucleolus [18]. The present findings confirm these observation in some extent as well as adding a new type of chromatin behavior referred to as association of chromosome regions or large-scale type-specific DNA assemblies in interphase. Together, this suggests the present approach to expand current possibilities of intrerphase genome organization studies and allows to identify new types of chromatin behavior.

Another technical key point for all the studies of chromosomes refers to their behavior during preparations of metaphase spreads or interphase nuclei including tissue processing, fixation and delivery on slides. Current concepts suggest these procedures to influence rather chromosome length and spreading of G-positive bands than chromatin behaviour as the positioning of chromosomes appears to be relatively conserved during the delivery of nuclei/metaphase suspension on slides [19]. Therefore, one can modulate chromatin behavior in living cells skipping sophisticated volumetric preparations. Considering this suggestion, it appears to be useful to monitor different conjunctions,

associations and assemblies of different DNA types and define the differences between volumetric and routine nucleus preparations. The approach proposed could be also applied for these aims.

In summary, a QFISH protocol may be not only applied for analysis of chromosome abnormalities and chromosomal mosaicism or identification of chromosome parental origin [4, 5, 15], but also for studying nuclear organization. Futhermore, the construction of 3D intensity profiles provides for additional information concerning DNA behavior in interphase being, therefore, a new technical milestone in chromatin behavior studies achieved. It is noteworthy, that recent molecular cytogenetic studies of the brain have proposed a new biomedical direction termed molecular neurocytogenetics (encompassing studies of chromosome numbers and behavior in the central nervous system), which requires additional technical tools to take the well-deserved place in current biomedecine [20]. Positive results, that were obtained here studying the adult human brain, present the approach described as an additional powerful technique for genetic and cell biology investigations of the human brain.

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АНАЛИЗ ПРОФИЛЯ ИНТЕНСИВНОСТИ СИГНАЛОВ ФЛЮОРЕСЦЕНТНОЙ ГИБРИДИЗАЦИИ *IN SITU* ДЛЯ ИЗУЧЕНИЯ ОРГАНИЗАЦИИ ГЕНОМА В ИНТЕРФАЗНЫХ ЯДРАХ КЛЕТОК ЧЕЛОВЕКА

Представлен метод построения двухмерных (2D) и трехмерных (3D) профилей интенсивности сигналов флюоресцентной гибридизации *in situ* (FISH), основанный на количественной FISH. Настоящая методика была использована для анализа расположения и распределения сигналов в интерфазных ядрах клеток различных соматических тканей человека. Использование 2D профилей интенсивности продемонстрировало возможность определения колокализации FISH-сигналов. Более того, предложенный подход позволил идентифицировать реплицированные сигналы, дать оценку эффективности гибридизации и сравнительный анализ вариации содержания ДНК специфических участков хромосом. Построение 3D профилей показало распределение интенсивности в пределах площади сигнала. Применение этой методики позволило определить сосредоточение различных типов последовательностей ДНК: классическая сателлитная и альфоидная ДНК; геннонасыщенные (G-положительные полосы) и генноненасыщенные (G-отрицательные полосы) участки хромосом. Кроме того, методика дала возможность оценить расположение хроматина в интерфазных ядрах как культивированных, так и некультивированных клеток. В результате исследования был сделан вывод о том, что предлагаемый подход является эффективной дополнительной методикой для изучения ядерной организации, специфики вариации и расположения последовательностей ДНК в интерфазных ядрах, а также поведения ядер при приготовлении хромосомных препаратов соматических клеток человека.

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АНАЛІЗ ПРОФІЛЮ ІНТЕНСИВНОСТІ СИГНАЛІВ ФЛУОРЕСЦЕНТНОЇ ГІБРИДИЗАЦІЇ *IN SITU* ДЛЯ ВИВЧЕННЯ ОРГАНІЗАЦІЇ ГЕНОМУ В ІНТЕРФАЗНИХ ЯДРАХ КЛІТИН ЛЮДИНИ

Представлено метод побудови двомірних (2D) та тримірних (3D) профілів інтенсивності сигналів флуоресцентної гібридизації *in situ* (FISH), що заснований на кількісній FISH. Наведена методика була використана для аналізу розташування та розподілу сигналів в інтерфазних ядрах клітин різних соматичних тканин людини. Використання 2D профілів інтенсивності продемонструвало можливість визначення локалізації FISH-сигналів. Більш того, даний підхід дозволив ідентифікувати репліковані сигнали, дати оцінку ефективності гібридизації та провести порівняльний аналіз варіації вмісту ДНК специфічних ділянок хромосом. Побудова 3D профілів показала розподіл інтенсивності у межах площі сигналу. Використання цієї методики дозволило визначити зосередження різних типів послідовностей ДНК: класична сателітна та альфоїдна ДНК; генонасичені (G-позитивні полоси) і генноненасичені (G-негативні полоси) ділянки хромосом. Крім цього, методика надала можливість оцінити розташування хроматина в інтерфазних ядрах як культивованих, так і некультивованих клітин. Зроблено висновок, що наведений підхід є ефективною додатковою методикою для вивчення ядерної організації, специфіки варіації та розташування послідовностей ДНК в інтерфазних ядрах, а також поведінки ядер при приготуванні хромосомних препаратів соматичних клітин людини.

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