# Chapter 8
# Chromosome Architecture Studied by High-Resolution FISH Banding in Three-Dimensionally Preserved Human Interphase Nuclei

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**Abstract** The impact of chromosome architecture in the formation of chromosome aberrations is a meanwhile well-established finding of interphase-directed molecular cytogenetic studies. Up to recent years, biomedical research of interphase chromosomes in their integrity was hindered by technical limitations. The introduction of three-dimensional suspension-based fluorescence in situ hybridization (S-FISH) in combination with microdissection-based engineered DNA probes and fluorescence multicolor chromosome banding (MCB) allowed studying interphase chromosome organization, numbers, and rearrangements in different kind of cells. Such studies already provided comprehensive information on the interphase architecture of normal human sperm, as well as first insights into the influence of chromosomal rearrangements on the 3D structure of the sperm nuclei. Also, the influence of additional chromosomal fragments present in a nucleus was successfully visualized by S-FISH. Finally, S-FISH supported the idea that disease-specific chromosomal translocations could be due to tissue specific genomic organization. Overall, S-FISH combined with MCB but also other DNA probes is a tool with high potential to resolve the influence of chromosomal imbalances and/or rearrangements on the interphase architecture, the latter being possibly a part of the epigenetic cell regulation, also being denominated as chromosomics.

## Introduction

In the interphase nucleus, chromosomes are located in specific regions, which are called "chromosome territories" (Cremer and Cremer 2001; Williams and Fisher 2003; Branco and Pombo 2006). Own multicolor banding (MCB)-based studies

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revealed that the chromosome shape itself is not lost in the interphase nucleus, and one can even identify "interphase chromosomes" instead of only chromosome territory, even irrespective of the cell cycle phase (Weise et al. 2002; Lemke et al. 2002).

Both chromosome size and gene density are discussed to have an important impact on the nuclear position of chromosomes. Small chromosomes preferentially locate close to the center of the nucleus, while large chromosomes can be found in the nuclear periphery (Sun et al. 2000; Bolzer et al. 2005). On the other hand, Croft et al. (1999) demonstrated a gene density-correlated radial arrangement of chromosomes in nuclei. Mainly gene-dense and early replicating chromatin can be found in the central part of the nucleus, while gene-poor and later replicating chromatin is located in nuclear periphery (Croft et al. 1999). Interestingly, this nuclear topological arrangement is conserved during primate evolution (Manvelyan et al. 2008a).

Here, we summarize the yet published applications of suspension-based fluorescence in situ hybridization (S-FISH) combined with FISH banding (Liehr et al. 2002, 2006), particularly the yet most used approach array-proven MCB (Weise et al. 2008). Besides, also other protocols were suggested for FISH studies in 3D-preserved nuclei (e.g., Walter et al. 2006). Also, recent studies showed that inter- and metaphase chromosomes preserve a genome-wide haploid order (Weise et al. 2016) and that this order is completely changed in senescent cells (Roediger et al. 2014). All these studies provide to the more and more emerging field of chromosomics, as predicted in 2005 by Prof. Uwe Claussen (Claussen 2005).

## S-FISH, the Method

Performing of a FISH experiment on human meta- and interphase cells after air-drying method is a well-established approach; it is routinely done as one- to multicolor-FISH test (Liehr et al. 2004a). However, the air-drying procedure of chromosome preparation, leading to well-spread metaphases under appropriate conditions, leads at the same time to flattening of the originally spherical interphase nuclei. Thus, interphase architecture is hard to be studied reliably on such kind of preparation (Hunstig et al. 2009), even though some basic insights can also be gained using such material for FISH banding (Weise et al. 2002; Lemke et al. 2002).

Still, there is an easy way to do studies in three-dimensionally (3D) preserved interphase nuclei obtained from routinely prepared cytogenetic preparations stored in Carnoy's fixative. One just needs to do the whole FISH procedure in cell suspension, and as a final step, the nuclei are placed on a polished concave slide before evaluation, immobilized in agarose. This approach for 3D-FISH analyses on totally spherical interphase nuclei, called suspension-based fluorescence in situ hybridization (S-FISH), was published first in 2002 (Steinhaeuser et al. 2002) and further developed and slightly modified later (Manvelyan et al. 2008a; Hunstig et al. 2009). Its principle is shown in Fig. 8.1.

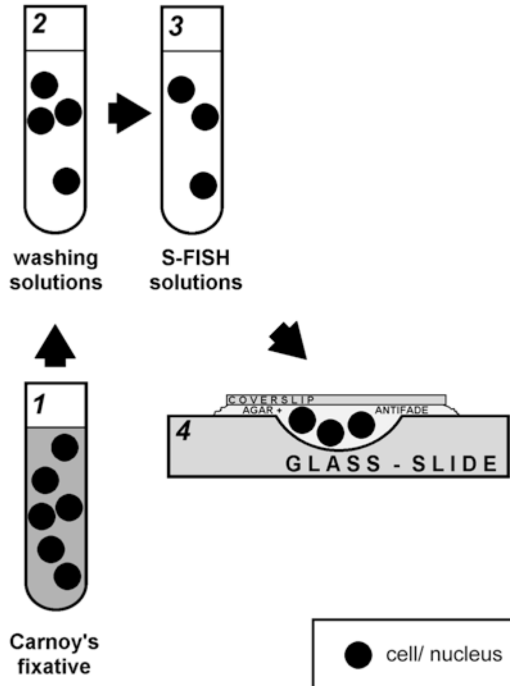


**Fig. 8.1** Schematic drawing of the suspension-based fluorescence in situ hybridization (S-FISH) procedure. Overall, S-FISH avoids this flattening and artificial swelling of the interphase nuclei, and the whole experiment is performed in suspension. A certain loss of cells during the washing steps is normal, shown here by the reduction of cells/nuclei from step 1 to step 4. In principle, Carnoy's fixative is replaced subsequently by solutions necessary for a FISH, and washing steps are included. Finally, the cells/nuclei are immobilized and counterstained in an agarose (AGAR) on a glass slide under a coverslip. The details of the protocol are described in Hunstig et al. (2009)

### *S-FISH: Which DNA Probes May Be Applied?*

For S-FISH, all available chromosome or chromosome region-specific DNA are principally suited. However, for application in S-FISH, at least double amount of the probe is necessary than for "normal" FISH experiments (Hunstig et al. 2009). To resolve the chromosome structure as a whole, single chromosome-directed FISH banding based on partial chromosome painting probes like in MCB is suited best (Weise et al. 2008). Besides, centromeric and/or locus-specific probes can be used as well for special questions (e.g., Manvelyan et al. 2009; Hunstig et al. 2009).

## Applications of S-FISH

Besides some studies done in comparative interphase cytogenetics of human and whitehanded gibbon and gorilla (Manvelyan et al. 2008a), S-FISH combined with MCB is mainly applied in the field of biomedical basic research of the human interphase nucleus. Here, still many questions are open and unanswered, mainly due to lack of suited methods, before introduction of S-FISH. Besides, more and more studies in other animals/species provide insights into the nuclear architecture (Karamysheva et al. 2017).

### *Human Sperm*

For the first time, the distribution of all human chromosomes in sperm was resolved comprehensively by S-FISH−/MCB studies. Strikingly, for the majority of the 24 human chromosomes, the distribution of the territories was alike as in lymphocytes; only the acrocentric chromosomes showed another location as in sperm, no nucleolus is formed (Manvelyan et al. 2008b). Thus, this nonrandom positioning must have a biological meaning. In other words, each chromosome needs to have a special position in the nucleus in order that the cell can work properly. Sperm are translationally inactive cells; however, they need to have chromosomes at the right places as soon as a sperm enters an oocyte and needs to become active again.

The study of Manvelyan et al. (2008b) showed a direct correlation of chromosome positions and their sizes, apart from chromosomes 1, 2, 6, 14, 18, 20, 21, and Y, i.e., large chromosomes were in the periphery, small in the center. Exactly those eight chromosomes not fitting in the correlation before perfectly aligned with gene density theory, i.e., gene-dense chromosomes were in the nuclear center, and gene-poor in the periphery.

There are also already other one studies in sperm of male with a chromosomal aberration (Bhatt et al. 2009; Karamysheva et al. 2015). Three males with paracentric inversion were studied, and no gross changes in the interphase positioning of the affected chromosomes were found. Here for sure, more studies on the influence of inborn rearrangements on the nuclear architecture of sperm, but also other in tissues, are necessary.

### *Different Tissues with Additional Chromosomal Fragments*

Additional chromosomal material present in the cell is suspected to alter or at least influence the chromosomal architecture. Besides complete trisomies as inborn or acquired aberrations, there is the possibility of partial trisomies induced either by derivative chromosomes or by the presence of a small supernumerary marker

chromosome (sSMC). The latter condition may be seen in 0.043% of newborn infants, 0.077% of prenatal cases, 0.433% of mentally retarded patients, and 0.171% of subfertile people (Liehr and Weise 2007). sSMC are defined as structurally abnormal chromosomes that cannot be identified or characterized unambiguously by conventional banding cytogenetics alone and are generally equal in size or smaller than a chromosome 20 of the same metaphase spread. sSMC are mostly detected unexpectedly in routine cytogenetics (Liehr et al. 2004b). Also, they are not easy to correlate with a specific clinical outcome as besides induction of genomic imbalance, mosaicism and other most often epigenetic factors can influence the phenotype of an sSMC carrier: Uniparental disomy, heterochromatization, and even their influence on the interphase architecture may play a role here. Also, a pilot study revealed some potential influence of sSMC presence on nuclear architecture recently (Karamysheva et al. 2015).

In a recent study (Klein et al. 2012), S-FISH revealed that an extra piece of DNA like an sSMC leads to gross rearrangements within the interphase nucleus, mainly concerning the sSMCs' normal sister chromosomes. Primarily, the position of the sSMC is influenced by and/or influencing the position of the homologous chromosomes. sSMC and one sister chromosome tend to colocalize; this seems to be driven mainly by the amount of euchromatin present in the sSMC. Also, the sSMC seems to take over the position of one normal sister chromosome. Thus, the remainder sister chromosome is displaced toward another location within the nucleus. These observations were made in B and T lymphocytes and/or skin fibroblasts.

## *Different Female Tissues and the Position of the X Chromosome*

S-FISH/MCB studies in buccal mucosa, B and T lymphocytes, and skin fibroblasts for the positioning of normal and derivative X chromosomes in female cells also may lead to interesting, yet impossible insights into the nuclear architecture. Preliminary yet unpublished results (Fig. 8.2) firstly confirmed that active and inactive X chromosomes are located in the cell periphery and that the inactive X chromosome colocalizes to big parts, even though not perfectly, with the Barr body. Interestingly, a dicentric X chromosome, leading to an almost complete trisomy X, altered the positioning of the two X chromosomes to each other, inducing a larger distance between both normal and derivative X chromosome compared to the normal cells. Thus, new insights may be obtained also by studying well-known phenomenon like X inactivation by the S-FISH approach.

## *Leukemia and the Positions of Chromosomes 8 and 21*

Nonrandom positioning of chromosomes in interphase nuclei is known to be of importance for genomic stability and formation of chromosome aberrations. So tissue specificity of chromosomal translocations could be due to tissue-specific

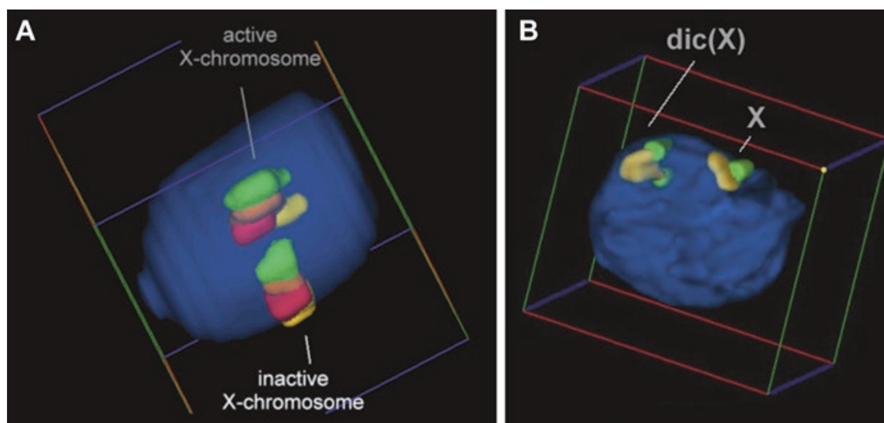


**Fig. 8.2** S-FISH results after application of X chromosome-specific DNA probe sets. (**a**) Active and inactive X chromosomes in a lymphocyte nucleus of a normal female labeled with an MCB-X probe set. (**b**) A normal (X) and derivative X chromosome (dic(X)) labeled with partial chromosome paints for Xp (green) and Xq (yellow) visualized in the fibroblast cell line GM15859 (Coriell). The female carrier had a constitutional karyotype 46,X,dic(X)(pter->q28::q28->pter)

genome organization (Meaburn et al. 2007; Brianna Caddle et al. 2007), and a positive correlation between spatial proximity of chromosomes/genes in interphase nuclei and translocation frequencies was shown (Bickmore and Teague 2002; Roix et al. 2003; Branco and Pombo 2006; Meaburn et al. 2007; Brianna Caddle et al. 2007; Grasser et al. 2008).

Manvelyan et al. (2008a, b) provided evidence that there might be an effect of specific chromosome positioning in myeloid bone marrow cells, i.e., a colocalization of chromosomes 8 and 21 could promote a translocation providing selective advantage of t(8;21) cells in AML-M2. Additional S-FISH studies confirmed that this is specifically true for AML patients having a trisomy 8 (Othman et al. 2012). Overall, studies to enlighten the nuclear position of tumor-related oncogenes, which are known to be activated by specific translocations are promising targets of future S-FISH-studies, as supported by recent comparable findings in thyroid cancer (Gandhi et al. 2009).

## S-FISH, Conclusions, and Perspectives

Overall, the combination of S-FISH and MCB for a three-dimensional analysis of chromosome position in interphase nucleus is a powerful tool, which can be accompanied by the use of locus-specific probes. The topological organization in interphase nucleus is nonrandom, and it becomes more and more obvious that there is a physiological reason behind that.

The already done and above summarized S-FISH studies in human show the potential of this approach for (i) genome-wide analysis of interphase architecture in yet not studied tissues (like done for sperm (Manvelyan et al. 2008b)), (ii) studies on architectural changes in nuclei with additional chromosomes or chromosomal material (like done for sSMC (Klein et al. 2012; Karamysheva et al. 2015) or the X chromosome), and (iii) analysis for the susceptibility of specific parts of the human genome for rearrangements due to colocalization (like done for the t(8;21) in AML (Manvelyan et al. 2009; Othman et al. 2012)). For sure, additional biomedical research aspect of interphase chromosomes may also be covered using the S-FISH/MCB approach, like recently the proof of interaction between distant chromosomal regions (Maass et al. 2018) and the description of nuclear architecture in hematopoietic stem cells (Grigoryan et al. 2018).

Overall, the approach discussed can be used not only based on human but also, if MCB probes are available for, based on probes from other species as already demonstrated by one example for murine mcb (Ktistaki et al. 2010). In conclusion, big advances in the field of chromosomics can be expected in the future from high-resolution FISH banding (MCB/mcb) in three-dimensionally preserved human interphase nuclei.

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