

Fluorescence *in situ* Hybridization General Principles and Clinical Application with Special Emphasis to Interphase Diagnostics

R. Raff and G. Schwanitz

*Institut für Humangenetik der Universität Bonn, Germany, Wilhelmstraße 31, 53111 Bonn, Germany
phone 00-49-(0)228 287 2338, Fax 00-49-(0)228 287 2545, email RRaff@gmx.de*

KEY WORDS Interphase FISH; mosaic analysis; centromere peculiarities; microdeletion; tumor karyotype.

ABSTRACT Fluorescence *in situ* hybridization (FISH) is a molecular-cytogenetic investigation method and thus covers a gap between classical cytogenetic and molecular-genetic techniques. By the broad spectrum of application possibilities it leads to important new developments in basic and applied cytogenetics. It enables the labeling of whole chromosomes and defined chromosome regions and furthermore gene localization with single copy probes. FISH is a technique that allows DNA sequences to be detected on metaphase chromosomes, in interphase nuclei, in a tissue section, or in blastomeres and gametes. In basic scientific research special fields of application comprise characterization of somatic cell hybrids, analyses of meiosis and of karyotype evolution. In clinical and tumor cytogenetic it helps to identify chromosome rearrangements, marker chromosomes, chromosome mosaicism and specific tumor cell lines. Fluorescence *in situ* hybridization receives a special importance for interphase cytogenetics, which mainly covers the development in this field.

INTRODUCTION

Fluorescence *in situ* hybridization offers the possibility to specifically mark individual chromosomes over their entire length or defined chromosome regions in meta- and interphase preparations (Fig. 1) (Chevret et al. 2000). Diagnostic possibilities have been increasing in many areas since hybridization signals can be diagnosed even in the three-dimensional structure of the nucleus. Interphase FISH discloses a new start in basic biomedical research. Areas of research dealing with the organization of the nucleus, analyses of meiosis, gene-expression or tumor evolution gained a new impetus leading to new experiments that contribute to the fundamental understanding of origin, inheritance and mechanism of chromosome aberrations.

Interphase FISH has gained importance in clinical practice because of the availability of

relevant information that is otherwise difficult or impossible to obtain. A suspected aneuploidy for example, can be analyzed with FISH examining degenerated tissue that either cannot be stimulated any longer to grow or solely exists in the way of formaline-fixed and paraffine-embedded tissue sections (Halder and Park 1999). An additional important advantage of interphase FISH is the rapid analysis of uncultivated cells in comparison to conventional chromosome investigations (Bryndorf et al. 2000). In the future the possibility of saving personal, time and material will play an increasing role since more and more DNA probes will be commercially available to advantageous prices.

In order to gain informations of the organization of the nucleus, its structure, architecture and the interaction of the chromosomes, interphase FISH became an irreplaceable technique (Cremer et al. 1996). During interphase chromosomes occupy distinct territories that can be exhibited in domains with the help of "whole chromosome" or "chromosome-arm specific paints" (wcp- and cap-probes) after *in situ* hybridization. It is known that there is a distinct arrangement of chromosome domains that are p- and q-arm- or band-specific (Solovei et al. 1999). Simultaneous multicolor labeling with wcp-, cap-, centromeric and telomeric probes could prove the pairing of homologous chromosome segments in the interphase-nuclei (Gamerding 1999). Three-dimensional microscopy (3D-spectral precision distance microscopy = SPDM) renders the exact measurement of distances of defined euchromatic markers in the nucleus up to a distance of 50 nm. The technique was developed in order to analyze the 3D-chromatin nanostructure of the bcr-region and the bcr/abl-fusion of the Philadelphia chromosome 22 in

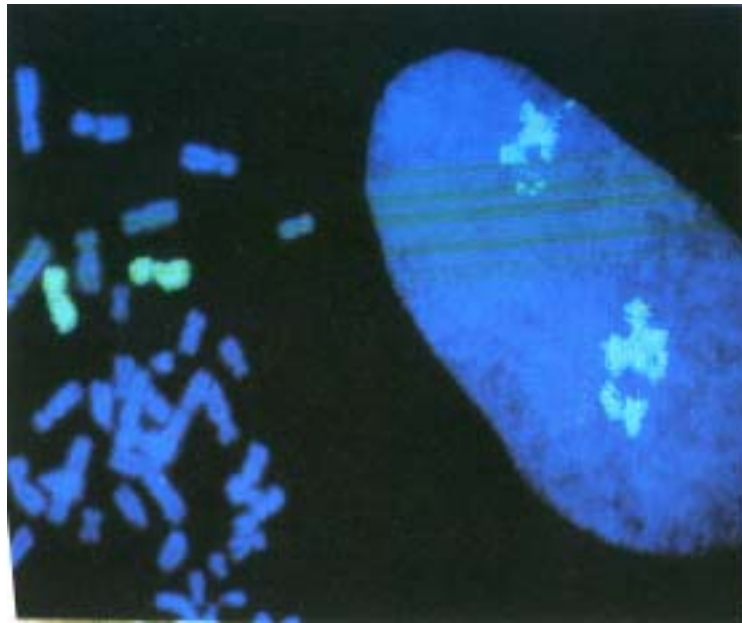


Fig. 1. Hybridization with wcp 11 (Oncor). The euchromatin of both homologous in metaphase is labeled, in interphase the two domains are shown; counterstain with DAPI

patients with chronic myeloid leukemia (CML) (Esa et al. 2000). Studies of the arrangement of chromosomal territories of the early and late replicating female X chromosome demonstrated that in contrary to preexisting opinions, replication in early and late S-phase is not limited to the surface but takes place outside of the territories as well (Visser et al. 1998). Movements in the nuclei can be demonstrated by the way of microinjection of fluorescence DNA probes into healthy cells as well as four-dimensional space-time images (Bornfleth et al. 1999). Results so far give rise to the assumption that certain chromatin areas move in a diffusion-like manner, more pronounced between different chromosomes than within the territory, alternating occasionally between accidental and targeted motions. Investigations of that kind will in the future lead to new disclosures about interactions among individual chromosomes in interphase nuclei and thus about fundamental mechanisms and correlations of gene activity, surface structure of chromosome territories and chromosome morphology. Comparison of the activities in

normal and pathological cells might produce new strategies for conventional treatment and gene-therapeutically onsets for numerous genetic diseases.

INVESTIGATION METHODS

FISH Procedure and DNA Probes

With FISH different types of sequences of the human genome are detectable. The euchromatic region of an entire chromosome or chromosome arm can be visualized by *whole chromosome paints* (wcp). These probes contain clones of DNA libraries of the specific chromosome. *Centromere specific probes* consist of highly repetitive sequences (α satellite DNA) that are present in the centromere of each chromosome. Repeat sequence probes are robust because the targets are large and repeated many times, which allows the hybridization to take place rapidly and produce a very large signal in meta- and interphase. *Unique sequence probes* have target regions that are not repeated in the genome and they are mainly used for gene

mapping, for analysis of microdeletion syndromes and in tumor genetics.

Performance of fluorescence in situ hybridization (FISH) consists of four fundamental steps: *denaturation* of chromosome- and probe-DNA, followed by *hybridization* of the existing single strands to form complementary double strands (renaturation) in several hours (4-16 h) at 37° C.

The DNA can hybridize nonspecifically to sequences which are partially but not entirely homologous to the probe sequence. Such hybrids are less stable than perfectly matched hybrids and can be dissociated by performing *stringent washes*. The stringency can be influenced by the concentration of destabilizing molecules such as formamid, by noncovalent cations, pH-factor and temperature. Finally, *detection* of the hybridized sequences is required, according to the fact that the DNA probe already contains nucleotides linked to a fluorescent dye (direct labeling) or if so-called immunogenic reporter molecules such as biotin or digoxigenin are applied, being accessible to specific antibodies that are conjugated to a fluorochrome (indirect labeling). The fluorescent dyes most frequently applied in both methods are fluorescein isothiocyanate (FITC, yellow) and tetra-methylrhodamin isothiocyanate (TRITC, red). By using two different reporter molecules labeled with different fluorescent dyes several regions in the genome can be analyzed simultaneously. Increasingly,

directly labeled probes are being used for FISH because of easy handling. The use of an indirectly labeled probe though enables amplification and thus increases the sensibility of the procedure.

Analyzed Cell Systems, Significance of Their Vitality and Pretreatment

In general, hybridization can be performed on all available tissues with cells containing nuclei. Problems can occur if the DNA in question exists in a degenerated fashion, e.g. in tissues of spontaneous abortions or stillbirths. Cytological preparations (Werner et al. 1997) as well as cultivated cell material (chromosomes in metaphase or interphase-nuclei) and uncultivated single-cell-preparations can be handled.

Application of Interphase FISH in Clinical Practice

Prenatal Diagnostics

One of the most important prerequisites for prenatal diagnostics is minimal risk as well as quick, valid and reliable results. The advantage of interphase FISH in this context is the rapid detection of selected chromosomal abnormalities, typically the ploidy status of chromosomes 13, 18, 21, X and Y (Fig. 2) (Pergament et al. 2000). Standard turnaround time for cytogenetic analysis of amniotic fluid samples averages 6-10 days because of the need to culture the cells,

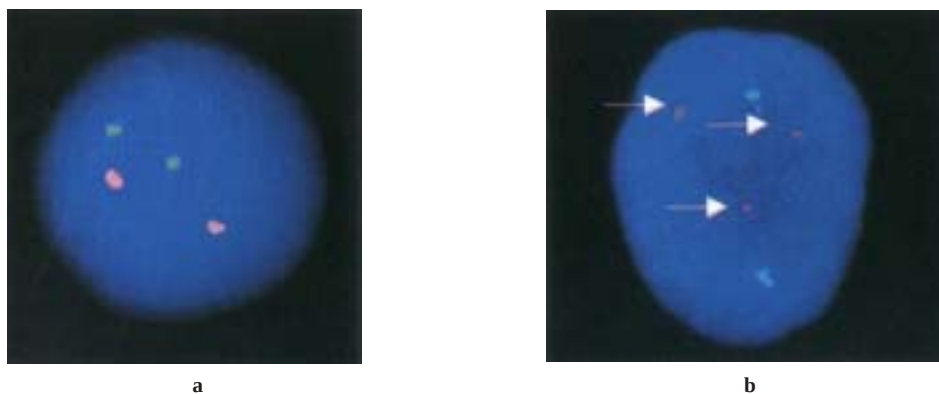


Fig. 2. Interphase FISH in uncultivated amniocytes (Vysis)

- a) DNA probes LSI 13 (13q14, green signal) and LSI 21 (21q22.21, red signal). Normal number of two signals for each probe
 b) DNA probes CEP 18 (D18Z1, violet signal) and CEP X (DXZ1, green signal). Normal number of two X-signals for a female karyotype. Trisomy 18 diagnosed by three signals

whereas interphase FISH provides a preliminary result within 24 hours. In cases of a pathological result, the affected parents have sufficient time to adapt the probable outcome of the diagnosis until the conventional chromosome analysis has been finished.

Interphase FISH following AC, CVS, FBS

Routinely, α -satellite DNA probes are being used for interphase diagnostics after *amniocentesis* (AC) for chromosomes 18, X and Y (Thein et al. 2000). Since 99,0 % of the centromere specific DNA of chromosomes 13 and 21 show homologous sequences, in addition DNA probes are being employed that consist of non-overlapping cosmid contigs of euchromatic regions of those two chromosomes. To their disadvantage, these probes show smaller hybridization signals than centromere probes in interphase nuclei. After labeling with different fluorochromes the DNA probes are analyzed in two or three combinations thus permitting hybridization of the cells on only one slide.

Several factors can influence the performance of interphase diagnostics of uncultivated amniocytes. Amniocentesis is done from the 14th to the 18th week of pregnancy. At that time a sufficient amount of cells for diagnostic purpose will be available. Since a certain quantity of these cells will be dead they will be removed with the first change of culture medium in cultivation but can cause distortion of hybridization results in interphase FISH after direct preparation.

The transabdominal puncture of the uterus for amniocentesis can cause contamination of amniotic fluid with maternal cells up to an amount of 20% (Hockstein et al. 1998), in bloody samples up to 50% (Nuß et al. 1994) and this may result in a false diagnosis. Hybridization of samples contaminated with blood therefore is considered critical.

The high mitotic activity in the cytotrophoblast of the chorionic villi allows the preparation of spontaneous mitoses and a first numeric analysis of the fetal karyotype and sex after *chorionic villi sampling* (CVS). In pathologic cases caused for example by aneuploidy chorionic villi frequently show an aberrant morphology and direct preparation does not provide with metaphases that can be analyzed. In such cases

and those of missing mitoses following long-term cultivation interphase FISH with DNA probes for chromosomes 13, 18, 21, X and Y can supply initial keys concerning the existence of the most common aneuploidies and of triploidies (Cai et al. 1999). By comparing diagnoses following FISH and conventional chromosome analysis in 2709 cases, a study of Bryndorf et al. (1996) could demonstrate that 57,0% of the pathologic karyotypes were detected with these probes but further chromosomes were involved in the aberrations of the remaining 43,0%.

A rapid aneuploidy screening can be done on blood smears after *fetal blood sampling* (FBS) in late pregnancy weeks or pregnancies with abnormal fetal development. A noninvasive method is offered by the analysis of fetal cells in the maternal circulation (Pertl and Bianchi 1999). Marking with magnetic reacting antibodies (magnetic activated cell sorting = MACS) and labeling with fluorescence dyes (fluorescence activated cell sorting = FACS) isolates fetal nucleated erythrocytes from maternal blood and makes them available for interphase diagnostics by FISH. This technique is being utilized in just a few specialized centers worldwide even though it was tested successfully in control studies (Rodriguez De Alba et al. 1999) as well as in pathologic pregnancies (de la Cruz et al. 1998).

Interphase FISH in Preimplantation Diagnostics

Preimplantation diagnostics involve methods that initially were developed for molecular-genetic analyses of monogenic diseases (Harper and Wells 1999). In vitro fertilization (IVF) in the meantime also comprehends molecular-cytogenetic analyses with FISH of individual blastomeres of the early embryo or of the polar body. Preimplantation diagnostics involving interphase FISH can decrease the risk to transfer an embryo with an unbalanced chromosome status (Munné et al. 1998) respectively a male embryo with an X-linked disease.

Postnatal Diagnostics

Interphase FISH is suitable for postnatal analyses on all tissues containing nucleated cells that can be prepared. No cultivation is required, so fixed tissues can be analyzed as well as

material that is difficult to cultivate. This further expands the range of diagnostics and includes cell systems such as buccal mucosa or cells of hair follicles (Lampel et al. 1993; Frias et al. 1996). In cultivated cells with a reduced mitotic activity or insufficient quality of chromosomes interphase FISH can produce additional information. By using centromere specific probes numerical changes can be analyzed thus making it possible to check *in-vitro* shifts of single cell lines in mosaic karyotypes and their distribution in different tissues (Fig. 3, 4). Furthermore it enables an analysis of structural chromosome disorder with interphase FISH. Microdeletions can be displayed in the interphase nucleus (Fig. 5) with specific single-copy probes (Novelli et al. 1999). FISH can also be used to diagnose duplications, for example the duplication of the chromosome region 17p11.2 resulting in the autosomal dominant neurodegenerative Charcot-Marie-Tooth disease. While metaphase chromosomes do not show the doubling of the reliable FISH signal, interphase FISH

renders the diagnosis in the decondensed euchromatin (Shaffer et al. 1999).

Tumor Diagnostics

Cultivation of tumor tissue for the purpose of cytogenetic analysis often proves to be very difficult caused by the pathological character of the tissue to be analyzed. The number of mitoses is low according to the type of tumor and chromosomes frequently are displayed in such a resolution that will not enable unequivocal structural analysis. Additionally, selection in cultivation of cell lines can influence the existing karyotype spectrum. Interphase hybridization, therefore, turned into an important component in diagnosis as well as prognosis and therapy of leukemia, lymphoma and solid tumors. Essentially, tumor characteristic chromosome disorders (aneuploidies, translocations, deletions) can be analyzed as well as the amplification of specific genes. For example, the amplification of the oncogene *her-2-neu* (ERBB2, localization on chromosome 17) in interphase cells can be a

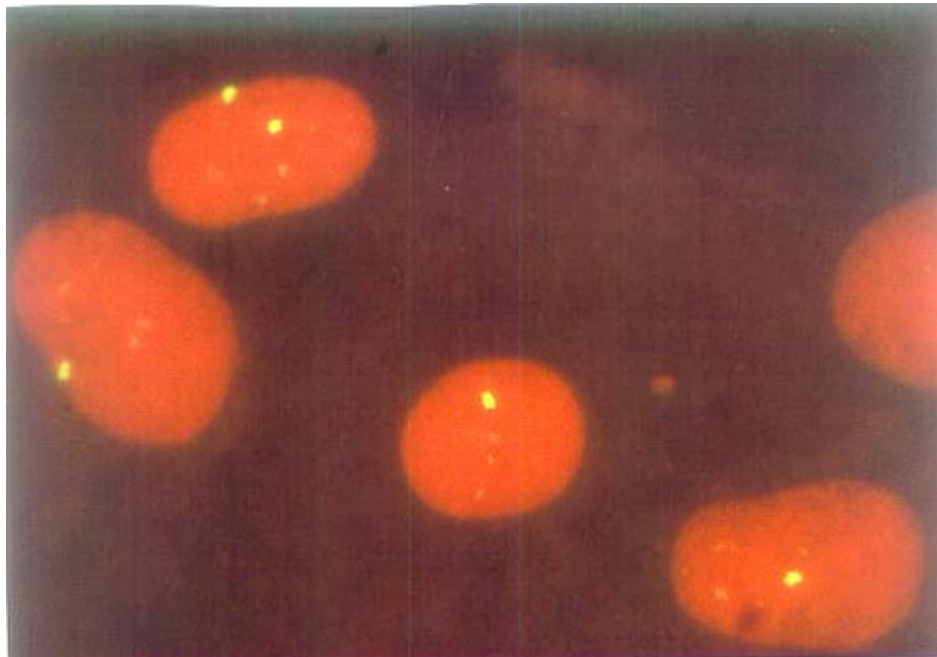


Fig. 3. Interphase analysis of a mosaic karyotype 45, X/46, XX. DNA probe DXZ1 (Oncor). Nuclei with one and two hybridization signals; counterstain with propidiumiodid

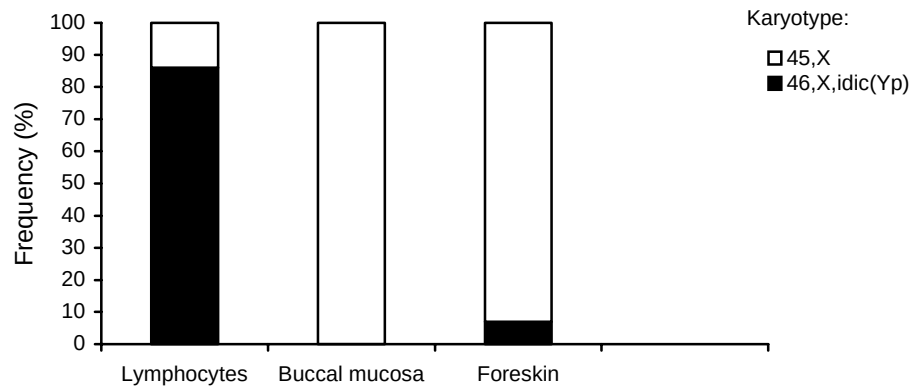


Fig. 4. Unequal distribution of two cell lines in different somatic tissues

considerable indicator for a specific type of breast cancer (Slamon et al. 1987). An increase in copy number of the oncogene is a manifestation of gene amplification and suggests poor prognosis for the patient. Further examples are

evidence of amplification for the *n-myc* gene in neuroblastoma or the *c-myc*-gene in mamma carcinoma. Tumor-characteristic translocations can be diagnosed with the help of interphase FISH: the most frequent evidence is the rearrangement

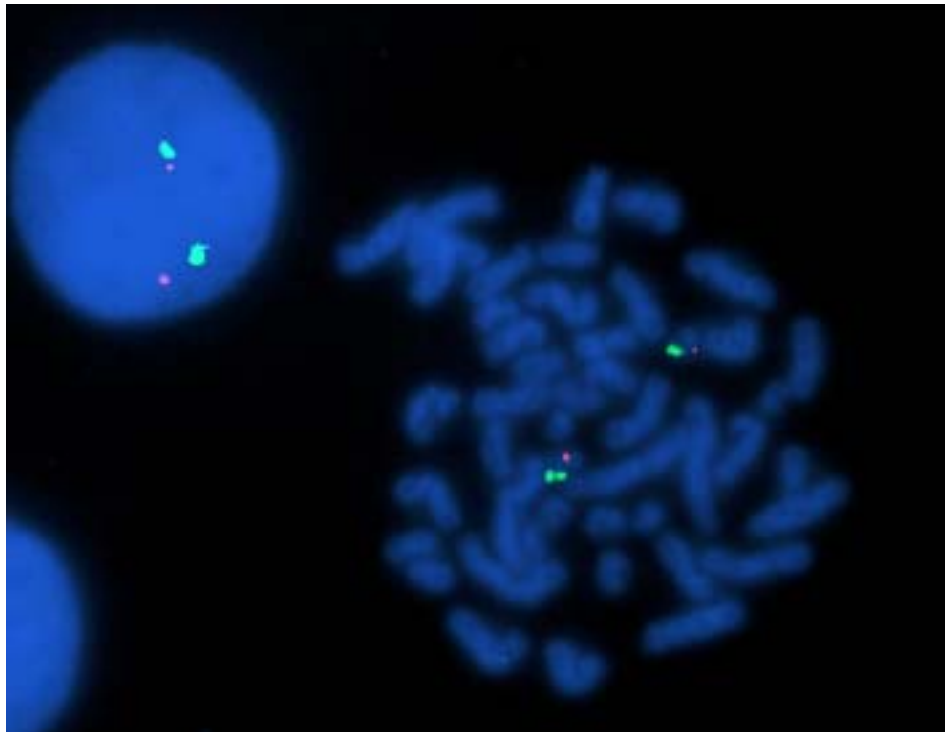


Fig. 5. Hybridization with single copy probe D22S75 (Oncor) in 22q11.2 (red signal) and the control cosmid in 22q13 (green signal). A microdeletion of the DiGeorge Syndrome critical region is excluded; counterstain DAPI

of the *bcr/abl*-genes (Fig. 6) occurring in the cells of almost all of the patients with chronic myeloid leukemia (CML) (Michalova et al. 2000). Another use of interphase FISH in tumor diagnostics are bone marrow transplant follow-up studies in cases with mixed sex donor-recipient pairs (van Tol et al. 1998).

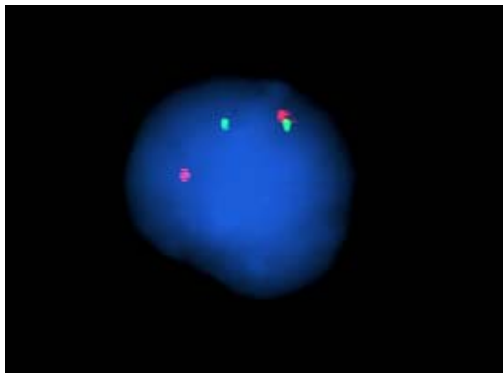


Fig. 6. Bone marrow cell of a patient with CML after hybridization with single copy probe *bcr/abl* in 9q34 (green signal) and 22q11.1 (red signal)(Oncor). A translocation 9/22 is proved in the patient; counterstain DAPI

The Significance of Centromere Structure and Position for Interphase FISH with Centromere Probes

Position of Centromeres in the Interphase Nucleus

Centromeric as well as telomeric regions frequently show association in interphase and form the so-called chromocenters. Gonosomes in par-

ticular hold a preferred position in the nucleus, a fact that has to be taken into consideration in analyses of gonosomal aberrations. Here, the analyzed cell type is also of special importance. In granulocytes, the inactivated X chromosome of the female appears as a drumstick-shaped extrusion of the nucleus, whereas the Y chromosome in the male forms the so-called sessile nodules.

Structural Peculiarities of the Centromeric Region

During the cell-cycle, centromeres have different appearances in G₁, S and G₂ phase (Fig. 7). While their morphology in G₀ and G₁ phase is the same they are decondensed during S phase and occupy a larger, not clearly defined area. The signals can overlay and in part of the cells they cannot be clearly analyzed. During G₂ phase, when centromeres following replication show double-signals that may not be differentiated well enough from an altered signal number, diagnostic problems may occur (Cannizzaro et al. 1997).

In females, signals for the centromere of the inactivated X chromosome are occasionally being altered. Apparently, inactivation of the second X chromosome will lead to various disturbances in the cell-cycle with increasing age (Mukherjee et al. 1998). Sometimes, permanent inactivation of a centromere occurs, leading either to unequal distribution of the inactive X chromosome, resulting in monosomy or trisomy of the X, or partial endoreduplication happens during S phase which possibly can lead to an amplification of the X chromosome in up to more than 20 copies.

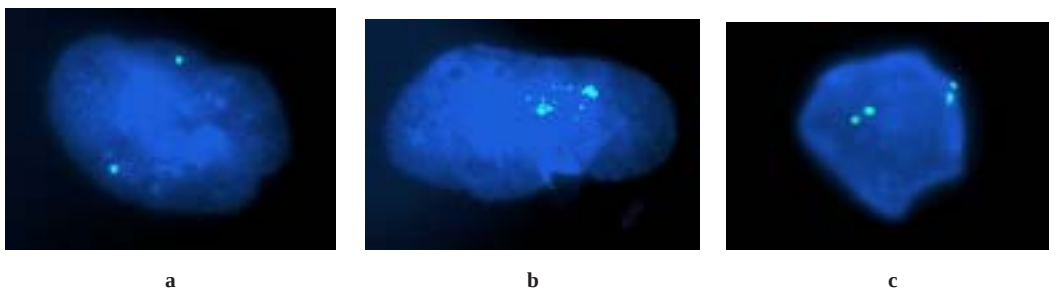
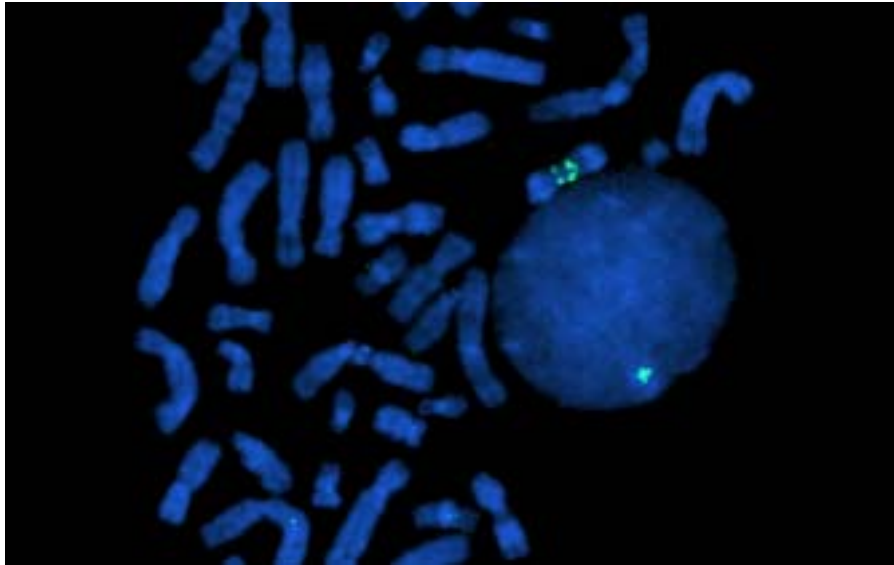
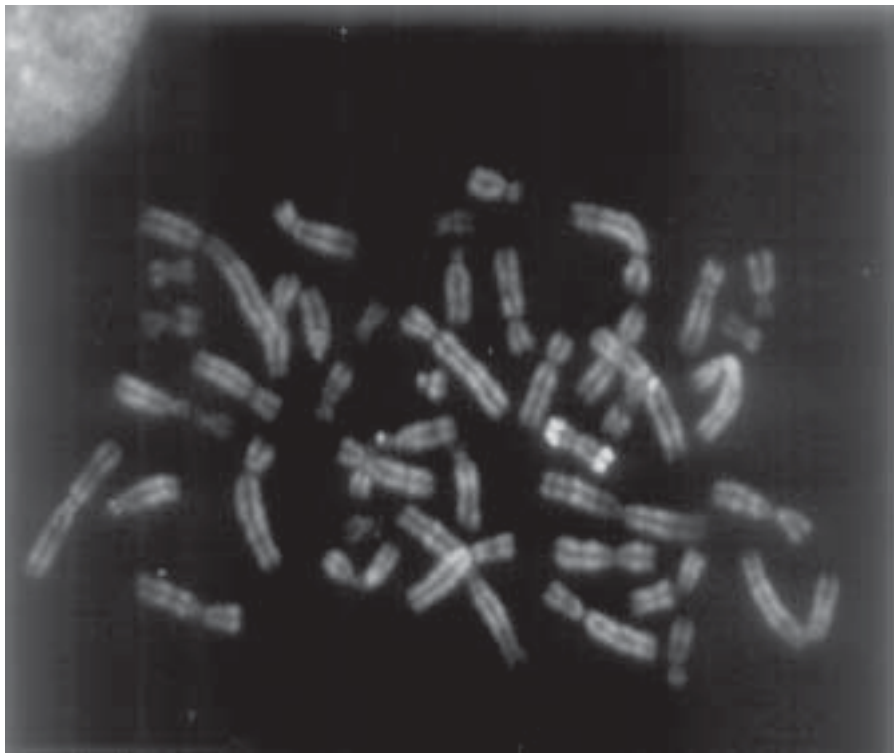


Fig. 7. Interphase nuclei in different phases of the cell cycle with DNA probe D12Z1 (Oncor)
a) G₁ phase b) S phase c) G₂ phase



a



b

Fig. 8. Mosaic karyotype 45, X/46, X, idic (Yq)
a) FISH with DNA probe DY3. Metaphase with two hybridization signals in the aberrant Y chromosome
b) Metaphase after QFQ banding

Analysis of Interphase Chromosomes with Pathological Karyotypes

After the diagnosis of a structural chromosome aberration by conventional cytogenetics, molecular-cytogenetic investigations often will follow. Analysis of chromosomes or chromosome regions with FISH is frequently performed with a combination of single copy and centromere probes presented in different colors. These combinations improve the reliability of the diagnosis. Additionally insight can be gained by interphase FISH on the morphology of defined chromosomes in the nucleus and their positions in normal as well as pathological karyotypes. The possibility to comprehend chromosomes in various places of the cell cycle lead to analyses of replication behavior of normally structured as well as aberrant chromosomes. The course of the cell cycle was studied for those chromosomes that showed a tendency to secondary selection *in vivo* and/or *in vitro* (Amiel et al. 2000). Also translocation chromosomes were analyzed, especially marker chromosomes in comparison to their normally structured homologous. Irregularities could be demonstrated and quantified (Koch et al. 1993).

Centromere Mutations

Mutations in centromeric regions can only be analyzed to their fullest extent with a combination of metaphase and interphase diagnostics thus comprehending functional particularities qualitatively and quantitatively. The ability to present euchromatin and heterochromatin of individual chromosomes separately with FISH allows detailed analyses of frequencies and types of mutations and preferred interchromosomal chromatin exchanges.

Centromere amplifications and partial deletions occur in very different frequencies. Only FISH investigations are able to analyze them further. Analyses of deletions proved that normal functions of the centromere are still possible even if its size is reduced to about 20%. In interphase diagnostics with centromere specific DNA probes such deletions give weak or missing hybridization signals and thus can lead to misdiagnoses in the nucleus (Verma et al. 1992; Marzais et al. 1999). Amplifications of the centromeric

regions are particularly frequent in chromosomes 18 and 20. The amount of an amplification can lead to a tenfold of the normal size without negative consequences such as anomalies of mitosis or premature cell death. These structurally aberrant chromosomes can continue through meiosis without secondary changes.

Findings of amplified centromeres in interphase FISH can lead to hybridization patterns that diverge from normal centromere signals and require further investigations.

Isochromosomes or whole arm translocations can occur if chromosomes perform transversal instead of a longitudinal separation during anaphase of meiosis or mitosis. If this "misdivision" occurs in an acrocentric chromosome the short arm can get lost. The phenotype usually does not change. Centromeric fissions as well as fusions played an important role in the evolution of the mammalian karyotype.

Centromere translocations occur in different autosomes as well as in gonosomes. Centric fusions of acrocentric chromosomes (13–15, 21 and 22) are especially frequent, and distinct combinations are preferred. Reasons are homologous sequences in the centromeric region (Nilsson et al. 1997). Specific hybridization could prove that in the fusion product centromeres can exclusively originate from one translocation partner but from both as well (Gravholt et al. 1992).

Marker chromosomes are caused by rearrangements within or between two or more chromosomes. Their centromere structure is monocentric, dicentric or, very rarely, multicentric. Entirely heterochromatic markers are mostly monocentric. They mainly originate from acrocentric chromosomes and then consist of the centromere, the pericentromeric heterochromatin (p11.1, in dicentrics with q11.1), the proximal short arm region (p12), the NOR-region (p12) and the satellites (p13). The majority of marker ring chromosomes is also monocentric and can contain partial euchromatic regions. Heterochromatic and euchromatic markers are dicentric if the breakpoint occurs at the end of a larger heterochromatic band, just like the proximal band of the short and the long arm of chromosome 15. One of the two centromeres has to be inactivated in a dicentric chromosome to enable a normal distribution of the chromatides in the anaphase

of mitosis (Fig. 8). Partial loss or increase of markers and formation of complex mosaic karyotypes will occur if inactivation is incomplete. Analyses of marker chromosomes without centromeric structure but with kinetochores are rare, but these structures are enabling a comparatively normal distribution of markers through mitosis. Rudimentary centromeric structures are then expected interstitially in the chromosome that developed in the course of karyotype evolution (Willard et al. 1998).

FISH was first performed on metaphase chromosomes prepared according to standard protocols. Introduction of interphase FISH increased the potential of this technique. Further development of fiber-FISH and the application of probe sets will lead to new investigation possibilities.

ACKNOWLEDGEMENT

We would like to thank Mrs. L. Kalz for her help with the manuscript.

REFERENCES

- Amiel A, Reish O, Gaber E, Kedar I, Diukman R, Fejgin M 2000. Replication asynchrony increases in women at risk for aneuploid offspring. *Chromosome Res*, **8**: 141-50.
- Bornfleth H, Edelmann P, Zink D, Cremer T, Cremer C 1999. Quantitative motion analysis of subchromosomal foci in living cells using four-dimensional microscopy. *Biophys J*, **77**: 2871-86.
- Bryndorf T, Christensen B, Vad M, Parner J, Carelli MP, Ward BE, Klinger KW, Bang J, Philip J 1996. Prenatal detection of chromosome aneuploidies in uncultured chorionic villus samples by FISH. *Am J Hum Genet*, **59**: 918-26.
- Bryndorf T, Lundsteen C, Lamb A, Christensen B, Philip J 2000. Rapid prenatal diagnosis of chromosome aneuploidies by interphase fluorescence in situ hybridization: a one-year clinical experience with high-risk and urgent fetal and postnatal samples. *Acta Obstet Gynecol Scand*, **79**: 8-14.
- Cai LS, Lim AS, Tan A 1999. Rapid one-day fluorescence in situ hybridization in prenatal diagnosis using uncultured amniocytes and chorionic villi. *Ann Acad Med Singapore*, **28**: 502-7.
- Cannizzaro LA, Shi G 1997. Fluorescent in situ hybridization (FISH) for DNA probes in the interphase and metaphase stages of the cell-cycle. *Methods Mol Biol*, **75**: 313-22.
- Chevret E, Volpi EV, Sheer D 2000. Mini review: form and function in the human interphase chromosome. *Cytogenet Cell Genet*, **90**: 13-21.
- Cremer C, Munkel C, Granzow M, Jauch A, Dietzel S, Eils R, Guan XY, Meltzer PS, Trent JM, Langowski J, Cremer T 1996. Nuclear architecture and the induction of chromosomal aberrations. *Mutat Res*, **366**: 97-116.
- de la Cruz F, Shifrin H, Elias S, Bianchi DW, Jackson L, Evans MI, Simpson JL, Holzgreve W, Klinger K 1998. Low false-positive rate of aneuploidy detection using fetal cells isolated from maternal blood. *Fetal Diagn Ther*, **13**: 380.
- Esa A, Edelmann P, Kreth G, Trakhtenbrot L, Amariglio N, Rechavi G, Hausmann M, Cremer C 2000. Three-dimensional spectral precision distance microscopy of chromatin nanostructures after triple-colour DNA labelling: a study of the BCR region on chromosome 22 and the Philadelphia chromosome. *J Microsc*, **199**: 96-105.
- Frias S, Ordaz G, Blanco B, Molina B, del Castillo V, Carnevale A 1996. Detection of aneuploidies using in situ hybridization in cells of the oral mucosa. *Rev Invest Clin*, **48**: 355-60.
- Gamerding U 1999. Clues for an ordered chromatin arrangement in associated domains of human homologous chromosomes. *Cytogenet Cell Genet*, **85**: 106.
- Gravholt CH, Friedrich U, Caprani M, Jorgensen AL 1992. Breakpoints in Robertsonian translocations are localized to satellite III DNA by fluorescence in situ hybridization. *Genomics*, **14**: 924-30.
- Halder A, Park Y 1999. Identification of the appropriate tissue from formalin fixed perinatal autopsy material for chromosomal ploidy detection by interphase FISH. *Indian J Med Res*, **110**: 102-6.
- Harper JC, Wells D 1999. Recent advances and future developments in PGD. *Prenat Diagn*, **19**: 1193-9.
- Hockstein S, Chen PX, Thangavelu M, Pergament E 1998. Factors associated with maternal cell contamination in amniocentesis samples as evaluated by fluorescent in situ hybridization. *Obstet Gynecol*, **92**: 551-6.
- Koch J, Fischer H, Askholm H, Hindkjaer J, Pedersen S, Kolvraa S, Bolund L 1993. Identification of a supernumerary der(18) chromosome by a rational strategy for the cytogenetic typing of small marker chromosomes with chromosome-specific DNA probes. *Clin Genet*, **43**: 200-3.
- Lampel S, Steilen H, Zang KD, Wullich B 1993. Sex chromatin in hair roots-25 years later: fluorescence in situ hybridization of hair root cells for detection of numerical chromosome aberrations. *Cytogenet Cell Genet*, **63**: 244-6.
- Marzais B, Vorsanova SG, Roizes G, Yurov YB 1999. Analysis of alphoid DNA variation and kinetochore size in human chromosome 21: evidence against pathological significance of alphoid satellite DNA diminutions. *Tsitol Genet*, **33**: 25-31.
- Michalova K, Zemanova Z, Cmunt E, Karban J, Brezinova J, Sindelarova L, Kurkova S, Kubcova S, Schwarz J 2000. The interphase fluorescence in situ hybridization (I-FISH) technique in patients with chronic lymphatic leukemia (CLL). *Cas Lek Cesk*, **139**: 334-8.
- Mukherjee AB, Costello C 1998. Aneuploidy analysis in fibroblasts of human premature aging syndromes by FISH during in vitro cellular aging. *Mech Ageing Dev*, **103**: 209-22.
- Munné S, Fung J, Cassel MJ, Marquez C, Weier HU 1998. Preimplantation genetic analysis of translocations: case-specific probes for interphase cell analysis. *Hum Genet*, **102**: 663-74.
- Nilsson M, Krejci K, Koch J, Kwiatkowski M, Gustavsson

- P, Landegren U 1997. Padlock probes reveal single-nucleotide differences, parent of origin and in situ distribution of centromeric sequences in human chromosomes 13 and 21. *Nat Genet*, **16**: 252-5.
- Novelli A, Sabani M, Caiola A, Digilio MC, Giannotti A, Mingarelli R, Novelli G, Dallapiccola B 1999. Diagnosis of DiGeorge and Williams syndromes using FISH analysis of peripheral blood smears. *Mol Cell Probes*, **13**: 303-7.
- Nuss S, Brebaum D, Grond-Ginsbach C 1994. Maternal cell contamination in amniotic fluid samples as a consequence of the sampling technique. *Hum Genet*, **93**: 121-4.
- Pergament E, Chen PX, Thangavelu M, Fiddler M 2000. The clinical application of interphase FISH in prenatal diagnosis. *Prenat Diagn*, **20**: 215-20.
- Pertl B, Bianchi DW 1999. First trimester prenatal diagnosis: fetal cells in the maternal circulation. *Semin Perinatol*, **23**: 393-402.
- Rodriguez De Alba M, Palomino P, Jurado A, Sanz R, Ibanez MA, Fernandez-Moya JM, Ayuso C, Diaz-Recasens J, Lahoz C, Ramos C 1999. Prenatal diagnosis on fetal cells obtained from maternal peripheral blood: report of 66 cases. *Prenat Diagn*, **19**: 934-40.
- Shaffer LG, Kennedy GM, Spikes AS, Lupski JR 1997. Diagnosis of CMT1A duplications and HNPP deletions by interphase FISH: implications for testing in the cytogenetics laboratory. *Am J Med Genet*, **69**: 325-31.
- Slamon D, Clark G, Wong S, Levin W, Ullrich A, McGuire W 1987. Human breast cancer: correlation of relapse and survival with amplification of HER-2/neu oncogene. *Science*, **244**: 707-712.
- Solovei BA, Cremer M, Habermann F, Karpf F, Tashiro S, Edelmann P, Eils R, Little G, Cremer C, Cremer P 1999. Three dimensional analysis of chromosome territories in normal and malignant human cell nuclei. *Cytogenet Cell Genet*, **85**: 19.
- Thein AT, Abdel-Fattah SA, Kyle PM, Soothill PW 2000. An assessment of the use of interphase FISH with chromosome specific probes as an alternative to cytogenetics in prenatal diagnosis. *Prenat Diagn*, **20**: 275-80.
- Tocharoentanaphol C, Parinayok R, Punthuwattana N, Kangwanpong D 2000. Fluorescence in situ hybridization: a rapid analysis to verify chromosome aberrations. *J Med Assoc Thai*, **83 Suppl 1**: S76-81.
- van Tol MJ, Langlois van den Bergh R, Mesker W, Ouwerkerk-van Velzen MC, Vossen JM, Tanke HJ 1998. Simultaneous detection of X and Y chromosomes by two-colour fluorescence in situ hybridization in combination with immunophenotyping of single cells to document chimaerism after sex-mismatched bone marrow transplantation. *Bone Marrow Transplant*, **21**: 497-503.
- Verma RS, Luke S 1992. Variations in alphoid DNA sequences escape detection of aneuploidy at interphase by FISH technique. *Genomics*, **14**: 113-6.
- Visser AE, Eils R, Jauch A, Little G, Bakker PJ, Cremer T, Aten JA 1998. Spatial distributions of early and late replicating chromatin in interphase chromosome territories. *Exp Cell Res*, **243**: 398-407.
- Werner M, Wilkens L, Aubele M, Nolte M, Zitzelsberger H, Komminoth P 1997. Interphase cytogenetics in pathology: principles, methods, and applications of fluorescence in situ hybridization (FISH). *Histochem Cell Biol*, **108**: 381-90.
- Willard HF 1998. Centromeres: the missing link in the development of human artificial chromosomes. *Curr Opin Genet Dev*, **8**: 219-25.