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# The DNA-Based Structure of Human Chromosome 5 in Interphase

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**In contrast to those of metaphase chromosomes, the shape, length, and architecture of human interphase chromosomes are not well understood. This is mainly due to technical problems in the visualization of interphase chromosomes in total and of their substructures. We analyzed the structure of chromosomes in interphase nuclei through use of high-resolution multicolor banding (MCB), which paints the total shape of chromosomes and creates a DNA-mediated, chromosome-region–specific, pseudocolored banding pattern at high resolution. A microdissection-derived human chromosome 5–specific MCB probe mixture was hybridized to human lymphocyte interphase nuclei harvested for routine chromosome analysis, as well as to interphase nuclei from HeLa cells arrested at different phases of the cell cycle. The length of the axis of interphase chromosome 5 was determined, and the shape and MCB pattern were compared with those of metaphase chromosomes. We show that, in lymphocytes, the length of the axis of interphase chromosome 5 is comparable to that of a metaphase chromosome at 600-band resolution. Consequently, the concept of chromosome condensation during mitosis has to be reassessed. In addition, chromosome 5 in interphase is not as straight as metaphase chromosomes, being bent and/or folded. The shape and banding pattern of interphase chromosome 5 of lymphocytes and HeLa cells are similar to those of the corresponding metaphase chromosomes at all stages of the cell cycle. The MCB pattern also allows the detection and characterization of chromosome aberrations. This may be of fundamental importance in establishing chromosome analyses in nondividing cells.**

## Introduction

Interphase chromosomes analyzed with currently available routine cytogenetic techniques do not exhibit any recognizable structures such as bands, centromeres, telomeres, or specific shapes. It has therefore been assumed that chromosomes in interphase are relatively decondensed (Comings 1968). Until now, the concept of condensation and decondensation of chromosomes during mitosis was well established and has profoundly influenced our understanding of the structure and function of chromosomes during mitosis. This concept implies that chromosomes in interphase are very long and condense after S phase. Clustering of chromatin loops results in condensation, giving very long prophase chromosomes, which show thousands of bands (Yunis 1981). These further condense to prometaphase, metaphase, and anaphase chromosomes. Decondensation takes place as cells return, via telophase, to interphase.

Surprisingly, there have been no scientific investigations directly confirming this dynamic concept—for example, by comparing the total length of chromosomes in interphase and metaphase. There is, however, indirect evidence, such as countless daily observations that harvesting chromosomes shortly after S phase results in elongated prophase and prometaphase chromosomes. Also, pictures of cells in G2 phase showing interlaced threads of elongated chromosomes that are sometimes much longer than the diameter of interphase cells are well known. Furthermore, the extent of compaction of chromosomes in interphase cells, estimated in both yeast (Guacci et al. 1994) and human lymphoma cell lines (Lawrence et al. 1988) by measuring the distances between fluorescently labeled DNA probes, was found to be significantly different when compared with mitotic chromosomes, with a two- to tenfold compaction of mitotic chromosomes in comparison with interphase chromosomes. In addition, the phenomenon of premature chromosome condensation (Johnson and Rao 1970) and results obtained from high-resolution chromosome preparations (Yunis 1976) provide further support for our understanding of chromosomes in interphase nuclei. It

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was observed that human prophase chromosomes can be very long, showing as many as 3,000 bands per haploid set (Yunis 1981). In addition, numerous investigations dealing with proteins such as topoisomerase II, HMC, SMC, H1, and H3, which have been confirmed to be involved in—or responsible for—chromosome condensation, have been published (for a review, see Koshland and Strunnikov [1996]). None of these data, however, can fully explain the speed with which chromosomes change their structure during mitosis or the fact that homologous metaphase chromosomes sometimes show extreme differences in size.

Experience with artificial stretching of human chromosomes (Claussen et al. 1994; Hliscs et al. 1997*a*) indicates that a significant chromosome-elongation process takes place during routine chromosome preparation. This leads to doubts as to whether chromosomes in interphase are indeed very long. Furthermore, microirradiation experiments (Cremer et al. 1982) and molecular cytogenetic investigations with whole-chromosome paints (Cremer et al. 1988; Lichter et al. 1988; Pinkel et al. 1988) or region-specific microdissection probes (Cremer et al. 1993; for review, see Chevret et al. 2000; Cremer and Cremer 2001) have confirmed a territorial organization of chromosomes in interphase nuclei that is more likely to be equivalent to short chromosomes in interphase.

To completely paint chromosomes in interphase, to measure their length, and to analyze their DNA-based banding structure, the microdissection-based high-resolution multicolor banding (MCB) technique was used. This is a FISH approach first described by Chudoba et al. (1999). The results show that chromosomes in interphase are not decondensed but are as short as metaphase chromosomes and show almost the same MCB pattern. This pattern can be used for the identification of small structural aberrations in chromosomes.

## Material and Methods

### *MCB on Lymphocyte Interphase Chromosome 5*

To examine the shape and banding pattern of interphase chromosomes and to measure their length, an MCB pattern was generated using a human chromosome 5–specific hybridization mixture on lymphocyte interphase nuclei from a normal male subject, as described elsewhere for metaphase chromosomes (Chudoba et al. 1999). Microscopic analysis was performed using an Axioplan II microscope (Zeiss) equipped with a CCD camera (Sony), an HBO 100 mercury lamp, and filter sets for 4,6-diamidino-2-phenylindole (DAPI), diethylaminocoumarine, fluorescein isothiocyanate, Cy3, Cy3.5, and Cy5. Images were captured and analyzed using the Isis imaging system (MetaSystems), which allows the level of the chromosome region–specific MCB resolution to be freely defined. Interphase chromosomes were analyzed at the 6-, 11-, 16-, and 21-band level. The lengths of the axes of routinely harvested and flattened interphase chromosomes were measured using the Isis imaging system. Statistical analyses were performed using the Levene test and the *t* test.

Chromosome elongation during drying of the fixative on the slide is mainly influenced by the humidity. Therefore, we also measured the length of interphase and metaphase chromosomes in half-spherical lymphocytes obtained by drying the fixative at a humidity of <7%. Fixed lymphocyte suspensions, routinely harvested for chromosome analysis, were dropped onto dry slides and dried under vacuum (DNA Speed Vac; Savant), without rotating the slides, at 22.4°C and a humidity of <7%. The MCB pattern of chromosome 5 was generated as described above. A laser scanning microscope (LSM 510 Meta; Zeiss) was used to measure the length and to analyze the shape of chromosome 5 at interphase and metaphase. Statistical analyses were performed using the Levene test and the *t* test.

### *Comparison of the Length of Metaphase and Interphase Chromosome 5*

The length of the center line of both chromosomes 5 from lymphocyte metaphases was measured using the Isis imaging system. The band resolution level was determined by counting the number of dark and light bands and by comparing the results with the number of bands on the ideograms published in the International System for Human Cytogenetic Nomenclature (Mittelman 1995). The relationship between the length of chromosome 5 and the number of GTG bands was determined by linear regression.

### *Cell Cycle–Specific MCB on HeLa Interphase Chromosome 5*

Synchronized HeLa cells were used to investigate cell cycle–specific aspects of chromosomal shapes and banding patterns of interphase chromosomes. HeLa cells synchronized in interphase were harvested every 2 h throughout the cell cycle, and 50 cells were analyzed at each time point. Logarithmically growing cells were synchronized by application of either a thymidine block, with subsequent arrest of the mitotic cells by $N_2O$ (Schmiady and Sperling 1981), or a double thymidine block without $N_2O$ treatment, to arrest the cells in the S phase. For the thymidine block, cells were cultured with 2 mM thymidine for 16 h, for a further 7 h in normal medium, and then were treated with $N_2O$ (4.2 kPa/cm$^2$) for 8.5 h. After release of the $N_2O$ block, the mitotic cells were harvested by gentle shaking and were plated into six 5-cm petri dishes. After 45 min, the cells had entered the G1 phase. Every 2 h, cells from one dish were harvested, incubated in hypotonic KCl, fixed in three changes of methanol/acetic acid (3:1), dropped onto wet slides, and air dried.

For the double thymidine block, which synchronizes cells between the early S phase and G2, cells were cultured with 2 mM thymidine for 16 h, for 11 h in normal medium, and for a further 12 h with 2 mM thymidine. After removal of the second thymidine block, the cells were harvested as described above. Calyculin A (10 nM; Sigma) was added to parallel cultures for 60 min before harvesting. The degree of synchrony (>90%) could be estimated in the prematurely condensed chromosomes in the course of the cell cycle.

## Results

### *MCB on Interphase Chromosome 5*

Nearly all interphase nuclei showed MCB patterns on chromosome 5 very similar to those of corresponding metaphase chromosomes at different, freely chosen, colored band levels (1, 6, 11, 16, and 21; fig. 1). Typically, both telomeric bands were visible, and the centromeric region did not show the characteristic constriction. Although a center line can be positioned as for metaphase chromosomes, interphase chromosomes are not as straight as metaphase chromosomes, being bent and sometimes folded. In 36 (34.0%) of the 106 nuclei analyzed, the complete MCB patterns of both chromosomes were visible. Sometimes very small colored bands, most often from the pericentromeric region, are not visible. Therefore, a complete banding pattern is defined as $\geqslant$10 colored bands being present at the 11-colored-band level, using the Isis software. This corresponds to an ~200-band level of G-banded chromosomes. The choice of a higher level, such as the 16- or 21-band level, for the definition of a completely banded chromosome would have resulted in the exclusion of too many chromosomes. Conversely, the six-band level is too low to characterize a chromosome 5 as being completely banded. Fifteen nuclei (14.2%) showed one completely banded chromosome 5, and one nucleus (0.9%) showed no MCB signal at all. In the remaining 54 nuclei (50.9%), the chromosome 5 MCB patterns were either incomplete, overlapping, or both. The similarity in shape between chromosome 5 in interphase and metaphase allows the measurement of the length of interphase chromosomes, defined as the length of the center line between both telomeric bands (fig. 1). In 67 of the 87 completely banded interphase chromosomes 5, the axes could be precisely positioned, and in the remaining 20, delineation of the axes was not possible, because of strong folding and loop configurations. The mean ± SD length of interphase chromosome 5 was 12.0 ± 2.3 $\mu$m.

Three-dimensional intact chromosomes 5 in half-spherical interphase nuclei revealed a gross banding pattern based on seven differently labeled microdissection libraries of the MCB probe mixture. The mean ± SD length of interphase chromosome 5 was 6.6 ± 1.0 $\mu$m ($n$ = 17), whereas metaphase chromosomes 5 (5.0 ± 1.4 $\mu$m; $n$ = 10) on the same slide were significantly shorter ($P < .002$).

### *Comparison between the Lengths of Interphase Chromosome 5 and Metaphase Chromosome 5*

To compare the length of interphase chromosomes to the band resolution of G-banded metaphase chromosomes, we first investigated whether a correlation exists between the length of metaphase chromosomes and their band level. The relationship (fig. 2) was highly significant ($P < .001$) and was used to determine the length of interphase chromosome 5, which was found to be as long as metaphase chromosome 5 at 600 G-band resolution.

### *Cell Cycle–Specific Banding Pattern of Interphase Chromosome 5 in HeLa Cells*

The MCB pattern of chromosome 5 in HeLa cells is present throughout the cell cycle (fig. 3). In contrast to G1 (fig. 3*A*), chromosomes in S and G2 phases are wider (fig. 3*B* and 3*C*) because of the replication-induced increase in the DNA content. Furthermore, the boundaries of chromosomes in S phase appear to be more diffusely marked when compared with those in G1 and G2. When compared with metaphase chromosomes, interphase chromosomes are wider at all stages of the cell cycle (fig. 3).

### *Identification of Chromosome Aberrations on Interphase Chromosomes*

The aberrations of chromosome 5 in HeLa cells are clearly visible in both metaphase (fig. 3*D*) and interphase (fig. 3*A*–*C*). The similarities in the banding patterns pose the question of whether the MCB of interphase chromosomes can be used for diagnostic purposes. To address this issue, cells from two subjects with known structural chromosome 5 aberrations were analyzed at interphase. Subject 1 was a child with congenital malformations and a duplicated interstitial insertion on 5q, present in cultivated lymphocytes (fig. 4*A*). Subject 2 was a patient with acute myeloid leukemia (AML M6), characterized by a 5q deletion present in cultivated bone marrow cells (fig. 4*B*). In both subjects, the 5q aberrations were clearly detectable (fig. 4), and the breakpoints, as previously determined on metaphase chromosomes (Lemke et al. 2001), were confirmed. Figure 4*A* also shows the folded structure of both chromosomes 5. The normal, clearly painted chromosome 5 is shown along with the aberrant chromosome 5 on which not all bands are visible. The aberrant chromosome 5 is characterized by the green band derived from 5q31 (*gray arrow*), which is inserted in 5q13, and is flanked by the ochre and white bands. In figure 4*B*, the deleted chromosome 5 of the 5q− syndrome can also be identified on the basis of its interphase banding pattern.

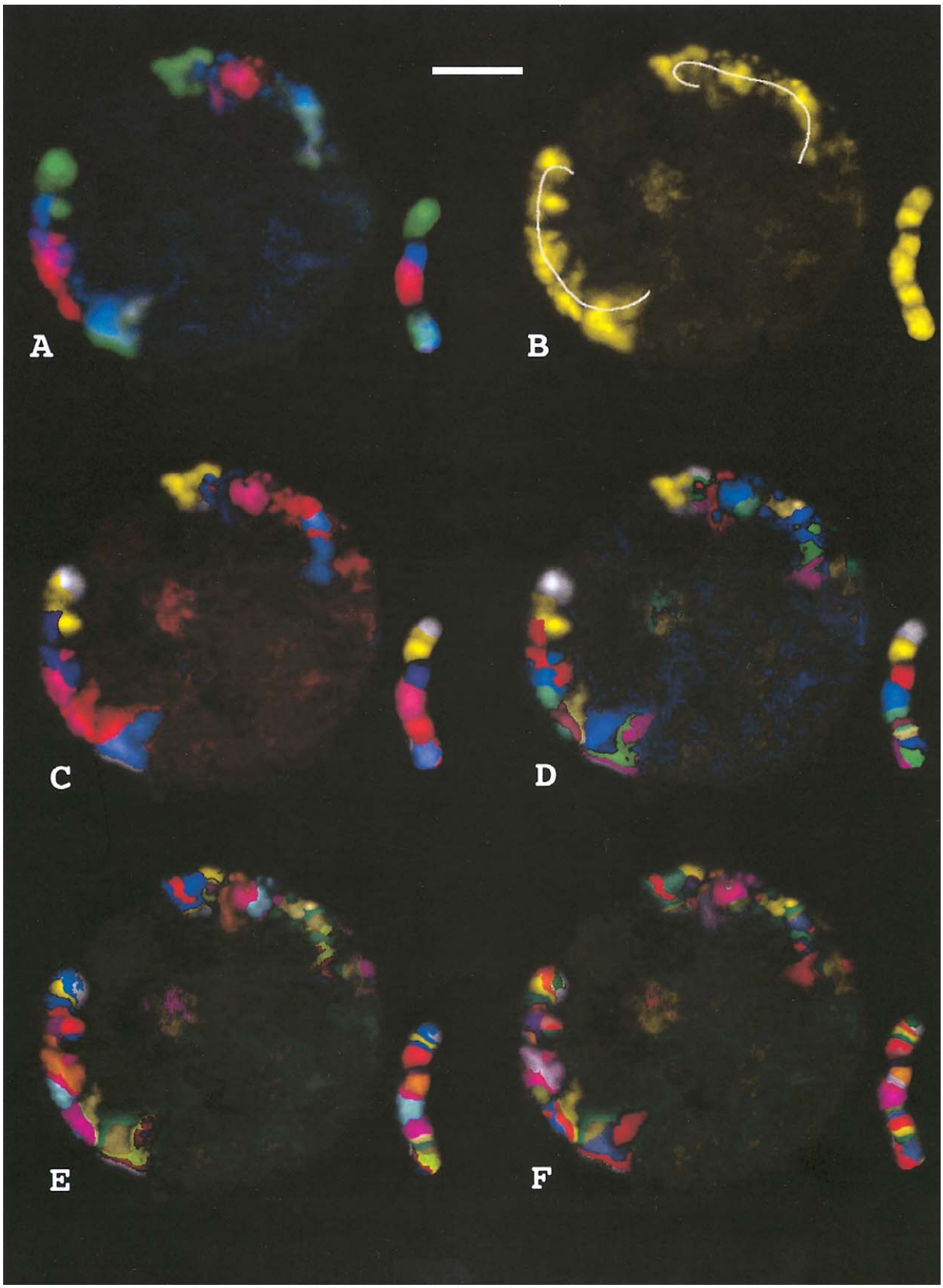


**Figure 1** Human chromosome 5 in a normal lymphocyte interphase nucleus at different MCB pattern resolutions. Both interphase chromosomes 5 are visible, showing the same color patterns as their corresponding metaphase chromosomes 5 on the right. Scale bar = 5 $\mu$m. *A,* Both chromosomes in the interphase nucleus hybridized with the MCB probe mixture. The different colors arise from the fluorescence signals taken with the individual filter combinations. DNA-based pseudocolors are not integrated. *B,* Both chromosomes 5, in one color. The MCB probe mixture has been used as a whole chromosome–painting probe. The lines represent the chromosome axes as center lines between the telomeric bands and were used to measure the length of the interphase chromosomes 5. *C, D, E,* and *F* show both chromosomes 5 in 6, 11, 16, and 21 different pseudocolors, respectively.

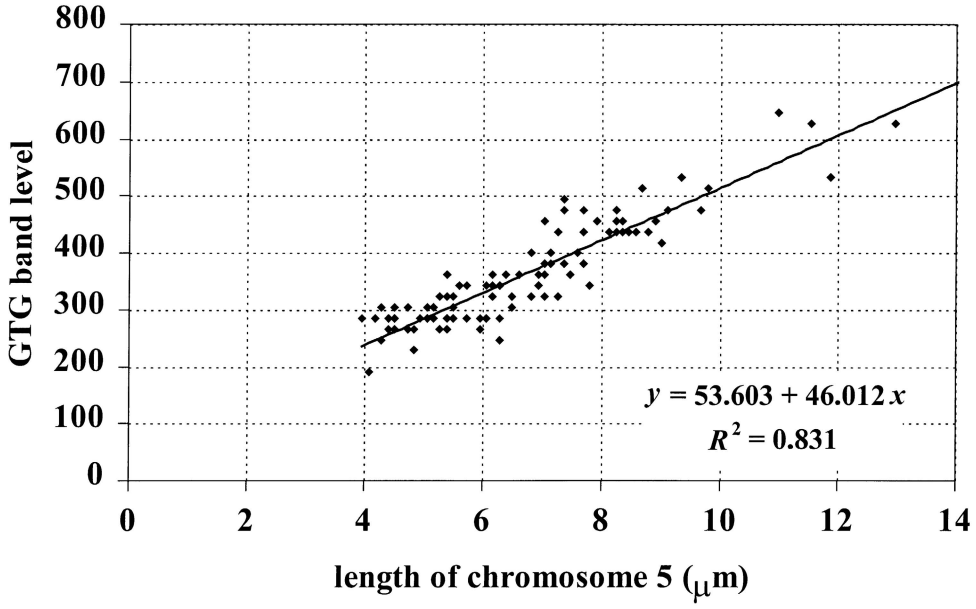


**Figure 2** Correlation between the length of metaphase chromosome 5 of lymphocytes and its GTG-band resolution (individual data points are represented by point markers; $n = 100$). The relationship is highly significant ($P < .001$), and $R^2$ confirms a linear relationship between the variables. Interphase chromosomes are an average of 12 $\mu$m in length and are therefore similar to metaphase chromosomes at the 600-band resolution level.

## Discussion

### *Length of Chromosomes in Interphase Nuclei*

Chromosomes in interphase are thought to (1) be much longer than chromosomes in prophase, (2) further condense to metaphase and anaphase chromosomes, and (3) become decondensed and longer after telophase. However, our results show that chromosomes in interphase are very similar in length to metaphase chromosomes. Therefore, doubts arise about the concept of chromosome condensation in general. We propose that all the convincing experiments published to date that deal with H3- and SMC-phosphorylation with respect to chromosome condensation (Gurley et al. 1978; Hirano 1999; Strunnikov and Jessberger 1999; Wei et al. 1999) may explain phenomena related to the formation and/or compaction of chromosome loops that influence the width of chromosomes in two dimensions (Weise et al. 2002) and probably their volume in three dimensions. The length of the chromosome axis, the third component of chromosome condensation (Koshland and Strunnikov 1996), is clearly not influenced as much, although this has not been investigated directly by FISH. Technical difficulties preventing such an investigation have been solved here by the use of MCB.

The distances between fluorescently labeled DNA probes on human lymphoma cells indicate as much as a tenfold compaction of mitotic chromosomes, when compared with interphase chromosomes (Lawrence et al. 1988). In addition, Yunis (1976, 1981) showed that prophase chromosomes are very long, in comparison with metaphase chromosomes. The discrepancy between these observations and our findings may be due to prophase chromosomes being artificially elongated during drying of fixative on the slide during chromosome preparation (Hliscs et al. 1997*b*). It also indicates that cell cycle–specific differences in the preparation-induced chromosome-elongation process may occur. In comparison with metaphase and interphase chromosomes, prophase chromosomes appear to be more sensitive to preparation-induced chromosome elongation. This elongation is a prerequisite for obtaining well-spread metaphases useful for chromosome analysis (Hliscs et al. 1997*b*). Chromosomes, routinely harvested for chromosome analysis shortly after completion of S phase, probably contain chromosome region–specific proteins that can easily be stretched during drying of the fixed cells on the slide, resulting in prophase chromosomes. Chromosomes harvested late after completing their S phase, however, are more resistant to preparation-induced chromosome stretching, leading to metaphase chromosomes that are relatively short. Chromosome-stretching experiments indicate that G-light bands are the stretchable units and that a hierarchy exists in their elasticity (Hliscs et al. 1997*a;* Küchler et al. 2001). Consequently, the stable G-band-

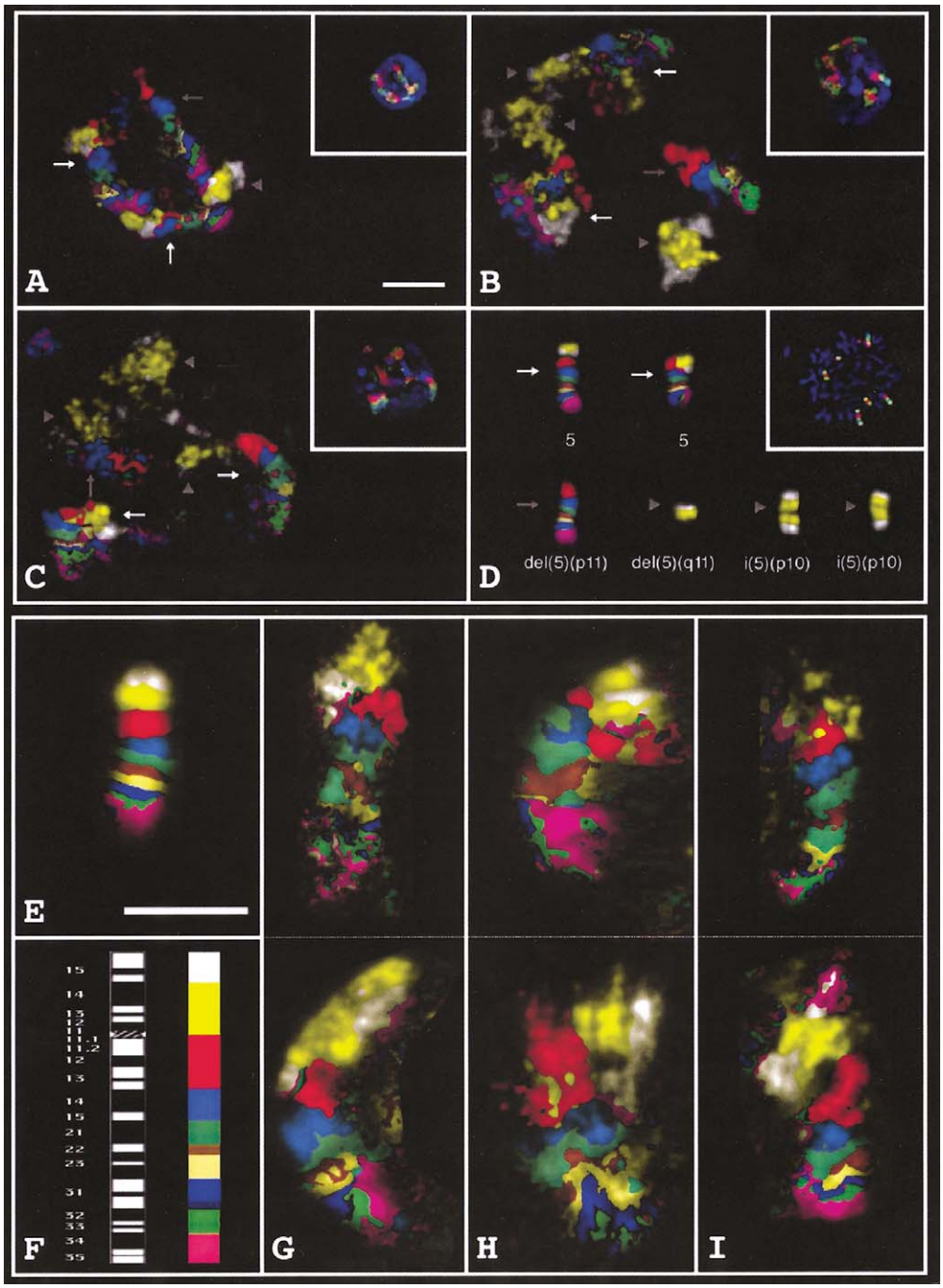


**Figure 3** Multicolor-banded human chromosomes 5 in G1, S, and G2 phases and in the metaphase stage of HeLa cells. Light arrows indicate the normal chromosomes; gray arrows indicate the chromosomes with a deletion of the short arm, del(5)(p11); gray triangles indicate the chromosomes with a deletion of the long arm, del(5)(q11), and the isochromosome 5p, i(5)(p10). Scale bars = 5 $\mu$m (scale bar in A is representative for A–D, and that in E is representative for E–I). *A,* MCB pattern of the normal and rearranged chromosomes 5 of a HeLa cell arrested at G1 of the cell cycle. Some chromosomes are close to each other by chance, and the MCB pattern is identical to that of the chromosomes in metaphase (see *D*). The same interphase nucleus is shown on the right, at lower magnification. The cell is DAPI-stained (*blue background*), and the different colors arise from the fluorescence signals taken with the individual filter combinations. *B,* An interphase nucleus shown arrested in S phase, comparable to *A*. *C,* An interphase nucleus shown arrested in G2 phase. Chromosome 5 in S phase is as long as in the G1 and G2 phases, but wider. In all stages of the cell cycle, the MCB patterns of chromosome 5 are identical. All HeLa-specific chromosome aberrations visible at the metaphase stage can also be identified on interphase chromosomes in the G1, S, and G2 phases. *E,* Metaphase chromosome 5 from HeLa cells at the same magnification (scale bar = 10 $\mu$m) as the two chromosomes in the G1 phase (*G*), the S phase (*H*), and the G2 phase (*I*). An idiogram of chromosome 5 (Mittelman 1995) and the corresponding colored bands are shown in *F*. The shape of the interphase chromosomes and the colored bands indicate similarities with metaphase chromosomes.

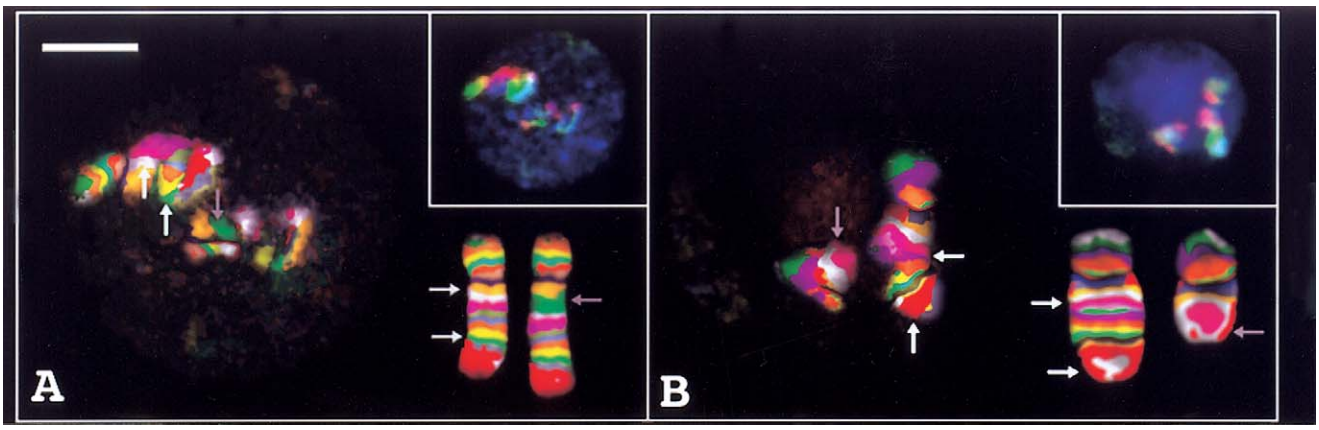


**Figure 4** Multicolor-banded interphase chromosomes 5 in two clinical cases of structural chromosome aberrations. Scale bar = 5 μm. *A,* Lymphocyte chromosomes 5 of a boy with congenital malformations, revealing a duplicated insertion of the green band derived from 5q31, between the ochre and white bands at 5q13. The green band on the short arm of chromosome 5 differs from the green color of band 5q31 and from that of the inserted band (*gray arrow*). Both chromosomes 5 shown are folded. The same cell, DAPI-stained, is shown on the top right, at lower magnification. The light arrows indicate the breakpoints on the normal chromosome 5 in the interphase (*left*) and metaphase stages (*right*). *B,* A chromosome 5 from cultivated bone marrow cells of a patient with leukemia (AML M6) with a 5q− syndrome. The light arrows indicate the breakpoints on the normal chromosomes 5 in the interphase (*left*) and metaphase stages (*right*), and the gray arrows indicate the rearranged chromosome 5 and its deletion breakpoint.

ing patterns occur, which provide the basis for most chromosome analyses.

The standard deviations of the length of flattened interphase chromosomes obtained after routine chromosome preparation and of intact three-dimensional chromosomes after preparation at reduced humidity are small. This indicates a stable length of interphase chromosomes. The mean length of flattened interphase chromosomes 5 obtained after routine chromosome preparation is 12 μm, about twice the length of those obtained after drying the fixative at a humidity of ⩽7%. Nearly identical results were found in a similar comparison of the length of metaphase chromosomes (Hliscs et al. 1997*b*). Metaphase chromosomes of the C group obtained after short-term evaporation of the fixative at 80°C were ~3 μm in length, and routinely harvested and dried metaphase chromosomes were ~5.6 μm.

Chromosome-stretching analysis (Hliscs et al. 1997*a;* Küchler et al. 2001) revealed that G-light bands represent chromosome regions that can be stretched. Chromosome stretching takes place during drying of the fixed suspension on the slide (Hliscs et al. 1997*b*) and, as discussed above, this is a prerequisite to obtaining metaphase spreads of sufficient quality for chromo some analyses. Without chromosome preparation–induced stretching, chromosomes are short and probably do not show any bands. The banding pattern may be regarded as a preparation-induced artifact, although this does not sufficiently reflect the fact that chromosome preparation leads to characteristic and highly reproducible morphological changes of chromosomes on which chromosome analyses are based. The biology behind the formation of these "reproducible artifacts" may be related to a chromosome region–specific fixed hierarchy in the potential of proteins of the chromatin to be stretched, but this has not been investigated. The methanol, acetic acid, and water of the fixative may interact with proteins of the chromatin, especially in G-light bands, thus resulting in their elongation. Following this interpretation, one may assume that routine chromosome preparation using fixative and drying of the fixed suspension on the slide induces a dramatic genomewide elongation of all chromosome regions in which housekeeping genes are located, leading to a banded metaphase spread. A similar mechanism may operate in living cells, restricted to single chromosome regions, to open the chromatin (G-light chromosome regions) for the transcription machinery, probably in a function-specific manner.

### *The Banded Structure of Interphase Chromosomes*

Ideas about the chromosomes in interphase nuclei have been influenced by the previous inability to visualize analyzable structures. We show for the first time, to our knowledge, that interphase chromosomes are structured, with a highly reproducible DNA-specific banding pattern. This will profoundly enhance our understanding of the architecture of the interphase nucleus. The existence of such a banding pattern can be regarded as a logical con-

sequence of the observation by Dietzel et al. (1998) that the chromosome arm domains in interphase nuclei show separate signals.

Some interphase chromosomes 5 show an incomplete MCB pattern. This partial loss of chromosome 5–specific MCB signals may be due to technical problems, because FISH techniques are predominantly adapted to metaphase chromosomes and not to interphase nuclei. This is supported by the observation that an MCB probe mixture of reduced hybridization quality resulted in increased partial loss of MCB signals. The margins of interphase chromosome 5 in S phase appear to be less sharply marked, which may also be a preparation-induced artifact. We decided not to investigate this in more detail.

Interphase nuclei in living cells are three-dimensional. Routine chromosome preparation, however, leads to flattened interphase nuclei, and significant alterations of the structure of chromosomes in interphase nuclei of cells flattened during preparation are expected. However, the similarity between the DNA-based banding pattern of flattened interphase chromosomes and that of metaphase chromosomes suggests that, in living cells, both three-dimensional intact interphase chromosomes and metaphase chromosomes may be more similar than expected. The preparation technique we used to gain information about three-dimensional intact interphase chromosomes led to half-spherical interphases, which are not optimal. In an attempt to preserve the three-dimensional chromatin structure of the interphase nuclei, we fixed cells with buffered formaldehyde; however, subsequent MCB did not give sufficient hybridization signals.

The DNA-based banding pattern of interphase chromosomes is very similar to that of metaphase chromosomes. This holds true not only for the G2 phase but also for the G1 and S phases. Consequently, the terms "chromosome territory" and "chromosome domain" will no longer be needed and can be replaced by the term "interphase chromosome." Chromosomes are chromosomes throughout the whole cell cycle, and ideas and concepts are needed in dealing with structural changes and reorganization of chromosomes during the cell cycle, with regard to functional aspects and tissue specificity. Several models for the organization and architecture of chromosomes in interphase nuclei have been discussed (for review, see Chevret et al. [2000] and Cremer and Cremer [2001]) that indicate that the basic information about interphase chromosomes available at present is not sufficient to define the correct model. Technical advances are needed, especially in FISH technology, to enable the dynamic changes in interphase nuclei to be visualized.

The position of genes inside chromosomes in interphase depends on the transcriptional activity of the gene (Dietzel et al. 1999). Active genes are located at, or close to, the surface of interphase chromosomes. Inactive genes are more central, suggesting that dynamic position changes probably interfere with functional aspects. The chromosome preparation–induced elongation mechanism described by Hliscs et al. (1997*b*), which probably takes place exclusively in G-light bands (Hliscs et al. 1997*a*) where the housekeeping genes are placed (Holmquist 1992), may have an equivalent in the interphase. The mechanisms to open chromatin structures for transcription could relocate structures from an internal to a more external position, depending upon whether the genes are active or inactive. For the human X chromosomes in interphase cells, Eils et al. (1996) showed that the active X chromosome has the larger and more irregular surface. An extreme variation of this is the observation that transcriptionally upregulated genes in the major histocompatibility complex on the short arm of chromosome 6 were found to be on an external loop, apparently outside the chromosome 6 territory (Volpi et al. 2000). Also, tissue-specific aspects of the same mechanism of transcriptional activation have been shown for the epidermal differentiation complex at 1q21 in keratinocytes, where the genes are active, and in lymphoblast interphase nuclei, where they are silenced (Williams et al. 2002). We therefore assume a hierarchy in the distance from actively transcribed chromosomal DNA to their interphase chromosome territory, which corresponds to the hierarchy of the splitting of bands into their sub-bands for pro-, prometa-, and metaphase chromosomes.

Chromosome 5 aberrations in HeLa interphase cells, such as i(p) or deletions of the p and q arms, are clearly visible (fig. 3). In clinical cases (fig. 4), chromosome 5 aberrations can also be detected. Therefore, we speculate that, in the future, the form of all human chromosomes, together with their substructures, will be visualized in three dimensions and will be used to detect chromosome aberrations in interphase nuclei. This opens new possibilities for cytogenetic investigations.

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