CONTENTS

DNA SEQUENCING

1 **Rapid DNA Sequencing Based Upon Single Molecule Detection**
Lloyd M. Davis, Frederic R. Fairfield, Carol A. Harger, James H. Jett, Richard A. Keller, Jong Hoon Hahn, Letitia A. Krakowski, Babetta L. Marrone, John C. Martin, Harvey L. Nutter, Robert L. Ratliff, E. Brooks Shera, Daniel J. Simpson, and Steven A. Soper

8 **Can the Scanning Tunneling Microscope Sequence DNA?**
S. M. Lindsay and M. Philipp

IN SITU HYBRIDIZATION

14 **DNA Sequence Mapping Using Electron Microscopy**
Sandya Narayanswami and Barbara A. Hamkalo

24 **Analysis of Genes and Chromosomes by Nonisotopic in situ Hybridization**
Peter Lichter, Ann L. Boyle, Thomas Cremer, and David C. Ward

CALENDAR

GATA 8(1): 24–35, 1991

Analysis of Genes and Chromosomes by Nonisotopic in situ Hybridization

PETER LICHTER,
ANN L. BOYLE,
THOMAS CREMER, and
DAVID C. WARD

Nonisotopic in situ hybridization is a powerful tool to analyze the organization of complex genomes. Current approaches utilizing this technique for the analysis of linear and spatial genome organizations are presented. Clinical applications of these approaches, which open new avenues for diagnosis of disease-related chromosomal changes, are also discussed.

Introduction

The contiguous DNA string within a chromosome provides the basis for the linear organization of the genetic material. Since the DNA extends roughly along the telomere–telomere axis of metaphase chromosomes, it is possible to order genes on the chromosomes. On the microscale, there is, however, the probability that the true spatial relationship of closely juxtaposed DNA sequences in metaphase chromosomes will be indeterminate, as the DNA is packaged in coiled superstructures. In interphase cell nuclei the chromatin of individual chromosomes is localized in distinct territories (see below), yet, to date, little is known about the spatial organization of the linear DNA molecule within such a chromosome domain. Since the interphase chromatin is more decondensed, a higher flexibility is expected with regard to the spatial organization of neighboring DNA regions.

The linear order of DNA segments within the genome can be established by a variety of methods. The most direct approach for mapping DNA sequences is in situ hybridization, which can be used to visualize targeted DNA sequences both in metaphase chromosomes and in interphase nuclei. Recently, nonisotopic in situ hybridization has become increasingly popular for this purpose. The development of nonisotopic labels for nucleic acid probes as well as some general applications of the methodology were recently reviewed elsewhere [1, 2]. Here we will focus on the application of nonisotopic in situ hybridization techniques in analyzing the linear and the spatial organization of complex genomes and their use as a tool in clinical cytogenetics. Details of other applications will be presented elsewhere in this journal [3–5].

Nonisotopic Labeling and Detection Systems

Various methods can be used to label nucleic acids nonisotopically. For applications involving in situ hybridization, enzymatic incorporation of nucleotides modified with biotin [6–9], digoxigenin [10], dinitrophenol [6, 11] or halogenated nucleotides (e.g., BrdU, FrdU) is usually preferred over chemical labeling techniques employing photoreactive compounds (photobiotin, photodigoxigenin, or photodinitrophenol) because of a higher labeling efficiency. However, other chemical modification schemes using acetylaminofluorene [12], mercuration [13, 14], or sulfonation [15] have been used successfully for sensitive nonradioactive detection of hybridized nucleic acid probes [16, 17]. Bound probes are also detectable as part of DNA–RNA hybrids, which are recognized by specific antihybrid antibodies [18, 19]. The visualization of hybridized probes can be achieved in several ways: (a) via fluorochromes analyzed by fluorescence microscopy, (b) via chemiluminescence detected by an emulsion overlay or directly by photon-counting devices (see below), or (c) via high-density colored precipitates generated by enzymatic assays or the use of metallic compounds, such as colloidal gold or silver, for visualization by phase contrast, Nomarski, or electron microscopy. All of the methods outlined above generally use indirect detection procedures. When fluorochromes are coupled directly to the probe molecules [20, and our own unpublished data], direct microscope examination of the signal is feasible. The sensitivity of each of the labeling/detection procedures re-

From the Institute for Viral Research, German Cancer Research Center (P.L.), and the Institute for Human Genetics and Anthropology (T.C.), Heidelberg, FRG; and the Department of Molecular Biophysics and Biochemistry (A.L.B.; D.C.W.) and the Department of Human Genetics (D.C.W.), Yale University School of Medicine, New Haven, Connecticut.

Address correspondence to: Dr. Peter Lichter, Institut für Virusforschung, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-6900 Heidelberg, FRG.

Received September 5, 1990; revised and accepted October 31, 1990.

© 1991 Elsevier Science Publishing Co., Inc., 655 Avenue of the Americas, New York, NY 10010
1050-3862/91/$03.50

GATA 8(1): 24–35, 1991

ported by various laboratories has varied widely. This variability, at least in part, may reflect the quality of the reagents used or the degree to which experimental parameters were optimized. Biotin and digoxigenin labeling combined with fluorescent detection are currently the most widely used procedures, because of the high sensitivity of detection and the commercial availability of the reagents. Fluorescent detection is generally preferred over enzymatic assays because of better spatial resolution, the ability to quantitate fluorescent signals by photon counting, and greater potential for simultaneous multiprobe analysis.

The potential of nonisotopic in situ hybridization procedures is significantly increased by multihybridization protocols enabling the simultaneous differential delineation of several target sequences. Whereas a large number of studies have included dual labeling techniques, recent developments in multiple fluorescence in situ hybridization enable the simultaneous visualization of three or more DNA regions [21, 22].

Optical Instrumentation and Digital Imaging

Refinements and new developments of optical instrumentation have also contributed significantly to the increased application of nonisotopic in situ hybridization techniques. Two features are of particular importance: the improved signal detection by increased sensitivity of optical instruments and the development of devices for three-dimensional (3D) microscopy. Although most of the advancements are based on fluorescent or chemiluminescent detection systems [23–25], the feasibility of a 3D analysis of chromatin in interphase cell nuclei of higher organisms by using transmission light microscopy in combination with digital image analysis has been recently demonstrated [26–28].

Digital imaging techniques are more and more being used to supplement conventional epifluorescence microscopy [25, 29]. Several reasons account for this development: (a) Very weak fluorescent signals can be documented more readily; image reproduction problems often occur when conventional photography is applied. (b) The potential of image processing greatly facilitates the analysis of a labeled object. Optical filtering techniques, such as thresholding, which enhances the signal to noise ratio, are particularly valuable. (c) Quantitative data on signal intensities or measurements of inter- and intrasignal distances can be easily assessed. (d) Handling and storing of images are easy to perform (however, convenient handling of multimegabyte images requires appropriate computer equipment).

Digital imaging microscopy is performed using sensitive camera systems or a laser scanner equipped with a photomultiplier. The most sensitive system to date, the cooled CCD (charged coupled device) camera, is a powerful instrument for biological research [30]. Because of its high efficiency in counting emitted photons over a broad spectral range of wavelengths, the CCD camera is the instrument of choice for two-dimensional (2D) analyses, e.g., for the analysis of fluorescent signals on metaphase chromosomes. However, when multiple images of a specimen are taken with different filter sets for multicolor analysis, each image must be registered accurately to guarantee the correct spatial presentation of overlaid (composite) images. The image registration problem is not a trivial one; the development of methods for its solution is an area of active research.

In 3D microscopy, a series of optical sections through a labeled specimen is obtained. Three-dimensional reconstruction and analysis of the object can be accomplished by applying appropriate computer software to a stack of digitized images. There is a considerable amount of out-of-focus fluorescence in each section detected by conventional image devices. High-quality optical sections for 3D microscopy require elimination of this out-of-focus fluorescence using sophisticated mathematical algorithms [31]. Laser scanning confocal microscopy [32–34] is designed to eliminate most of the out-of-focus fluorescence prior to the optical detection device. In this way, optical sections of high quality are obtained directly, reducing the amount of mathematical operations required for 3D microscopy. In conclusion, commercially available confocal laser scanning microscopes can be used directly for both 2D and 3D digital imaging microscopy, whereas the available CCD camera systems are superior in signal detection, but further development is required for routine application to 3D microscopy.

Recent Developments in Nonisotopic in situ Hybridization

The sensitivity of detecting nucleic acid target sequences nonisotopically has increased consider-

© 1991 Elsevier Science Publishing Co., Inc., 655 Avenue of the Americas, New York, NY 10010

ably over the past decade. Initially, small DNA probes could be readily used only when target sequences were present in higher copy numbers, as for example satellite DNA [35, 36], including alphoid repeats (for review, see [37]) or unique sequences on polytene chromosomes [38]. During the ensuing years, technical improvements were developed in numerous laboratories that enhanced the speed, reproducibility, and spatial resolution over that of the original autoradiographic procedures. Parameters influencing the hybridization efficiency and signal specificity were more precisely defined and optimized (e.g. [39–43]). Subsequent reports of mapping single copy genes in genomes of high complexity varied in labeling efficiency. An increasing number of studies, however, has now demonstrated a highly specific delineation of unique DNA in mammalian genomes [44–56]. Mapping of unique DNA of a complexity as low as 1 kb has been reported.

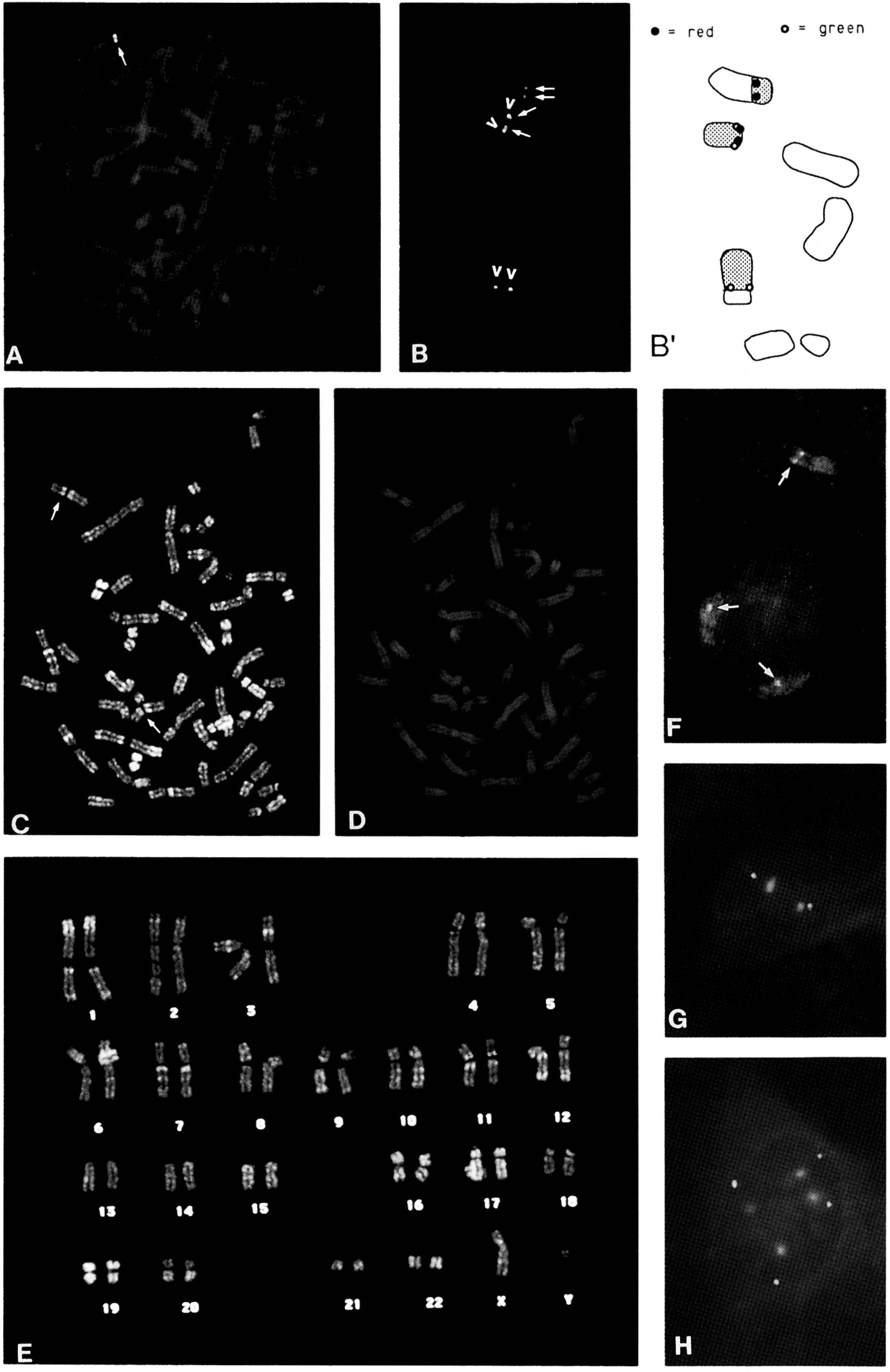
Considerably longer probes are likely to contain interspersed repetitive sequences resulting in additional signals throughout the genome of higher organisms. Protocols designed to suppress the hybridization signals from these repetitive sequence elements by using appropriate competitor DNA have been published [57–59]. Total genomic DNA is easy to prepare and is usually sufficient as competitor DNA. When high concentrations of competitor are needed, the Cot1 DNA fraction [60] selectively enriched in highly repetitive DNAs is preferred, because it does not compete with the unique sequence fraction of the probe [57]. The suppression hybridization procedure does not require the identification and isolation of single-copy sequences within the probe, thus substantially decreasing the efforts necessary to map a genomic DNA fragment. This procedure, which we refer to as *c*hromosomal *i*n *s*itu *s*uppression (CISS) hybridization, is being used to map mammalian sequences cloned in plasmid, bacteriophage, cosmid, or YAC (yeast artificial chromosome) vectors [11, 57, 61–66]. An example using YAC DNA is shown in Figure 1A.

When complex probe sets, such as complete DNA libraries derived from sorted human chromosomes, are used as probes [58, 59, 67], this technique is referred to as "chromosome painting" [59], since individual chromosomes or subchromosomal regions are visualized in a highly specific manner.

Background may become a significant problem if a large fraction of DNA sequences in the probe

Figure 1. Delineation of DNA sequences on human metaphase chromosomes and in interphase nuclei. **A.** A gel-purified YAC containing human DNA sequences (kindly provided by Terry Lerner, Integrated Genetics, Framingham, MA) was labeled with biotin and hybridized under suppression hybridization conditions to normal human chromosomes (46, XY). Detection via FITC-conjugated to avidin reveals the localization of the probe (see arrow) on the long arm of the X chromosome. Chromosomes are counterstained with propidium iodide. Chromosome identification was achieved by DAPI-banding (not shown). **B.** Characterization of a chromosomal breakpoint within 11p. Metaphase of a somatic hybrid cell line containing the relevant parts of the normal chromosome 11 as well as of the two derivatives of a translocation t(2;11) of a Potter's facies syndrome patient, after hybridization with two cosmid probes of the central part of 11p. The probes, designated 13-4A and 4-4B, were labeled with biotin and dinitrophenol and detected via FITC (arrowheads) and rhodamine (arrows), respectively. They are close together on the normal chromosome (near the center), but separated by the breakpoint as indicated by the single labels on each derivative chromosome (top and bottom). On each chromosome the target sites on both chromatids are labeled. The chromosomal counterstain is not shown, but the outline of the chromosomes is shown in the schematic drawing in **B′**, where the chromosome-11 material is shown as shadowed region (in this particular subculture, the human chromosomes are not complete, as, for example, on the normal chromosome 11 the terminal portion of the short arm is missing). **C–E.** Simultaneous visualization of a fluorescently labeled cosmid probe and chromosome banding. (C) Digoxigenin-labeled cosmid probe J1-2 and a biotin-labeled DNA probe containing four Alu repeats were cohybridized and detected via rhodamine and FITC, respectively. Note the R-banding-like pattern of the Alu probe signal. Chromosome identification and band assignment can be easily performed by using Alu banding. Cosmid J1-2 had been mapped by FLpter values (see text) to a chromosome region corresponding to 11p11.2. This is indeed the cytological map position, as the signal is within the brightly stained band 11p11.2 (see arrows). (D) DAPI stain of the metaphase shown in C. Note that the DAPI stain is producing a G-banding pattern in heat-denatured chromosomes, even without further treatment (such as distamycin). Whereas Alu probes do not label centromeres and several pericentromeric regions, the DAPI counterstain reveals the full chromosome continuum (compare C and D). (E) Complete karyotype of the Alu-banded metaphase in C (46, XY). Note that the quality of the Alu banding is superior to the DAPI banding. **F.** Simultaneous visualization of the X-chromosome domain (hybridization of digoxigenin-labeled X-chromosome library DNA and detection via rhodamine) and the X-chromosomal gene for dystrophin (hybridization of biotin-labeled probe cpT1 and detection via FITC, see arrows) in a normal female lymphocyte nucleus as well as in a metaphase plate, which is partly shown with one X chromosome (courtesy of Thomas Ried, Heidelberg, FRG). **G and H.** Position of the subcentromeric heterochromatic region 1q12 (hybridization of acetylaminofluorene-labeled probe pUC1.77 and detection via rhodamine) and the telomeric band 1p36 (hybridization of biotin-labeled probe p1-79 and detection via FITC) of human chromosome 1 in normal human fibroblasts (for methodological details see [147]). Note that the orientation and spatial extension of the short arm of chromosome 1 can be easily recognized in diploid (G) and tetraploid (H) nuclei after dual color interphase chromosome banding using these probes. The pictures in B–E are part of a collaborative study published recently [11], F is part of a study by Ried and coworkers [159], and pictures in G and H are from a study by Thomas Cremer and Laura Manuelidis (Yale University) (unpublished). The digitized images (A–E) were obtained by using a confocal laser scanning microscope (A and B) or a cooled CCD camera (C–E). The pictures in F–H were taken by conventional photography using a standard epifluorescence microscope.

© 1991 Elsevier Science Publishing Co., Inc., 655 Avenue of the Americas, New York, NY 10010



© 1991 Elsevier Science Publishing Co., Inc., 655 Avenue of the Americas, New York, NY 10010

does not contribute to a specific signal, for example in chromosome library DNA cloned in phage lambda Charon 4A [68], where the vector to insert ratio is large (e.g., 10:1), or in YAC DNA present in yeast cells in addition to a whole yeast genome complement. In such cases, it might be preferable to gel-purify the phage DNA inserts or the YAC DNA to eliminate lambda sequences or yeast DNA, respectively. Recently, new chromosome libraries have been constructed in plasmid vectors, where the vector to insert ratio is lowered [69], and these libraries may be preferable to the phage libraries, which have been used so far for chromosome painting.

Cosmids are particularly useful probes, as they can delineate ~90% of the target sites without significant background noise. Thus, as applied to gene mapping (see below), almost every metaphase spread is informative, since most spreads give four signals (i.e., on both chromosome homologs and both chromatids). This greatly reduces the statistical analysis required. As the probe complexity decreases, the percentage of delineated target sequences decreases as well. When the chromosomal target is smaller than ~2 kb, the percentage of metaphases exhibiting highly specific signals is usually reduced to <50% (see, e.g., [55, 56]). Although a variety of high-sensitivity in situ hybridization protocols have been published (for citations, see above; the procedures used in our laboratories are described explicitly in [11, 58, 70]), it is agreed that certain factors are critical for a successful hybridization. Problems arise mainly by neglecting one of the following factors: purity and concentration of the DNA template, control of the labeling reaction (such as labeling efficiency and the actual size of the probe molecules after the labeling), high-quality preparations of chromosomes and nuclei, control of the conditions for optimal denaturation of the target DNA, and quality control of reagents used during the whole procedure.

New Approaches for Generation of Probe Sets

For many cytogenetic applications (see below), probe sets for chromosome painting at an intermediate level, e.g., subchromosomal regions of one or more chromosome bands in length, would be preferable to DNA libraries from whole sorted chromosomes. Such probes are presently largely restricted to the centromeric and pericentromeric heterochromatin and some other blocks of tandemly repeated DNA located on the chromosome arms, but are still missing for most other chromosomal subregions. In this context, three approaches seem to be promising: (a) Contiguous cloned DNA covering long genomic regions, such as one or more YAC clones or sets of contiguous cosmids, respectively, will be increasingly available as part of the research in the human genome initiative. (b) DNA probe sets obtained by cloning microdissected chromosomal material can be used to paint desired chromosomal subregions of humans in a targeted fashion [71]. In addition, this method could in principle also be applied to delineate chromosomes and chromosomal subregions of any species without the need to sort chromosomes. (c) Somatic hybrid cell DNA can be used as a probe for chromosome painting. The total DNA of a hybrid cell line can be used under suppression hybridization conditions and hybridized back to normal metaphases of one "parental" species, thus the chromosomal content of this species in the hybrid line is immediately apparent [72, 73]. However, variable concentrations of species-specific DNA in hybrid lines as well as cross-homologies of certain sequences can affect the quality of such an analysis. To avoid these problems, PCR amplification of species-specific sequences in the hybrid DNA has been carried out by using species-specific oligomer primers targeting interspersed repetitive DNA elements [74, 75]. The resultant PCR products can be prepared at little expense and provide efficient probe sets for chromosome painting without the necessity of cloning [76, 77]. Careful selection of hybrid lines with small chromosomal fragments, such as radiation-reduced or chemically reduced hybrids, will allow the generation of painting probes for a wide range of chromosomal subregions.

Rapid Mapping of DNA Sequences

The utility of CISS hybridization in combination with digital imaging microscopy to map large numbers of genomic DNA fragments with speed and precision has been demonstrated [11]. For this, probes were ordered along a chromosome by using mapping coordinates defined by the fraction of the distance between the signal and a reference point (arbitrarily chosen as the end of the short arm of the chromosome, pter) to the total length of the chromosome (FLpter, fractional length of chromosome with reference point pter). It should

© 1991 Elsevier Science Publishing Co., Inc., 655 Avenue of the Americas, New York, NY 10010

be noted that the ISCN banding ideograms [78] are not normalized relative to the fractional length of chromosomes, so that FLpter values cannot be extrapolated from these ideograms to give map coordinates as a band locus. This approach can be used however, to map the relative chromosomal position of multiple probes. The precision of this mapping strategy is greatly dependent on the condensation of the chromosome; significantly greater resolution is obtained with elongated (prometaphase) chromosomes.

Parameters influencing the variability of chromosomal length must be carefully considered, since chromatin condensation is nonuniform. Chromosome length polymorphisms must be taken into account when more than one chromosome homolog is analyzed, because such variability, e.g., of heterochromatin blocks, chromosome satellites, or even "normal" interindividual length differences [79, 80], can be considerable. These potential limitations can be circumvented by using comparable chromosomes of one individual [11] or by carefully measuring a variable region to normalize the linear measurements [55].

Inclusion of chromosome banding, the major means of identifying chromosomes, reduces the influence of length polymorphisms in the analysis, since selected smaller distances, defined by the border of bands, could be used as reference points [65]. Furthermore, the simultaneous banding interlocks such mapping data in terms of classical cytogenetic terminology (see Figure 1C–E). With fluorescent signal detection, conventional Giemsa or Wright banding is time consuming, since the analysis of the signal and the banding is carried out in two consecutive steps with separate photographic processes. Simultaneous detection of chromosome banding by Giemsa staining, following BrdU incorporation and staining with Hoechst 33258, has been reported [46]. Banding methods that can be applied in parallel to fluorescence probe detection, and do not require a relocation of chromosomes, are preferable. Chromomycin (modified from [45]), quinacrine (our own unpublished data), Hoechst 33258 [49], or DAPI banding [67, 81] (see, e.g., Figure 1D) are easily combined with the hybridization/detection procedure. In our hands, differential staining of chromosomes with propidium iodide, resulting in R banding, was of variable quality, but recent studies reported propidium iodide banding after BrdU incorporation and demonstrated the usefulness of this technique for mapping [82, 83]. Due to morphological changes during chromosome denaturation, the quality of subsequent banding may be variable and less than optimal. Fortunately, alternative procedures are available. Replication banding following incorporation of BrdU into cellular DNA late in S phase [84], which gives predominantly an R-banding pattern, has been applied successfully in DNA mapping studies [27, 65]. DNA probes for interspersed repetitive elements cause distinct banding patterns on metaphase chromosomes [11, 85, 86] and can be used to produce a simultaneous in situ hybridization banding profile. Alu repeats, which also generate an R-banding-like pattern [87–89], are very useful for human chromosome identification. Both cloned Alu sequences [11] and PCR products generated from an Alu primer [86] have been reported. An example of a cosmid mapping in combination with Alu banding is shown in Figure 1C–E. To date, neither L1 clones nor L1 PCR products have generated reproducible G banding in humans. In contrast, the L1 sequence of mouse is particularly effective in generating a banding pattern very similar to Giemsa dark bands [85]. Interestingly, of the two major Alu-like repeat families in mouse DNA, only the B2 family gives a useful R-banding pattern on murine chromosomes.

Additional confirmation of chromosome assignment can be achieved by cohybridization with a probe or probe set known to map to the target chromosome of interest (preferably labeled in a different way). The latter approach is particularly useful when analyzing multiple clones derived from a monochromosomal hybrid cell line or from a chromosome library prepared after flow sorting.

The relationship of two or more probes can be analyzed with higher resolution if the probes are hybridized simultaneously on the same chromosome using multicolor detection procedures [65, and our own unpublished results]. Another route to the rapid acquisition of mapping information is to exploit translocation chromosomes or panels of hybrid cell lines containing fragments of a single chromosome of interest. Mapping a probe on rearranged chromosomes [90] in relation to known chromosomal breakpoints results in a quick yes or no answer regarding the relative order of probes [62]. An example of this is shown in Figure 1B.

To improve the mapping resolution further, chromosomal DNA can be analyzed in more decondensed states, as for example after premature chromosome condensation, chromosome shat-

© 1991 Elsevier Science Publishing Co., Inc., 655 Avenue of the Americas, New York, NY 10010

tering, or in interphase nuclei. The possibility that distances between two targets located at different sites of the same chromosome can be quantitatively assessed directly in interphase nuclei has first been demonstrated by Rappold and coworkers using isotopic in situ hybridization [91]. Surprisingly, these and other data (see below) indicated a considerably less decondensed state of interphase chromosomes than previously thought. More recently, "interphase chromatin mapping" has been dramatically improved using nonisotopic protocols for the mapping of DNA probes separated by distances ranging from 30 kb up to ~1 Mb [47, 61, 65]. Following multiple distance measurements, the highest likelihood of probe order was established [61, 65], which was in complete agreement with mapping data obtained by other methods. In contrast, ordering probes on metaphase chromosomes is not based on distance measurement, but on ordering along the longitudinal axis of the chromosome. Although simultaneously hybridized probes as close to each other as 100 kb reveal spatially resolvable signals such probes cannot be ordered along the longitudinal axis of chromosomes, since they usually occur within the same latitude of a chromatid. Analyzing multiple paired signals, we have been able to order probes on chromosomes in the 1-Mb range (unpublished data), whereas others were unable to establish an order at that resolution [65]. This might reflect the fact that the 1-Mb range represents the border of resolution for metaphase mapping and the resolution might be influenced not only by chromosome length, but also by variations of chromatin conformation at different chromosomal sites. Mapping resolution of interphase and metaphase chromatin is further described elsewhere [5].

Finally, it should be mentioned that CISS hybridization provides an extremely powerful tool for the comparative mapping of individual sequences contained in cosmid or YAC clones (our unpublished data). In addition, blocks of genetic material that are syntenic to humans can be identified unequivocally in other primate species by chromosome painting with DNA libraries from sorted human chromosomes or appropriate regional probe sets [92].

Nuclear Topography

Although early cytologists such as Carl Rabl, Theodor Boveri, and Eduard Strasburger suggested a territorial organization of interphase chromosomes (for review, see [93]), this view was disputed in the 1960s and early 1970s. By this time, it had become obvious that the length of the chromatin fiber constituting an individual chromosome exceeds the diameter of the nucleus manyfold. Accordingly, extremely different types of arrangements of such a fiber can theoretically be envisaged. More importantly, electron microscopic studies of cell nuclei had failed to distinguish individual chromosome domains [94, 95]. Unequivocal evidence for the existence of a territorial interphase chromosome organization in somatic mammalian cell nuclei was provided by laser microbeam studies [96–98] and more recently by in situ hybridization experiments. Individual chromosome domains have been directly visualized in cell nuclei of interspecies mammalian somatic hybrid cells after in situ hybridization with total genomic DNA of one species [42, 99–102]. Preliminary 3D analyses of complete human chromosomes in somatic hybrid cell nuclei have been reported [103, 104]. The painting of individual chromosomes in normal and aneuploid cells further established that the same territorial organization of chromosomes occurs in nonhybrid cells [58, 59, 67] (see Figure 1F). In situ hybridization experiments have also confirmed a territorial interphase chromosome organization for plant species [105, 106].

Nonradioactive in situ hybridization to specific chromosome targets and 3D microscopy have been recently applied by several groups for the 3D analysis of the nuclear topography in human cells [27, 104, 107–109]. Delineation of the heterochromatic region 1q12 in different human cell types serves as an illustrative example of how the concept of cell-type-specific chromatin arrangements is presently being tested (see Figure 1G and H). Manuelidis and Borden [27] using a 3D approach observed that one 1q12 region was frequently associated with the nucleolus in nuclei of large neurons, while the corresponding region of the homologous chromosome was found to be either associated with the nucleolus as well, or with the nuclear periphery. In glial cell nuclei, however, both 1q12 regions were generally associated with the nuclear envelope. Two-dimensional analyses of the relative arrangements of the two 1q12 regions in nuclei of lymphocytes, amniotic fluid cells, and fibroblasts as performed by Emmerich and coworkers [110] indicated a highly variable and possibly random arrangement. Van

© 1991 Elsevier Science Publishing Co., Inc., 655 Avenue of the Americas, New York, NY 10010

Dekken and coworkers [108, 109] noticed in 3D analyses that the 1q12 regions were preferentially associated with the nuclear envelope of spherical and polymorphic hemopoietic nuclei. Again the relative positions of the two regions in the periphery of spherical nuclei could not be distinguished from a random distribution pattern. In contrast, Arnoldus and coworkers [111], using a 2D approach, observed a clear spatial association of the two regions in most nuclei of human cerebellum, but not in nuclei of human cortex. While these data collections may appear disparate, they could reflect a variable but cell-type-specific arrangement of chromatin.

The idea of a functionally important role of intranuclear positioning of chromatin at various levels in replication, transcription, and differentiation has been debated extensively (for reviews and discussion, see [94, 112–125]). Three sets of observations seem to be of particular interest in this context. First, terminal differentiation of cells may be correlated with specific chromatin movements within the cell nucleus [126–130]. Second, the 3D structure and arrangement of chromatin may also be altered by pathological influences [28, 131–133]. Third, recent evidence has suggested the nonrandom distribution of nonchromosomal nuclear structures, such as RNP particles [134]. In addition, fluorescence in situ hybridization experiments have demonstrated that RNA transcripts are accumulated and transported in a topologically highly ordered manner [135, 136].

In general, the extent to which DNA domain organization—DNA loops of up to many hundred kilobases in length, heterochromatin blocks, nucleoli, telomeres and centromeres, chromosome bands, or even complete chromosomes—influences fundamental nuclear processes is still ill defined. Mathog and Sedat [137] have concluded that the observed organization of polytene chromosomes in salivary gland nuclei is inconsistent with the idea that position of the chromosomes in the polytene nucleus plays a major role in the normal genetic regulation of euchromatic loci. However, comprehensive data for many cell types in various species are needed before a general picture may emerge.

The interpretation of data obtained by in situ hybridization and 3D microscopy has to take into account inherent methodological limitations. First, it is still not known to what extent chromatin distributions observed after various fixation and in situ hybridization procedures reflect the in vivo situation. Second, in spite of great improvements in 3D microscopy of chromosomes [137–139], there are still limitations that impair the precision with which the shape, size, and distribution of intranuclear chromosome targets can be measured. Third, it is far from trivial to create 3D models of chromosome distribution in order to decide for or against a random distribution. Despite these difficulties, we expect that the ability to visualize virtually any chromosomal region in cell nuclei by in situ hybridization, and further advancements of 3D microscopy, will greatly enhance our knowledge about the organization of the cell nucleus in the near future.

Clinical Applications

In spite of the present uncertainties concerning the finite intracellular organization of chromatin, the fact that the DNA of each chromosome can be recognized as a distinct entity in the cell nucleus offers the opportunity to exploit in situ hybridization for the evaluation of chromosome aberrations not only in mitotic cells, but also in cycling or noncycling interphase cells, and even in terminally differentiated cells. Examples of some recent applications in clinical cytogenetics, prenatal diagnosis, and the evaluation of the carrier status of disease genes will be discussed below. Tumor cytogenetics, one of the most promising fields of application, will be discussed in more detail elsewhere (T. Cremer et al., in preparation). For other applications such as chromosome aberration detection in biological dosimetry [140–143], the reader is referred to the literature.

The concept of analyzing chromosome aberrations in interphase nuclei started with the detection of numerical aberrations of the sex chromosomes by staining of the Barr and Y bodies, respectively [144, 145]. The use of chromosome DNA probes to generalize this concept has been termed "interphase cytogenetics" [146]. Although the number of chromosomes that can be analyzed simultaneously is limited to the number of different reporter/detector combinations available, a distinct advantage of the method is that it can be carried out in cases where metaphase chromosomes are difficult, if not impossible, to prepare, as for example in the case of material from many solid tumors.

Chromosome-specific repetitive DNA probes are ideal for the detection of aneuploidies, be-

© 1991 Elsevier Science Publishing Co., Inc., 655 Avenue of the Americas, New York, NY 10010

cause they label their target regions with very high efficiencies and the focal signals can be easily evaluated in metaphase as well as in interphase nuclei by conventional microscopy [21, 22, 146–155]. For example, these probes have been used to detect aneuploidies in amniotic fluid cells of trisomies (e.g., trisomy 18) and aneuploidies of specific tumors such as gliomas, bladder and breast carcinomas, and various leukemias. However, for the detection of structural changes, the repetitive probes are of limited use.

Chromosome painting is a more powerful tool for the analysis of structural chromosome aberrations. It is very often extremely difficult to assign small pieces of rearranged chromosomal material by banding techniques, whereas painting of the chromosome in question visualizes even small translocated material of the targeted chromosome [156]. Chromosome painting can be used for detecting numerical as well as structural aberrations of a particular chromosome (e.g., chromosome 21 aberrations diagnostic for Down's syndrome) [48, 59, 67, 69, 157].

Smaller DNA probes are very useful for the analysis of structural changes in defined chromosomal subregions. One of the most challenging tasks of cytogenetic analysis is the identification of small deletions, especially when they appear to be submicroscopic. Quantitative Southern blot analysis has been a means of detecting such deletions. Alternative methods include analysis of large DNA fragments by using pulsed-field gel electrophoresis. Conventional isotopic in situ hybridization using DNA probes relevant for a suspected deleted region requires a statistical analysis that does not distinguish between a deleted allele or a hybridization experiment of lower efficiency. In contrast, the high efficiency of nonisotopic in situ hybridization with cloned genomic DNA fragments permits such an analysis not only on metaphase chromosomes, but also in interphase nuclei. The usefulness of this approach has been demonstrated for detecting a deletion of the ankyrin gene resulting in a subtype of hereditary spherocytosis [158] as well as for the demonstration of the carrier status of women with deletions in the dystrophin gene [159]. Diagnostic applications for other genetic diseases including microdeletions, such as retinoblastoma, DiGeorge's syndrome, or the 4p-minus syndrome, can be predicted.

Single genomic probes are also successfully used for determining the representation of a particular chromosome band in a cell population. In certain diagnostic applications, such as the detection of trisomy 21, small probes are more informative than painting of whole chromosomes, because the more focal signals are easier to quantitate [48, 160]. Furthermore, single probes flanking or spanning a chromosomal breakpoint can be used to detect a diagnostically important breaking event [161–163] or to characterize further a breakpoint region [11, 164, 165]. An example of a breakpoint in the short arm of chromosome 11 from a Potter's facies syndrome patient was previously reported and is shown in Figure 1B. This approach seems particularly useful in analyzing specific translocations in blood cell tumors [162, 163, 165]. The details, implications, and prospects of this application of nonisotopic in situ hybridization are reviewed elsewhere in this journal [3].

In summary, we have demonstrated here the various ways by which in situ hybridization is being applied to establish the genetic composition and organization of complex genomes and how probe sets derived from such studies are being increasingly applied to address important clinical questions.

References

1. Raap AK, Dirks RW, Jiwa NM, Nederlof PM, van der Ploeg M: in Modern Pathology of AIDS and Other Retroviral Infections (Racz P, Haase AT, Gluckman JC, eds). Basel, S Karger, 1990, pp 17–28
2. Lichter P, Ward DC: Nature 345:93–95, 1990
3. Gray JW: Genet Anal Techn Appl 8, 1991 (in press)
4. Hamkalo BA: Genet Anal Techn Appl 8:1–10, 1991 [this issue]
5. Lawrence JB: Genet Anal Techn Appl 8, 1991 (in press)
6. Langer PR, Waldrop AA, Ward DC: Proc Natl Acad Sci USA 78:6633–6637, 1981
7. Brigati DJ, Myerson D, Leary JJ, et al.: Virology 126: 32–50, 1983
8. Lo Y-MD, Mehal WZ, Fleming KA: Nucleic Acids Res 16:8719, 1988
9. Weier H-UG, Segraves R, Pinkel D, Gray JW: J Histochem Cytochem 38:421–426, 1990
10. Kessler C, Höltke HJ, Seibl R, Burg J, Mühlegger K: Biol Chem Hoppe-Seyler 371:917–927, 1990
11. Lichter P, Tang CC, Call K, et al.: Science 247:64–69, 1990
12. Landegent JE, Jansen in de Wal N, Baan RA, Hoeijmakers JHJ, van der Ploeg M: Exp Cell Res 153:61–72, 1984
13. Dale RMK, Martin E, Livingston DC, Ward DC: Biochemistry 14:2447–2457, 1975
14. Hopman AHN, Wiegant J, Tesser GI, van Duijn P: Nucleic Acids Res 14:6471–6488, 1986

© 1991 Elsevier Science Publishing Co., Inc., 655 Avenue of the Americas, New York, NY 10010

15. Verdlov ED, Monastyrskaya GS, Guskova LI, Levitan TL, Sheichenko VI, Budowsky EI: Biochim Biophys Acta 340:153–165, 1974
16. Landegent JE, Jansen in de Wal N, van Ommen G-J, et al.: Nature 317:175–177, 1985
17. Hopman AHN, Wiegant J, van Duijn P: Exp Cell Res 169:357–368, 1987
18. Rudkin GT, Stollar BD: Nature 265:472–473, 1977
19. Bauman JGJ, Wiegant J, Borst P: Exp Cell Res 138:485–490, 1980
20. Dirks RW, van Gijlswijk RP, Tullis RH, et al.: J Histochem Cytochem 38:467–473, 1990
21. Nederlof PM, Robinson D, Abuknesha R, et al.: Cytometry 10:20–27, 1989
22. Nederlof PM, van der Flier S, Wiegant J, et al.: Cytometry 11:126–131, 1990
23. Arndt-Jovin DJ, Robert-Nicoud M, Kaufman SJ, Jovin TM: Science 230:247–256, 1985
24. Agard DA, Hiraoka Y, Shaw P, Sedat JW: Methods Cell Biol 30:353–377, 1989
25. Jovin TM, Arndt-Jovin DJ: Annu Rev Biophys Biophys Chem 18:271–308, 1989
26. Erhardt A, Zinser G, Komitowski D, Bille J: Appl Optics 24:194–200, 1985
27. Manuelidis L, Borden J: Chromosoma 96:397–410, 1988
28. Borden J, Manuelidis L: Science 242:1687–1691, 1988
29. Jones SJ, Taylor ML, Baarslag MW, et al.: J Microsc 158:235–248, 1990
30. Hiraoka Y, Sedat JW, Agard DA: Science 238:36–41, 1987
31. Agard DA, Sedat JW: Nature 302:676–681, 1983
32. Brakenhoff GJ, Blom P, Barends P: J Microsc 117:219–232, 1979
33. Cremer C, Cremer T: Microscopica Acta 81:31–44, 1978
34. Shotton DM: J Cell Sci 94:175–206, 1989
35. Hutchison NJ, Langer-Safer PR, Ward DC, Hamkalo BA: J Cell Biol 95:609–618, 1982
36. Manuelidis L, Langer-Safer PR, Ward DC: J Cell Biol 95:619–625, 1982
37. Willard HF, Waye JS: Trends Genet 3:192–198, 1987
38. Langer-Safer PR, Levine M, Ward DC: Proc Natl Acad Sci USA 79:4381–4385, 1982
39. Manuelidis L: Focus 7:4–8, 1985
40. Lawrence JB, Singer RH: Nucleic Acids Res 13:1777–1799, 1985
41. Albertson DG: EMBO J 4:2493–2498, 1985
42. Pinkel D, Straume T, Gray JW: Proc Natl Acad Sci USA 83:2934–2938, 1986
43. Raap AK, Marijnen JGJ, Vrolijk J, van der Ploeg M: Cytometry 7:235–242, 1986
44. Garson JA, van den Berghe JA, Kemshead JT: Anal Biochem 15:4761–4770, 1987
45. Ambros PF, Karlic HI: Chromosoma 77:251–254, 1987
46. Bhatt B, Burns J, Flannery D, McGee JO: Nucleic Acids Res 16:3951–3961, 1988
47. Lawrence JB, Villnave CA, Singer RH: Cell 52:51–61, 1988
48. Lichter P, Cremer T, Tang CC, Watkins PC, Manuelidis L, Ward DC: Proc Natl Acad Sci USA 85:9664–9668, 1988
49. Albertson DG, Fishpool R, Sherrington P, Nacheva E, Milstein C: EMBO J 7:2801–2805, 1988
50. Albertson DG, Sherrington PD, Rabbitts PH: Hum Genet 83:127–132, 1989
51. Cherif D, Bernard O, Berger R: Hum Genet 81:358–362, 1989
52. Viegas-Pequignot E, Dutrillaux B, Magdelenat H, Coppey-Moisan M: Proc Natl Acad Sci USA 86:582–586, 1989
53. Viegas-Pequignot E, Li ZL, Dutrillaux B, Apiou F, Paulin D: Hum Genet 83:33–36, 1989
54. Takahashi E, Hori T, Lawrence JB, et al.: Jinrui Idengaku Zasshi 34:307–311, 1989
55. Shaw J, Lichter P, Driesel AJ, Williamson R, Chamberlain S: Cytogenet Cell Genet 53:221–224, 1990
56. Giuffra LA, Lichter P, Wu J, et al.: Genomics 8:340–346, 1990
57. Landegent JE, Jansen in de Wal N, Dirks RW, Baas F, van der Ploeg M: Hum Genet 77:366–370, 1987
58. Lichter P, Cremer T, Borden J, Manuelidis L, Ward DC: Hum Genet 80:224–234, 1988
59. Pinkel D, Landegent J, Collins C, et al.: Proc Natl Acad Sci USA 85:9138–9142, 1988
60. Britten RJ, Kohne DE: Science 161:529–540, 1968
61. Trask B, Pinkel D, van den Engh G: Genomics 5:710–717, 1989
62. Kievits T, Dauwerse JG, Wiegant J, et al.: Cytogenet Cell Genet 53:134–136, 1990
63. Trask BJ, Hamlin JL: Genes Dev 3:1913–1925, 1989
64. Ruiz JC, Wahl GM: Mol Cell Biol 10:3056–3066, 1990
65. Lawrence JB, Singer RH, McNeil JA: Science 249:928–932, 1990
66. Wada M, Little RD, Abidi F, et al.: Am J Hum Genet 46:95–106, 1990
67. Cremer C, Lichter P, Borden J, Ward DC, Manuelidis L: Hum Genet 80:235–246, 1988
68. van Dilla MA, Deaven LL, Albright KL, et al.: Biotechnology 4:537–552, 1986
69. Fuscoe JC, Collins CC, Pinkel D, Gray JW: Genomics 5:100–109, 1989
70. Boyle AL: in Current Protocols in Molecular Biology (Ausubel FM, Brent R, Kingston RE, et al., eds). New York, John Wiley and Sons, 1991 (in press)
71. Lengauer C, Eckelt A, Weith A, et al.: Cytogenet Cell Genet 1991 (in press)
72. Kievits T, Devilee P, Wiegant J, et al.: Cytometry 11:105–109, 1990
73. Boyle AL, Lichter P, Ward DC: Genomics 7:127–130, 1990
74. Nelson DL, Ledbetter SA, Corbo L, et al.: Proc Natl Acad Sci USA 86:6686–6690, 1989
75. Ledbetter SA, Nelson DL, Warren ST, Ledbetter DH: Genomics 6:475–481, 1990
76. Lichter P, Ledbetter SA, Ledbetter DH, Ward DC: Proc Natl Acad Sci USA 87:6634–6638, 1990
77. Lengauer C, Riethman H, Cremer T: Hum Genet 86:1–6, 1990

© 1991 Elsevier Science Publishing Co., Inc., 655 Avenue of the Americas, New York, NY 10010

78. Harnden DG, Path FRC, Klinger HP: ISCN 1985. An International System for Human Cytogenetic Nomenclature. Basel, S Karger, 1985
79. Trask B, van den Engh G, Gray JW: Am J Hum Genet 45:753–760, 1989
80. Trask B, van den Engh G, Mayall B, Gray JW: Am J Hum Genet 45:739–752, 1989
81. Cherif D, Le Coniat M, Suarez HG, Bernheim A, Berger R: Cancer Genet Cytogenet 33:245–249, 1988
82. Fan Y-S, Davis LM, Shows TB: Proc Natl Acad Sci USA 87:6223–6227, 1990
83. Cherif D, Julier C, Delattre O, Derré J, Lathrop GM, Berger R: Proc Natl Acad Sci USA 87:6639–6643, 1990
84. Vogel W, Autenrieth M, Speit G: Hum Genet 72:129–132, 1986
85. Boyle AL, Ballard SG, Ward PC: Proc Natl Acad Sci USA 87:7757–7761, 1990
86. Baldini A, Ward DC: submitted, 1991
87. Manuelidis L, Ward DC: Chromosoma 91:28–38, 1984
88. Korenberg JR, Rykowski MC: Cell 53:391–400, 1988
89. Moyzis RK, Torney DC, Meyne J, et al.: Genomics 4: 273–289, 1989
90. Buluwela L, Albertson DG, Sherrington P, Rabbitts PH, Spurr N, Rabbitts TH: EMBO J 7:2003–2010, 1988
91. Rappold GA, Cremer T, Hager HD, Davies KE, Müller CR, Yang T: Hum Genet 67:317–325, 1984
92. Wienberg J, Jauch A, Stanyon R, Cremer T: Genomics 8:347–350, 1990
93. Cremer T: Von der Zellenlehre zur Chromosomentheorie. Naturwissenschaftliche Erkenntnis und Theorienwechsel in der frühen Zell- und Vererbungsforschung. Heidelberg, Springer Verlag, 1985
94. Comings DE: Am J Hum Genet 20:440–460, 1968
95. Wischnitzer S: Int Rev Cytol 34:1–48, 1973
96. Zorn C, Cremer C, Cremer T, Zimmer J: Hum Genet 35: 83–89, 1976
97. Cremer T, Cremer C, Baumann H, et al.: Hum Genet 60: 46–56, 1982
98. Cremer T, Baumann H, Nakanishi K, Cremer C: Chromosomes Today 8:203–212, 1984
99. Schardin M, Cremer T, Hager HD, Lang M: Hum Genet 71:281–287, 1985
100. Durnam DM, Gelinas RE, Myerson D: Somatic Cell Mol Genet 11:571–577, 1985
101. Manuelidis L: Hum Genet 71:288–293, 1985
102. Hopman AHN, Wiegant J, Raap AK, Landegent JE, van der Ploeg M, van Duijn P: Histochemistry 85:1–4, 1986
103. Trask B, van den Engh G, Pinkel D, et al.: Hum Genet 78:251–259, 1988
104. van Dekken H, Pinkel D, Mullikin J, Trask B, van den Engh G, Gray J: J Cell Sci 94:299–306, 1989
105. Schwarzacher T, Leitch AR, Bennett MD, Heslop-Harrison JS: Ann Bot 64:315–324, 1989
106. Leitch AR, Mosgöller W, Schwarzacher T, Bennett MD, Heslop-Harrison JS: J Cell Sci 95:335–341, 1990
107. Popp S, Scholl HP, Loos P, et al.: Exp Cell Res 189:1–12, 1990
108. van Dekken H, van Rotterdam A, Jonker RR, van der Voort HTM, Brakenhoff GJ, Baumann JGJ: Cytometry 11:570–578, 1990
109. van Dekken H, van der Voort HTM, Brakenhoff GJ, Baumann JGJ: Cytometry 11:579–585, 1990
110. Emmerich P, Loos P, Jauch A, et al.: Exp Cell Res 181:126–140, 1989
111. Arnoldus EPJ, Peters ACB, Bots GTAM, Raap AK, van der Ploeg M: Hum Genet 83:231–234, 1989
112. Vogel F, Schroeder TM: Humangenetik 25:265–297, 1974
113. Heslop-Harrison JS, Bennett MD: J Embryol Exp Morphol 83(Suppl):51–73, 1984
114. Blobel G: Proc Natl Acad Sci USA 82:8527–8529, 1985
115. Jackson DA: Trends Biochem Sci 11:249–252, 1986
116. Gasser SM, Laemmli UK: Trends Genet 3:16–22, 1987
117. Hilliker AJ, Appels R: Exp Cell Res 185:297–318, 1989
118. Belmont AS, Braunfeld MB, Sedat JW, Agard DA: Chromosoma 98:129–143, 1989
119. Manuelidis L, Chen TL: Cytometry 11:8–25, 1990
120. Chung H-MM, Shea C, Fields S, Taub RN, van der Ploeg LHT: EMBO J 9:2611–2619, 1990
121. Montag M, Spring H, Trendelenburg MF, Kriete A: J Microsc 158:225–233, 1990
122. Minden JS, Agard DA, Sedat JW, Alberts BM: J Cell Biol 109:505–516, 1989
123. Hiraoka Y, Minden JS, Swedlow JR, Sedat JW, Agard DA: Nature 342:293–296, 1989
124. Mills AD, Blow JJ, White JG, Amos WB, Wilcock D, Laskey RA: J Cell Sci 94:471–477, 1989
125. Arndt-Jovin DJ, Robert-Nicaud M, Jovin TM: J Microsc 157:131–139, 1990
126. Graham GA: Anat Rec 119:469–491, 1954
127. Schmid M, Krone W: Chromosoma 56:327–347, 1976
128. Manuelidis L: Proc Natl Acad Sci USA 81:3123–3127, 1984
129. Manuelidis L: Ann NY Acad Sci 450:205–221, 1985
130. Brinkley BR, Brenner SL, Hall JM, Tousson A, Balczon RD, Valdivia MM: Chromosoma 94:309–317, 1986
131. Barr M, Bertram EG: J Anat 85:171–181, 1951
132. Bertram EG: in The Sex Chromatin (Moore KL, ed). Philadelphia, London, W.B. Saunders Company, 1966, pp 162–172
133. Brasch K: Biochem Cell Biol 68:408–426, 1990
134. Ringertz N, Hadlaczky G, Hallman H, Nyman U, Petterson I, Sharp GC: J Cell Sci (Suppl) 4:11–28, 1986
135. Lawrence JB, Singer RH, Marselle LM: Cell 57:493–502, 1989
136. Spector DL: Proc Natl Acad Sci USA 87:147–151, 1990
137. Mathog D, Sedat JW: Genetics 121:293–311, 1989
138. Hochstrasser M, Sedat JW: J Cell Biol 104:1455–1469, 1987
139. Hochstrasser M, Sedat JW: J Cell Biol 104:1471–1482, 1987
140. Lucas JN, Tenjin T, Straume T, et al.: Int J Radiat Biol 56:35–44, 1989
141. Meyne J, Littlefield LG, Moyzis RK: Mutat Res 226:75–79, 1989
142. Cremer T, Popp S, Emmerich P, Lichter P, Cremer C: Cytometry 11:110–118, 1990

© 1991 Elsevier Science Publishing Co., Inc., 655 Avenue of the Americas, New York, NY 10010

143. Popp S, Remm B, Hausmann M, et al.: Kerntechnik 55:204–210, 1990
144. Barr ML, Bertram EG: Nature 163:676–677, 1949
145. Pearson PL, Bobrow M, Vosa CG: Nature 226:78–80, 1970
146. Cremer T, Landegent J, Brückner A, et al.: Hum Genet 74:346–352, 1986
147. Cremer T, Tesin D, Hopman AHN, Manuelidis L: Exp Cell Res 176:199–220, 1988
148. Hopman AHN, Ramaekers FCS, Raap AK, et al.: Histochemistry 89:307–316, 1988
149. Devilee P, Thierry RF, Kievits T, et al.: Cancer Res 48:5825–5830, 1988
150. Hopman AHN, Poddighe PJ, Smeets WAGB, et al.: Am J Pathol 135:1105–1117, 1989
151. Viegas-Pequignot E, Jeanpierre M, Dutrillaux AM, Gerbault-Seureau M, Muleris M, Dutrillaux B: Hum Genet 81:311–314, 1989
152. Nederlof PM, van der Flier S, Raap AK, et al.: Cancer Genet Cytogenet 42:87–98, 1989
153. Anastasi J, Le Beau MM, Vardiman JW, Westbrook CA: Am J Pathol 136:131–139, 1990
154. Kolluri RV, Manuelidis L, Cremer T, Sait S, Gezer S, Raza A: Am J Hematol 33:117–122, 1990
155. Giwercman A, Hopman AHN, Ramaekers FCS, Skakkebaek NE: Am J Pathol 136:497–502, 1990
156. Popp S, Jauch A, Qui JY, Smialek B, Cremer T, Becher R: Cancer Genet Cytogenet 1991 (in press)
157. Jauch A, Daumer C, Lichter P, Murken J, Schroeder-Kurth T, Cremer T: Hum Genet 85:145–150, 1990
158. Lux SE, Tse WT, Menninger JC, et al.: Nature 345:736–739, 1990
159. Ried T, Mahler V, Vogt P, et al.: Hum Genet 85:581–586, 1990
160. Lichter P, Jauch A, Cremer T, Ward DC: in Molecular Genetics of Chromosome 21 and Down Syndrome (Patterson D, ed). New York, Alan R. Liss, 1990, pp 69–78
161. Dauwerse JG, Kievits T, Beverstock GC, et al.: Cytogenet Cell Genet 53:126–128, 1990
162. Arnoldus EPJ, Wiegant J, Noordemeer IA, et al.: Cytogenet Cell Genet 54:108–111, 1990
163. Tkachuk D, Westbrook C, Andreef M, et al.: Science 250:559–562, 1990
164. Mengle-Gaw L, Albertson OG, Sherrington PD, Rabbitts TH: Proc Natl Acad Sci USA 85:9171–9175, 1988
165. Rowley JD, Diaz MO, Espinosa III R, et al.: Proc Natl Acad Sci USA 87:9358–9362, 1990

© 1991 Elsevier Science Publishing Co., Inc., 655 Avenue of the Americas, New York, NY 10010