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Centromeres in Cell Division, Evolution, Nuclear Organization and Disease

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Abstract As the spindle fiber attachment region of the chromosome, the centromere has been investigated in a variety of contexts. Here, we will review current knowledge about this unique chromosomal region and its relevance for proper cell division, speciation, and disease. Understanding the three-dimensional organization of centromeres in normal and tumor cells is just beginning to emerge. Multidisciplinary research will allow for new insights into its normal and aberrant nuclear organization and may allow for new therapeutic interventions that target events linked to centromere function and cell division. J. Cell. Biochem. 104: 2040–2058, 2008. © 2008 Wiley-Liss, Inc.

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The study of the cell nucleus and its components has a long history that began in the 19th century and is closely interlaced with the history of cell biology and genetics. An important contribution made by previous generations of researchers was the careful and detailed observations and illustrations of cells, nuclei, and chromosomes. Prior to photographic representations and digital imaging, hand-drawn representations guided the research community. Continued progress in the field was made possible by the critical analyses by these pioneers on cells of many species. This work greatly benefited from the development of microscopes, photography and imaging. Phase contrast, polarization, differential interference contrast, fluorescence and electron microscopy and live cell imaging—to name just a few—have given us higher resolutions and further knowledge gain. The foundation for our current studies was laid by the forerunners of cell biology and genetics through their dedication and precise observations.

The nucleus was first described by Robert Brown in 1833. In 1873, Fol, Bütschli, and Strasburger discovered "karyokinetic figures"—today called the mitotic apparatus [for review see, Gourret, 1995]. In 1882, Walther Flemming described the process of mitosis for the first time in detail. During cell division, he observed a "stainable substance of the nucleus" that separates into thread-like strings. Hence, he coined the term "mitosis" from Greek for "thread." Six years later, these threads were defined as chromosomes by Heinrich Waldeyer [1888] [http://www.laskerfoundation.org/news/gnn/timeline/timeline_top.html; Cremer, 1985]. Flemming had used aniline dyes to stain and then visualize these "threads" and commented "we will designate as chromatin that substance, in the nucleus, which upon treatment

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with dyes known as nuclear stains does absorb the dye" (1882) (Fig. 1). Due to the staining method and his gift of detailed observation, Flemming was able to state that the threads—chromosomes—split along their length into two identical halves (sister chromatid separation). He published these findings in *Zellsubstanz, Kern und Zelltheilung* (1882) [for review see also, Rieder and Khodjakov, 2003]. Although Flemming observed cell divisions in detail, the centromere, the focus of this article, was not yet described. Instead, he showed chromosomes with multiple spindle fibers attaching all over the length of the chromosome [Flemming, 1882; Cremer, 1985]. Although, Boveri [1902, 1914, 1929] had postulated that chromosome and centrosome errors contribute to the development of malignant tumors, the centromere was also unknown to him.

The name "centromere" was given to the primary constriction of the chromosome by Darlington [1936a]. He defined it as the "site of spindle fiber attachment" [1936a]. "The centromeres are active in moving the chromosomes on the spindle" [Darlington, 1936b]. In Darlington's time, the centromere was thus already recognized for its key role in cell division; spindle attachment and chromosome movements were described in detail and depicted in hand-drawn figures. Importantly, naturally occurring or irradiation-induced mis-divisions of the centromere were already described [McClintock, 1932, 1933; Darlington, 1936b]. The resulting consequences of centromere mis-division were illustrated for the chromosome constitution of the resulting daughter cells, and hypotheses for the mechanisms leading to this event were discussed in great detail [Darlington, 1936b]. Some of these questions are still valid today and have not been resolved. These include aspects of the regulation and timing of sister chromatid separation

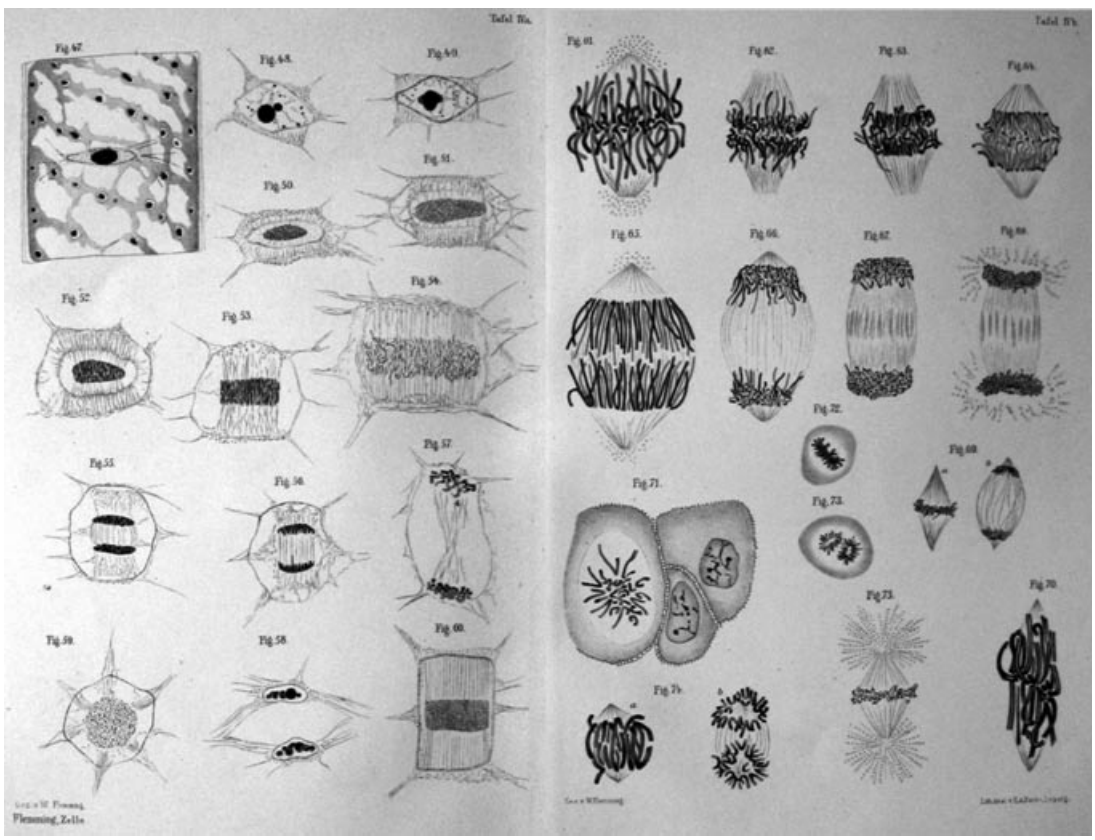

Fig. 1. Cell division cycles. Historical drawings by Walther Flemming (1843–1905) showing for the first time cell division that he termed mitosis and ''threats'' (later called chromosomes) that are evenly distributed during mitosis to daughter cells. The drawings represent the green algae *Spirogyra* (Figs. 47–60), the plants *Lilium corceum* (Fig. 61), *Lilium corceum* (Figs. 62–68), *Iris sibirica* (Fig. 69), and *Lilium tigrinum* (Fig. 70), the human cornea (Figs. 71–73), testes of salamander (Figs. 74, 75), and the egg of the sea urchin *Toxopneustes lividus* (Fig. 75). All images are reproduced from Flemming's book *Zellsubstanz, Kern und Zelltheilung*, 1882.

and centromere division and consequences of their dysregulation, the mechanisms of iso-chromosome formation, and the effects of mis-segregation on the subsequent cell divisions, cell lineages and the organism.

THE CENTROMERE

The centromere, cytogenetically is the primary constriction of each chromosome and is defined as the chromosomal region that will determine the formation of the kinetochore and sister chromatid cohesion during cell division. Through the kinetochore, the chromosome interacts with the spindle microtubules and ensures chromatid segregation [Villasante et al., 2007]. Crucial to proper chromosome segregation is the bi-orientation of each chromosome. Each sister kinetochore is positioned on the opposite sides of the centromere and attaches to microtubules derived from the opposite spindle pole [Loncarek et al., 2007]. Failure to perform this bi-orientation, leads to syntelic chromosomes whose two kinetochores attach to one spindle pole. This error can be corrected through the force of astral microtubules, and this event is dependent on the presence of functional centrosomes [Loncarek et al., 2007].

The DNA sequence associated with all primate centromeres is the alpha-satellite DNA. It consists of tandem repeats of 171 base pairs (defining one monomer), and it is AT rich [Choo, 1997]. A large fraction of these monomers is organized into higher order repeats, with a repeat unit ranging in size from 3 to 5 Mb. The difference in sequence between these units is $<2\%$ [Alkan et al., 2007]. The evolutionary analysis of primate centromeric DNA showed that gibbon alpha-satellite sequences have only limited relationship with human sequences. In contrast, human and chimpanzee higher order centromeric repeats show a common origin [Alkan et al., 2007]. A very detailed description of a human functional centromere is presented for the X chromosome by Schueler et al. [2001].

Centromeres are surrounded by pericentromeres. These are chromosomal regions rich in transposons, retroelements, pseudogenes, and expressed genes [Saffery et al., 2003; Hall et al., 2006]. All of these elements contribute to the dynamic evolution of these sequences as shown for four *Brassica* species [Hall et al., 2006]. Centric breakage and fusion has been observed in Robertsonian translocation chromosomes of wheat and rye [Zhang et al., 2001]. Pericentric inversions in humans are often associated with decreased male fertility [Collodel et al., 2006; Chantot-Bastaraud et al., 2007, for review, see Anton et al., 2005], cancer [Mathew et al., 2002; Anelli et al., 2005; Pedrazzini et al., 2006], mental retardation [Ramadevi et al., 2002; Ulucan et al., 2006] and other diseases such as: [epilepsy: Grosso et al., 2004; cleft lip: Beiraghi et al., 2003; schizophrenia and learning disability: Hampson et al., 1999]. Such inversions may also be associated with duplications and deletions [López-Exposito et al., 2006; Patil and Phadke, 2007].

It is thus not surprising that pericentromeric regions are both hotspots for recombination events during evolution [Yue et al., 2005] and in cancer [Shaw and Lupski, 2004; Jamet et al., 2005]. In this context, we note that DNA double-strand-break hotspots are positioned near centromeres and telomeres [Blitzblau et al., 2007]. Mapping of the meiotic single-stranded DNA molecules revealed hotspot breakpoints in pericentromeric regions as well as within $\sim$100 kb region at the telomeric ends of chromosomes. Thus, evolutionary hotspots at pericentromeres are also hotspots of DNA double-strand-breaks during meiosis and sites of genomic instability in cancer.

Centromere Evolution, Speciation, Structural Abnormalities and Cancer

The centromere contributes to speciation. There are many examples but, we will focus on two, the speciation of macropodine marsupials [Bulazel et al., 2007; Metcalfe et al., 2007] and mice [Capanna and Castiglia, 2004]. Examining centromere sequence composition and breakpoint reuse in wallabies, wallaroos and kangaroos, Bulazel et al. [2007] described species-specific shifts in the composition of centromere sequences. Metcalfe et al. [2007] who studied four marsupial hybrids found that all hybrids exhibited a low frequency of de novo rearrangements. The type of centromere instability described included the amplification of alpha satellites and of a transposable elements, de novo whole chromosome arm rearrangements and changes in chromatin structure.

In mice, speciation is commonly achieved with telocentric chromosomes that display a high grade of homogenization. There is greater than 99% sequence identity between

the telocentric sequences for all mouse chromosomes, leading to frequent recombinations and stable evolutionary maintenance. This genetic similarity however, also enables the possibility of Robertsonian (Rb) translocations [Garagna et al., 2001; Kalitsis et al., 2006]. Rb translocation chromosomes were first discovered in 1916 in grasshoppers by W R B Robertson [for review, see Gardner and Sutherland, 2004]. Mice with Rb translocation chromosomes exist in nature [Nachman and Searle, 1995; Gazave et al., 2003], and these translocations are also common in some laboratory mice. In nature, whenever they occur in hybrid zones, the mice that are Rb translocation chromosome carriers often display reduced fertility. This is due to reduced chiasmata formation and meiotic recombination leading to spermatogonic impairment when mating with non-carriers or carriers of different types of Rb chromosomes [Bidau et al., 2001; Merico et al., 2003]. Rb chromosomes contribute to non-disjunction and to transmission ratio distortion (TRD) [Underkoffler et al., 2005; Schulz et al., 2006].

End-to-end fusions of human acrocentric chromosomes generate human Robertsonian (Rb) translocation chromosomes. Rb translocations in humans constitute the most common structural genetic abnormalities in aborted fetuses and newborns [Jacobs, 1981; Nielsen and Wohlert, 1991; Kim and Shaffer, 2002]. Moreover, human Rb translocation chromosomes have been found as acquired or constitutional genetic lesions in hematological cancers [Qian et al., 2002; Welborn, 2004], in solid tumors [Padilla-Nash et al., 2001; Bayani et al., 2003], and at the onset of acute myelogenous leukemia [Shimokawa et al., 2004].

Cancer cells follow evolutionary principles when they develop into highly malignant tumors. Work by Darai et al. [2005] illustrates this in that the breakpoints in human tumors from chromosome 3p21.3 region coincides with the evolutionary breakpoints seen in *Caenorhabditis elegans*, *Drosophila melanogaster*, *Fugu rubripes*, *Gallus gallus*, *Mus musculus*, *Rattus norvegicus*, and *Canis familiaris*. CER1, a 2.4 Mb region at 3p21.3, shows breakpoints clustered within 200 kb of the telomeric and centromeric borders. The frequent involvement of centromeric regions in genomic instability of cancers is also discussed in a recent review by Bayani et al. [2007]. Examples for such centromeric involvement is seen in prostate cancer [Beheshti et al., 2000; Vukovic et al., 2007] and osteosarcomas [Bayani et al., 2003].

It is evident that centromeres are dynamic structures that permit evolutionary remodeling of chromosomes, and that facilitates speciation. The centromeres are also substrates for rearrangements that are associated with structural genetic abnormalities in cancer where, in the same fashion as evolution, selection occurs at the cellular level in an accelerated fashion.

Centromeric Nucleosomes

Mammalian centromeres are defined based on their epigenetic higher order chromatin organization [Taddei et al., 2001]. The centromeres of all species examined to date have a universal histone composition consisting of CENP-A (CenH3), a histone H3 variant, histone H4, histone H2A and H2B [Dalal et al., 2007]. Centromeric histones do not contain the usual histone H3 but the CENP-A (CenH3) [Dalal et al., 2007]. The centromeric nucleosome organization is also unique in that its histones do not show an octamer organization but rather form a hemisome with one molecule each of CENP-A, H4, H2A, and H2B [Dalal et al., 2007]. The histone variant H2A.Z is found interspersed in between subdomains of CENP-A [Greaves et al., 2007]. Orthaus et al. [2008] recently illustrated the association of CENP-A and CENP-B in living human cells using fluorescence resonance energy transfer (FRET), a technique that allows one to visualize the close association of target molecules that are found in the vicinity of 10 nm.

Functional Studies Addressing the Role of Specific Proteins in Centromere Biology, Cell Division and Development

Functional understanding of centromeric histones, the role of the securing-separase complex and of epigenetic modifications at the centromere is due to specific mouse models that addressed the role of centromeric histones in the context of a living organism. As well, study of a rare human syndrome, ICF (immunodeficiency, centromere instability, facial abnormalities), helped to elucidate the crucial role of epigenetic modifications at the centromere for normal cellular functions and development.

CENP-A, CENP-B, and CENP-C Functions

Using gene targeting, Howman et al. [2000] describe that CENP-A$^{-/-}$ mice do not survive

beyond embryonic day E6.5, while CENP-A$^{-/+}$ mice are viable and fertile. CENP-A$^{-/-}$ embryos displayed mitotic problems such as the formation of micro- and macronuclei, chromosome hypercondensation, nuclear bridges, and blebbing with chromatin fragmentation. In the absence of CENP-A, CENP-B and CENP-C are dispersed throughout the nucleus and are not localized at centromeres. Using a partially functional CENP-A GFP fusion protein, Kalitsis et al. [2003] confirmed that CENP-A is required for embryonal development. CENP-A-GFP homozygote mice die at day E10.5 of embryonic development showing much aneuploidy, missegregation of chromosomes and increased apoptosis [Kalitsis et al., 2003]. In this context, Black et al. [2004] showed that CENP-A confers a unique structural rigidity to the centromeric nucleosome. The disruption of CENP-B is not lethal, but mice with disrupted CENP-B have lower body and testis weight [Hudson et al., 1998]. CENP-C heterozygous mice are fertile and viable however, CENP-C null mice are not viable. Developmental problems including mitotic arrest, morphological degeneration, micronuclei and aberrant chromosome segregation patterns that become apparent at the morula stage [Kalitsis et al., 1998]. Recent data from Shen et al. [2007] indicated that mutations in PTEN prevented the normal physical association with CENP-C and this caused centromere instability resulting in centromere breakage and chromosomal translocations.

The Separase/Securing-Complex and Centromeric Division

Kumada et al. [2006] addressed the functional importance of mammalian separase and securin proteins for cell division and found that separase is required for the early development of mice. In the absence of separase, centromeres are unable to separate in mitosis and remain continuously linked in interphase. Karyotype analysis showed that two to four chromatids were linked at the centromere. Because these were all derived from the inability of the centromeres to separate, all centromerically clustered chromosomes contained either diploid or quadruple copies of the same chromosome. Heterozygous separase mice are viable, but separase null mice stop their development at day E3.5. Culture of 1–3 day blastocytes indicated that the overall cell number in separase$^{-/-}$ blastocytes was lower, and that the size of individual cells was twice that of separase heterozygous mice. Moreover, the absence of separase induced increased ploidy, aberrant numbers of centrosomes, and growth retardation. When homozygous securin$^{null/null}$ mice, which is not lethal, were crossed with heterozygous securin$^{null/+}$ separase$^{null/+}$ mice the securin$^{null/null}$separase$^{null/+}$ combination was embryonically lethal.

Centromeric Fusions in the Immunodeficiency, Centromeric Region Instability, Facial Anomalies (ICF) Syndrome

ICF is a rare recessive genetic disorder [Choo, 1997] with only 50 reported cases throughout the world [Ehrlich et al., 2006]. This immunodeficiency and chromosome instability syndrome is generally lethal before adulthood due to epigenetic changes. The identification of mutations in DNA methyltransferase 3B (DNMT3B) located on chromosome 20q11-13 by Xu et al. [1999] led to the mechanistic understanding of the disease. In ICF syndrome, the centromeric satellites are almost completely unmethylated in all tissues, whereas these regions are heavily methylated at cytosine residues in normal cells [Xu et al., 1999]. Thus, the absence of a functional DNMT3B methyltransferase causes the elongation of juxtacentromeric heterochromatin. In metaphase, such defects give these chromosomal regions a thread-like appearance. In interphase cells, these regions show self-associations. Blebs and micronuclei containing these sequences are also common. Chromosomal abnormalities are most common for the classical satellites 2 and 3 at the juxtacentromeric regions of chromosomes 1, 9, and 16. These three chromosomes are frequently fused to each other in centromeric clusters.

Hypomethylation of Centromeric Alpha Satellite Regions in Cancer

Cancer cells frequently display hypomethylation of satellite regions, and this is commonly associated with rearrangements in these regions [Qu et al., 1999a,b; Ehrlich et al., 2006]. Hypomethylation of satellite 2 DNA is found in ovarian cancers including cystadenomas, low malignant potential tumors and epithelial carcinomas [Qu et al., 1999a]. The authors reported a statistical correlation between the extent of hypomethylation and the degree of malignancy. The study by Widschwendter et al.

[2004] examined DNA methylation and ovarian cancer (115 ovarian tumors) and found that hypomethylation of satellite 2 of chromosome 1 increased from non-neoplastic to neoplastic tumors with poor prognosis. Fifteen 5′regions of genes implicated in ovarian cancer were also examined. They found hypermethylation of CDH13 (16q24) and RNR1 (13p12) were strongly correlated with hypomethylation of satellite 2 DNA. Another example is in Wilm's tumor, where there are rearrangements in hypomethylated pericentromeric heterochromatin of chromosomes 1 and 16 with classical satellite 2 DNA [Qu et al., 1999b]. All of the above findings are highly relevant for our understanding of aberrant methylation at centromeric regions and the consequences thereof. These data, however, have to be viewed in the context of global genomic changes in methylation patterns—and epigenetic changes in general—during carcinogenesis [Virmani et al., 2001; D'Alessio and Szyf, 2006; Esteller, 2007].

NEOCENTROMERES

Neocentromeres are functional centromeres that are situated at non-centromeric regions and they are able to assemble a functional kinetochore [Amor and Choo, 2002]. The first neocentromere was described by du Sart et al. [1997], and since then another 60 examples of neocentromeres have been characterized [Amor and Choo, 2002]. In a study that focused on greater than 20 functionally important kinetochore-associated proteins, Saffery et al. [2000] showed that all proteins investigated but one, CENP-B, are present at both centromeres and neocentromeres. CENP-B is thus missing at neocentromeres. Neocentromeres do not carry alpha-satellite DNA [Amor and Choo, 2002] and they are frequently found on marker chromosomes in cancer. They can also be experimentally induced in *Drosophila* (ibid.). Occasionally, neocentromeres are observed in individuals with stable karyotypes [Amor and Choo, 2002; Amor et al., 2004; Ventura et al., 2004]. They can play an important role for chromosome and karyotype evolution [Amor et al., 2004; Warburton, 2004].

An example in karyotype evolution is a neocentromere located at chromosome 4p21.3 [Amor et al., 2004]. The family that carries this neocentromere has transmitted it to the second generation and shows a normal and stable karyotype. The normal centromere on chromosome 4 is inactivated. The neocentromere is fully functional in binding CENP-A, CENP-C, CENP-E, and CENP-I, Bub1 and HP1, a Swi6 homologue. However, the levels of CENP-A are reduced and a moderate reduction in sister chromatid cohesion was noted suggesting the possibility of subtle structural and functional differences between the neocentromere and the normal centromere. Ventura et al. [2004] reported on two human neocentromeres. The one found at 3q26 is a chromosomal region where a new centromere evolved from a common ancestor of Old World Monkeys about 25–40 million years ago. The other neocentromere was found at 3q24 with inactivation of the normal centromere and this new chromosome 3 was stably transmitted to the next generation.

Centromeres and the Nuclear Matrix

Are centromeres freely moving through the nucleus or are they attached to some nuclear structure in a regulated fashion?

He and Brinkley showed in [1996] that individual centromeres/prekinetochores form stable associations with the nuclear matrix. They proposed that the arrangement of individual centromeres within the nucleus may have influenced the occurrence of specific fusion and translocation events during evolution. In addition, they speculate that this centromere/kinetochore complex may play a role during chromosome movements and associations in the interphase nucleus. Sumer et al. [2003] described, tested and confirmed a 2.5 Mb region with enhanced scaffold/matrix attachment properties at a human neocentromere. Thus, we conclude that centromeres have the potential to attach to the nuclear matrix. Whether they attach to it all the time or only during specific phases of the cell cycle is presently not known.

Nuclear Organization of Centromeres During the Cell Cycle

The three-dimensional (3D) organization of centromeres in the interphase nucleus has been studied in the context of cellular differentiation, cell cycle, embryonic development, and cellular transformation. Studying human diploid fibroblasts using anti-centromere antibody staining and laser scanning confocal microscopy, Bartholdi [1991] described that centromere positions vary during the cell cycle. In G1, the

author describes centromeres associated with nucleoli or fused in chromocentres, with very few single centromeres. The latter are dispersed throughout the nucleus. In S phase, the fused centromeres dispersed into single centromere signals and formed distinct patterns (rings or lines). In prophase, the fluorescent centromere signals were found to condense into distinct double spots.

The analysis of human lymphocytes [Weimer et al., 1992] also indicated a cell-cycle specific organization of centromeres in the interphase nucleus. Using anti-centromere antibodies (ACAs) from patients with CREST syndrome, these authors described a peripheral positioning of centromeres in G0 and G1, which weakened in S phase. G2 cells had a random distribution of centromeres.

Solovei et al. [2004] investigated a series of human cell types and found differences in centromere positions in cycling and postmitotic cells. The cells they examined included G_0 peripheral blood lymphocytes, terminally differentiated monocytes, cycling, PHA-stimulated lymphocytes, diploid lymphoblastoid cells, normal fibroblasts, and neuroblastoma (SH-EP) cells. They used immunostaining, confocal microscopy and 3D image analysis and observed that the localization of the centromeres was similar in all cell types, with a peripheral location in G_0 cells. In G1 and early S, the centromeres were found in clusters at the periphery of the nucleus, in late S/G2, the centromeres declustered and migrated towards the center of the nucleus.

Our work [Sarkar et al., 2007] described the 3D organization of centromeres in mouse lymphocytes during the cell cycle. We found centromere localization is cell cycle dependent. The centromeres were mainly found in the peripheral regions in G1- and G2-arrested lymphocytes whereas a more general distribution throughout the nucleus was found during logarithmic growth. To measure the 3D organization of centromeres, we developed a software program, named CentroView [Sarkar et al., 2007]. We used a 3D-centromere FISH approach rather than the anti-centromere fluorescent immunohistochemistry. Image acquisition was performed using deconvolution microscopy [Schaefer et al., 2001] and Zeiss AxioVision on an Axioplan 2 microscope (Zeiss). Sampling sizes were 200 nm in z and 107 nm in x and y axes.

From the data presented above, we concluded that there is agreement of cell-cycle dependent centromere distribution in interphase nuclei of all human and mouse cells examined so far. It is important to note that a cell-type specific centromere organization has also been described in plants. Work by Fang and Spector [2005] showed by live cell imaging of centromere-specific histone H3-GFP, that centromeres of transgenic Arabidopsis lines are positioned at the nuclear periphery during interphase. The position of duplicated sister-centromeres is cell-type dependent. They are found clustered in root epidermal cells and dispersed in leaf epidermal cells.

Differentiation and Distribution of Centromeres in the Interphase Nucleus

Beil et al. [2002 and 2005] studied the organization of centromeres during differentiation of the promyelocytic leukemia cell line NB4. The authors used the CREST serum to stain centromeres and confocal microscopy to assess centromere positions. Induction of differentiation by retinoic acid in NB4 cells induces an alternative nuclear distribution of centromeres compared with undifferentiated cells. The differentiated cells exhibited a decreased number of centromere clusters (chromocentres) and the distance between them increased from 350 to 800 nm.

Differentiating mouse T cells display an altered organization of centromeres when compared to their non-differentiated counterparts [Kim et al., 2004]: While undifferentiated cells have centromeres localized within the nuclear interior, differentiated CD4+ and CD8+ T cells have their centromere positioned more to the periphery. Similarly, human embryonic stem cells have a smaller portion of their centromeres located at the nuclear periphery compared with differentiated cells [Wiblin et al., 2005].

We conclude that differentiation requires altered nuclear centromere positions which may be linked to the global remodeling of nuclear functions during this process such as changes in transcription of genes and cessation of replication. The nuclear periphery seems to be the preferred region occupied by centromeres when cells differentiate. This is also the preferred position of centromeres in G0 and G1 cells (see above) and thus probably the position that is most conceivable with a resting and end-stage differentiated cells.

Remodeling of Centromere Positions During Mouse Embryongenesis

Mouse embryos remodel their nuclear organization as they progress from the one-cell-stage to the blastocyst stage of embryonic development. This remodeling includes both the centromeres and the pericentric heterochromatin [Martin et al., 2006a]. Martin et al. [2006b] also showed nuclear remodeling during nuclear transfer. During early embryonic development, after the transfer of ES cell chromosomes into mouse ooplasm, centromeres adopted the same nuclear organization as seen in normal mouse embryos.

Remodeling of Centromere Positions During Cellular Transformation

Centromere repositioning in the interphase nucleus contributes to the remodeling of the nucleus when cells become transformed. There is a significant redistribution of centromere positions in nuclei of immortalized and transformed lymphocytes as compared with normal nuclei [Sarkar et al., 2007]. While centromeres of normal mouse lymphocytes assume a peripheral position in interphase nuclei, those of tumor cells redistribute towards a more central nuclear position (Fig. 2). These changes are highly significant [Sarkar et al., 2007]. The remodeling of centromere positions can be induced through conditional c-Myc oncoprotein expression in otherwise diploid mouse PreB lymphocytes and is dependent on the *myc box II* region [Guffei et al., 2007]. The remodeling of centromere positions enables the development of mouse Robertsonian (Rb) translocation chromosomes after conditional c-Myc deregulation [Guffei et al., 2007]. The latter are also observed in cells with constitutive c-Myc deregulation [McCormack et al., 1998; Guffei et al., 2007].

Centromeres and their pericentric regions are evolutionary dynamic. These features strongly enhance the likelihood that spatially repositioned centromeres can then interact with each allowing increased recombination, breakage and fusion events. We conclude that centromere remodeling during cellular transformation may permit the occurrence of specific chromosomal rearrangements and favor new gene expression and replication profiles as centromeres (and thus chromosomes and their respective genes) are more frequently localized in the nuclear interior. The nuclear interior regions are associated with both transcription

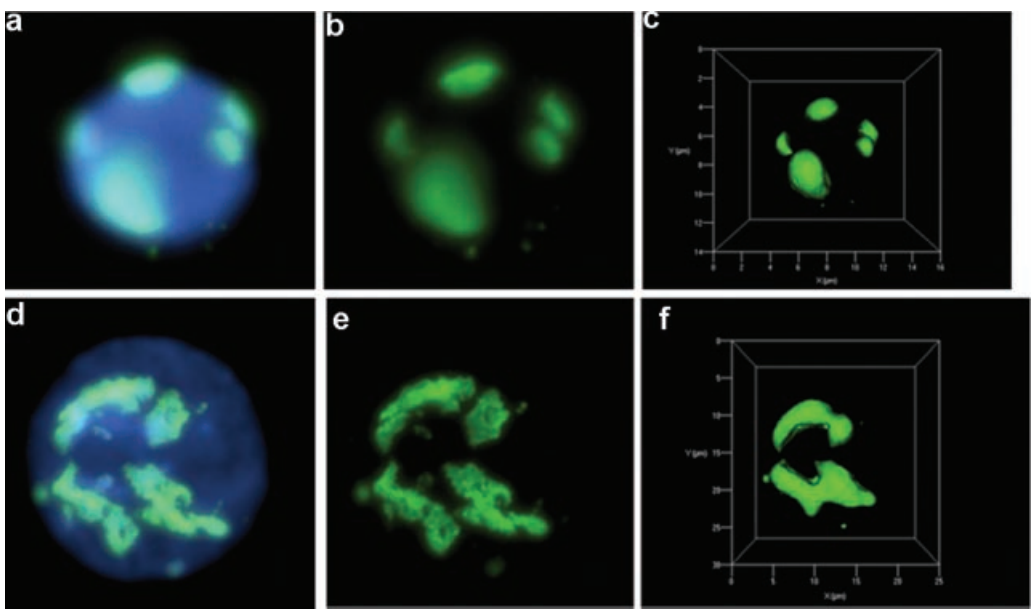

Fig. 2. Nuclear organization of centromeres in normal and tumor cells. The peripheral organization of centromeres (green) in normal mouse lymphocyte nuclei (blue) is shown in **a**–**c**. Two-dimensional (2D) images of normal nuclei and centromeres are shown in (a) and (b), and the three-dimensional (3D) organization of centromeres is shown in (c). The nuclear organization of centromeres (green) in mouse tumor cell (plasmacytoma) nuclei (blue) are shown in figures (**d**–**f**). Representative 2D images are given in (d) and (e), and the 3D representation of the same nucleus is shown in (f). Note the peripheral organization of the centromeres in normal cells and the central location in tumor cells [for details, see text and Sarkar et al., 2007].

and replication [Casolari et al., 2005; Lanctôt et al., 2007].

It is too early to say whether the nuclear matrix interactions with centromeres are altered during centromere remodeling or whether the methylation status of the centromeric regions is altered under these conditions. It is also important to assess whether centromere remodeling occurs during cellular transformation of different cell lineages. This would allow one to conclude whether or not centromere remodeling is a general phenomenon of cellular transformation or just applicable to the transformation of lymphocytes. It is most important not to gather a series of different tumor cell lines to answer this question, but to work with primary cells of different lineages and follow them as they are transformed. Only a rigorous approach in the selection of appropriate model systems will give us the answers to the above questions.

Treatments Targeting the Centromere and Cell Division Cycles

Due to the key role of centromeres in normal cell division and in cancer, it is not surprising that much effort is and has been devoted to inhibiting centromere function. The concept behind these approaches is that a non-functional centromere/kinetochore will induce a cell cycle checkpoint and thus inhibit the spread of malignant cells.

There are general approaches, such as the use of taxanes and vinca alkaloids that target the microtubules [Kelling et al., 2003]. However, Schmidt and Bastians [2007] noted that the targeting of mitosis also affects non-cancerous cells and thus has significant side effects. Thus novel anti-mitotic drugs that target non-microtubule structures, such as mitotic kinesins and Aurora or polo-like kinases, are being tested in clinical trials as well [Liu et al., 2006; Strebhardt and Ullrich, 2006; Schmit and Ahmad, 2007; Schmidt and Bastians, 2007]. Mitosis is also being targeted by inhibitors of farnesyl transferase, histone deacetylase and Hsp90 [for review, see Sudakin and Yen, 2007].

Several recent studies have looked at the effects of inhibiting histone deacetylation (HDAC) on centromere function and cell division. Taddei et al. [2001] investigated the impact of histone deacetylase inhibition on the disruption of pericentromeric heterochromatin and on centromere function. The authors found that pericentromeric regions will relocate to the nuclear periphery. HP1 is not retained at the centromere and defects in chromosome segregation occur. This process is reversible when the drug is removed [Taddei et al., 2001]. The authors concluded that underacetylation of pericentromeric heterochromatin is necessary for centromere function and localization within the nucleus. Moreover, HP1 will only associate with heterochromatin when the pericentromeric region is underacetylated. Of note is the finding of Gilchrist et al. [2004], who did not observe a relocation of centromeres by the inhibition of histone deacetylases. The reason for these different results is not known at this time.

In a recent study, Magnaghi-Jaulin et al. [2007] showed that inhibition of HDAC causes premature sister chromatid separation in cells when the mitotic spindle assembly checkpoint (SAC) is already activated. As a result, SAC and the cyclin-dependent kinase 1 are inactivated, histone H3 dephosphorlyated and MAD2 is lost from the kinetochore [Magnaghi-Jaulin et al., 2007].

Sumer et al. [2004] described that the inhibition of histone deacetylases led to a shift in binding of CENP-A and a reduced size of the scaffold/matrix attachment region (S/MAR) both at a neocentromere and at a native centromere. Treatment with DNA-intercalating drug distamycin A further reduced the S/MAR domain and centromere proteins binding and increased chromosome mis-segregation. These treatments did not alter the transcriptional competence for 47 underlying genes tested by the group.

A word of caution: while the goal of mitosis and centromere targeted therapies is to disrupt further cell divisions of aberrant cells, some of these treatments will affect normal cells, while others will select for "survivors" that manage to escape the treatment regime and will exit mitosis. Such cells are expected to be aneuploid and highly malignant. Thus, while the concept of inhibiting mitosis is excellent, the consequences of generating a few survivors is detrimental, unless a combination treatment can be sought that will target such cells as well.

Treatments Targeting the Centromere–Matrix Interaction

As summarized above, there is experimental evidence for a centromere–matrix interaction

[He and Brinkley, 1996; Sumer et al., 2003]. Is it possible to use this interaction to inhibit cell division in tumor cells? First, the nature of the interaction of the centromere with the nuclear matrix in cancer cells needs to be investigated. Since centromeres alter their positions in cancer cells [Sarkar et al., 2007] with consequences for genome stability [Guffei et al., 2007], it is likely that the associations of centromeres with the matrix is dynamic and an adaptable event. In other words, centromeres can be altered and possibly modulated according to the selective pressures under which a transformed cell finds itself. Since there is a precedence for a reduced size of the centromere-associated S/MAR [Sumer et al., 2004], therefore centromere attachment to the matrix may be less stable in transformed cells. Although the attachment of the centromere may be different in nature, cell division still continues. Moreover, the new organization of the centromeres is not concordant with that of a resting or differentiated cell and is different from that of a cycling cell [Sarkar et al., 2007]. The novel nuclear compartmentalization of centromeres in transformed cells may allow for new targeted treatments. This will only be possible after we learn more about the altered interaction of centromeres with the nuclear matrix in cancer cells.

Several lines of evidence suggest that the nuclear matrix may be a good substrate for anti-cancer therapies. For example, Pienta and Lehr [1993] demonstrated that estramustine, an estradiol-nitrogen mustard conjugate that binds to the nuclear matrix, and etoposide, a topoisomerase II inhibitor showed promise in treating hormone-refractory prostate cancer cells. DNA synthesis at the nuclear matrix was inhibited and tumor cell growth was inhibited in vitro in the Mat-LyLu rat prostate carcinoma cell line, the PC-3 human prostate adenocarcinoma cell line and in vivo in the Dunning Copenhagen rat adenocarcinoma prostate model. Estramustine has been part of phase II clinical trials and used in combination with other drugs for androgen-independent prostate cancer [Clark et al., 2001; Millikan et al., 2003; Dimopoulos et al., 2004; Thall et al., 2007]. Catapano et al. [1996] have discussed the possibility of using the nuclear matrix as target for anti-cancer therapy. Roti et al. [1998] promote the concept of hyperthermic killing of cancer cells by targeting the matrix and this notion was further promoted by Coffey et al. [2006].

It may be feasible to target CENP-A, a component of the centromeric nucleosome that is not found in the nucleosome of other chromosomal regions [Dalal et al., 2007].

As we learn more about nuclear matrix association with chromosomes and centromeres, it may be feasible to design specific treatments that target this interaction in a way that renders cell division impossible. The best approaches may be those that succeed to target only cancer cells, using the cancer-cell restricted expression of inhibitors, cancer-cell restricted delivery of small molecules [Sun et al., 2008; Tyler et al., 2007] and/or intrabodies [Williams and Zhu, 2006] that target any of the centromere–matrix and mitotic machinery-associated components within the cancer cell.

Imaging Considerations for the Centromere

The study of the nuclear organization of centromeres is now possible due to recent advances in imaging techniques. To enable the detailed analysis that such complex structures require, a variety of measurement methods are generally applied. In this section, we provide a short overview of the most commonly used methodologies that have already led to significant discoveries in the field and describe other methods that will have potential application for future refinement of analysis.

CONVENTIONAL FLUORESCENCE MICROSCOPY

Fluorescence microscopy has grown to become a central tool for imaging centromere organization within the nucleus. However, there are still limitations that should be well understood. To date, the most common optical microscopy methods are based on wide-field or confocal microscopy. These methods provide a spatial resolution of about 200–250 nm in the image plane and 400–500 nm along the optical axis [Garini et al., 2005]. Both methods provide the ability to measure the three dimensional nucleus by acquiring set of images at different focal planes. This has become an established technique for investigating the structural and functional characteristics of cells [Wolf, 2007]. With the development of high-efficiency detectors based on charged coupled devices (CCD) cameras, it is possible to observe even single fluorescent molecules in the nucleus [Xie et al.,

2006]. Since centromeres are larger structures, each can be labeled with many fluorescent molecules. It can therefore be observed with very short exposure times that minimize photobleaching and phototoxicity and allow imaging for very long periods of time when live-imaging is performed [Stephens and Allan, 2003; Elf et al., 2007]. Additionally, these methods provide the ability to detect multiple centromeres simultaneously. This is possible due to spectral and color imaging methods that now allow the simultaneous detection of many probes [Bolzer et al., 2005; Garini et al., 2006].

The use of microscopic techniques for quantitative assessment of centromere distribution requires careful data analysis. Imaging systems introduce blurring effects of the true signals, creating artificially expanded representation of the signals. This blurring is due to the physical limitations of the optical microscope and it is governed by the blurring function called the point spread function (PSF) of the system [Garini et al., 2005]. This has particular relevance when centromeres are close to each other or when aggregates of centromeric DNA are formed. In these cases, the collective signal from each aggregate result from the summed effect of the signal from all labeled DNA in the aggregate. When two aggregates are spatially adjacent, the artificially blurred regions from each can overlap and sum to create regions of signal intensity that are typically only slightly less than the signal intensity observed within the aggregates themselves, creating the appearance that the two aggregates actually collectively form a larger aggregate [Sarkar et al., 2007]. If the metric used to assess centromere distribution is sensitive to aggregates, it is important that controls be used to characterize these effects, which should then be incorporated into the analysis.

One method of avoiding misclassification of aggregates due to blurring is to use a multilevel thresholding approach [Sarkar, 2007]. In such an approach, primary segmentation of distinct centromere regions can be performed using an automatic thresholding technique such as the Isodata algorithm [Ridler and Calvard, 1978]. To separate artificially connected regions, a second threshold is then applied based upon a user-given parameter expressed as some multiple of the original threshold. This parameter requires calibration based upon the imaging system and deconvolution parameters used, and is set based upon visual agreement of the observed region separation by the user. Blurred regions will fall below the appropriate threshold while the majority of signal from the aggregates will remain above. Once the regions have been separated at the higher threshold, a signal is assigned to each region that is proportional to its relative contribution to the artificially connected region observed at the lower threshold. In this way, the approach allows for accurate region separation without introducing bias into the quantification of region intensities. However, it is important to note that the technique is ineffective if the blur effects are extensive enough to cause the artificial connections to have intensity values similar to those observed within the regions themselves.

Structural differences between samples must also be considered. Analysis of spatial distribution may be performed using concentric shells between the nuclear center and boundary, for example, describing the position of each centromere signal by their radius along the line connecting the nucleus center and the boundary (Fig. 3). Such methods have been successfully used [Wiblin et al., 2005]; however, their validity relies on consistency between the inherent assumptions about nuclear geometry and the physical geometry of the samples. Depending upon the preparation and physical processing involved, samples may vary widely in physical geometry, and alternative methods that are independent of such differences may be needed. One solution is to determine the center of each segmented region (weighted by intensity), and determine the position of each center along a radial line projected from the nuclear center to the nuclear boundary (Fig. 4) [Sarkar, 2007]. In studying centromere distribution across multiple samples, this position may be expressed as a percent distance from the nuclear center, where 100% represents the nuclear periphery. For each sample, the sum of the background-corrected intensities off all pixels within each region may then be divided by the total sum of these intensities across all regions in the image, resulting in a normalization of the total signal intensity for each sample to a total of 1, and the total intensity in each observed centromere region being expressed as a fractional amount representing its contribution to the total signal intensity. Such normalization ensures that larger

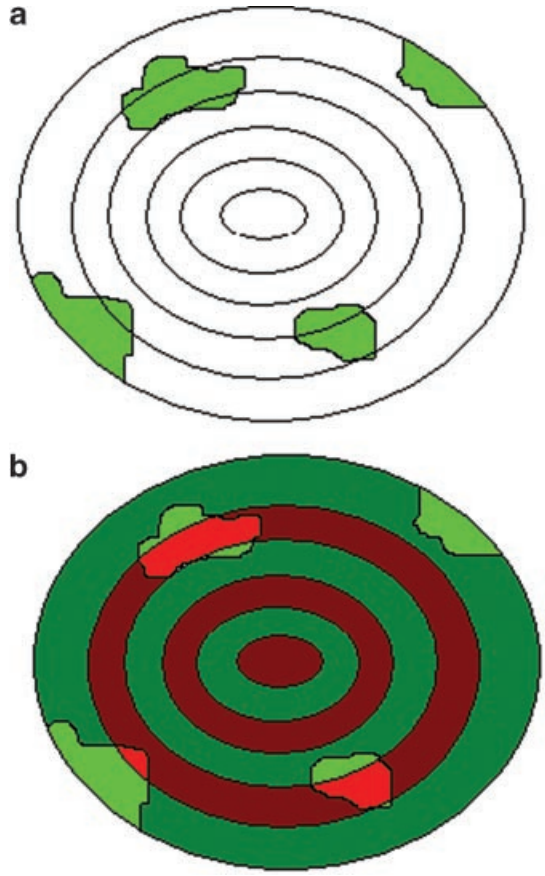

Fig. 3. Illustration of a concentric shells approach. **a**: The nuclear territory is divided into several concentric shells, each at a fixed radial distance. **b**: Centromere signal in each shell is measured and binned as appearing at the known radial distance of the shell. Centromere signal regions spanning multiple shells are divided amongst the shells proportionately.

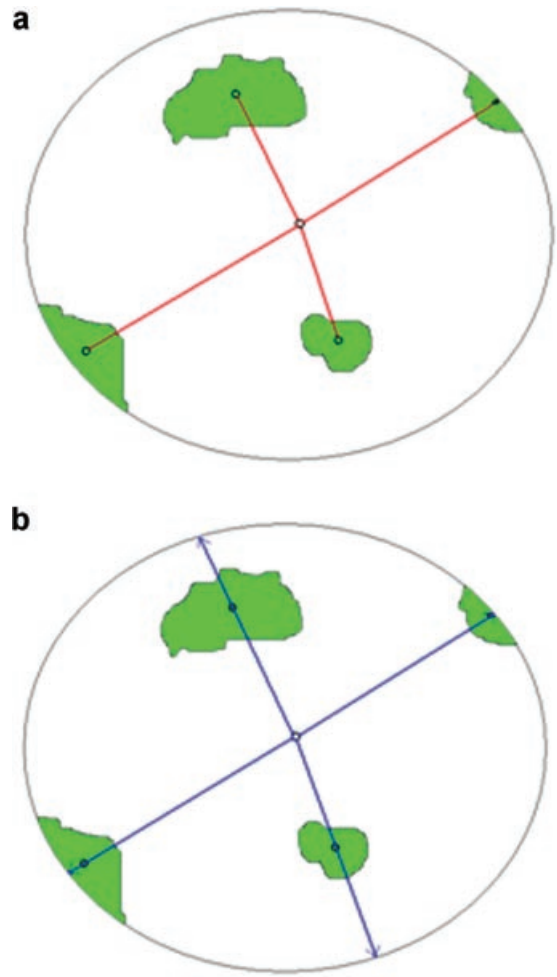

Fig. 4. Illustration of a radial projection approach. **a**: The center of mass of each centromere signal region is determined, after which the lengths of the radial arms from the nuclear center to each center of mass is calculated. **b**: Each radial arm is then further projected to the nuclear boundary, and the radial distance of the signal region is calculated relative to the nuclear boundary.

segmented regions that comprise multiple centromeres are represented with respect to their proportional signal output, as opposed to being equated with smaller regions composed of fewer or single centromeres. In addition, data may then be directly combined across samples in order to observe spatial distribution trends, since each sample is normalized to the same standard. This method thus allows for effective estimation of spatial distribution without making assumptions about nuclear shape.

The dependence of the final data on image analysis algorithms to account for such issues represents a current challenge for the standardization of fluorescence microscopy as a tool for quantitative assessment of the organization of centromeres. To this end, efforts should always be made to establish relationships between the parameters of the imaging system and the analytic parameters used in the assessment of the images, and should be applied towards the development of standardized imaging protocols and analysis methods. Without such standardization, it is difficult to compare data across studies since the variation in methods will be represented in the data as well. Developing such standardized techniques demands a strong understanding of the imaging system and underlying physical factors in order to develop appropriate processing and analysis algorithms that will allow reliable and valid conclusions to be drawn from the data.

FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET) MICROSCOPY

FRET is a powerful technique for studying molecular interaction in single cells [Rieder and Khodjakov, 2003; Rheenen et al., 2004; Orthaus et al., 2007]. This method provides signal sensitivity to intra- and intermolecular distances in the 1–10 nm range with a spatial resolution that significantly overcome that seen in typical conventional optical microscopy [Jares-Erijman and Jovin, 2003]. Also, it allows in vivo and in vitro measurements of protein–protein interactions within cells [Chen et al., 2007]. In a recent study using FRET it was confirmed that the two kinetochore proteins CENP-A and CENP-B were colocalized in vivo at centromeres [Orthaus et al., 2007].

The major approaches used to assess FRET are based on emission measurement and on acceptor photobleaching [Berney and Danuser, 2003]. The quantitative estimation of the observed signals could be made by determination of the FRET index or the transfer efficiency, however a direct comparison between results obtained with different indices can be difficult [Berney and Danuser, 2003]. The one essential requirement for stable FRET measurement is strong spectral overlap of the donor and acceptor and this introduces substantial background in the registered signals [Berney and Danuser, 2003; Chen et al., 2007]. The other major sources of background noise that should be considered are autoflorescence, detector noise, spectral sensitivity variation in both acceptor and donor channels [Chen et al., 2007].

Novel High Resolution Optical Microscopy Methods

In the last few years, there have been successful developments of a number of high resolution methods that overcome the traditional optical diffraction limit. Each of these methods uses a different physical and optical principle and an excellent spatial resolution of 50 nm and better is achieved [Garini et al., 2005]. One of these methods, photo-activated localization microscopy PALM measures small well-apart fluorescent probes and registers the intensity at the center of the PSF. When many images are measured and registered, a high-resolution image emerges [Betzig et al., 2006; Patterson et al., 2007]. To ensure the coverage of the whole object volume, the fluorescent probes are turned on and off by photoactivation. At this time the method is extremely slow, but has the potential to become faster in the future. Another method is saturation emission depletion (STED) that makes use of a nonlinear depletion effect of an excited spot such that a very small volume at the center of the excited spot is fluorescent, when scanning the image point-by-point, a high resolution image is achieved [Hell, 2003; Westphal et al., 2003]. A third method is structured illumination microscopy, in which two powerful integrating beams of light forms a structured light illumination with a well-defined interference pattern. Measurement of a few images and correct analysis of the data results in an improved resolution. When nonlinear effects are combined with the interference pattern, an even better resolution is achieved [Gustafsson, 2005]. These methods will surely enable the study of high-resolution optical microscopy of centromeres in the nucleus.

Alternative Methods for Centromere Organization Measurements

Alterative imaging modalities that may be used to study the organization of centromeres include techniques such as electron microscopy (EM) and synchrotron based transmission X-ray microscopy (TXM). Transmission electron microscopy (TEM) provides a resolution of $\sim$2–8 nm [Ottensmeyer et al., 1978; Koster et al., 1997; Steven and Aebi, 2003] allowing the observation of fine structural details of the investigated samples. The thickness of the samples is a crucial parameter as the electrons are strongly scattered or absorbed rather than transmitted from the matter [Egerton, 2006], which limits the sectioning of the specimen ($<0.5\,\mu$m). The propagation of the electron beam in a vacuum requires water from the sample to be removed or immobilized. The cryofixation methods for specimen preparation such as high-pressure freezing and freeze-substitution offers preservation of the ultrastructure of the cells, which is close to their native state [McDonald, 2007]. The combination of tomographic imaging principles with TEM can allow 3D reconstruction of centromeres within the nucleus. The resolution of the reconstructed volume depends on the number of registered 2D projections, which are restricted by the specificity of the specimen holder and stage [Adam et al., 2005; Lebbink et al., 2007]. However, a suitable

compromise should be made between achieving higher resolution and radiation damage to the sample [Egerton et al., 2004; Lebbink et al., 2007]. To date the application of EM combined with serial section reconstructions to centromere studies confirms the importance of centromere geometry for mitosis. It has been shown that the shape of the centromere can be a major factor in spindle formation, as the proper centromere organization promotes bipolarity [Loncarek et al., 2007].

The measurement techniques relayed by synchrotron based transmission X-ray microscopy (STXM) offer a good agreement between resolution, thickness of the investigated specimen and accumulated structural damage. In the spectral region of 2.34–4.38 nm, the so-called "water window" between the absorption edges of carbon and oxygen (corresponding to 284–543 EV), linear absorption coefficients of organic matter and water are significantly different [Meyer-Ilse et al., 2001; Adam et al., 2005]. Thus, the measurement of whole, hydrated cells (up to 10 μm) without any chemical fixation for contrast enhancement is possible [Weis et al., 2000; Meyer-Ilse et al., 2001; Paunesku et al., 2006]. The cellular architecture and different cell organelles are registered in close to their native stage, and the application of markers (conjugated gold colloids) allows identification of specific targets of interest in the cell [Gros et al., 2005]. The reported resolution down to 30 nm, together with the possibility of 360° tomographic measurement and golden nano-particles labeling [Weis et al., 2000; Larabell and Gros, 2004] will allow for high precision 3D reconstruction for the study of the nuclear organization of centromeres in the future.

CONCLUSIONS

Centromeres are unique chromosomal structures that permit proper chromosome segregation. They are evolutionary hotspots, carry unique centromeric chromatin and a specific nucleosome organization. The pericentric regions contribute to genomic plasticity during speciation and disease. The nuclear positions that centromeres occupy during embryonic development, differentiation, cell cycle, and transformation are key to functional requirements of the cell that require further elucidation. Future work will further examine the interplay of centromere topology and function during development, differentiation and tumorigenesis.

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REFERENCES

Adam J-F, Moy J-P, Susini J. 2005. Table-top water window transmission x-ray microscopy: Review of the key issues, and conceptual design of an instrument for biology. Rev Sci Instrum 76:091301.

Alkan C, Ventura M, Archidiacono N, Rocchi M, Sahinalp SC, Eichler EE. 2007. Organization and evolution of primate centromeric DNA from whole-genome shotgun sequence data. PLoS Comput Biol 3(9):1807–1818.

Amor DJ, Choo KH. 2002. Neocentromeres: Role in human disease, evolution, and centromere study. Am J Hum Genet 71(4):695–714. Epub 2002 Aug 26. Review.

Amor DJ, Bentley K, Ryan J, Perry J, Wong L, Slater H, Choo KH. 2004. Human centromere repositioning "in progress". Proc Natl Acad Sci USA 101(17):6542–6547. Epub 2004 Apr 14.

Anelli L, Albano F, Zagaria A, Liso A, Cuneo A, Mancini M, Liso V, Rocchi M, Specchia G. 2005. Pericentric chromosome 8 inversion associated with the 5′RUNX1/3′CBFA2T1 gene in acute myeloid leukemia cases. Ann Hematol 84(4):245–249. Epub 2004 Nov 16.

Anton E, Blanco J, Egozcue J, Vidal F. 2005. Sperm studies in heterozygote inversion carriers: A review. Cytogenet Genome Res 111(3–4):297–304. Review.

Bartholdi MF. 1991. Nuclear distribution of centromeres during the cell cycle of human diploid fibroblasts. J Cell Sci 99(Pt 2):255–263.

Bayani J, Zielenska M, Pandita A, Al-Romaih K, Karaskova J, Harrison K, Bridge JA, Sorensen P, Thorner P, Squire JA. 2003. Spectral karyotyping identifies recurrent complex rearrangements of chromosomes 8, 17, and 20 in osteosarcomas. Genes Chromosomes Cancer 36(1):7–16.

Bayani J, Selvarajah S, Maire G, Vukovic B, Al-Romaih K, Zielenska M, Squire JA. 2007. Genomic mechanisms and measurement of structural and numerical instability in cancer cells. Semin Cancer Biol 17(1):5–18. Epub 2006 Oct 26. Review.

Beheshti B, Karaskova J, Park PC, Squire JA, Beatty BG. 2000. Identification of a high frequency of chromosomal rearrangements in the centromeric regions of prostate cancer cell lines by sequential giemsa banding and spectral karyotyping. Mol Diagn 5:23–32.

Beil M, Dürschmied D, Paschke S, Schreiner B, Nolte U, Bruel A, Irinopoulou T. 2002. Spatial distribution patterns of interphase centromeres during retinoic acid-induced differentiation of promyelocytic leukemia cells. Cytometry 47(4):217–225.

Beil M, Fleischer F, Paschke S, Schmidt V. 2005. Statistical analysis of the three-dimensional structure of centromeric heterochromatin in interphase nuclei. J Microsc 217(Pt 1):60–68.

Beiraghi S, Zhou M, Talmadge CB, Went-Sumegi N, Davis JR, Huang D, Saal H, Seemayer TA, Sumegi J. 2003. Identification and characterization of a novel gene disrupted by a pericentric inversion inv(4)(p13.1q21.1) in a family with cleft lip. Gene 309(1):11–21.

Berney C, Danuser G. 2003. FRET or No FRET: A quantitative comparison. Biophys J 84:3992–4010.

Betzig E, Patterson GH, Sougrat R, Lindwasser OW, Olenych S, Bonifacino JS, Davidson MW, Lippincott-Schwartz J, Hess HF. 2006. Imaging intracellular fluorescent proteins at nanometer resolution. Science 313:1642–1645.

Bidau CJ, Giménez MD, Palmer CL, Searle JB. 2001. The effects of Robertsonian fusions on chiasma frequency and distribution in the house mouse (Mus musculus domesticus) from a hybrid zone in northern Scotland. Heredity 87(Pt 3):305–313.

Black BE, Foltz DR, Chakravarthy S, Luger K, Woods VL, Jr., Cleveland DW. 2004. Structural determinants for generating centromeric chromatin. Nature 430(6999): 578–582.

Blitzblau HG, Bell GW, Rodriguez J, Bell SP, Hochwagen A. 2007. Mapping of meiotic single-stranded DNA reveals double-stranded-break hotspots near centromeres and telomeres. Curr Biol 17(23):2003–2012.

Bolzer A, Kreth G, Solovei I, Koehler D, Saracoglu K, Fauth C, Müller S, Eils R, Cremer C, Speicher MR, Cremer T. 2005. Three-dimensional maps of all chromosomes in human male fibroblast nuclei and prometaphase rosettes. PLOS Biol 3:1–17.

Boveri T. 1902. Über mehrpolige Mitosen als Mittel zur Analyse des Zellkerns. Verh Phys Med Gesellschaft Würzburg 35:67–90.

Boveri T. 1914. Zur Frage der Entstehung maligner Tumoren. Jena: Fischer.

Boveri T. 1929. The origin of malignant tumors. Translated by Marcella Boveri. Baltimore: The Williams & Wilkens Company.

Bulazel KV, Ferreri GC, Eldridge MD, O'Neill RJ. 2007. Species-specific shifts in centromere sequence composition are coincident with breakpoint reuse in karyotypically divergent lineages. Genome Biol 8(8): R170.

Capanna E, Castiglia R. 2004. Chromosomes and speciation in Mus musculus domesticus. Cytogenet Genome Res 105(2–4):375–384.

Casolari JM, Brown CR, Drubin DA, Rando OJ, Silver PA. 2005. Developmentally induced changes in transcriptional program alter spatial organization across chromosomes. Genes Dev 19(10):1188–1198.

Catapano CV, Carbone GM, Fernandes DJ. 1996. The nuclear matrix as a target for cancer therapy. Ann Oncol 7(7):659–666. Review. No abstract available.

Chantot-Bastaraud S, Ravel C, Berthaut I, McElreavey K, Bouchard P, Mandelbaum J, Siffroi JP. 2007. Sperm-FISH analysis in a pericentric chromosome 1 inversion, 46,XY,inv(1)(p22q42), associated with infertility. Mol Hum Reprod 13(1):55–59. Epub 2006 Oct 31.

Chen Y, Mauldin JP, Day RN, Periasamy A. 2007. Characterization of spectral FRET imaging microscopy for monitoring nuclear protein interactions. J Microsc 228:139–152.

Choo KH. 1997. Centromere DNA dynamics: Latent centromeres and neocentromere formation. Am J Hum Genet 61(6):1225–1233.

Clark PE, Peereboom DM, Dreicer R, Levin HS, Clark SB, Klein EA. 2001. Phase II trial of neoadjuvant estramustine and etoposide plus radical prostatectomy for locally advanced prostate cancer. Urology 57(2):281–285.

Coffey DS, Getzenberg RH, DeWeese TL. 2006. Hyperthermic biology and cancer therapies: A hypothesis for the "Lance Armstrong effect". JAMA 296(4):445–448.

Collodel G, Moretti E, Capitani S, Piomboni P, Anichini C, Estenoz M, Baccetti B. 2006. TEM, FISH and molecular studies in infertile men with pericentric inversion of chromosome 9. Andrologia 38(4):122–127.

Cremer T. 1985. Von der Zellenlehre zur Chromosomentheorie. Berlin Heidelberg New York Tokyo: Springer Verlag.

Dalal Y, Furuyama T, Vermaak D, Henikoff S. 2007. Structure, dynamics, and evolution of centromeric nucleosomes. Proc Natl Acad Sci USA 104(41):15974–15981. Epub 2007 Sep 24. Review.

D'Alessio AC, Szyf M. 2006. Epigenetic tête-à-tête: The bilateral relationship between chromatin modifications and DNA methylation. Biochem Cell Biol 84(4):463–476. Review.

Darai E, Kost-Alimova M, Kiss H, Kansoul H, Klein G, Imreh S. 2005. Evolutionarily plastic regions at human 3p21.3 coincide with tumor breakpoints identified by the "elimination test". Genomics 86(1):1–12.

Darlington CD. 1936a. The external mechanisms of chromosomes. I—The scope of the enquiry. Proc R Soc Lond B Biol Sci 121(No. 823):264–273.

Darlington CD. 1936b. Misdivision and the genetics of the centromere. J Genet XXVII:341–364.

Dimopoulos MA, Kiamouris C, Gika D, Deliveliotis C, Giannopoulos A, Zervas A, Alamanis C, Constantinidis C, Koutsilieris M. 2004. Combination of LHRH analog with somatostatin analog and dexamethasone versus chemotherapy in hormone-refractory prostate cancer: A randomized phase II study. Urology 63(1):120–125.

du Sart D, Cancilla MR, Earle E, Mao JI, Saffery R, Tainton KM, Kalitsis P, Martyn J, Barry AE, Choo KH. 1997. A functional neo-centromere formed through activation of a latent human centromere and consisting of non-alpha-satellite DNA. Nat Genet 16(2):144–153.

Egerton RF. 2006. Physical principles of electron microscopy. US: Springer.

Egerton RF, Li P, Malac M. 2004. Radiation damage in the TEM and SEM. Micron 35:399–409.

Ehrlich M, Woods CB, Yu MC, Dubeau L, Yang F, Campan M, Weisenberger DJ, Long T, Youn B, Fiala ES, Laird PW. 2006. Quantitative analysis of associations between

DNA hypermethylation, hypomethylation, and DNMT RNA levels in ovarian tumors. Oncogene 25(18):2636–2645.

Elf J, Li G-W, Xie XS. 2007. Probing transcription factor dynamics at the single-molecule level in a living cell. Science 316:1191–1193.

Esteller M. 2007. Cancer epigenomics: DNA methylomes and histone-modification maps. Nat Rev Genet 8(4):286–298. Epub 2007 Mar 6. Review.

Fang Y, Spector DL. 2005. Centromere positioning and dynamics in living Arabidopsis plants. Mol Biol Cell 16(12):5710–5718. Epub 2005 Sep 29.

Flemming W. 1882. Zellsubstanz, Kern und Zelltheilung. Leipzig.

Garagna S, Marziliano N, Zuccotti M, Searle JB, Capanna E, Redi CA. 2001. Pericentromeric organization at the fusion point of mouse Robertsonian translocation chromosomes. Proc Natl Acad Sci USA 98(1):171–175.

Gardner RJM, Sutherland GR. 2004. Chromosome abnormalities and genetic counseling. Cary, North Carolina, U.S.A: Oxford University Press.

Garini Y, Vermolen BJ, Young IT. 2005. From micro to nano: Recent advances in high-resolution microscopy. Curr Opin Biotechnol 16:3–12.

Garini Y, Young IT, McNamara G. 2006. Spectral imaging: Principles and applications. Cytometry 69A:735–747.

Gazave E, Catalan J, Ramalhinho Mda G, Mathias Mda L, Nunes AC, Dumas D, Britton-Davidian J, Auffray JC. 2003. The non-random occurrence of Robertsonian fusion in the house mouse. Genet Res 81(1):33–42.

Gilchrist S, Gilbert N, Perry P, Bickmore WA. 2004. Nuclear organization of centromeric domains is not perturbed by inhibition of histone deacetylases. Chromosome Res 12(5):505–516.

Gourret JP. 1995. Modelling the mitotic apparatus. From the discovery of the bipolar spindle to modern concepts. Acta Biotheor 43(1-2):127–142.

Greaves IK, Rangasamy D, Ridgway P, Tremethick DJ. 2007. H2A.Z contributes to the unique 3D structure of the centromere. Proc Natl Acad Sci USA 104(2):525–530. Epub 2006 Dec 28.

Gros MAL, McDermott G, Larabell CA. 2005. X-ray tomography of whole cells. Curr Opin Struct Biol 15:593–600.

Grosso S, Pucci L, Farnetani M, Di Bartolo RM, Galimberti D, Mostardini R, Anichini C, Balestri M, Morgese G, Balestri P. 2004. Epilepsy and electroencephalographic findings in pericentric inversion of chromosome 12. J Child Neurol 19(8):604–608.

Guffei A, Lichtensztejn Z, Gonçalves Dos Santos Silva A, Louis SF, Caporali A, Mai S. 2007. c-Myc-dependent formation of Robertsonian translocation chromosomes in mouse cells. Neoplasia 9(7):578–588.

Gustafsson MGL. 2005. Nonlinear structured-illumination microscopy: Wide-field fluorescence imaging with theoretically unlimited resolution. Proc Natl Acad Sci USA 102:13081–13086.

Hall AE, Kettler GC, Preuss D. 2006. Dynamic evolution at pericentromeres. Genome Res 16(3):355–364. Epub 2006 Feb 6.

Hampson RM, Malloy MP, Mors O, Ewald H, Flannery AV, Morten J, Porteous DJ, Muir WJ, Blackwood DH. 1999. Mapping studies on a pericentric inversion (18) (p11.31 q21.1) in a family with both schizophrenia and learning disability. Psychiatr Genet 9(3):161–163.

He D, Brinkley BR. 1996. Structure and dynamic organization of centromeres/prekinetochores in the nucleus of mammalian cells. J Cell Sci 109(Pt 11):2693–2704.

Hell SW. 2003. Toward fluorescence nanoscopy. Nat Biotechnol 21:1347–1355.

Howman EV, Fowler KJ, Newson AJ, Redward S, MacDonald AC, Kalitsis P, Choo KH. 2000. Early disruption of centromeric chromatin organization in centromere protein A (Cenpa) null mice. Proc Natl Acad Sci USA 97(3):1148–1153.

Hudson DF, Fowler KJ, Earle E, Saffery R, Kalitsis P, Trowell H, Hill J, Wreford NG, de Kretser DM, Cancilla MR, Howman E, Hii L, Cutts SM, Irvine DV, Choo KH. 1998. Centromere protein B null mice are mitotically and meiotically normal but have lower body and testis weights. J Cell Biol 141(2):309–319.

Jacobs PA. 1981. Mutation rates of structural chromosome rearrangements in man. Am J Hum Genet 33(1):44–54.

Jamet D, Marzin Y, Douet-Guilbert N, Morel F, Le Bris MJ, Herry A, Banzakour S, Bourquard P, Morice P, Abgrall JF, Berthou C, De Braekeleer M. 2005. Jumping translocations in multiple myeloma. Cancer Genet Cytogenet 161(2):159–163.

Jares-Erijman E, Jovin T. 2003. FRET imaging. Nat Biotechnol 21:1387–1395.

Kalitsis P, Fowler KJ, Earle E, Hill J, Choo KH. 1998. Targeted disruption of mouse centromere protein C gene leads to mitotic disarray and early embryo death. Proc Natl Acad Sci USA 95(3):1136–1141.

Kalitsis P, Fowler KJ, Earle E, Griffiths B, Howman E, Newson AJ, Choo KH. 2003. Partially functional Cenpa-GFP fusion protein causes increased chromosome missegregation and apoptosis during mouse embryogenesis. Chromosome Res 11(4):345–357.

Kalitsis P, Griffiths B, Choo KH. 2006. Mouse telocentric sequences reveal a high rate of homogenization and possible role in Robertsonian translocation. Proc Natl Acad Sci USA 103(23):8786–8791. Epub 2006 May 26.

Kelling J, Sullivan K, Wilson L, Jordan MA. 2003. Suppression of centromere dynamics by Taxol in living osteosarcoma cells. Cancer Res 63(11):2794–2801.

Kim SR, Shaffer LG. 2002. Robertsonian translocations: Mechanisms of formation, aneuploidy, and uniparental disomy and diagnostic considerations. Genet Test 6(3):163–168.

Kim SH, McQueen PG, Lichtman MK, Shevach EM, Parada LA, Misteli T. 2004. Spatial genome organization during T-cell differentiation. Cytogenet Genome Res 105(2–4):292–301.

Koster AJ, Grimm R, Typke D, Hegerl R, Stoschek A, Walz J, Baumeister W. 1997. Perspectives of molecular and cellular electron tomography. J Struct Biol 120:276–308.

Kumada K, Yao R, Kawaguchi T, Karasawa M, Hoshikawa Y, Ichikawa K, Sugitani Y, Imoto I, Inazawa J, Sugawara M, Yanagida M, Noda T. 2006. The selective continued linkage of centromeres from mitosis to interphase in the absence of mammalian separase. J Cell Biol 172(6):835–846.

Lanctôt C, Cheutin T, Cremer M, Cavalli G, Cremer T. 2007. Dynamic genome architecture in the nuclear space: Regulation of gene expression in three dimensions. Nat Rev Genet 8(2):104–115.

Larabell CA, Gros MAL. 2004. X-ray tomography generates 3-D reconstructions of the yeast, saccharomyces cerevisiae, at 60-nm resolution. Mol Biol Cell 15:957–962.

Lebbink MN, Geerts WJC, Krift TPvd, Bouwhuis M, Hertzberger LO, Verkleij AJ, Koster AJ. 2007. Template matching as a tool for annotation of tomograms of stained biological structures. J Struct Biol 158:327–335.

Liu X, Lei M, Erikson RL. 2006. Normal cells, but not cancer cells, survive severe Plk1 depletion. Mol Cell Biol 26(6):2093–2108.

Loncarek J, Kisurina-Evgenieva O, Vinogradova T, Hergert P, La Terra S, Kapoor TM, Khodjakov A. 2007. The centromere geometry essential for keeping mitosis error free is controlled by spindle forces. Nature 450(7170): 745–749.

López-Exposito I, Guillén-Navarro E, Bafallíu JA, Bernabé MC, Escalona A, Fuster C. 2006. Duplication 19q13-qter and deletion 19p13-pter arising from an inversion (19)(p13.3q13.3) of maternal origin. Eur J Med Genet 49(6):511–515. Epub 2006 Jun 23.

Magnaghi-Jaulin L, Eot-Houllier G, Fulcrand G, Jaulin C. 2007. Histone deacetylase inhibitors induce premature sister chromatid separation and override the mitotic spindle assembly checkpoint. Cancer Res 67(13):6360–6367.

Martin C, Beaujean N, Brochard V, Audouard C, Zink D, Debey P. 2006a. Genome restructuring in mouse embryos during reprogramming and early development. Dev Biol 292(2):317–332.

Martin C, Brochard V, Migné C, Zink D, Debey P, Beaujean N. 2006b. Architectural reorganization of the nuclei upon transfer into oocytes accompanies genome reprogramming. Mol Reprod Dev 73(9):1102–1111.

Mathew S, Dalton J, Riedley S, Spunt SL, Hill DA. 2002. Complex t(X;18)(p11.2;q11.2) with a pericentric inversion of the X chromosome in an adolescent boy with synovial sarcoma. Cancer Genet Cytogenet 132(2):136–140.

McClintock B. 1932. A correlation of ring-shaped chromosomes with variegation. Proc Natl Acad Sci USA 18:677–681.

McClintock B. 1933. The association of non-homologous parts of chromosomes at mid-prophase of meiosis in *Zea Mays*. Z Zellforsch 19:191–237.

McCormack SJ, Weaver Z, Deming S, Natarajan G, Torri J, Johnson MD, Liyanage M, Ried T, Dickson RB. 1998. Myc/p53 interactions in transgenic mouse mammary development, tumorigenesis and chromosomal instability. Oncogene 16(21):2755–2766.

McDonald K. 2007. Cryopreparation methods for electron microscopy of selected model systems. Methods Cell Biol 79:23–56.

Merico V, Pigozzi MI, Esposito A, Merani MS, Garagna S. 2003. Meiotic recombination and spermatogenic impairment in Mus musculus domesticus carrying multiple simple Robertsonian translocations. Cytogenet Genome Res 103(3–4):321–329.

Metcalfe CJ, Bulazel KV, Ferreri GC, Schroeder-Reiter E, Wanner G, Rens W, Obergfell C, Eldridge MD, O'Neill RJ. 2007. Genomic instability within centromeres of interspecific marsupial hybrids. Genetics 177(4):2507–2517.

Meyer-Ilse W, Hamamoto D, Nair A, Lelievre SA, Denbeaux G, Johnson L, Pearson AL, Yager D, Legros MA, Larabell CA. 2001. High resolution protein localization using soft X-ray microscopy. J Microsc 201:395–403.

Millikan R, Thall PF, Lee SJ, Jones D, Cannon MW, Kuebler JP, Wade J III, Logothetis CJ. 2003. Randomized, multicenter, phase II trial of two multicomponent regimens in androgen-independent prostate cancer. J Clin Oncol 21(5):878–883.

Nachman MW, Searle JB. 1995. Why is the house mouse karyotype so variable? Trends Ecol Evol 10:397–402.

Nielsen J, Wohlert M. 1991. Chromosome abnormalities found among 34,910 newborn children: Results from a 13-year incidence study in Arhus, Denmark. Hum Genet 87(1):81–83.

Orthaus S, Biskup C, Hoffmann B, Hoischen C, Ohndorf S, Benndorf K, Diekmann S. 2008. Assembly of the inner kinetochore proteins CENP-A and CENP-B in living human cells. Chembiochem 9(1):77–92.

Ottensmeyer FP, Bazett-Jones DP, Hewitt JJ, Price GB. 1978. Structure analysis of small proteins by electron microscopy: Valinomycin, bacitracin and low molecular weight cell growth stimulators. Ultramicroscopy 3:303–313.

Padilla-Nash HM, Heselmeyer-Haddad K, Wangsa D, Zhang H, Ghadimi BM, Macville M, Augustus M, Schröck E, Hilgenfeld E, Ried T. 2001. Jumping translocations are common in solid tumor cell lines and result in recurrent fusions of whole chromosome arms. Genes Chromosomes Cancer 30(4):349–363.

Patil SJ, Phadke SR. 2007. Pericentric inversion causing duplication and deletion of chromosome region 13q22 –> qter in the offspring. Am J Med Genet A 143(1):82–84.

Patterson GH, Betzig E, Lippincott-Schwartz J, Hess HF. 2007. Developing photoactivated localization microscopy (PALM). 4th IEEE ISBI: From Nano to Macro. 940–943.

Paunesku T, Vogt S, Maser J, Lai B, Woloschak G. 2006. X-Ray fluorescence microprobe imaging in biology and medicine. J Cell Biochem 99:1489–1502.

Pedrazzini E, Cerretini R, Noriega MF, Narbaitz M, Palacios MF, Negri P, Bengió R, Slavutsky I. 2006. Inversions of chromosomes 2 and 6 in mantle cell lymphoma. Cytogenetic, FISH, and molecular studies. Cancer Genet Cytogenet 167(2):164–167.

Pienta KJ, Lehr JE. 1993. Inhibition of prostate cancer growth by estramustine and etoposide: Evidence for interaction at the nuclear matrix. J Urol 149(6):1622–1625.

Qian J, Xue Y, Sun J, Guo Y, Pan J, Wu Y, Wang W, Yao L. 2002. Constitutional Robertsonian translocations in (9;22)-positive chronic myelogenous leukemia. Cancer Genet Cytogenet 132(1):79–80.

Qu G, Dubeau L, Narayan A, Yu MC, Ehrlich M. 1999a. Satellite DNA hypomethylation vs. overall genomic hypomethylation in ovarian epithelial tumors of different malignant potential. Mutat Res 423(1–2):91–101.

Qu GZ, Grundy PE, Narayan A, Ehrlich M. 1999b. Frequent hypomethylation in Wilms tumors of pericentromeric DNA in chromosomes 1 and 16. Cancer Genet Cytogenet 109(1):34–39.

Ramadevi AR, Naik U, Dutta U, Srikanth, Prabhakara K. 2002. De novo pericentric inversion of chromosome 4, inv(4)(p16q12) in a boy with piebaldism and mental retardation. Am J Med Genet 113(2):190–192.

Rheenen Jv, Langeslag M, Jalink K. 2004. Correcting confocal acquisition to optimize imaging of fluorescence

resonance energy transfer by sensitized emission. Biophys J 86:2517–2529.
Ridler TW, Calvard S. 1978. Picture thresholding using an iterative selection method. IEEE Transactions on Systems, Man and Cybernetics 8:630–632.
Rieder CL, Khodjakov A. 2003. Mitosis through the microscope: Advances in seeing inside live dividing cells. Science 300:91–96.
Roti JL, Kampinga HH, Malyapa RS, Wright WD, vander-Waal RP, Xu M. 1998. Nuclear matrix as a target for hyperthermic killing of cancer cells. Cell Stress Chaperones 3(4):245–255. Review.
Saffery R, Irvine DV, Griffiths B, Kalitsis P, Wordeman L, Choo KH. 2000. Human centromeres and neocentromeres show identical distribution patterns of >20 functionally important kinetochore-associated proteins. Hum Mol Genet 9(2):175–185.
Saffery R, Sumer H, Hassan S, Wong LH, Craig JM, Todokoro K, Anderson M, Stafford A, Choo KH. 2003. Transcription within a functional human centromere. Mol Cell 12(2):509–516.
Sarkar R, Guffei A, Vermolen BJ, Garini Y, Mai S. 2007. Alterations of centromere positions in nuclei of immortalized and malignant mouse lymphocytes. Cytometry A 71(6):386–392.
Schaefer LH, Schuster D, Herz H. 2001. Generalized approach for accelerated maximum likelihood based image restoration applied to three-dimensional fluorescence microscopy. J Microsc 204(Pt 2):99–107.
Schmidt M, Bastians H. 2007. Mitotic drug targets and the development of novel anti-mitotic anticancer drugs. Drug Resist Update 10(4–5):162–181. Epub 2007 Jul 31. Review.
Schmit TL, Ahmad N. 2007. Regulation of mitosis via mitotic kinases: New opportunities for cancer management. Mol Cancer Ther 6(7):1920–1931. Review.
Schueler MG, Higgins AW, Rudd MK, Gustashaw K, Willard HF. 2001. Genomic and genetic definition of a functional human centromere. Science 294(5540):109–115.
Schulz R, Underkoffler LA, Collins JN, Oakey RJ. 2006. Nondisjunction and transmission ratio distortion of Chromosome 2 in a (2.8) Robertsonian translocation mouse strain. Mamm Genome 17(3):239–247. Epub 2006 Mar 3.
Shaw CJ, Lupski JR. 2004. Implications of human genome architecture for rearrangement-based disorders: The genomic basis of disease. Hum Mol Genet 13(Spec No 1):R57–R64.
Shen WH, Balajee AS, Wang J, Wu H, Eng C, Pandolfi PP, Yin Y. 2007. Essential role for nuclear PTEN in maintaining chromosomal integrity. Cell 128(1):157–170.
Shimokawa T, Sakai M, Kojima Y, Takeyama H. 2004. Acute myelogeneous leukemia (M5a) that demonstrated chromosomal abnormality of robertsonian 13;21 translocation at onset. Intern Med 43(6):508–511. Review.
Solovei I, Schermelleh L, Düring K, Engelhardt A, Stein S, Cremer C, Cremer T. 2004. Differences in centromere positioning of cycling and postmitotic human cell types. Chromosoma 112(8):410–423. Epub 2004 Jun 9.
Stephens DJ, Allan VJ. (2003) Light microscopy techniques for live cell imaging. Science 300:82–86.
Steven AC, Aebi U. 2003. The next ice age: Cryo-electron tomography of intact cells. Trends Cell Biol 13:107–110.
Strebhardt K, Ullrich A. 2006. Targeting polo-like kinase 1 for cancer therapy. Nat Rev Cancer 6(4):321–330. Review.
Sudakin V, Yen TJ. 2007. Targeting mitosis for anti-cancer therapy. BioDrugs 21(4):225–233. Review.
Sumer H, Craig JM, Sibson M, Choo KH. 2003. A rapid method of genomic array analysis of scaffold/matrix attachment regions (S/MARs) identifies a 2.5-Mb region of enhanced scaffold/matrix attachment at a human neocentromere. Genome Res 13(7):1737–1743.
Sumer H, Saffery R, Wong N, Craig JM, Choo KH. 2004. Effects of scaffold/matrix alteration on centromeric function and gene expression. J Biol Chem 279(36): 37631–37639. Epub 2004 Jun 25.
Sun L, Li D, Dong X, Yu H, Dong JT, Zhang C, Lu X, Zhou J. 2008. Small-molecule inhibition of Aurora kinases triggers spindle checkpoint-independent apoptosis in cancer cells. Biochem Pharmacol 75:1027–1034.
Taddei A, Maison C, Roche D, Almouzni G. 2001. Reversible disruption of pericentric heterochromatin and centromere function by inhibiting deacetylases. Nat Cell Biol 3(2):114–120.
Thall PF, Logothetis C, Pagliaro LC, Wen S, Brown MA, Williams D, Millikan RE. 2007. Adaptive therapy for androgen-independent prostate cancer: A randomized selection trial of four regimens. J Natl Cancer Inst 99(21):1613–1622. Epub 2007 Oct 30.
Tyler RK, Shpiro N, Marquez R, Eyers PA. 2007. VX-680 inhibits Aurora A and Aurora B kinase activity in human cells. Cell Cycle 6(22):2846–2854. Epub 2007 Aug 27.
Ulucan H, Akin R, Kösem M, Gül D. 2006. De novo pericentric inversion of chromosome 5 in a girl with mental retardation and unilateral ear malformation. Am J Med Genet A 140(3):298–299.
Underkoffler LA, Mitchell LE, Abdulali ZS, Collins JN, Oakey RJ. 2005. Transmission ratio distortion in offspring of mouse heterozygous carriers of a (7.18) Robertsonian translocation. Genetics 169(2):843–848. Epub 2004 Nov 1.
Ventura M, Weigl S, Carbone L, Cardone MF, Misceo D, Teti M, D'Addabbo P, Wandall A, Björck E, de Jong PJ, She X, Eichler EE, Archidiacono N, Rocchi M. 2004. Recurrent sites for new centromere seeding. Genome Res 14(9):1696–1703.
Villasante A, Abad JP, Méndez-Lago M. 2007. Centromeres were derived from telomeres during the evolution of the eukaryotic chromosome. Proc Natl Acad Sci USA 104(25):10542–10547. Epub 2007 Jun 8.
Virmani AK, Rathi A, Sathyanarayana UG, Padar A, Huang CX, Cunnigham HT, Farinas AJ, Milchgrub S, Euhus DM, Gilcrease M, Herman J, Minna JD, Gazdar AF. 2001. Aberrant methylation of the adenomatous polyposis coli (APC) gene promoter 1A in breast and lung carcinomas. Clin Cancer Res 7(7):1998–2004.
Vukovic B, Beheshti B, Park P, Lim G, Bayani J, Zielenska M, Squire JA. 2007. Correlating breakage-fusion-bridge events with the overall chromosomal instability and in vitro karyotype evolution in prostate cancer. Cytogenet Genome Res 116(1–2):1–11.
Warburton PE. 2004. Chromosomal dynamics of human neocentromere formation. Chromosome Res 12(6):617–626. Review.

Weimer R, Haaf T, Krüger J, Poot M, Schmid M. 1992. Characterization of centromere arrangements and test for random distribution in G0, G1, S, G2, G1, and early S' phase in human lymphocytes. Hum Genet 88(6):673–682.

Weis D, Schneider G, Niemann B, Guttmann P, Rudolph D, Schmahl G. 2000. Computed tomography of cryogenic biological specimens based on X-ray microscopic images. Ultramicroscopy 84:185–197.

Welborn J. 2004. Acquired Robertsonian translocations are not rare events in acute leukemia and lymphoma. Cancer Genet Cytogenet 151(1):14–35.

Westphal V, Kastrup l, Hell SW. 2003. Lateral resolution of 28 nm (l/25) in far-field fluorescence microscopy. Appl Phys B 77:377–380.

Wiblin AE, Cui W, Clark AJ, Bickmore WA. 2005. Distinctive nuclear organization of centromeres and regions involved in pluripotency in human embryonic stem cells. J Cell Sci 118:3861–3868.

Widschwendter M, Jiang G, Woods C, Müller HM, Fiegl H, Goebel G, Marth C, Müller-Holzner E, Zeimet AG, Laird PW, Ehrlich M. 2004. DNA hypomethylation and ovarian cancer biology. Cancer Res 64(13):4472–4480.

Williams BR, Zhu Z. 2006. Intrabody-based approaches to cancer therapy: Status and prospects. Curr Med Chem 13(12):1473–1480. Review.

Wolf DE. 2007. Fundamentals of fluorescence and fluorescence microscopy. Methods Cell Biol 81:63–91.

Xie XS, Yu J, Yang Y. 2006. Living cells as test tubes. Science 312:228–230.

Xu GL, Bestor TH, Bourc'his D, Hsieh CL, Tommerup N, Bugge M, Hulten M, Qu X, Russo JJ, Viegas-Péquignot E. 1999. Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. Nature 402(6758):187–191.

Yue Y, Grossmann B, Ferguson-Smith M, Yang F, Haaf T. 2005. Comparative cytogenetics of human chromosome 3q21.3 reveals a hot spot for ectopic recombination in hominoid evolution. Genomics 85(1):36–47.

Zhang P, Friebe B, Lukaszewski AJ, Gill BS. 2001. The centromere structure in Robertsonian wheat-rye translocation chromosomes indicates that centric breakage-fusion can occur at different positions within the primary constriction. Chromosoma 110(5):335–344.