Mechanisms of Development 120 (2003) 1217–1230

www.elsevier.com/locate/modo

Gene expression and nuclear architecture during development and differentiation

Wallace F. Marshall*

Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT 06520, USA

Accepted 8 May 2003

Abstract

Development requires a precise program of gene expression to be carried out. Much work has focussed on the regulatory networks that control gene expression, for example in response to external cues. However, it is important to recognize that these regulatory events take place within the physical context of the nucleus, and that the physical position of a gene within the nuclear volume can have strong influences on its regulation and interactions. The first part of this review will summarize what is currently known about nuclear architecture, that is, the large-scale three-dimensional arrangement of chromosome loci within the nucleus. The remainder of the review will examine developmental processes from the point of view of the nucleus.

Keywords: Anaphase; Chromatin; Chromosome; Gene gating; Heterochromatin; In situ hybridization; Matrix attachment region; Meiosis; Nuclear envelope; Nuclear lamin; Nuclear matrix; Nuclear pore; Nucleolus; Rabl configuration; RNA localization; Robertsonian translocation; Silencing; Telomere; Transvection

1. Introduction to nuclear architecture

1.1. Dynamic organization of chromosomes

Work over the last few decades has made it clear that individual chromosome loci are non-randomly arranged within the nucleus, indicating that there is indeed a defined three-dimensional (3D) nuclear architecture. However, it has also become clear that interphase chromatin is somewhat mobile, but that this mobility is constrained. The combination of defined positioning and constrained diffusion leads to a dynamic view of nuclear architecture in which interactions between loci are regulated by purely mechanical means (Marshall, 2002). This dynamic structural organization of the nucleus provides the physical context for the changes in gene expression that drive development.

Although the full ensemble of molecular interactions that establishes nuclear architecture is still being determined, two particularly important mechanisms have emerged that play dominant roles in positioning loci within the nucleus: (a) interactions with the nuclear envelope (NE) and (b) persistence of the mitotic chromosome arrangement.

1.2. Nuclear envelope interactions

There can be no doubt whatsoever that particular loci are non-randomly associated with the nuclear envelope (NE) while other loci are either randomly arranged relative to the NE or else are non-randomly located within the nuclear interior (Murray and Davies, 1979; Marshall et al., 1996; Manuelidis and Borden, 1988; Chung et al., 1990; Hoefers et al., 1993; Vourc'h et al., 1993; Hochstrasser et al., 1986). For instance, careful mapping of loci spanning the left arm of chromosome 2 in *Drosophila* revealed specific NE association sites (Fig. 1A) spaced roughly 1–2 Mb apart, thus dividing the chromosome into a series of megabase-sized loops (Marshall et al., 1996).

Proximity of a locus with the NE might indicate a tethering or targeting interaction of the particular locus with some molecular component of the NE, but it could just as well arise indirectly by some other mechanism of positioning loci that fortuitously places some loci near the NE. For example, if some loci were excluded from the nuclear

* Present address: Department of Biochemistry & Biophysics, University of California San Francisco, San Francisco, CA94143, USA.
E-mail address: wmarshall@biochem.ucsf.edu (W.F. Marshall).

0925-4773/$ - see front matter © 2003 Published by Elsevier Ireland Ltd.
doi:10.1016/j.mod.2003.05.001

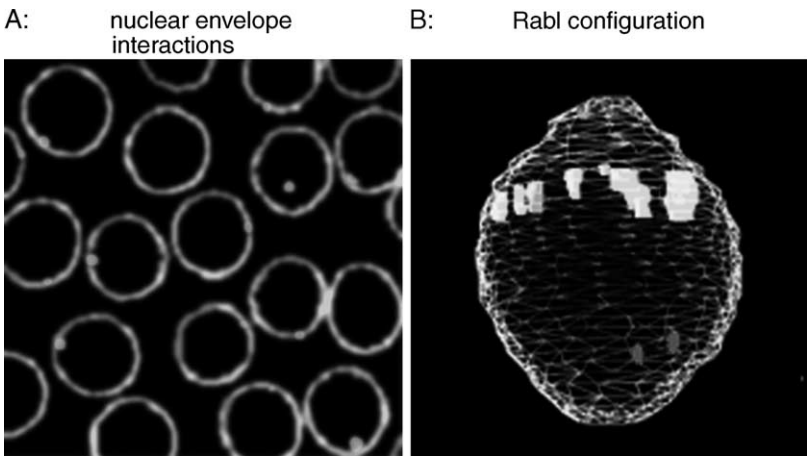

Fig. 1. Nuclear organization in *Drosophila* embryos. (A) Specific sites in the genome interact with the nuclear envelope. Image shows fluorescent in situ hybridization (FISH) on cycle 13 embryos using a heterochromatin repeat probe for the AATAC repeat (red) co-stained with antibodies specific for nuclear lamins (green). In essentially all cells, this particular simple-sequence repeat block is located at the nuclear envelope. Image taken from Marshall et al. (1996). (B) Interphase chromosomes are polarized according to their mitotic arrangement. Image shows dual-label FISH on early *Drosophila* embryos using two probes: (green) probe specific for a pericentromeric heterochromatin repeat block AAGAG, (red) probe specific for the euchromatic *bw* locus which is located distally on the chromosome arm. In this side-view, the nucleus is oriented with the former spindle pole located at the top of the image. As predicted by the retention of mitotic chromosome arrangement, centromere-proximal loci are clustered near the top of the nucleus. Image taken from Dernburg et al. (1996).

interior because they cannot interact with some internal structure, this would force them to be non-randomly near to the NE. There is, however, substantial evidence that NE localization does in fact involve an actual physical interaction between chromatin and the NE. This has been most clearly demonstrated for polytene chromosomes, whose large size has allowed a variety of mechanical approaches to be applied in order to show that the chromosomes really are physically adhering to the inner face of the NE at discrete sites (Quick, 1980; Hill and Whytock, 1993; Skaer et al., 1976). For interphase chromatin in non-polytene cells, direct mechanical manipulation is not feasible, but passive observations of chromatin motion in vivo have clearly indicated that NE-associated loci are more highly constrained that internally located loci, suggesting that it is the NE localized sites that are physically tethered and the interior sites that are forced into their position by a process of physical exclusion (Heun et al., 2001; Chubb et al., 2002).

Given that a subset of loci are targeted to the NE, one would then expect that the intrinsic rigidity of the chromatin, simple steric exclusion, and perhaps most importantly entropic forces would drive the rest of the chromosome into a non-random radial positioning, in which even loci not actually attached to the NE will tend to occupy specific radial distances relative to the surface. This non-random radial positioning has been most systematically measured in *Drosophila* early embryos (Marshall et al., 1996), but clearly also occurs in vertebrates (Croft et al., 1999; Cremer et al., 2001; Carvalho et al., 2001) and plants (ten Hoopen et al., 1999). Because of such non-random radial positioning, interactions between loci will always be non-random, even for loci not specifically tethered to a structure like the NE.

1.3. Persistence of mitotic chromosome arrangement

Chromatin is really nothing more than a very complicated polymer, and like any polymer, one would expect the path of the chromatin to follow a random walk through space. In order to deform the chromatin polymer into a defined arrangement, one has to apply some sort of force. Anaphase provides just such a force, by pulling on the kinetochores. During anaphase, the centromeric regions are brought together at the spindle pole while the telomeres trail behind, thus drawing the chromosome arms out much the same way stretching a rubber band elongates the originally random-walk polymer of the rubber molecules. When the chromatin decondenses in telophase, this arrangement is retained, yielding a polarized nucleus in which telomeres are at one side of the nucleus and centromeres at another (Fig. 1B). This ‘Rabl Configuration’, named after its discoverer Carl Rabl, has been clearly demonstrated using fluorescence in situ hybridization (FISH) in a wide range of cell types including insects, fungi, vertebrates, and plants (Marshall et al., 1996; Cremer et al., 1982; Funabiki et al., 1993; Haaf and Ward, 1995; Wilkie et al., 1999; Jin et al., 2000; Cowan et al., 2001; Shaw et al., 2002). Not just the centromeres and telomeres, but all the intervening chromatin, is non-randomly arranged by this process. This has been explicitly documented in *Drosophila* embryos, in which loci spanning the left arm of chromosome 2 were found to be position along the nuclear axis in an order precisely matching their linear order along the chromosome

(Marshall et al., 1996). Since the Rabl arrangement is established during mitosis, and then gradually randomized by slow chromatin diffusing during interphase, it is most dramatic in cells that are dividing actively, and tends to become less obvious in cells that have arrested in the cell cycle. For instance it has been clearly shown in *Drosophila* that the strong Rabl configuration seen in early embryos becomes disrupted in cells that have exited the cell cycle later in development (Dernburg et al., 1996). The Rabl arrangement thus represents a facet of nuclear architecture that varies during development, thus producing a clear difference in nuclear architecture as a function of cell cycle arrest.

Another effect of mitosis on nuclear architecture is the separation of chromosomes into spatially disjoint territories as a result of condensation during prophase. When the chromatin decondenses in telophase, this spatial separation is initially retained, thus depositing the chromosomes in non-overlapping regions within the nucleus. This separation of interphase chromosomes into distinct territories has been well documented in many organisms (reviewed in Cremer and Cremer, 2001).

NE interactions thus provide a non-random radial position, while the Rabl configuration provides a non-random axial position. If one then imagines the position of a locus within the nucleus defined in a set of spherical coordinates, one can see that a given locus will tend to lie within a donut-shaped sub-region of the nucleus. Whether there is a third axis of positioning, such that the angular relation between two loci within a given annulus is non-random, is much less clear. Although there have been reports of non-random angular positioning of chromosomes in metaphase (Nagele et al., 1995), other reports have failed to detect this organization in similar circumstances (Leitch et al., 1994; Allison and Nestor, 1999). This is clearly a possibility that requires further investigation.

Before we leave the discussion of mitotic chromosome structure, it is worth pointing out that, just as the interphase organization of chromosomes will, to some degree, reflect their organization at the preceding mitosis, it is equally the case that mitotic chromosomes may, to some extent, also reflect their organization in the preceding interphase. It is possible, then, that studies showing that mitotic chromosomes have specific defined shapes during metaphase, may be telling us something about the physical arrangement of those same chromosomes in the previous interphase (Houtsmuller et al., 2000). The exact relation between interphase chromosome shape and mitotic chromosome shape is an interesting question that deserves more detailed examination.

1.4. Interphase chromatin movement

Many chromosome interactions, such as recombination, clearly require chromosomes to move within the nucleus. And yet, the maintenance of a defined nuclear architecture requires that chromatin does NOT move to a significant extent. How is the chromatin movement that underlies biological function reconcilable with the high degree of spatial organization that is observed? We are left with a koan-like paradox: how can chromatin move, and yet not move?

It has long been appreciated that interphase chromosomes were fairly immobile, based on pioneering observations of Boveri on the similarity of chromosome arrangements in successive mitoses in *Ascaris* embryos (Boveri, 1909). Modern studies using photobleaching and other biophysical approaches have confirmed that long-range chromosome movements tend not to occur during interphase (Cremer et al., 1982; Selvin et al., 1990; Robinett et al., 1996; Abney et al., 1997; Zink et al., 1998; Sun and Yokota, 1999). These studies, however, were limited in their spatial resolution and restricted to analysis of large chunks of bulk chromatin.

When movement of individual loci was measured in living cells using the much higher resolution technique of sub-micron single-particle tracking (Marshall et al., 1997), it was found that interphase chromatin does in fact undergo substantial and rapid diffusion, but this diffusion is constrained such that a given locus can only diffuse within a small sub-region of the nucleus. In both yeast and in *Drosophila* embryos, individual chromatin loci moved with a diffusion constant of approximately 10^{-11} cm^2/s due to thermally driven Brownian motion. But this diffusion was confined to a sub-region with a radius of 0.3 μm for yeast and 0.9 μm for *Drosophila*, equivalent to 1–5% of the nuclear volume (Marshall et al., 1997). Following this first analysis of chromatin diffusion in yeast and *Drosophila* embryos, subsequent analyses have confirmed the result that interphase chromatin can diffuse within a confined region in a wide range of cell types and developmental stages including mammals and plants (Masuzawa et al., 2000; Vazquez et al., 2001; Edelmann et al., 2001; Heun et al., 2001; Chubb et al., 2002; Kato, personal communication). Extensive analysis of chromatin movement in *Drosophila* revealed that chromatin movement was most highly constrained when viewed over very short time scales. On longer time scales, chromatin is free to diffuse over larger distances (Vazquez et al., 2001). This could mean that chromatin diffusion at short time scales is constrained by interaction with a structure (for example the nuclear lamina) that itself undergoes slow random movements. Nuclear pores turn over at a slow but detectable rate (Daigle et al., 2001), so if chromatin were constrained by interactions with nuclear pore complexes (NPCs) (see below), then slow rearrangement of the nuclear pores would allow the relaxation of chromatin constraint on long time scales. The idea that the constraint on chromatin diffusion is predominantly supplied by the NE is supported by the fact that diffusion of chromatin loci associated with the NE is

significantly more constrained than other loci (Heun et al., 2001; Chubb et al., 2002). This was most clearly shown by Chubb and co-workers (2002), who showed that NE-associated chromatin has a diffusion constant identical to other chromatin, but a much smaller region of confinement within the nucleus.

Perhaps the most tantalizing possibility concerning chromosome motion comes from recent hints that chromatin motion can in some cases be highly directional and processive. This has been directly observed by tracking centromeres in animal culture cells (Shelby et al., 1996). A somewhat more indirect result is that genes in *Drosophila* embryos undergo a large vertical shift in position during the mid-blastula transition (Gunewardena and Rykowski, 2000). Because heterochromatin also begins condensing during this period, it seems likely that when the heterochromatin condenses, it stretches the euchromatic arms, thus producing an apparently directed motion.

1.5. Molecular interactions that specify nuclear architecture

Given that the NE appears to play a key role in tethering chromatin during interphase, and given also that the interaction of specific loci with the NE is one of the most obvious and clear-cut features of nuclear architecture, we naturally ask the question, which part of the NE is responsible for binding chromatin? The nuclear lamins, which form a network on the inner surface of the NE, have been shown capable of binding specific DNA sequences in vitro (Luderus et al., 1994; Baricheva et al., 1996). Lamin proteins can also bind histones (Taniura et al., 1995; Goldberg et al., 1999). Moreover, lamin-associated proteins other than the lamins themselves have been found that can bind DNA or chromatin (Ye and Worman, 1994; Lopez and Wolfner, 1997; Furukawa, 1999; Gindullis et al., 1999; Duband-Goulet and Courvalin, 2000). Although these studies are based on purely in vitro binding assays, raising obvious questions about biological relevance, a careful 3D microscopy investigation revealed a high frequency of contact between chromatin and nuclear lamin fibers (Paddy et al., 1990) implying that chromatin–lamin interactions are likely to be important in intact cells. Besides playing a role in interphase nuclear organization, lamins are also involved in re-assembling the nucleus around chromosomes during telophase, so one must be careful to distinguish between chromatin–lamin interactions that are involved solely in nuclear re-assembly and those that play a role in interphase organization. For example in *Drosophila* embryos, the loci that interact with the NE in interphase are distinct from those that interact with the NE during nuclear re-assembly in telophase (Marshall et al., 1996).

Although nuclear lamins together with their associated proteins are the primary structural element of the NE, they may not be the only candidates for chromatin interaction molecules. In particular, NPCs have been proposed to interact with chromatin as well. In vitro studies have revealed an NPC protein that faces the nuclear lumen and that can bind DNA (Sukegawa and Blobel, 1993). Another possible NPC–chromatin interaction is suggested by reports that yKu70, a telomere protein, can associate with the nucleoporin Nup145 via interaction of both proteins with a third protein, Mlp2 (Galy et al., 2000). It has been reported that Mlp2, Nup145, and yKu70 are all needed for telomeres to associate with the NE in vivo (Galy et al., 2000; Laroche et al., 1998), but other reports indicate that Mlp2 is not required for telomere–NE interactions (Hediger et al., 2002a). Moreover, if yeast nuclear pores are clustered within the plane of the nuclear membrane in response to a specific mutation, the position of telomeres does not change as a consequence, further arguing against a direct association between telomeres and nuclear pores (Hediger et al., 2002b). Thus, the question of whether or not nuclear pores mediate specific interactions between chromatin and the NE remains controversial.

The NE has received the lion's share of attention in determining possible interactions with chromatin, probably because NE–chromatin associations are one of the most visually obvious features of nuclear architecture. This is partly because the NE is a clearly defined structure that is simple to visualize, allowing one to easily compare the location of a given chromosome locus with the NE (Marshall et al., 1997). Moreover, in vivo chromatin mobility studies suggest that NE interactions are the primary interaction that constrains chromatin motion (Chubb et al., 2002). However, this does not preclude the possibility that chromatin might also be constrained by interactions with other structures located in the nuclear interior. An internal nuclear superstructure called the nuclear matrix was first defined as the insoluble residue that remains after isolated nuclei are heavily extracted (Berezney and Coffey, 1974), and has been visualized in nuclei using special resinless embedding procedures for electron microscopy (Capco et al., 1982). Various in vitro experiments have identified specific DNA elements called matrix attachment regions (MARs) or scaffold attachment regions (SARs) that can bind to such nuclear matrix preparations, suggesting that these elements could mediate an interaction of specific chromosome sites with this internal nuclear structure (see for example Gasser and Laemmli, 1986). Moreover, the genes and proteins interacting with the nuclear matrix appear to vary during development and differentiation (Stein et al., 1994).

The main problem with the nuclear matrix concept is that it is defined in purely operational terms as an entity that is produced by certain extraction procedures. This raises the serious concern that the whole thing might simply be an artifact of the preparation procedure. It is certainly true that nuclear matrix preparations contain within them proteins whose localization in vivo are known to be spatially distinct, such as topoisomerase II and lamins, which indicates that either the 'matrix' is actually composed of several different structures that happen to be insoluble under similar

conditions, or else that the associations of these proteins are actually created by the matrix extraction procedure. In either case, it raises serious doubts about the validity of many nuclear matrix preparations as indicators of a single bona fide in vivo structure. Moreover, some proteins reported to be contained in the nuclear matrix or scaffold are clearly rather soluble in vivo. For example, although topoisomerase II is often considered to be part of the nuclear scaffold, it has been clearly shown that topoisomerase II can undergo substantial exchange onto and off of chromosomes when visualized in vivo (Swedlow et al., 1993), arguing that even if it is part of a matrix of some sort, this matrix must be highly dynamic and not at all the rigid structure that the term matrix might suggest. Perhaps the best evidence that there is an internal nuclear structure in living cells comes from studies of the *Drosophila* centrosome proteins CP60 and CP190. These proteins appear to form a network within nuclei of living *Drosophila* embryos when imaged at high resolution, and importantly this network persists in the shape of the nucleus even after the NE has broken down and the chromosomes have congressed to the metaphase plate (Oegema et al., 1997). CP60 and CP190 are components of the nuclear matrix defined biochemically, and thus may represent a true matrix that exists as a coherent structure in living cells. However, it is worth noting that chromosomes are able to congress freely through this matrix during prometaphase, without deforming the matrix, implying that the underlying interactions must be highly labile in order to allow passage of the large chromosomes. In any case, these studies suggest that the nuclear matrix may remain a viable concept, and that the key will be to analyze it using careful studies in living cells in order to avoid concerns about in vitro artifacts.

2. Gametes and fertilization

We now consider the role that nuclear architecture plays during development. Development begins when two gametes fuse to form a zygote. We therefore begin our discussion with gene regulation and nuclear architecture in gametes.

Sperm chromatin, in both animals and plants, is for the most part transcriptionally inactive and highly condensed. During spermatogenesis, the usual histone complement of chromatin is replaced by sperm specific proteins, including specialized histone variants as well as non-histone proteins such as protamines, leading to a highly condensed chromatin structure (reviewed in Fuentes-Mascorro et al., 2000). In addition to their role in chromatin compaction, these sperm-specific proteins may also have a strong influence on nuclear architecture within the sperm head. For instance, a sperm-specific variant of histone H2B has been shown to be part of a sperm-specific telomere-binding complex (Gineitis et al., 2000), which has been proposed to play a role in anchoring sperm telomeres to the NE. This interaction may explain the dramatic arrangement of chromosomes in the sperm nucleus, with all centromeres clustered in the nuclear interior and all telomeres on the nuclear surface (Zalensky et al., 1995).

Oocytes also tend to have a distinct nuclear architecture. For instance in *Spisula* the chromosomes, which are arrested in meiosis, are condensed onto the NE leaving the lumen of the nucleus apparently empty. Given that so many oocytes arrest during meiotic divisions, it is likely that much of the nuclear architecture seen in these cells is a remnant of the specific nuclear architecture established during meiosis as an aid to chromosome pairing (see below). However, this also means that before zygotic gene expression can begin, meiosis must complete and then the chromatin must be remodeled into its normal interphase configuration.

Following fertilization, the maternal and paternal pronuclei must fuse to form the nucleus of the zygote. This pronuclear fusion (also called karyogamy) has been studied most intensively in yeast, although mutants that interfere with pronuclear fusion in metazoan development have also been described (Mathe et al., 1998). After the pronuclei have fused, the parental genomes remain spatially separated for several divisions, implying that chromosomes do not undergo substantial changes in position either during interphase (see above) or during the intervening mitoses (Conklin, 1901; Moenkhaus, 1904).

3. Early embryogenesis: axis specification and cleavage divisions

The main goals of early development can, it seems, be accomplished with only a few zygotic genes: in general transcriptional activity in early embryos of metazoans is extremely low, and early development relies mainly on proteins and mRNA produced in the mother and stored in the oocyte. The zygotic chromosomes, therefore, are mainly just being carried along for the ride. Yet the nucleus, as a physical entity, does in fact play important roles in early development.

One of the hallmark features of early development is the asymmetric accumulation of specific proteins and mRNAs in different regions of the embryo. In most cases, the asymmetric accumulation of a small number of different molecules underlies the initial events of axis specification. Current work has focussed mainly on the role of cytoplasmic structures (actin cables, microtubules, etc.) in RNA and protein localization, however, nuclear architecture could contribute to this process as well. It is clear that the oocyte nucleus contains molecules necessary for the early cleavage divisions. This was demonstrated in microsurgical experiments by Yatsu (1905) who showed that when *Cerebratulus* eggs were cut into pieces, a piece lacking a nucleus could form asters provided that the cutting was done after germinal vesicle (i.e. NE) breakdown, implying that some factor necessary for cell division is released from the nucleus. Thus, the nucleus seems to play a role in cleavage by providing a compartment to sequester proteins needed for the cleavage divisions.

Moreover, there is substantial evidence that the position of the nucleus can influence the local concentration of molecules in the vicinity of the nucleus. The locally exported factors can include molecules involved directly in cleavage, for example in the yeast *S. pombe* it is currently thought that the nucleus defines the position of cleavage by the local export of the plekstrin homology domain protein mid1 (Bahler et al., 1998). But perhaps more interestingly, the nucleus can also locally export specific mRNA messages, thereby leading to a local increase in message accumulation. For instance, the asymmetric accumulation of Gurken mRNA appears to reflect the local export of this transcript from the oocyte nucleus once it has reached the site that will define the future anterodorsal end of the embryo. That the nucleus contributes directly to the asymmetric localization of Gurken is further supported by the fact that a mutant disrupting the primary *Drosophila* nuclear lamin gene Dm(0) causes defects in Gurken mRNA localization and leads to a defect in dorsal–ventral patterning (Guillemin et al., 2001). Mutations that displace the nucleus lead to a corresponding loss of proper dorsal–ventral axis specification (Lei and Warrior, 2000; Guichet et al., 2001).

It thus seems clear that the position of the nucleus within the cell can play a role in mRNA localization. A further level of complexity arises when one considers that, because of the defined nuclear architecture, such as the Rabl arrangement, different genes may lie closer to different regions of the NE. One might therefore suspect that transcripts could be preferentially exported through a sub-region of the NE near where the gene is found. Moreover, since mRNA must be exported through NPCs, it has been suggested that active genes could be targeted to the NE in order to facilitate transcript export, an hypothesis known as gene gating (Blobel, 1985). If gene gating brings a gene to the NE near the site of future message accumulation, then locally increased export through the nearby nuclear pores could explain the ultimate asymmetric transcript localization. This is a beautiful and elegant concept, but unfortunately appears not to be true in reality. A careful study of many pair-rule genes in *Drosophila*, whose transcripts are specifically localized relative to the nucleus during early embryonic development, revealed no relation between the position of the gene in the nucleus and the position of transcript accumulation (Wilkie et al., 1999). It thus appears that the primary role of the nucleus in dictating RNA localization will depend on the position of the nucleus as a whole, rather than on the position of individual genes within the nucleus.

4. Differentiation and morphogenesis

4.1. Nuclear architecture changes during differentiation

Comparatively few zygotic genes are expressed during early development in metazoa. But as cleavage divisions wind down and morphogenesis and differentiation begin, zygotic gene expression programs are triggered that ultimately dictate the individual identities of different cell types. The activation of zygotic gene expression is accompanied by, and in some cases apparently dependent on, a conversion in the cell cycle from the rapid divisions of cleavage which lack G1 and G2, to more normal cell cycles characteristic of later development (Edgar and Schubiger, 1986). In plants the situation is slightly more complex and it appears that zygotic genes are activated very early in development, but only from the maternally supplied genome, indicating that the paternal genome is initially silenced and must be restructured later on in plant development (Vielle-Calzada et al., 2000), although the plant sperm chromatin undergoes most of its decondensation, and simultaneously permits expression of some paternally encoded genes, immediately upon fertilization (Scholten et al., 2002).

Because differentiation tends to be effectively irreversible, large-scale alterations in chromatin often accompany these dramatic changes in gene expression. The most visually striking change in chromatin organization is heterochromatinization, in which simple-sequence repeat tracts become assembled into specialized heterochromatin structures. Heterochromatin is generally not seen during early development, only forming later when differentiation starts. It would not be surprising if the large changes in gene expression and chromatin packing that occur during differentiation might give rise to corresponding changes in nuclear organization. Indeed, the specific cell fate adopted leads to obviously different nuclear organizations. The close relation between cell identification and nuclear architecture is best appreciated by the fact that nuclei from different cell types in the same organism often have obviously different nuclear architectures. (Hochstrasser and Sedat, 1987; Manuelidis and Borden, 1988; Leitch et al., 1994; Dernburg et al., 1996; Skalnikova et al., 2000). Changes in nuclear architecture are also observed in cell lines induced to undergo differentiation (Chaly and Munro, 1996; Bartova et al., 2002). For example, in rat L6E9 myoblasts, centromeres switch position and become NE associated when the cells are induced to differentiate (Chaly and Munro, 1996). What drives these changes during development? In some cases, changes in nuclear architecture are likely to reflect cell type-specific changes in nuclear proteins. For example, germ line cells express specialized forms of nuclear lamins that could result in a different organization within germ line nuclei (Benavente and Krohne, 1985). Changes in chromatin structure downstream of genetic regulatory networks might also cause chromosomes to reorganize within the nucleus. Recent experiments show that alteration in DNA methylation and histone acetylation can also cause changes in interphase chromosome arrangement (Santos et al., 2002).

In addition to activating new programs of gene expression, another process that accompanies developmental progression is exit from the cell cycle. This occurs

at different times in different developing tissues, and for stem cell populations it may be postponed indefinitely. Because the mitotic chromosome arrangement is maintained for only a finite time after a cell enters G1, one would expect that this arrangement (including such consequences as the Rabl configuration) would be most dramatic in dividing cells and would be reduced, or lost altogether, in quiescent cells, thus producing a radical change in the position of genes within the nucleus. Certainly it is true in *Drosophila* that the Rabl configuration which is so dramatic in the rapidly dividing cells of early embryos (Marshall et al., 1996) is almost entirely lost in imaginal disc cells (Dernburg et al., 1996). It has been directly demonstrated in mammalian cells that chromosome positions change as proliferating cells become quiescent (Vourc'h et al., 1993; Hulspas et al., 1994; Bridger et al., 2000). Another set of data supporting the variation of nuclear architecture on cell cycle timing is provided by studies showing that chromosome positions are dramatically rearranged in nuclei of cells ectopically expressing the cyclin dependent kinase inhibitor $p21^{WAF}$(Linares-Cruz et al., 1998). Thus, the cessation of the cell cycle that accompanies differentiation can itself cause dramatic changes in nuclear architecture.

4.2. Gene regulation by heterochromatin domains in the nucleus

What is the functional role of the nuclear architecture changes seen during development and differentiation? Because large changes in zygotic transcription accompany the changes in nuclear architecture, it raises the possibility that these structural rearrangements may in fact play a causal role in changing gene expression patterns. But this conclusion is based on pure correlation, and to avoid falling into the fallacy of *post hoc ergo propter hoc*, we should ask whether there is any direct evidence for a role of nuclear architecture in regulating gene expression.

One of the most clear-cut correlations between gene expression and nuclear organization comes from studies of gene silencing by heterochromatin. Heterochromatin tends to aggregate into one or a few large domains called chromocenters, thus defining a set of structural landmarks against which the position of a given gene can be determined. This self-aggregation of heterochromatin in large domains is observed in both animals and in plants (Fransz et al., 2002). By analyzing gene position versus heterochromatin, it has now been shown that when euchromatic genes become silenced by heterochromatic insertions (a phenomenon called position effect variegation in *Drosophila*) the genes shift from their normal position and become associated with the large heterochromatin domains (Dernburg et al., 1996; Csink and Henikoff, 1996; Brown et al., 1997; Francastel et al., 1999; Grogan et al., 2001). An extremely interesting and informative experiment has recently been reported in which the heterochromatin protein HP1 was artificially targeted to reporter gene loci. These genes, which became silenced, were embedded in polytene chromosomes and unable to relocate within the nucleus, and yet they still established a physical interaction with other silenced loci as judged by the presence of chromatin strands connecting the loci to other HP1 enriched regions (Li et al., 2003). The correlation between silencing and position is thus extremely strong in such cases, but at least based on such evidence it remains just a correlation. It has, however, been shown that localization of a gene into a heterochromatin domain can be sufficient to silence the gene: when a euchromatic reporter gene was artificially targeted to the heterochromatin domain in *Drosophila* by flanking chromosome pairing sites, the gene became silenced (Sass and Henikoff, 1999). Other experiments suggest that localization into the heterochromatin domain may be necessary as well as sufficient: chromosome rearrangements that impede localization of a gene to the heterochromatin domain have the effect of reducing silencing (Talbert et al., 1994; Henikoff et al., 1995). A simple model to explain these results is that the heterochromatin domain creates a 'bad neighborhood' for transcription, that exerts a silencing effect on euchromatic genes brought into this region (although one must bear in mind that there are also specific heterochromatic genes that are only properly expressed when embedded in heterochromatin).

Another well known feature of silencing by heterochromatin is variegation, in which genes are silent in some cells and active in others, with similar expression in neighboring cells. This spatial inhomogeneity indicates that gene activity is inherited in a clonal fashion, with occasional switches, during development. Interestingly, the rate of switching, as reflected in the spatial coarseness of the variegated pattern, appears to vary as a function of developmental stage (Weiler and Wakimoto, 1998), possibly reflecting differences in cell cycle time or chromosome dynamics. One might expect that if the gene state is dictated by nuclear position, that this state should be randomized every time the cell divides due to chromosome rearrangements during mitosis, but recent studies have suggested that chromosomes retain their relative positions during mitosis (Gerlich et al., 2003).

One interesting aspect of silencing by heterochromatin is the function of insulators and boundary elements, sequences that can protect nearby euchromatic genes from silencing by heterochromatin. For example, the gypsy transposon contains an insulator that protects genes from silencing by nearby heterochromatin and whose function requires the Su(hw) and mod(mdg4) proteins. There is some evidence that insulator function may involve nuclear architecture. both Su(hw) and mod(mdg) localize to the NE, as does the gypsy insulator itself (Gerasimova et al., 2000). Su(hw) mutants that compromise insulator function cause the insulator sequence to dissociate from the NE, implying that Su(hw) protein tethers insulator sequences to the NE, and more importantly, that this tethering could be critical in

insulator function. A similar ability of proteins that interact with the NE to block silencing has also been documented in yeast (Ishii et al., 2002).

If gene silencing by heterochromatin requires movement of the gene into the heterochromatin domain, then sticking the gene onto the NE might impede its subsequent movement into the heterochromatin domain. It would be interesting to know when in the cell cycle insulators recruit their targets to the NE. Given that heterochromatin tends to flank the centromeres (most dramatically seen in *Drosophila*), then the anaphase alignment of chromosomes would remove most euchromatic genes from the vicinity of the heterochromatin. Recruitment of a gene into the silenced domain would then require looping back within the nucleus, in opposition to the Rabl configuration. If insulators anchored their targets onto the NE early in G1, they might then be able to block or delay the subsequent association of the site with the heterochromatin domain.

Although the relation between boundary element/insulator activity and NE interactions is consistent with silencing via recruitment to a nuclear sub-domain, we must bear in mind that many studies of silencing indicate that heterochromatin spreads in a processive manner along the chromatin. It is less obvious how attaching a site to the NE could block such spreading in cis. One way to reconcile the views is that spreading might simply reflect the fact that once one region becomes part of the silenced domain, then neighboring regions of chromatin are physically constrained to be near the silenced domain and hence more likely to become silenced than regions farther away. In this manner, regions of the chromatin will become recruited into the silenced domain in a progressive manner that would strongly resemble processive spreading. In thinking about factors and DNA elements that block silencing, an important distinction has recently been drawn between insulators/boundary elements, which act as structural blockades preventing the spread of heterochromatin, and desilencing elements which act on a region of euchromatin to antagonize its silencing (Ishii and Laemmli, 2003). Proteins which appear to antagonize silencing include histone variants (Meneghini et al., 2003), some transcription factors (Ishii and Laemmli, 2003), and histone-modifying enzymes (Ishii and Laemmli, 2003; reviewed in Narlikar et al., 2002). Thus the distinction between boundary elements and desilencing activities is more than just semantic hair-splitting: while boundary elements/insulators seem likely to act via large-scale structural effects, such as preventing recruitment to nuclear compartments, desilencing activities are more likely to act through conventional molecular-scale interactions.

4.3. Gene regulation by nuclear envelope interactions

In addition to playing a role as a physical tether to block gene recruitment to heterochromatin, the NE might play an even more direct role in gene regulation. There has been some controversy as to whether the inner surface of the nuclear envelope (NE) constitutes an active or an inactive compartment within the nucleus. The first evidence for a connection between gene expression and the NE came from an experiment done by Weintraub and co-workers, who demonstrated an increased nuclease sensitivity of NE-associated chromatin, suggesting that active genes with an open chromatin conformation are specifically localized to the NE (Hutchison and Weintraub, 1985). On the other hand, imaging of transcription sites by in situ hybridization within the nucleus showed that active genes are mainly localized in the interior of the nucleus rather than at the NE (Carter et al., 1993). Insertion of a block of heterochromatin into a euchromatic position is sufficient to locate the whole region onto the NE (Dernburg et al., 1996). FISH studies of individual chromosomes have found that gene-poor chromosomes with reduced overall transcription tend to be NE associated (Croft et al., 1999; Cremer et al., 2001; Boyle et al., 2001). Furthermore, strong activation of a reporter locus in mammalian cells caused the locus to dissociate from the NE and move to the nuclear interior (Tumbar and Belmont, 2001). It thus seems most likely that NE-associated chromatin may tend to be transcriptionally silent. Does the NE play a causal role in this silencing?

The key to understanding the connection between gene expression and NE is the question of causality: are silent genes recruited to the NE because they became silent, or do the genes become silent because they became recruited to the NE? When a reporter gene in yeast was artificially targeted to the NE by means of an NE-interacting DNA binding fusion protein, the gene became silenced (Andrulis et al., 1998), although a later study showed that not all NE-associated proteins share this property and that some proteins involved in nuclear transport, such as the importin-β protein Cse1, actually can serve to protect flaking genes from silencing when artificially targeted near the gene (Ishii et al., 2002). As mentioned above, mutations in the telomere-binding protein dimer yKu70/yKu80 and the NPC associated proteins Mlp1/Mlp2 have been reported to dissociate telomeres away from the NE (Galy et al., 2000; Laroche et al., 1998), although other studies found that Mlp mutants had no such effect (Hediger et al., 2002a) calling into question the role that nuclear pores might play in telomere attachment. Another NE-associated protein, Esc1, also appears to be involved in attachment of some silent chromatin regions to the NE (Andrulis et al., 2002). It has also been shown that SIR proteins themselves can play a role in telomere attachment to the NE during some parts of the cell cycle (Hediger et al., 2002a). Taken together, these studies indicate that we still have much to learn about the precise molecular interactions that underlie chromatin–NE association, but at least they strongly suggest a role for NE attachment in silencing.

If targeting a gene to the NE really does cause it to become silenced, how is this silencing carried out? The simplest possibility is that there could be proteins associated

with the NE which are capable of repressing gene expression. Interestingly, the SIR3 and SIR4 silencing proteins in yeast are associated with the NE (Gotta et al., 1996; Palladino et al., 1993), suggesting that genes associated with the NE may simply experience a higher local concentration of silencing proteins. Most of these studies have taken place in yeast, however, and it will be interesting to see whether the NE also plays a direct silencing role in metazoans or plants.

One potential example of gene silencing by the NE in metazoa comes from studies of the lamin-associated protein 2 beta (LAP2β), an integral membrane protein found in the NE (Nili et al., 2001). LAP2β binds the protein germ cell-less (GCL) which is turn can bind the transcription factor E2F–DP. When LAP2β and GCL were expressed ectopically, reduced transcriptional induction by the E2F–DP complex was observed. One possible interpretation of this result could be that Lap2β and GCL could recruit E2F-regulated genes to the NE where they would become silenced. This system needs more careful examination since it may provide the first major inroad into understanding gene silencing by the NE in metazoa.

4.4. Regulation of chromosome interactions by nuclear architecture

Another aspect of nuclear architecture that impinges on gene expression is interactions between widely separated loci. For example in the phenomenon of transvection, a promotor on one chromosome can drive transcription of a coding region on another chromosome (Wu and Morris, 1999). Although transvection is best characterized in *Drosophila* it seems to occur in other organisms including flowering plants as well (Coen and Carpenter, 1988). Barring some sort of unknown action at a distance, transvection should require the interacting sites to move into physical contact. If genes occupy non-random positions within the nucleus, then their probability of coming into contact by constrained diffusion should likewise be highly non-random.

The strong influence of nuclear architecture on chromosome interactions has been dramatically and clearly demonstrated in a study of the establishment of somatic homolog pairing (Fung et al., 1998), which is best known in *Drosophila* but also occurs in humans and yeast (LaSalle and Lalande, 1996; Burgess et al., 1999). A time-resolved analysis of the kinetics of initiation of somatic pairing in *Drosophila* (Fung et al., 1998) showed that the loci which paired first were the ones that started out close to each other within the nucleus as a result of a non-random position in the nuclear interior, while loci that started out farther apart tended to pair later. In fact, the kinetics of pairing could be predicted exactly assuming that chromatin moved strictly by constrained diffusion and taking into account the known nuclear architecture in the *Drosophila* embryo (Fung et al., 1998).

This study of homolog pairing leads to a general model for the role of nuclear architecture in regulating chromosome interactions: because chromatin diffusion is constrained to a small sub-region of the nucleus, only loci whose regions of confinement overlap can interact. Loci that start out far apart cannot interact, since their regions of confinement do not overlap, thus precluding physical contact. Hence, the relative positions between loci, as dictated by the nuclear architecture, play a decisive role in determining which interactions can and cannot occur. Thus, as our understanding of the role that transvection and related processes play in developmental regulation of gene expression increases, we are likely to find that nuclear architecture becomes increasingly important.

Transvection is a reversible interaction between loci, but in some cases, development in particular tissues or cell types involves physical rearrangements of chromosomes. Obvious examples include the V(D)J recombination events that give rise to a repertoire of immunoglobulins, or the site-specific recombination involved in mating type switching in yeast. In all these cases, even more obviously that with transvection, physically separated loci must come into direct contact in order to recombine. One would therefore expect that the same influence of nuclear architecture as was seen for homolog pairing should also be seen for chromosome rearrangements. Evidence that nuclear architecture can in fact influence such rearrangements comes from studies on accidental rearrangements that occur in disease processes like cancer.

Studies of radiation induced recombination and DNA break repair show that the pattern of interaction between loci is highly non-random (Hilliker, 1985; Sachs et al., 2000) and some of this non-uniformity could in principle result from non-random position of the interacting chromosomal regions. Robertsonian translocations, spontaneous reciprocal translocations of whole chromosome arms, provide a further example of the influence that nuclear organization might have on chromosome interactions. The distribution of breakpoints in such Robertsonian translocations is non-random: translocations having both breakpoints on acrocentric chromosomes contain nucleolar organizing regions (NORs) that occur with a much higher frequency than expected (Therman et al., 1989). Since the NOR regions are embedded in the nucleolus, loci proximal to the NOR will tend to congregate around the periphery of the nucleolus. As a result, acrocentric chromosomes with NORs will tend to be non-randomly close together in the nucleus relative to other chromosomes, and hence will be more likely to recombine with each other than with other chromosomes. Chromosomes lacking an NOR will not experience this bias and hence will tend to recombine more randomly with arbitrary regions of the genome. As a result, only the NOR containing chromosomes demonstrate the bias in recombination frequencies.

From the point of view of human health, one of the most important effects of nuclear architecture occurs in chromosome translocations that lead to Leukemia and other cancers. A translocation between the BCR and ABL loci on

chromosomes 22 and 9 gives rise to a translocation called the Philadelphia chromosome. The majority of patients with chronic myelogenous leukemia (CML) contain this rearrangement in their genomes. The breakpoint encodes a novel fusion protein called BCR–ABL, which plays a direct role in leukemia. But why is this particular rearrangement so common? From our discussion of the role of nuclear organization on chromosome interactions, one might expect that perhaps the BCR and ABL loci interact with high frequency because their nuclear positions tend to coincide, thus making spontaneous recombination in that region more likely than between other loci. As predicted by such considerations, studies of nuclear architecture have in fact shown that BCR and ABL loci are non-randomly close together in the nucleus, potentially leading to an increased tendency for these particular loci to interact with each other during recombination events (Kozubek et al., 1999; Neves et al., 1999). Another specific rearrangement involved in cancer development involves the RET and H4 loci. An inversion with breakpoints in RET and H4 is found in many cases of radiation-induced thyroid cancer, for example in victims of the Chernobyl disaster. As with BCR and ABL, RET and H4 are non-randomly close together in normal cells, again suggesting that the spatial proximity of these loci may bias them to recombine with each other (Nikiforova et al., 2000).

4.5. Role of nuclear positioning in morphogenesis

There is thus substantial evidence that nuclear architecture plays an important role in gene regulation during differentiation. However, development does not end when a tissue-specific set of genes is activated. In order to produce an organism, cells undergo complex morphogenetic processes, and in these processes the nucleus also plays an important part. During brain development, the apparent migration of newly minted neurons out towards the cortex is likely to be driven largely by movement of the cells nucleus, a type of motility called nucleokinesis that requires microtubule (Morris, 2000). *Drosophila* nuclear lamin mutants that showed defective Gurken mRNA localization in the oocyte (see above), also showed defects in the directed outgrowth of cytoplasmic extensions during tracheal formation in *Drosophila*, suggesting that local export from migrating nuclei may influence morphogenesis of tracheal outgrowths and perhaps other branching structures as well (Guillemin et al., 2001). There is also a long-standing observation in plants that nuclei tend to move near the sites of polarized cell growth (Haberlandt, 1887). Thus, movements of the entire nucleus appear to play important roles in directed morphogenesis as well as early axis specification.

5. Germ line and meiosis

Meiosis and gametogenesis involves major changes in gene expression patterns. First, there is a set of meiosis-specific genes that are expressed in cells that undergo meiosis (Chu et al., 1998). Many of these genes encode products involved in meiotic chromosome pairing and recombination. The other important gene expression program of gametogenesis is the production and storage of maternally encoded mRNA, that will supply most of the mRNA needed for early cleavage when the zygotic genome is relatively inactive transcriptionally. In the *Drosophila* egg chamber, a single germ line cell undergoes clonal expansion to produce a single oocyte attached in a syncytium to a collection of additional germ-line derived nurse cells that are employed to produce proteins and mRNA transcripts, which are then transported into the oocyte (reviewed in Riechmann and Ephrussi, 2001). mRNA stored in the oocyte is generally not translated until development begins. The mechanisms that keep maternally encoded mRNA from being translated until after mating are reviewed elsewhere (Richter et al., 1990). In addition to the deliberate accumulation of maternally encoded messages, it has also been proposed that the specific chromatin configuration in meiosis may allow a general increase in transcription at many loci having nothing to do with meiosis or gametogenesis and that these unwanted transcripts are packaged in a translationally repressed form in order to prevent accumulation of high levels of undesired proteins (Kleene, 2001).

The unusually high levels of transcription that occur in gametogenesis have in many cases necessitated the development of unusual nuclear architectures. Most dramatic are the lampbrush chromosomes, seen both in amphibians and in some insects, in which the chromosomes assemble into huge looping structures within the nucleus. Interesting, in some cases it seems that different loci develop into morphologically distinct lampbrush structures, providing visually suggestive evidence that the morphology of the lampbrush loops somehow correlates with their DNA sequence organization (Hess, 1970; Bonaccorsi et al., 1988; Hochstenbach et al., 1993; Reugels et al., 2000).

From the point of view of nuclear architecture, meiosis is probably the most fascinating process in biology. Prior to the onset of meiosis, homologous chromosomes are in general not spatially associated with each other. As meiosis begins, the chromosomes start to condense and assemble a protein based core structure called the axial element. Once the chromosomes have condensed, they begin to pair, first at a few discrete sites and eventually over their entire length (Gillies, 1975; Rasmussen, 1976; Zickler, 1977; Scherthan et al., 1992; Dawe et al., 1994).

Studies of meiotic pairing show that chromosomes often move over very large distances in the nucleus in order to pair. How can homologous chromosomes locate each other in the crowded environment of the nucleus? First of all, the very fact that pairing generally occurs after condensation

means that at the time when chromosomes must locate each other, the chromatin is far less entangled that it would be if they had to pair during interphase. Moreover, there appear to be actively driven chromosome rearrangements that probably facilitate orderly chromosome interactions. This is dramatically illustrated by the formation of a structure called the Bouquet. Early in meiosis, telomeres are seen to cluster to one limited region of the NE. With the telomeres clustered in this one place, the chromosome arms loop out, thus leading to a configuration that resembles a bouquet of flowers. Because nuclear pores also cluster in the region where the telomeres cluster, and because many studies have shown that telomeres can interact with nuclear pores (Laroche et al., 1998; Galy et al., 2000), it is probably the case that either the telomere clustering drives the nuclear pore clustering, or else the nuclear pore clustering drives the telomere clustering. Recent evidence in plants has shown that telomere clustering can be disrupted without an effect on nuclear pore clustering, suggesting that bouquet formation is normally driven by nuclear pore clustering rather than vice versa (Cowan et al., 2002). The mechanism that clusters the nuclear pores during meiosis remains unknown, although it is interesting to note that in *C. elegans*, the P-granules that segregate to the germ line interact with the NE and appear to become associated with clusters of nuclear pores (Pitt et al., 2000). It is thus conceivable that aggregation of nuclear pores could be driven by such an interaction, and this could then, in turn, drive telomere clustering if the telomeres remained attached to the clustering nuclear pores. Regardless of the mechanism by which it occurs, the meiotic bouquet is predicted to facilitate chromosome pairing by bringing the sub-telomeric regions into close proximity. How the individual homologous chromosomes recognize each other is not, however, understood at this time.

After meiosis is completed, the final gametes are produced and sent out into the world to seek each other out. If mating is successful, the pronuclei will fuse, and once again begins the cycle of development. We have seen that at each stage of this process, there are specific changes in gene expression accompanied by, and possibly reliant on, specific changes in nuclear architecture. Clearly, our understanding of the role that nuclear organization plays is still at a very rudimentary stage. The relatively recent revelation of specific DNA elements, such as insulators, that can change the position of flanking genes within the nucleus, together with the identification of specific proteins required to establish and maintain nuclear architecture, will provide the molecular tools needed to determine the function of interphase chromosome arrangements. Combining genetic and reverse genetic manipulations to modify these molecular determinants, with advanced imaging methods to observe the consequences in living cells, ought to finally resolve many of the long-standing questions about the role that nuclear architecture plays in gene expression and development.

Acknowledgements

W.F.M. was funded by a Leukemia and Lymphoma Society Special Fellowship.

References

Abney, J.R., Cutler, B., Fillbach, M.L., Axelrod, D., Scalettar, B.A., 1997. Chromatin dynamics in interphase nuclei and its implications for nuclear structure. J. Cell Biol. 137, 1459–1468.

Allison, D.C., Nestor, A.L., 1999. Evidence for a relatively random array of human chromosomes on the mitotic ring. J. Cell Biol. 145, 1–14.

Andrulis, E.D., Neiman, A.M., Zappulla, D.C., Sternglanz, R., 1998. Perinuclear localization of chromatin facilitates transcriptional silencing. Nature 394, 592–595.

Andrulis, E.D., Zappulla, D.C., Ansari, A., Perrod, S., Laiosa, C.V., Gartenberg, M.R., Sternglanz, R., 2002. Esc1, a nuclear periphery protein required for Sir4-based plasmid anchoring and partitioning. Mol. Cell Biol. 22, 8292–8301.

Bahler, J., Steever, A.B., Wheatley, S., Wang, Y., Pringle, J.R., Gould, K.L., McCollum, D., 1998. Role of polo kinase and Mid1p in determining the site of cell division in fission yeast. J. Cell Biol. 143, 1603–1616.

Baricheva, E.A., Berrios, M., Bogachev, S.S., Borisevich, I.V., Lapik, E.R., Sharakhov, I.V., et al., 1996. DNA from *Drosophila melanogaster* β-heterochromatin binds specifically to nuclear lamins in vitro and the nuclear envelope in situ. Gene 171, 171–176.

Bartova, E., Kozubek, S., Jirsova, P., Kozubek, M., Gajova, H., Lukasova, E., et al., 2002. Nuclear structure and gene activity in human differentiated cells. J. Struct. Biol. 139, 76–89.

Benavente, R., Krohne, G., 1985. Change of karyoskeleton during spermatogenesis of *Xenopus*, expression of lamin LIV, a nuclear lamina protein specific for the male germ line. Proc. Natl Acad. Sci. USA 82, 6176–6180.

Berezney, R., Coffey, D.S., 1974. Identification of a nuclear protein matrix. Biochem. Biophys. Res. Commun. 60, 1410–1417.

Blobel, G., 1985. Gene gating, a hypothesis. Proc. Natl Acad. Sci. USA 82, 8527–8529.

Bonaccorsi, S., Pisano, C., Puoti, F., Gatti, M., 1988. Y chromosome loops in *Drosophila melanogaster*. Genetics 120, 1015–1034.

Boveri, T., 1909. Die blastomerenkerne von *Ascaris megalocephala* und die Theorie der Chromosomenindividualität. Archiv für Zellforschung 3, 181–268.

Boyle, S., Gilchrist, S., Bridger, J.M., Mahy, N.L., Ellis, J.A., Bickmore, W.A., 2001. The spatial organization of human chromosomes within the nuclei of normal and emerin-mutant cells. Hum. Mol. Genet. 10, 211–219.

Bridger, J.M., Boyle, S., Kill, I.R., Bickmore, W.A., 2000. Re-modelling of nuclear architecture in quiescent and senescent human fibroblasts. Curr. Biol. 10, 149–152.

Brown, K.E., Guest, S.S., Smale, S.T., Hahm, K., Merkenschlager, M., Fisher, A.G., 1997. Association of transcriptionally silent genes with Ikaros complexes at centromeric heterochromatin. Cell 91, 845–854.

Burgess, S.M., Kleckner, N., Weiner, B.M., 1999. Somatic pairing of homologs in budding yeast, existence and modulation. Gene Dev. 13, 1627–1641.

Capco, D.G., Wan, K.M., Penman, S., 1982. The nuclear matrix, three-dimensional architecture and protein composition. Cell 29, 847–858.

Carter, K.C., Bowman, D., Carrington, W., Fogarty, K., McNeil, J.A., Fay, F.S., Lawrence, J.B., 1993. A three-dimensional view of precursor messenger RNA metabolism within the mammalian nucleus. Science 259, 1330–1335.

Carvalho, C., Pereira, H.M., Ferreira, J., Pina, C., Mendonca, D., Rosa, A.C., Carmo-Fonseca, M., 2001. Chromosomal G-dark bands

determine the spatial organization of centromeric heterochromatin in the nucleus. Mol. Biol. Cell 12, 3563–3572.
Chaly, N., Munro, S.B., 1996. Centromeres reposition to the nuclear periphery during L6E9 myogenesis in vitro. Exp. Cell Res. 223, 274–278.
Chu, S., DeRisi, J., Eisen, M., Mulholland, J., Botstein, D., Brown, P.O., Herskowitz, I., 1998. The transcriptional program of sporulation in budding yeast. Science 282, 699–705.
Chubb, J.R., Boyle, S., Perry, P., Bickmore, W.A., 2002. Chromatin motion is constrained by association with nuclear compartments in human cells. Curr. Biol. 12, 439–445.
Chung, H.M., Shea, C., Fields, S., Taub, R.N., Van de Ploeg, L.H., Tse, D.B., 1990. Architectural organization in the interphase nucleus of the protozoan *Typanosoma brucei*, location of telomeres and mini-chromosomes. Eur. Mol. Biol. Org. J. 9, 2611–2619.
Coen, E.S., Carpenter, R., 1988. A semi-dominant allele, niv-525, acts in trans to inhibit expression of its wild-type homologue in *Antirrhinum majus*. Eur. Mol. Biol. Org. J. 7, 877–883.
Conklin, E.G., 1901. The individuality of the germ nuclei during the cleavage of the egg of crepidula. Biol. Bull. 2, 257–265.
Cowan, C.R., Carlton, P.M., Cande, W.Z., 2001. The polar arrangement of telomeres in interphase and meiosis. Rabl organization and the bouquet. Plant Physiol. 125, 532–538.
Cowan, C.R., Carlton, P.M., Cande, W.Z., 2002. Reorganization and polarization of the meiotic bouquet-stage cell can be uncoupled from telomere clustering. J. Cell Sci. 115, 3757–3766.
Cremer, T., Cremer, C., Baumann, H., Lüdtke, E.K., Sperling, K., Teuber, V., Zorn, C., 1982. Rabl's model of the interphase chromosome arrangement tested in Chinese hamster cells by premature chromosome condensation and laser-UV-microbeam experiments. Hum. Genet. 60, 46–56.
Cremer, M., von Hase, J., Volm, T., Brero, A., Kreth, G., Walter, J., et al., 2001. Non-random radial higher-order chromatin arrangements in nuclei of diploid human cells. Chromosome Res. 9, 541–567.
Croft, J.A., Bridger, J.M., Boyle, S., Perry, P., Teague, P., Bickmore, W.A., 1999. Differences in the localization and morphology of chromosomes in the human nucleus. J. Cell Biol. 145, 1119–1131.
Csink, A.K., Henikoff, S., 1996. Genetic modification of heterochromatic association and nuclear organization in *Drosophila*. Nature 381, 529–531.
Daigle, N., Beaudouin, J., Hartnell, L., Imreh, G., Hallberg, E., Lippincott-Schwartz, J., Ellenberg, J., 2001. Nuclear pore complexes form immobile networks and have a very low turnover in live mammalian cells. J. Cell Biol. 154, 71–84.
Dawe, R.K., Sedat, J.W., Agard, D.A., Cande, W.Z., 1994. Meiotic chromosome pairing in maize is associated with a novel chromatin organization. Cell 76, 901–912.
Dernburg, A.F., Broman, K.W., Fung, J.C., Marshall, W.F., Philips, J., Agard, D.A., Sedat, J.W., 1996. Perturbation of nuclear architecture by long-distance chromosome interactions. Cell 85, 745–759.
Duband-Goulet, I., Courvalin, J.C., 2000. Inner nuclear membrane protein LBR preferentially interacts with DNA secondary structures and nucleosomal linker. Biochemistry 39, 6483–6488.
Edelmann, P., Bornfleth, H., Zink, D., Cremer, T., Cremer, C., 2001. Morphology and dynamics of chromosome territories in living cells. Biochim. Biophys. Acta 1551, M29–M40.
Edgar, B.A., Schubiger, G., 1986. Parameters controlling transcriptional activation during early *Drosophila* development. Cell 44, 871–877.
Francastel, C., Walters, M.C., Groudine, M., Martin, D.I., 1999. A functional enhancer suppresses silencing of a transgene and prevents its localization close to centromeric heterochromatin. Cell 99, 259–269.
Fransz, P., De Jong, J.H., Lysak, M., Castiglione, M.R., Schubert, I., 2002. Interphase chromosomes in *Arabidopsis* are organized as well defined chromocenters from which euchromatin loops emanate. Proc. Natl Acad. Sci. USA 99, 14584–14589.
Fuentes-Mascorro, G., Serrano, H., Rosado, A., 2000. Sperm chromatin. Arch. Androl. 45, 215–225.
Funabiki, H., Hagan, I., Uzawa, S., Yanagida, M., 1993. Cell cycle-dependent specific positioning and clustering of centromeres and telomeres in fission yeast. J. Cell Biol. 121, 961–976.
Fung, J.C., Marshall, W.F., Dernburg, A., Agard, D.A., Sedat, J.W., 1998. Homologous chromosome pairing in *Drosophila melanogaster* proceeds through multiple independent initiations. J. Cell Biol. 141, 5–20.
Furukawa, K., 1999. LAP2 binding protein 1 (L2BP1/BAF) is a candidate mediator of LAP2-chromatin interaction. J. Cell Sci. 112, 2485–2492.
Galy, V., Olivo-Marin, J.C., Scherthan, H., Dove, V., Rascalou, N., Nehrbass, U., 2000. Nuclear pore complex in the organization of silent telomeric chromatin. Nature 403, 108–112.
Gasser, S.M., Laemmli, U.K., 1986. Cohabitation of scaffold binding regions with upstream/enhancer elements of three developmentally regulated genes of *D. melanogaster*. Cell 46, 521–530.
Gerasimova, T.I., Byrd, K., Corces, V.G., 2000. A chromatin insulator determines the nuclear localization of DNA. Mol. Cell 6, 1025–1035.
Gerlich, D., Beaudouin, J., Kalbfuss, B., Daigle, N., Eils, R., Ellenberg, J., 2003. Global chromosome positions are transmitted through mitosis in mammalian cells. Cell 112, 751–764.
Gillies, C.B., 1975. An ultrastructural analysis of chromosomal pairing in maize. Carlsberg Res. Commun. 40, 135–162.
Gindullis, F., Peffer, N.J., Meier, I., 1999. MAF1, a novel plant protein interacting with matrix attachment region binding protein MFP1, is located at the nuclear envelope. Plant Cell. 11, 1755–1768.
Gineitis, A.A., Zalenskaya, I.A., Yau, P.M., Bradbury, E.M., Zalensky, A.O., 2000. Human sperm telomere-binding complex involves histone H2B and secures telomere membrane attachment. J. Cell Biol. 151, 1591–1597.
Goldberg, M., Harel, A., Brandeis, M., Rechsteiner, T., Richmond, T.J., Weiss, A.M., Gruenbaum, Y., 1999. The tail domain of lamin Dm0 binds histones H2A and H2B. Proc. Natl Acad. Sci. USA 96, 2852–2857.
Gotta, M., Laroche, T., Formenton, A., Maillet, L., Scherthan, H., Gasser, S.M., 1996. The clustering of telomeres and colocalization with Rap1, Sir3, and Sir4 proteins in wild-type *Saccharomyces cerevisiae*. J. Cell Biol. 134, 1349–1363.
Grogan, J.L., Mohrs, M., Harmon, B., Lacy, D.A., Sedat, J.W., Locksley, R.M., 2001. Early transcription and silencing of cytokine genes underlie polarization of T helper cell subsets. Immunity 14, 205–215.
Guichet, A., Peri, F., Roth, S., 2001. Stable anterior anchoring of the oocyte nucleus is required to establish dorsoventral polarity of the *Drosophila* egg. Dev. Biol. 237, 93–106.
Guillemin, K., Williams, T., Krasnow, M.A., 2001. A nuclear lamin is required for cytoplasmic organization and egg polarity in *Drosophila*. Nat. Cell Biol. 3, 848–851.
Gunewardena, S., Rykowski, M.C., 2000. Direct evidence for interphase chromosome movement during the mid-blastula transition in *Drosophila*. Curr. Biol. 10, 285–288.
Haaf, T., Ward, D.C., 1995. Rabl orientation of CENP-B box sequences in *Tupaia belangeri* fibroblasts. Cytogenet. Cell Genet. 70, 258–262.
Haberlandt, G., 1887. Über die Beziehungen zwischen Funktion und Lage des Zellkerns, Fischer Verlag, Jena.
Hediger, F., Neumann, F.R., Van Houwe, G., Dubrana, K., Gasser, S.M., 2002a. Live imaging of telomeres: yKu and Sir proteins define redundant telomere-anchoring pathways in yeast. Curr. Biol. 12, 2076–2089.
Hediger, F., Dubrana, K., Gasser, S.M., 2002b. Myosin-like proteins 1 and 2 are not required for silencing or telomere anchoring, but act in the Tel1 pathway of telomere length control. J. Struct. Biol. 140, 79–91.
Henikoff, S., Jackson, J.M., Talbert, P.B., 1995. Distance and pairing effects on the brown Dominant heterochromatic element in *Drosophila*. Genetics 140, 1007–1017.
Hess, O., 1970. Genetic function correlated with unfolding of lampbrush loops by the Y chromosome in spermatocytes of *Drosophila hydei*. Mol. Gen. Genet. 106, 328–346.

Heun, P., Laroche, T., Shimada, K., Furrer, P., Gasser, S.M., 2001. Chromosome dynamics in the yeast interphase nucleus. Science 294, 2181–2186.

Hill, R.J., Whytock, S., 1993. Cytological structure of the native polytene salivary-gland nucleus of *Drosophila melanogaster*—a microsurgical analysis. Chromosoma 102, 446–456.

Hilliker, A.J., 1985. Assaying chromosome arrangement in embryonic interphase nuclei of *Drosophila melanogaster* by radiation induced interchanges. Genet. Res. 47, 13–18.

Hochstenbach, R., Wilbrink, M., Suijkerbuijk, R., Hennnig, W., 1993. Localization of the lampbrush loop pair Nooses on the Y chromosome of *Drosophila hydei* by fluorescence in situ hybridization. Chromosoma 102, 546–552.

Hochstrasser, M., Sedat, J.W., 1987. Three-dimensional organization of *Drosophila melanogaster* interphase nuclei. I. Tissue-specific aspects of polytene nuclear architecture. J. Cell Biol. 104, 1455–1470.

Hochstrasser, M., Mathog, D., Gruenbaum, Y., Saumweber, H., Sedat, J.W., 1986. Spatial organization of chromosomes in the salivary gland nuclei of *Drosophila melanogaster*. J. Cell Biol. 102, 112–123.

Hoefers, C., Baumann, P., Hummer, G., Jovin, T.M., Arndt-Jovin, D.J., 1993. The localization of chromosome domains in human interphase nuclei. Three-dimensional distance determinations of fluorescence in situ hybridization signals from confocal laser scanning microscopy. Bioimaging 1, 96–106.

ten Hoopen, R., Montijn, B.M., Veuskens, J.T., Oud, O.J., Nanninga, N., 1999. The spatial localization of T-DNA insertions in petunia interphase nuclei: consequences for chromosome organization and transgene insertion sites. Chromosome Res. 7, 611–623.

Houtsmuller, A.B., Oud, J.L., Montijn, M.B., Worring, M., Smeulders, A.W., Nanninga, N., 2000. Chromosome no. 1 of *Crepis capillaris* shows defined 3D-shapes in mitotic prophase. Chromosome Res. 8, 243–252.

Hulspas, R., Houtsmuller, A.B., Krijtenburg, P.J., Bauman, J.G., Nanninga, N., 1994. The nuclear position of pericentromeric DNA of chromosome 11 appears to be random in G0 and non-random in G1 human lymphocytes. Chromosoma 103, 286–292.

Hutchison, N., Weintraub, H., 1985. Localization of DNAase I-sensitive sequences to specific regions of interphase nuclei. Cell 43, 471–482.

Ishii, K., Laemmli, U.K., 2003. Structural and dynamic functions establish chromatin domains. Mol. Cell. 11, 237–248.

Ishii, K., Arib, G., Lin, C., Van Houwe, G., Laemmli, U.K., 2002. Chromatin boundaries in budding yeast, the nuclear pore connection. Cell 109, 551–562.

Jin, Q.W., Fuchs, J., Loidl, J., 2000. Centromere clustering is a major determinant of yeast interphase nuclear organization. J. Cell Sci. 113, 1903–1912.

Kleene, K.C., 2001. A possible meiotic function of the peculiar patterns of gene expression in mammalian spermatogenic cells. Mech. Dev. 106, 3–23.

Kozubek, S., Lukásová, E., Marecková, A., Skalníková, M., Kozubek, M., Bártová, E., et al., 1999. The topological organization of chromosomes 9 and 22 in cell nuclei has a determinative role in the induction of t(9, 22) translocations and in the pathogenesis of t(9,22) leukemias. Chromosoma 108, 426–435.

Laroche, T., Martin, S.G., Gotta, M., Gorham, H.C., Pryde, F.E., Louis, E.J., Gasser, S.M., 1998. Mutation of yeast Ku genes disrupts the subnuclear organization of telomeres. Curr. Biol. 8, 653–656.

LaSalle, J.M., Lalande, M., 1996. Homologous association of oppositely imprinted chromosomal domains. Science 272, 725–728.

Lei, Y., Warrior, R., 2000. The *Drosophila* Lissencephaly1 (DLis1) gene is required for nuclear migration. Dev. Biol. 226, 57–72.

Leitch, A.R., Brown, J.K., Mosgoller, W., Schwarzacher, T., Heslop-Harrison, J.S., 1994. The spatial localization of homologous chromosomes in human fibroblasts at mitosis. Hum. Genet. 93, 275–280.

Li, Y., Danzer, J.R., Alvarez, P., Belmont, A., Wallrath, L.L., 2003. Effects of tethering HP1 to euchromatic regions of the *Drosophila* genome. Development 130, 1817–1824.

Linares-Cruz, G., Bruzzoni-Giovanelli, H., Alvaro, V., Roperch, J.P., Tuynder, M., Schoevaert, D., et al., 1998. p21WAF-1 reorganizes the nucleus in tumor suppression. Proc. Natl Acad. Sci. USA 95, 1131–1135.

Lopez, J.M., Wolfner, M.F., 1997. The developmentally regulated *Drosophila* embryonic nuclear lamina protein 'Young Arrest' fs(1)Ya is capable of associating with chromatin. J. Cell Sci. 110, 643–651.

Luderus, M.E., den Blaauwen, J.L., de Smit, O.J., Compton, D.A., van Driel, R., 1994. Binding of matrix attachment regions to lamin polymers involves single-stranded regions and the minor groove. Mol. Cell Biol. 14, 6297–6305.

Manuelidis, L., Borden, J., 1988. Reproducible compartmentalization of individual chromosome domains in human CNS cells revealed by in situ hybridization and three-dimensional reconstruction. Chromosoma 96, 397–410.

Marshall, W.F., 2002. Order and disorder in the nucleus. Curr. Biol. 12, R185–R192.

Marshall, W.F., Dernburg, A.F., Harmon, B., Agard, D.A., Sedat, J.W., 1996. Specific interactions of chromatin with the nuclear envelope, positional determination within the nucleus in *Drosophila melanogaster*. Mol. Biol. Cell 7, 825–842.

Marshall, W.F., Straight, A., Marko, J.F., Swedlow, J., Dernburg, A., Belmont, A., et al., 1997. Interphase chromosomes undergo constrained diffusional motion in living cells. Curr. Biol. 7, 930–939.

Manuelidis, L., Borden, J., 1988. Reproducible compartmentalization of individual chromosome domains in human CNS cells revealed by in situ hybridization and three-dimensional reconstruction. Chromosoma 96, 397–410.

Masuzawa, N., Urata, Y., Yagi, K., Ashihara, T., 2000. Constrained, random, and independent motion of texas-red labeled chromatin in living interphase PtK2 cells. Acta Histochem. Cytochem. 33, 419–427.

Mathe, E., Boros, I., Josvay, K., Li, K., Puro, J., Kaufman, T.C., Szabad, J., 1998. The Tomaj mutant alleles of alpha Tubulin67C reveal a requirement for the encoded maternal specific tubulin isoform in the sperm aster, the cleavage spindle apparatus and neurogenesis during embryonic development in *Drosophila*. J. Cell Sci. 111, 887–896.

Meneghini, M.D., Wu, M., Madhani, H.D., 2003. Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. Cell 112, 725–736.

Moenkhaus, W.J., 1904. The development of the hybrids between *Fundulus heteroclitus* and *Medidia notata* with especial reference to the behavior of the maternal and paternal chromatin. Am. J. Anat. 3, 29–65.

Morris, N.R., 2000. Nuclear migration. From fungi to the mammalian brain. J. Cell Biol. 148, 1097–1101.

Murray, A.B., Davies, H.G., 1979. Three-dimensional reconstruction of the chromatin bodies in the nuclei of mature erythrocytes from the newt *Triturus cristatus*, the number of nuclear envelope-attachment sites. J. Cell Sci. 35, 59–66.

Nagele, R., Freeman, T., McMorrow, L., Lee, H.Y., 1995. Precise spatial positioning of chromosomes during prometaphase, evidence for chromosomal order. Science 270, 1831–1835.

Narlikar, G.J., Fan, H.Y., Kingston, R.E., 2002. Cooperation between complexes that regulate chromatin structure and transcription. Cell 108, 475–487.

Neves, H., Ramos, C., da Silva, M.G., Parreira, A., Parreira, L., 1999. The nuclear topography of ABL, BCR, PML, and RAR alpha genes, evidence for gene proximity in specific phases of the cell cycle and stages of hematopoietic differentiation. Blood 93, 1197–1207.

Nikiforova, M.N., Stringer, J.R., Blough, R., Medvedovic, M., Fagin, J.A., Nikiforov, Y.E., 2000. Proximity of chromosomal loci that participate in radiation-induced rearrangements in human cells. Science 290, 138–141.

Nili, E., Cojocaru, G.S., Kalma, Y., Ginsberg, D., Copeland, N.G., Gilbert, D.J., et al., 2001. Nuclear membrane protein LAP2beta mediates transcriptional repression alone and together with its binding partner GCL (germ-cell-less). J. Cell Sci. 114, 3297–3307.

Oegema, K., Marshall, W.F., Sedat, J.W., Alberts, B.M., 1997. Two proteins that cycle asynchronously between centrosomes and nuclear structures, *Drosophila* CP60 and CP190. J. Cell Sci. 110, 1573–1583.

Paddy, M.R., Belmont, A.S., Saumweber, H., Agard, D.A., Sedat, J.W., 1990. Interphase nuclear envelope lamins form a discontinuous network that interacts with only a fraction of the chromatin in the nuclear periphery. Cell 62, 89–106.

Palladino, F., Laroche, T., Gilson, E., Axelrod, A., Pillus, L., Gasser, S.M., 1993. SIR3 and SIR4 proteins are required for the positioning and integrity of yeast telomeres. Cell 75, 543–555.

Pitt, J.N., Schisa, J.A., Priess, J.R., 2000. P granules in the germ cells of *Caenorhabditis elegans* adults are associated with clusters of nuclear pores and contain RNA. Dev. Biol. 219, 315–333.

Quick, P., 1980. Junctions of polytene chromosomes and the inner nuclear membrane. Experientia 36, 456–457.

Rasmussen, S.W., 1976. The meiotic prophase in *Bombyx mori* females analyzed by three dimensional reconstructions of synaptonemal complexes. Chromosoma 54, 245–293.

Reugels, A.M., Kurek, R., Lammermann, U., Bunemann, H., 2000. Mega-introns in the dynein gene DhDhc7(Y) on the heterochromatic Y chromosome give rise to the giant threads loops in primary spermatocytes of *Drosophila hydei*. Genetics 154, 759–769.

Richter, J.D., Paris, J., McGrew, L.L., 1990. Maternal mRNA expression in early development, regulation at the 3′ end. Enzyme 44, 129–146.

Riechmann, V., Ephrussi, A., 2001. Axis formation during *Drosophila* oogenesis. Curr. Opin. Genet. Dev. 11, 374–383.

Robinett, C.C., Straight, A.F., Li, G., Wilhelm, C., Sudlow, G., Murray, A., Belmont, A.S., 1996. In vivo localization of DNA sequences and visualization of large-scale chromatin organization using lac operator/repressor recognition. J. Cell Biol. 135, 1685–1700.

Sachs, R.K., Hlatky, L.R., Trask, B.J., 2000. Radiation-produced chromosome aberrations, colourful clues. Trends Genet. 16, 143–146.

Santos, A.P., Abranches, R., Stoger, E., Beven, A., Viegas, W., Shaw, P.J., 2002. The architecture of interphase chromosomes and gene positioning are altered by changes in DNA methylation and histone acetylation. J. Cell Sci. 115, 4597–4605.

Sass, G.L., Henikoff, S., 1999. Pairing-dependent mislocalization of a *Drosophila* brown gene reporter to a heterochromatic environment. Genetics 152, 595–604.

Scherthan, H., Loidl, J., Schuster, T., Schweizer, D., 1992. Meiotic chromosome condensation and pairing in *Saccharomyces cerevisiae* studied by chromosome painting. Chromosoma 101, 590–595.

Scholten, S., Lorz, H., Kranz, E., 2002. Paternal mRNA and protein synthesis coincides with male chromatin decondensation in maize zygotes. Plant J. 32, 221–231.

Selvin, P.R., Scalettar, B.A., Langmore, J.P., Axelrod, D., Klein, M.P., Hearst, J.E., 1990. A polarized photobleaching study of chromatin reorientation in intact nuclei. J. Mol. Biol. 214, 911–922.

Shaw, P.J., Abranches, R., Paula Santos, A., Beven, A.F., Stoger, E., Wegel, E., Gonzalez-Melendi, P., 2002. The architecture of interphase chromosomes and nucleolar transcription sites in plants. J. Struct. Biol. 140, 31–38.

Shelby, R.D., Hahn, K.M., Sullivan, K.F., 1996. Dynamic elastic behavior of alpha-satellite DNA domains visualized in situ in living human cells. J. Cell Biol. 135, 545–557.

Skaer, R.J., Whytock, S., Emmines, J.P., 1976. Intranuclear electrophoresis of the chromatin of living cells. J. Cell Sci. 21, 479–496.

Skalnikova, M., Kozubek, S., Lukasova, E., Bartova, E., Jirsova, P., Cafourkova, A., et al., 2000. Spatial arrangement of genes, centromeres and chromosomes in human blood cell nuclei and its changes during the cell cycle, differentiation and after irradiation. Chromosome Res. 8, 487–499.

Stein, G.S., van Wijnen, A.J., Stein, J.L., Lian, J.B., Bidwell, J.P., Montecino, M., 1994. Nuclear architecture supports integration of physiological regulatory signals for transcription of cell growth and tissue-specific genes during osteoblast differentiation. J. Cell Biochem. 55, 4–15.

Sukegawa, J., Blobel, G., 1993. A nuclear pore complex protein that contains zinc finger motifs, binds DNA, and faces the nucleoplasm. Cell 72, 29–38.

Sun, H.B., Yokota, H., 1999. Correlated positioning of homologous chromosomes in daughter fibroblast cells. Chromosome Res. 7, 603–610.

Swedlow, J.R., Sedat, J.W., Agard, D.A., 1993. Multiple chromosomal populations of topoisomerase II detected in vivo by time-lapse, three-dimensional wide-field microscopy. Cell 73, 97–108.

Talbert, P.B., LeCiel, C.D., Henikoff, S., 1994. Modification of the *Drosophila* heterochromatic mutation brown Dominant by linkage alterations. Genetics 136, 559–571.

Taniura, H., Glass, C., Gerace, L., 1995. A chromatin binding site in the tail domain of nuclear lamins that interacts with core histones. J. Cell Biol. 131, 33–44.

Therman, E., Susman, B., Denniston, C., 1989. The nonrandom participation of human acrocentric chromosomes in Robertsonian translocations. Ann. Hum. Genet. 53, 49–65.

Tumbar, T., Belmont, A.S., 2001. Interphase movements of a DNA chromosome region modulated by VP16 transcriptional activator. Nat. Cell Biol. 3, 134–139.

Vazquez, J., Belmont, A.S., Sedat, J.W., 2001. Multiple regimes of constrained chromosome motion are regulated in the interphase *Drosophila* nucleus. Curr. Biol. 11, 1227–1239.

Vielle-Calzada, J.P., Baskar, R., Grossniklaus, U., 2000. Delayed activation of the paternal genome during seed development. Nature 404, 91–94.

Vourc'h, C., Taruscio, D., Boyle, A.L., Ward, D.C., 1993. Cell cycle-dependent distribution of telomeres, centromeres, and chromosome-specific subsatellite domains in the interphase nucleus of mouse lymphocytes. Exp. Cell Res. 205, 142–151.

Weiler, K.S., Wakimoto, B.T., 1998. Chromosome rearrangements induce both variegated and reduced, uniform expression of heterochromatic genes in a development-specific manner. Genetics 149, 1451–1464.

Wilkie, G.S., Shermoen, A.W., O'Farrell, P.H., Davis, I., 1999. Transcribed genes are localized according to chromosomal position within polarized *Drosophila* embryonic nuclei. Curr. Biol. 9, 1263–1266.

Wu, C.T., Morris, J.R., 1999. Transvection and other homology effects. Curr. Opin. Genet. Dev. 9, 237–246.

Yatsu, N., 1905. The formation of centrosomes in enucleated egg-fragments. J. Exp. Zool. 2, 287–312.

Ye, Q., Worman, H.J., 1994. Primary structure analysis and lamin B and DNA binding of human LBR, an integral protein of the nuclear envelope inner membrane. J. Biol. Chem. 269, 11306–11311.

Zalensky, A.O., Allen, M.J., Kobayashi, A., Zalenskaya, I.A., Balhorn, R., Bradbury, E.M., 1995. Well-defined genome architecture in the human sperm nucleus. Chromosoma 103, 577–590.

Zickler, D., 1977. Development of the synaptonemal complex and the 'recombination nodules' during meiotic prophase in the seven bivalents of the fungus *Sordaria macrospora* Auersw. Chromosoma 61, 289–316.

Zink, D., Cremer, T., Saffrich, R., Fischer, R., Trendelenburg, M.G., Ansorge, W., Stelzer, E.H., 1998. Structure and dynamics of human interphase chromosome territories in vivo. Hum. Genet. 102, 241–251.