Cell. Mol. Life Sci. 64 (2007) 131–138
1420-682X/07/020131-8
DOI 10.1007/s00018-006-6465-0

Cellular and Molecular Life Sciences

Regulation of telomere movement by telomere chromatin structure

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Online First 10 January 2007

Abstract. Beyond their role in replication and chromosome end capping, telomeres are also thought to function in meiotic chromosome pairing, meiotic and mitotic chromosome segregation as well as in nuclear organization. Observations in both somatic and meiotic cells suggest that the positioning of telomeres within the nucleus is highly specific and believed to be dependent mainly on telomere interactions with the nuclear envelope either directly or through chromatin interacting proteins. Although little is known about the mechanism of telomere clustering, some studies show that it is an active process. Recent data have suggested a regulatory role for telomere chromatin structure in telomere movement. This review will summarize recent studies on telomere interactions with the nuclear matrix, telomere chromatin structure and factors that modify telomere chromatin structure as related to regulation of telomere movement.

Keywords. Telomere chromatin structure, telomere binding factors, ATM, telomere nuclear matrix interactions, telomere movement.

Introduction

Telomeres are structurally and functionally complex. They consist of an array of simple DNA repeats at the extreme end of the chromosome, with a more complex array of repeats adjacent to it. A large number of proteins have been identified that bind to telomeric DNA repeats or to the protein complexes that are built at the chromosome end. Telomeric repeats are believed to be an essential and sufficient *cis*-element for telomere function. Telomeres share several features characteristic of heterochromatin, but information about their structure is still incomplete. Chromatin structure is an important factor for determining protein-DNA interactions, with consequences for DNA metabolism and transcription control [1–4]. Since the emergence of the nucleosome model of chromatin, there has been considerable progress in elucidating how chromatin structure at the level of nucleosome organization can either repress or potentiate transcription [5, 6]. Although telomeres do not contain any active genes, telomere chromatin structure could influence the interaction of telomeres with the nuclear matrix and expression of genes in subtelomeric regions. In the mammalian interphase nucleus, telomeres appear to be attached to the filamentous nuclear matrix [7], while in budding yeast cells, telomeres are clustered in a few perinuclear chromatin domains [8]. In many cells chromosome arms are sequestered within the nucleus, and they may be aligned from telomere to centromere. This arrangement may be maintained in part by associations of telomeres with each other and with the nuclear envelope (Fig. 1). There is reason to believe that the resulting nuclear domains are important for establishing or maintaining chromatin structure and transcriptional activity. Dramatic changes in chromosomal position within the nucleus may occur, for example, at the beginning of meiosis. Telomeres may play a profound role in these movements, which are critical for meiotic recombination and segregation. Here we present an overview of the telomere chromatin structure in the biology of the cell with an emphasis on telomere movement.

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Telomere chromatin structure

Telomeric DNA is mostly composed of double-stranded TTAGGG repeats, which are necessary for telomere

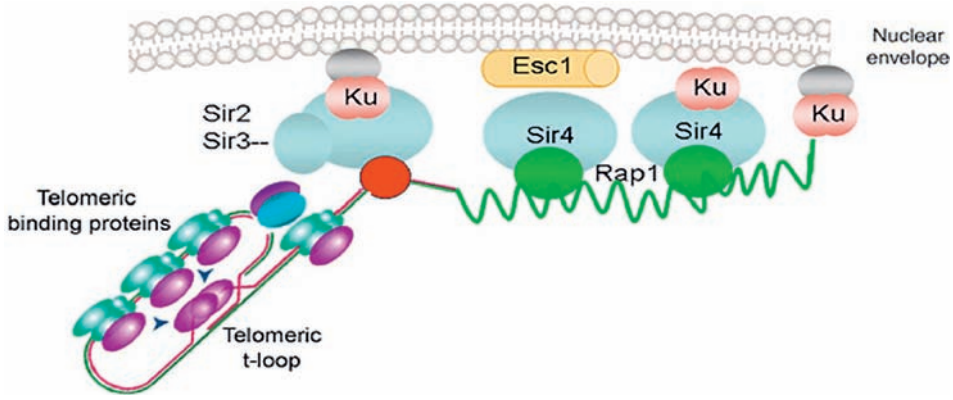

Figure 1. Model representing the interaction of telomeres with the nuclear matrix.

function in somatic cells [9, 10]. The termini of human telomeres carry an overhang (~300 nucleotides) of single-stranded 3′ DNA [10]. Telomere movement certainly will be dependent upon their stability and recognition of physiological signals generated during transcription, replication and segregation. Telomeres and telomere-capping proteins allow cells to distinguish natural chromosome ends from damaged DNA. Disruption of telomeric function can trigger a DNA damage response, including p53-dependent apoptosis [11]. Overloading of DNA repair activities can also threaten the integrity of chromosome ends, thereby leading to extensive genome instability [12, 13]. Telomeric proteins stabilize telomeres by protecting the single-stranded overhang from degradation or by remodeling the telomeres into a t-loop structure [13, 14]. Invasion of the single-stranded overhang into the adjacent double-stranded telomeric tract forms the t-loop structure. At the ends of human chromosomes, three interconvertible states may exist: t-loops, POT1 capping and engagement with telomerase [3]. The telomeric complex contains a number of proteins, and it will be important to investigate which members of the complex have a direct role in meiosis and telomere movement. Proteins such as TRF1, TRF2, Rap1, Ndj1 and Taz1 are found at meiotic telomeres, and genetic studies indicate that these proteins are important for meiotic telomere functions. TRF1, TRF2 and Rap1 form a complex with three other telomeric proteins, POT1, TIN2 and TPP1 (referred to as shelterin), which allows cells to distinguish telomeres from sites of DNA damage [15]. Without the protective activity of shelterin, telomeres are no longer hidden from the DNA damage surveillance mechanism, and chromosome ends are inappropriately processed as double-strand breaks by DNA repair pathways which could lead to telomere dysfunction (Fig. 2) [16]. The current data argue that shelterin is not a static structural component of the telomere. Instead, shelterin is emerging as a protein complex with DNA remodeling activity that acts together with several associated DNA repair factors to change the structure of the telomeric DNA, thereby protecting chromosome ends, and thus regulate telomere movement. A key point to address in the future is how these proteins are involved either directly or indirectly in meiotic telomere movement and functional organization in mitotic cells.

Telomeric sites are implicated in establishing functional nuclear chromatin organization. Studies of the dynamic behavior of telomeric DNA repeats in living human osteosarcoma U2OS cells indicated a majority of telomeres

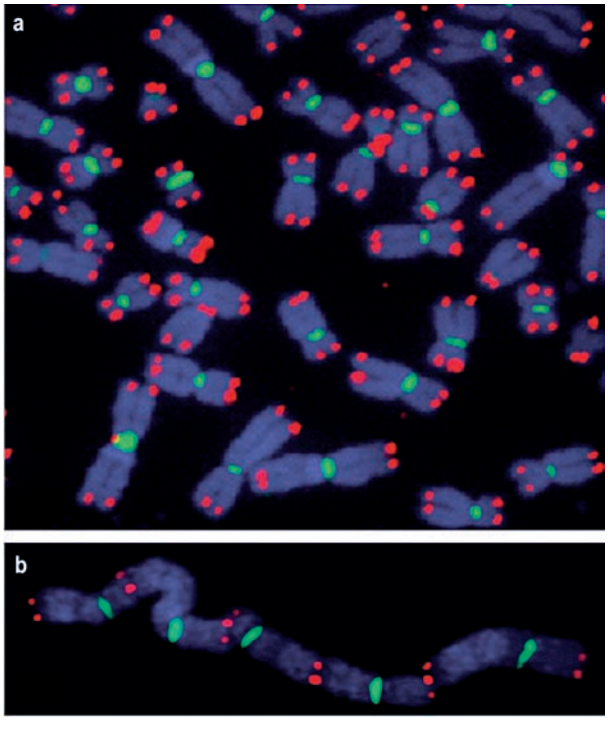

Figure 2. Telomere FISH analysis showing human metaphase chromosomal spreads. Telomere signals are red and centromere signals are green. (*a*) represents the metaphase section of control 293 cells and (*b*) the metaphase of 293 cells with expression of mutant TRF2$^{\Delta B\Delta M}$ and knockdown of hRad9 (note almost all chromosomes undergo telomere fusion).

had constrained diffusive movement, while individual telomeres demonstrated significant directional movement [17]. Intriguingly, a subfraction of telomeres were shown to associate and dissociate, suggesting that *in vivo* telomere clusters are not static but dynamic structures. This phenomenological observation, although mechanistically vague, might have important biological/functional implications. Telomeres have also been observed to associate with promyelocytic leukemia (PML) bodies, with a subset of telomeres again having a higher degree of mobility [17]. Association of telomeres with PML bodies may explain the high incidence of chromosomal rearrangements in somatic cell subtelomeric regions [18, 19] and for recombination-based interchromosomal telomeric DNA exchanges in ALT cells [20].

Status of nucleosomal organization of telomere chromatin

Chromatin structure is an important factor regulating protein-DNA interactions, and there is a difference between nucleosomal organization of bulk DNA as compared with telomeres, where telomeric histone H4 is hypoacetylated (reviewed in [21, 22]). Interestingly, ataxia-telangiectasia (A-T) cells show relatively higher levels of micrococcal nuclease (MNase) digestion of chromatin as compared with normal cells [23]. In fact, cells from normal control individuals have a telomere chromatin MNase digestion nucleosomal ladder, each containing partials up to seven subunits, whereas A-T cells revealed a less extensive MNase periodicity, and telomeric nucleosomal arrays with up to three subunits are detected. These results suggest that telomeric nucleosome arrays in A-T cells are less uniformly spaced or extend over a smaller region than arrays of normal cells [23]. Ataxia-telangiectasia mutated (ATM) has been shown to be associated with chromatin, and a fraction of it is detected in nuclear aggregates [24–26]. However, it is not clear how inactivation of ATM could lead to altered nucleosomal periodicity. As mentioned above, the telomeric histone H4 is hypoacetylated, and ATM does interact with chromatin-modifying factors [27]. It is possible that ATM regulates nucleosomal periodicity, which could influence telomere movement.

Telomere association with nuclear matrix

Cytological observation of telomere associations with the nuclear matrix has a long history, as Rabl described the proximity of telomeres to the nuclear envelope (Fig. 1) in amphibia as early as 1885 [28]. However, it is still unclear whether the relationship between telomeres and the nuclear envelope is a passive consequence of anaphase chromosome orientation or the result of an active process that maintains an ordered nuclear architecture during interphase. In meiosis, remarkable chromosomal reorganization and telomere clustering is a universally conserved feature of the pre-pachytene 'bouquet' stage of meiosis (for details see [29]). Several lines of evidence establish that one of the most likely functions of the bouquet is to ensure the efficient initiation of synapsis of homologous chromosomes.

Direct evidence that chromosomes are restricted to subdomains in *Drosophila melanogaster* interphase nuclei comes from examination of the arrangement of polytene chromosomes. Individual chromosome arms never intertwine, and telomeres tend to cluster at the side of the nucleus opposite the chromocenter [30]. With probes that paint whole chromosomes, it was shown that individual chromosomes occupy distinct positions [31, 32]. The organization of telomeres in embryonic diploid *D. melanogaster* nuclei has also been investigated; again, a highly polarized configuration was revealed [31, 33, 34].

Biochemical evidence for interaction of telomeres with the nuclear matrix

Mammalian telomeres are packaged in telomere-specific chromatin [21, 22]. Human and mouse cell lines have their telomeric tracts attached to the nuclear matrix, which is a proteinaceous subnuclear fraction [23, 35, 36]. Telomere-length homeostasis in yeast requires the binding of a protein along the telomeric tract [37–39], and changes in the telomeric protein complex influence the stability of chromosome ends [40]. In mammals, a nuclear matrix binding site occurs at least once in every kilobase pair of the telomere tract [36], suggesting that mammalian telomeres have frequent, multiple interactions with the nuclear matrix (Fig. 1).

Factors linked with telomere movement

Telomeres tend to form associations with each other. These associations have been implicated in the formation of nuclear domains that may be important for transcriptional regulation, sister chromatid pairing at mitosis and for homologous meiotic synapsis. These functions do involve telomere movement, which may depend on the status of telomere structure.

Telomere clustering is often adjacent to the nuclear envelope, although it is distributed independent of nuclear pores. It contributes to the global positioning of interphase chromosomes. The clustering of telomeres in budding yeast contributes to the repression of subtelomeric chromatin, conferring a telomere position effect (TPE), which resembles the position-effect variegation (PEV) nucleated by centromeric repeats in flies. Indeed, it is thought that the mechanisms that bring fission and budding yeast telomeres together are likely to provide paradigms for other repeat-dependent interactions. Telomere nuclear envelope association occurs through two redun-

dant mechanisms: one dependent on the Ku heterodimer and the second on the silencing factor Sir4. Sir4 has the 'partitioning and anchoring domain' (PAD) [41], which is adjacent to the lamin-like carboxyl terminus of Sir4 and binds specifically to the protein Esc1 (Establishes Silent Chromatin) [42]. Esc1 is a large acidic protein localized along the inner nuclear membrane at interpore spaces. *In vivo* a non-silent telomere can be localized at the nuclear envelope through interaction with Ku; and being thus placed in a zone enriched for Sir proteins, this telomere is thought to have a better chance to become repressed [4]. When targeted to DNA, the Ku80 subunit is able to relocalize DNA to the nuclear periphery in a manner independent of Sir4 or Esc1 [8, 43]. The efficiency of Ku80-mediated anchorage varies between G1 and S phases of the cell cycle, being stronger in G1. This mechanism may reflect a link between telomere anchoring and telomere end protection. Once the Sir-dependent chromatin is established, silencing itself will reinforce the perinuclear localization through the Sir4 anchoring pathway. In this way, the formation of silent chromatin is self-reinforcing and contributes to the spatial positioning of telomeres.

In mammalian cells, several protein factors influence the telomere/nuclear matrix interaction, such as inactivation of ATM or 14-3-3-sigma or ectopic expression of hTERT [23, 44, 45]. For example, inactivation of ATM function was found to enhance the frequency of chromosome end associations, telomere loss and increased telomere nuclear matrix interactions [23, 46]. Interestingly both somatic and germ cells deficient in ATM function had more than 80% of the telomeric DNA attached to the nuclear matrix whereas in control cells only about 50% is attached to the nuclear matrix [23, 47]. It is not clear at present how inactivation of ATM enhances the association of telomeres with the nuclear matrix. It is possible that inactivation of ATM modifies the telomere chromatin structure, the nuclear matrix or even an intermediate protein factor linking the two. Recently, several chromatin-modifying factors interacting with ATM have been reported. The most promising ATM interacting chromatin modifying candidate that could influence telomere chromatin structure is hMOF, the human ortholog of the *Drosophila* MOF gene (males absent on the first), encoding a protein with histone acetyltransferase activity, and whose target substrate is lysine 16 (K16) of histone H4 [27].

Role of telomere-interacting proteins in telomere movement

Telomeres interact not only with the matrix and nuclear envelope, but also with each other (Figs. 1, 3). For example, yeast cells immunostained with antibodies against Rap1p exhibit fewer spots than the expected number of telomeres, suggesting telomere clustering [48]. Rap1p is localized to the nuclear periphery [48] and colocalizes with Sir3p, Sir4p and with Y′ telomere-associated DNA [49, 50]. Genetic and biochemical evidence strongly suggest that Rap1p, Sir3p and Sir4p form a multiprotein complex. Rap1p has a dispersed nuclear staining in *sir3* and *sir4* mutants; the normal focal pattern of Sir3p staining is diffuse in a *sir4* mutant. Similarly, Sir4p staining is no longer punctate in a *sir3* mutant. Telomeres, however, are still clustered in these mutants as detected by the telomere-associated DNA sequence Y′. One explanation is that proteins other than Sir3p and Sir4p may be involved in the localization of the telomeres [51]. According to the model of Maillet et al. [50] compartmentalization enables bifunctional proteins such as Rap1p and Abf1p, which can both activate and repress transcription, to perform both functions simultaneously in the same nucleus. Additionally, peripheral localization of telomeres helps to anchor the chromosomes, preventing their reorganization during interphase [52].

Although telomeres themselves may not mediate chromosome segregation, the separation of telomeres during cell division creates a special problem for the segregational system [53]. Evidence that *cis*-acting functions are required for the separation of telomeres has been obtained for *Tetrahymena*. Altering the telomerase RNA template in *Tetrahymena* from GGGGTT to GGGGTTTT can create a block in anaphase chromosome separation in the micronucleus [54]. Cytological analysis revealed a failure of telomere separation of sister chromatids. Stretched chromosomes were often seen as one continuous fiber passing through the midzone of the spindle. Such observations propose that telomeres on sister chromatids are normally associated until metaphase and that the defective telomeres prevent telomere separation. Thus, a specific element of the telomere repeat may be required in *cis* to mediate chromatid separation. Intensive research is ongoing to identify such *cis* elements and determine whether these modify the telomere chromatin structure. For example, mutations in the *UbcD1* gene, which encodes a class I ubiquitin-conjugating enzyme, cause telomere-telomere attachments during both mitosis and male meiosis [55]. In these mutants, telomeres associate inappropriately with the telomeres of their sister chromatids and with telomeres of both homologous and nonhomologous chromosomes. The Sir4p of yeast telomeres also binds the deubiquitinating enzyme Ubp3p, suggesting that telomeric protein ubiquitination is a general phenomenon [56].

More interesting is the possibility that telomeres may be involved in adherence of sister chromatids to one another until anaphase. Normal telomere-telomere associations, seen cytologically in a variety of organisms, could be mediated through single-stranded DNA tails [57] or telomere proteins. The latter include the TBP proteins of ciliates, the Rap1 or Sir proteins of yeast, and the TRF of mammals [9]. There are three ways in which

proteins could mediate telomere-telomere associations [58]. First, dimers or multimers of telomere-binding proteins might be able to associate simultaneously with two or more telomeres. Second, proteins may link telomeres indirectly, through a third element such as a component of the nuclear envelope. Several potential receptors for chromatin have been identified that localize specifically to the inner nuclear membrane during interphase. Lamin-binding proteins LAP2 and LBR associate rapidly with chromosomes and the reassembling nuclear envelope during anaphase and telophase. LBR binds to two human homologs of the *D. melanogaster* heterochromatin protein HP1, which is also found at telomeres. The third variation suggests that telomere proteins might facilitate DNA-DNA interactions between telomeres. Oligonucleotides of single-stranded, G-rich, telomeric DNA from ciliates, vertebrates and yeast form alternative DNA structures *in vitro* that depend on non-canonical base pairing of the guanines [59]. The *beta*-subunit of the *Oxytricha* telomere protein and Rap1p of yeast have the ability to fold or stabilize telomeric DNA in G-quartet structures that mediate telomere-telomere association *in vitro* [57]. Linear plasmids form telomere-telomere interactions *in vitro*, similar to those on molecules isolated from yeast, but only if their ends have a TG1-3 tail. Wellinger et al. [60] argue that telomere-telomere interactions involve duplex DNA held together by G:G base pairs, rather than a triple helix or G quartet. TG1-3 tails and telomere-telomere interactions were detected *in vivo* in strains lacking telomerase, suggesting that telomerase-independent mechanisms generate TG1-3 tails at the end of S-phase by cell cycle-regulated degradation of the C1-3A strand. The resulting single-stranded tails are a potential substrate for telomerase, other telomere-binding proteins and can influence telomere movement.

Chromatin-modifying factors and telomere chromatin structure

In higher eukaryotic cells, a portion of the transcriptionally inactive heterochromatin, including that of telomeres, is associated with a structure called the nuclear matrix (Fig. 1) [61–63]. Conserved heterochromatin proteins (HPs), which contain a characteristic chromodomain, play a critical role in establishing and maintaining these heterochromatic domains [64]. Heterochromatin proteins are localized on three different chromosomal sites [65]. In mammals, chromodomain-containing proteins appear to be either structural components of large macromolecular chromatin complexes or proteins involved in remodeling the chromatin structure. The isoforms of HP1 (HP1α, HP1β and HP1γ), heteromers that have been shown to be associated with nucleosomal core histones [66] and to reduce transcription of nearby promoters when directly tethered to DNA [67]. HP1 has been suggested to be a conserved component of the highly compact chromatin of centromeres and telomeres in *Drosophila* [68]. In addition, mutation of HP1 results in telomeric fusions [69], and ectopic overexpression results in increased end associations [70]. It is thought that the proteins encoded by the HP1 class of the conserved chromobox genes are primarily involved in the packaging of chromosomal domains into a repressive heterochromatic state. Thus, it is possible that HP1 proteins may have a role, too, in the regulation of telomere movement.

Regulation of telomere movement in meiosis

Telomeres have also been considered key structures of meiotic chromosomes [47, 52, 71, 72]. Meiosis is a specialized cell division that ensures the proper segregation of genetic material and formation of viable haploid gametes. During early meiotic prophase telomeres redistribute and accumulate at a limited sector of the nuclear envelope to form a chromosomal bouquet as telomeres gather near the centrosome (for reviews, see [7]). The most critical events of meiosis occur during prophase I, when homologous chromosomes become aligned (prealign), synapse (pair) and recombine with each other. A number of studies suggest that bouquet formation mediates prealignment of homologues and thereby facilitates synapsis (reviewed in [29]). ATM has been shown to be involved with the dispersal of telomeres from the telomere clusters in spermatocytes, as the inactivation of ATM results in the aberrant accumulation of cells with clustered telomeres (Fig. 3) [47]. The only known telomeric proteins that have been implicated in bouquet formation are the products of Taz1 in fission yeast [73, 74] and Ndj1/Tam1 in budding yeast [75–77]. Whether such proteins are involved in the regulation of telomere structure during telomere movement is yet unknown, as the mechanisms by which cells collect telomeres from disparate regions of the nucleus and pull each chromosome into the bouquet have remained elusive.

Abundant cytological evidence suggests that the localization of telomeres during meiosis is distinct from that ob-

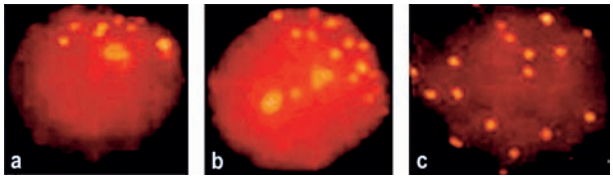

Figure 3. Spermatocytes showing telomere clustering and subsequent separation of telomeres in the postleptotene stage of meiosis prophase I as detected by the TTAGGG probe and propidium iodide used as counterstain. (*a*) Spermatocytes of control mice at the leptotene/zygotene stage. (*b*, *c*) Spermatocytes showing increased telomere signal numbers; such telomere dispersal at the pachytene was rarely seen in $Atm^{-/-}$ spermatocytes.

served in somatic nuclei. In meiotic cells telomeres cluster at zygotene to form a so-called bouquet. The bouquet arrangement of chromosomes has been noted in a variety of organisms [52]. This nuclear arrangement is likely to be functionally linked to the process of homologous pairing and synapsis. During the period in which telomeres form a cluster, there is also evidence that they are tightly attached to the nuclear envelope and possibly interact with the cytoskeleton. The most dramatic evidence for the importance of these associations is chromosomal pairing in the fission yeast [78], where prophase chromosome movement apparently is directed by the telomeres. A gene *NDJ1* encodes a telomere-associated protein required for meiotic chromosome segregation in *S. cerevisiae* [76]. This protein accumulates at the telomeres during meiotic prophase, and its absence results in high levels of failed meiotic chromosome segregation. The *ndj1* mutant phenotype includes delays in the formation of synaptonemal complex axial elements, in synapsis and in the first meiotic division; loss of telomeric localization of Rap1p; reduced levels of sporulation and spore viability; and distributive segregation of linear heterologs. However, there is no effect of the absence of Ndj1p on the segregation of telomere-less ring chromosomes. This means that the mentioned protein is not required for meiotic chromosome separation *per se*, but rather that Ndj1p is essential to separate chromosomes that have telomeres.

Significance of telomere clustering

The mechanism of telomere clustering and the gene products needed to bring it about have remained difficult to address, as it has not been possible to directly observe telomere clustering in living cells. Currently, only a few mutants exist that would aid in the identification of proteins associated with telomere movement during bouquet formation in mutant meiocytes in several model organisms (including maize, mouse, and budding and fission yeast). Results from studies of mutants indicate that the function of the meiotic bouquet is actually to make meiotic prophase much faster and more efficient.

Perspectives

The maintenance of interchromosomal order for proper function in the nucleus has been a topic of great interest [79], but research in this area has moved slowly. This has resulted in a lack of knowledge about how chromosomes are moved around in the interphase somatic nucleus and by an inability to pinpoint when such movements occur. The bouquet is a special and very dramatic example of establishment of chromosome domain polarity within the nucleus of meiotic cells, and unlike somatic chromosomal rearrangements, its functional importance is well established, although how the telomere movements are regulated is yet unclear. Further progress in our understanding of the regulation of telomere movement by identifying the factors involved in telomere chromatin structure changes and the machinery responsible for bringing telomeres into close proximity on the nuclear envelope will have implications not only for our understanding of meiosis and chromosome homology search, but also for our understanding of how chromosome domains are set up and maintained within the somatic interphase nucleus.

Acknowledgements. The work is supported by NS34746 and CA10445.

1 Pruss, D., Reeves, R., Bushman, F. D. and Wolffe, A. P. (1994) The influence of DNA and nucleosome structure on integration events directed by HIV integrase. J. Biol. Chem. 269, 25031–25041.
2 Pruss, D., Bushman, F. D. and Wolffe, A. P. (1994) Human immunodeficiency virus integrase directs integration to sites of severe DNA distortion within the nucleosome core. Proc. Natl. Acad. Sci. USA 91, 5913–5917.
3 Wallrath, L. L., Lu, Q., Granok, H. and Elgin, S. C. (1994) Architectural variations of inducible eukaryotic promoters: preset and remodeling chromatin structures. Bioessays 16, 165–170.
4 Otten, A. D. and Tapscott, S. J. (1995) Triplet repeat expansion in myotonic dystrophy alters the adjacent chromatin structure. Proc. Natl. Acad. Sci. USA 92, 5465–5469.
5 Wolffe, A. P. (1994) Transcription: in tune with the histones. Cell 77, 13–16.
6 Wolffe, A. P., Wong, J. and Pruss, D. (1997) Activators and repressors: making use of chromatin to regulate transcription. Genes Cells. 2, 291–302.
7 Scherthan, H. (2006) Telomere attachment and clustering during meiosis (this issue).
8 Taddei, A. and Gasser, S. M. (2004) Multiple pathways for telomere tethering: functional implications of subnuclear position for heterochromatin formation. Biochim. Biophys. Acta 1677, 120–128.
9 de Lange, T. (2002) Protection of mammalian telomeres. Oncogene 21, 532–540.
10 Pandita, T. K. (2002) Telomeres and telomerase. Encyc. Cancer 4, 355–362.
11 Karlseder, J., Broccoli, D., Dai, Y., Hardy, S. and de Lange, T. (1999) p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. Science 283, 1321–1325.
12 Blackburn, E. H. (2000) Telomere states and cell fates. Nature 408, 53–56.
13 de Lange, T. (2004) T-loops and the origin of telomeres. Nat. Rev. Mol. Cell Biol. 5, 323–329.
14 Blackburn, E. H. (2001) Switching and signaling at the telomere. Cell 106, 661–673.
15 de Lange, T. (2005) Shelterin: the protein complex that shapes and safeguards human telomeres. Genes Dev. 19, 2100–2110.
16 Pandita, R. K., Sharma, G. G., Laszlo, A., Hopkins, K. M., Davey, S., Chakhparonian, M., Gupta, A., Wellinger, R. J., Zhang, J., Powell, S. N., Roti Roti, J. L., Lieberman, H. B. and Pandita, T. K. (2006) Mammalian rad9 plays a role in telomere stability, s- and g2-phase-specific cell survival, and homologous recombinational repair. Mol. Cell Biol. 26, 1850–1864.
17 Molenaar, C., Wiesmeijer, K., Verwoerd, N. P., Khazen, S., Eils, R., Tanke, H. J. and Dirks, R. W. (2003) Visualizing telomere dynamics in living mammalian cells using PNA probes. EMBO J. 22, 6631–6641.

18 Flint, J., Wilkie, A. O., Buckle, V. J., Winter, R. M., Holland, A. J. and McDermid, H. E. (1995) The detection of subtelomeric chromosomal rearrangements in idiopathic mental retardation. Nat. Genet. 9, 132–140.
19 van Overveld, P. G., Lemmers, R. J., Deidda, G., Sandkuijl, L., Padberg, G. W., Frants, R. R. and van der Maarel, S. M. (2000) Interchromosomal repeat array interactions between chromosomes 4 and 10: a model for subtelomeric plasticity. Hum. Mol. Genet. 9, 2879–2884.
20 Dunham, M. A., Neumann, A. A., Fasching, C. L. and Reddel, R. R. (2000) Telomere maintenance by recombination in human cells. Nat. Genet. 26, 447–450.
21 Pandita, T. K. (2001) The role of ATM in telomere structure and function. Radiat. Res. 156, 642–647.
22 Pandita, T. K. and Dhar, S. (2000) Influence of ATM function on interactions between telomeres and nuclear matrix. Radiat. Res. 154, 133–139.
23 Smilenov, L. B., Dhar, S. and Pandita, T. K. (1999) Altered telomere nuclear matrix interactions and nucleosomal periodicity in ataxia telangiectasia cells before and after ionizing radiation treatment. Mol. Cell Biol. 19, 6963–6971.
24 Gately, D. P., Hittle, J. C., Chan, G. K. and Yen, T. J. (1998) Characterization of ATM expression, localization, and associated DNA-dependent protein kinase activity. Mol. Biol. Cell 9, 2361–2374.
25 Andegeko, Y., Moyal, L., Mittelman, L., Tsarfaty, I., Shiloh, Y. and Rotman, G. (2001) Nuclear retention of ATM at sites of DNA double strand breaks. J. Biol. Chem. 276, 38224–38230.
26 Scherthan, H., Jerratsch, M., Dhar, S., Wang, Y. A., Goff, S. P. and Pandita, T. K. (2000) Meiotic telomere distribution and Sertoli cell nuclear architecture are altered in Atm- and Atm-p53-deficient mice. Mol. Cell Biol. 20, 7773–7783.
27 Gupta, A., Sharma, G. G., Young, C.S. H., Agarwal, M., Smith, E. R., Paull, T. T., Lucchesi, J. C., Khanna, K. K., Ludwig, T. and Pandita, T. K. (2005) Involvement of human MOF in ATM function. Mol. Cell. Biol. 25, 5292–5305.
28 Rabl, C. (1885) Über Zellteilung. Morphol. Jahrbuch 10, 214–330.
29 Scherthan, H. (2006) Meiotic Telomeres. In: Telomeres, 2nd edn., pp. 225–260, de Lange, T., Lundblad, V. and Blackburn, E. (eds.), CSH Press, Cold Spring Harbor, New York.
30 Hochstrasser, M., Mathog, D., Gruenbaum, Y., Saumweber, H. and Sedat, J. W. (1986) Spatial organization of chromosomes in the salivary gland nuclei of Drosophila melanogaster. J. Cell Biol. 102, 112–123.
31 Marshall, W. F., Dernburg, A. F., Harmon, B., Agard, D. A. and 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.
32 Dernburg, A. F., Broman, K. W., Fung, J. C., Marshall, W. F., Philips, J., Agard, D. A. and Sedat, J. W. (1996) Perturbation of nuclear architecture by long-distance chromosome interactions. Cell 85, 745–759.
33 Johansen, K. M., Johansen, J., Baek, K. H. and Jin, Y. (1996) Remodeling of nuclear architecture during the cell cycle in Drosophila embryos. J. Cell Biochem. 63, 268–279.
34 Hiraoka, Y., Agard, D. A. and Sedat, J. W. (1990) Temporal and spatial coordination of chromosome movement, spindle formation, and nuclear envelope breakdown during prometaphase in Drosophila melanogaster embryos. J. Cell Biol. 111, 2815–2828.
35 de Lange, T. (1992) Human telomeres are attached to the nuclear matrix. EMBO J. 11, 717–724.
36 Luderus, M. E., van Steensel, B., Chong, L., Sibon, O. C., Cremers, F. F. and de Lange, T. (1996) Structure, subnuclear distribution, and nuclear matrix association of the mammalian telomeric complex. J. Cell Biol. 135, 867–881.
37 Lustig, A. J., Kurtz, S. and Shore, D. (1990) Involvement of the silencer and UAS binding protein RAP1 in regulation of telomere length. Science 250, 549–553.
38 Kyrion, G., Boakye, K. A. and Lustig, A. J. (1992) C-terminal truncation of RAP1 results in the deregulation of telomere size, stability, and function in Saccharomyces cerevisiae. Mol. Cell Biol. 12, 5159–5173.
39 McEachern, M. J. and Blackburn, E. H. (1995) Runaway telomere elongation caused by telomerase RNA gene mutations. Nature 376, 403–409.
40 Zakian, V. A. (1995) Saccharomyces telomeres: function, structure and replication. In: Telomeres, CSH Press, Cold Spring Harbor, New York.
41 Ansari, A. and Gartenberg, M. R. (1997) The yeast silent information regulator Sir4p anchors and partitions plasmids. Mol. Cell Biol. 17, 7061–7068.
42 Andrulis, E. D., Zappulla, D. C., Ansari, A., Perrod, S., Laiosa, C. V., Gartenberg, M. R. and Sternglanz, R. (2002) Esc1, a nuclear periphery protein required for Sir4-based plasmid anchoring and partitioning. Mol. Cell Biol. 22, 8292–8301.
43 Taddei, A., Hediger, F., Neumann, F. R., Bauer, C. and Gasser, S. M. (2004) Separation of silencing from perinuclear anchoring functions in yeast Ku80, Sir4 and Esc1 proteins. EMBO J. 23, 1301–1312.
44 Dhar, S., Squire, J. A., Hande, M. P., Wellinger, R. J. and Pandita, T. K. (2000) Inactivation of 14–3-3sigma influences telomere behavior and ionizing radiation-induced chromosomal instability. Mol. Cell Biol. 20, 7764–7772.
45 Sharma, G. G., Gupta, A., Wang, H., Scherthan, H., Dhar, S., Gandhi, V., Iliakis, G., Shay, J. W., Young, C. S. and Pandita, T. K. (2003) hTERT associates with human telomeres and enhances genomic stability and DNA repair. Oncogene 22, 131–146.
46 Smilenov, L. B., Morgan, S. E., Mellado, W., Sawant, S. G., Kastan, M. B. and Pandita, T. K. (1997) Influence of ATM function on telomere metabolism. Oncogene 15, 2659–2665.
47 Pandita, T. K., Westphal, C. H., Anger, M., Sawant, S. G., Geard, C. R., Pandita, R. K. and Scherthan, H. (1999) Atm inactivation results in aberrant telomere clustering during meiotic prophase. Mol. Cell Biol. 19, 5096–5105.
48 Klein, F., Laroche, T., Cardenas, M. E., Hofmann, J. F., Schweizer, D. and Gasser, S. M. (1992) Localization of RAP1 and topoisomerase II in nuclei and meiotic chromosomes of yeast. J. Cell Biol. 117, 935–948.
49 Gotta, M. and Gasser, S. M. (1996) Nuclear organization and transcriptional silencing in yeast. Experientia 52, 1136–1147.
50 Maillet, L., Boscheron, C., Gotta, M., Marcand, S., Gilson, E. and Gasser, S. M. (1996) Evidence for silencing compartments within the yeast nucleus: a role for telomere proximity and Sir protein concentration in silencer-mediated repression. Genes Dev. 10, 1796–1811.
51 Konkel, L. M., Enomoto, S., Chamberlain, E. M., McCune-Zierath, P., Iyadurai, S. J. and Berman, J. (1995) A class of single-stranded telomeric DNA-binding proteins required for Rap1p localization in yeast nuclei. Proc. Natl. Acad. Sci. USA 92, 5558–5562.
52 Dernburg, A. F., Sedat, J., Cande, W. Z. and Bass, H. W. (1995) The cytology of telomeres. In: Telomeres, pp. 295–338, Blackburn, E. H. and Greider, C. W. (eds.), CSH Press, Cold Spring Harbor, New York.
53 Hawley, R. S. (1997) Unresolvable endings: defective telomeres and failed separation. Science 275, 1441–1443.
54 Kirk, K. E., Harmon, B. P., Reichardt, I. K., Sedat, J. W. and Blackburn, E. H. (1997) Block in anaphase chromosome separation caused by a telomerase template mutation. Science 275, 1478–1481.
55 Cenci, G., Rawson, R. B., Belloni, G., Castrillon, D. H., Tudor, M., Petrucci, R., Goldberg, M. L., Wasserman, S. A. and Gatti, M. (1997) UbcD1, a Drosophila ubiquitin-conjugating

enzyme required for proper telomere behavior. Genes Dev. 11, 863–875.
56 Moazed, D. and Johnson, D. (1996) A deubiquitinating enzyme interacts with SIR4 and regulates silencing in *S. cerevisiae*. Cell 86, 667–677.
57 Henderson, E. (1995) Telomere DNA structure. In: Telomeres, pp. 11–34, Blackburn, E. H. and Greidcer, C. W. (eds.), CSH Press, Cold Spring Harbor, New York.
58 Gilson, E., Laroche, T. and Gasser, S. M. (1993) Telomeres and the functional architecture of the nucleus. Trends Cell. Biol. 3, 128–134.
59 Sundcquist, W. I. (1993) Conducting the G-quartet. Curr. Biol. 3, 893–895.
60 Wellinger, R. J., Ethier, K., Labrecque, P. and Zakian, V. A. (1996) Evidence for a new step in telomere maintenance. Cell 85, 423–433.
61 Mathog, D., Hochstrasser, M., Gruenbaum, Y., Saumweber, H. and Sedat, J. (1984) Characteristic folding pattern of polytene chromosomes in Drosophila salivary gland nuclei. Nature 308, 414–421.
62 Blobel, G. (1985) Gene gating: a hypothesis. Proc. Natl. Acad. Sci. USA 82, 8527–8529.
63 Pandita, T. K. (2002) ATM function and telomere stability. Oncogene 21, 611–618.
64 Wang, S., Guo, M., Ouyang, H., Li, X., Cordon-Cardo, C., Kurimasa, A., Chen, D. J., Fuks, Z., Ling, C. C. and Li, G. C. (2000) The catalytic subunit of DNA-dependent protein kinase selectively regulates p53-dependent apoptosis but not cell-cycle arrest. Proc. Natl. Acad. Sci. USA 97, 1584–1588.
65 Chevillard, C., Reik, W., McDermott, M., Fontes, M., Mattei, M. G. and Singh, P. B. (1993) Chromosomal localization of human homologs of the Drosophila heterochromatin protein 1 (HP1) gene. Mamm. Genome 4, 124–126.
66 Zhao, T., Heyduk, T., Allis, C. D. and Eissenberg, J. C. (2000) Heterochromatin protein 1 binds to nucleosomes and DNA in vitro. J. Biol. Chem. 275, 28332–28338.
67 Cryderman, D. E., Tang, H., Bell, C., Gilmour, D. S. and Wallrath, L. L. (1999) Heterochromatic silencing of Drosophila heat shock genes acts at the level of promoter potentiation. Nucleic Acids Res. 27, 3364–3370.
68 James, T. C., Eissenberg, J. C., Craig, C., Dietrich, V., Hobson, A. and Elgin, S. C. (1989) Distribution patterns of HP1, a heterochromatin-associated nonhistone chromosomal protein of Drosophila. Eur J. Cell Biol. 50, 170–180.
69 Fanti, L., Giovinazzo, G., Berloco, M. and Pimpinelli, S. (1998) The heterochromatin protein 1 prevents telomere fusions in Drosophila. Mol. Cell 2, 527–538.
70 Sharma, G. G., Hwang, K. K., Pandita, R. K., Gupta, A., Dhar, S., Parenteau, J., Agarwal, M., Worman, H. J., Wellinger, R. J. and Pandita, T. K. (2003) Human heterochromatin protein 1 isoforms HP1(Hsalpha) and HP1(Hsbeta) interfere with hTERT-telomere interactions and correlate with changes in cell growth and response to ionizing radiation. Mol. Cell Biol. 23, 8363–8376.
71 Ashley, T. (1994) Mammalian meiotic recombination: a reexamination. Hum. Genet. 94, 587–593.
72 Moens, P. B. and Pearlman, R. E. (1990) Telomere and centromere DNA are associated with the cores of meiotic prophase chromosomes. Chromosoma 100, 8–14.
73 Cooper, J. P., Watanabe, Y. and Nurse, P. (1998) Fission yeast Taz1 protein is required for meiotic telomere clustering and recombination. Nature 392, 828–831.
74 Nimmo, E. R., Pidoux, A. L., Perry, P. E. and Allshire, R. C. (1998) Defective meiosis in telomere-silencing mutants of Schizosaccharomyces pombe. Nature 392, 825–828.
75 Chua, P. R. and Roeder, G. S. (1997) Tam1, a telomere-associated meiotic protein, functions in chromosome synapsis and crossover interference. Genes Dev. 11, 1786–1800.
76 Conrad, M. N., Dominguez, A. M. and Dresser, M. E. (1997) Ndj1p, a meiotic telomere protein required for normal chromosome synapsis and segregation in yeast. Science 276, 1252–1255.
77 Rockmill, B. and Roeder, G. S. (1998) Telomere-mediated chromosome pairing during meiosis in budding yeast. Genes Dev. 12, 2574–2586.
78 Chikashige, Y., Ding, D. Q., Funabiki, H., Haraguchi, T., Mashiko, S., Yanagida, M. and Hiraoka, Y. (1994) Telomere-led premeiotic chromosome movement in fission yeast. Science 264, 270–273.
79 Bickmore, W. A. and Chubb, J. R. (2003) Dispatch. Chromosome position: now, where was I? Curr Biol 13, R357–R359.