The Polar Arrangement of Telomeres in Interphase and Meiosis. Rabl Organization and the Bouquet[1]

Carrie R. Cowan, Peter M. Carlton, and W. Zacheus Cande*

Departments of Plant and Microbial Biology (C.R.C., W.Z.C.) and Molecular and Cell Biology (P.M.C., W.Z.C.), University of California, Berkeley, California 94720

It has long been appreciated that chromosomes do not lay passively in the nucleus, but are dynamically reorganized to suit the cell's needs. Chromosomes condense into compact bodies for cell division, decondense in interphase to allow gene expression, and pair along their lengths during meiosis in preparation for the reductional division. Chromosome behavior can be broadly classified into two types: autonomous and nonautonomous. Autonomous behavior is seen at the chromosome level and below, and involves only the chromosomes themselves, as in the examples given above. Nonautonomous behavior involves physical interactions between the chromosomes and the rest of the cell and results in global changes in the organization of the chromosomes. The best known example of nonautonomous chromosome behavior may be the orchestration of chromosome movement at mitosis through interaction with the spindle microtubules. However, in recent years the study of chromosome behavior in interphase and meiotic prophase has revealed many roles for global, nonautonomous reorganization of chromosomes within the nucleus.

Mitotic chromosomes rely on centromere-based organization, whereas interphase and meiotic prophase cells appear to have chosen telomeres as the sites of interaction with the rest of the cell. Many descriptive studies have revealed the special positioning of telomeres in cells at certain times. In particular, during both interphase and meiotic prophase, telomeres are often located at the nuclear periphery in limited regions or clusters. Plants have played an important role in many of these studies because of their superior cytological features. In each case, the telomere organization can be thought of as imposing order on the chromosomes. This review will discuss the mechanisms by which telomeres are positioned and maintained, and the possible functions of the polar arrangement of the telomeres.

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* Corresponding author; e-mail zcande@uclink4.berkeley.edu; fax 510–643–6791.

TELOMERE STRUCTURE

Telomeres are specialized chromosome regions with many peculiar features. First and most obvious among these features is the property of being the physical ends of the linear DNA molecules that make up chromosomes. Second, in the majority of organisms studied, telomeres contain stretches of simple DNA repeats, added by telomerase as a way to ensure against the gradual erosion of chromosome length through canonical DNA replication. Third, there is evidence that some nucleotides in telomeres are chemically modified. The final peculiar feature of telomeres is that they are known to be specifically associated with many proteins that contribute to their maintenance and to telomere-specific behaviors such as transcription silencing.

In most organisms, the telomeric DNA consists of many repeats of a simple sequence added by telomerase. Though the telomeric DNA repeats differ slightly between organisms, they have in common a strand with several G bases running 5′ to 3′ toward the end of the chromosome (Pryde et al., 1997). The total length of telomeric DNA can be as small as 36 bp (ciliates) or as long as 15 kb (mammals; Richards et al., 1993). Another feature of telomeric regions in many organisms is the presence of repetitive DNA families adjacent to the telomeric DNA (Pryde et al., 1997). These sub-telomeric regions are often cytologically heterochromatic. For example, the ends of rye (*Secale cereale*) chromosomes are visibly condensed throughout most stages of the cell cycle.

Proteins that function to maintain the chromatin organization necessary for appropriate behavior of telomeres have been identified by various means. For a summary of telomeric proteins and the organisms in which they have been identified, see Table I. Direct in vitro binding to the telomere repeat led to the identification of Tbf1p (TTAGGG binding factor) from *Saccharomyces cerevisiae* (Brigati et al., 1993), TRF1 from humans (Chong et al., 1995), and Arabidopsis proteins (Zentgraf, 1995). A myb-related motif in Tbf1 that is essential for telomere binding (the telobox; Bilaud et al., 1996) pointed to homologous proteins in maize (*Zea mays*), parsley (*Petroselinum crispum*), and humans, all of which have been shown to bind telomere-like sequences. Putative homologs were also found in rice (*Oryza sativa*) and Arabidop-

Table I. *Proteins involved in proper telomere structure and their distribution among kingdoms*

A + indicates that at least one example of the protein class has been identified in the specified kingdom. Blanks indicate that no examples have been reported. Telomerase is responsible for adding telomere repeats to the ends of chromosomes. Telomere length regulators prevent overelongation of telomeres by telomerase, either directly or indirectly. Telobox family proteins are defined by a myb-related motif involved in telomeric DNA binding, and some have been shown to act as negative regulators of telomere length. HP1 family proteins contain chromo- and shadow-chromodomains, and are involved in heterochromatin maintenance. The Ku heterodimer is an abundant telomere protein thought to protect the telomeres from overshortening, recombination, etc. It will be interesting to discover whether plants have examples from all classes.

Protein Family	Plants	Animals	Fungi	Ciliates
Telomerase	+	+	+	+
Telomere Length Regulator		+	+	+
Telobox Family	+	+	+	
HP1 Family		+	+	+
Ku Heterodimer		+	+	

sis (Bilaud et al., 1996). Rap1, the major structural protein at *S. cerevisiae* telomeres, was identified by its DNA-binding properties at the silent mating type locus, only later being shown to function at telomeres (Buchman et al., 1988). The Ku heterodimer is an abundant telomere-binding protein with a role in telomere protection (Bertuch and Lundblad, 1998). A subset of telomere-binding proteins has been found to play an essential role in proper telomere length maintenance, preventing overelongation by telomerase. Negative regulators of telomere length include Rap1p from both *S. cerevisiae* and humans (Kyrion et al., 1993; Li et al., 2000), Taz1p from *Schizosaccharomyces pombe* (Cooper et al., 1997), and TRF proteins from humans (van Steensel and De Lange, 1997). Mutations that result in loss of transcriptional silencing at the telomeres identified various telomere structure proteins: the Sir proteins from *S. cerevisiae* (Hennig, 1999), Swi6 from *S. pombe* (Ekwall et al., 1995), and Su(var)2–5/HP1 from *Drosophila melanogaster* (Eissenberg and Elgin, 2000). Mutations leading to the end-to-end fusions of chromosomes have elucidated proteins important for protecting telomeres against double-strand break repair and recombination. Telomere fusions result from mutations in *D. melanogaster* HP1 (Eissenberg and Elgin, 2000) and human TRF2 (van Steensel et al., 1998). Additional protein complexes with specialization at the telomeres are nucleosomes, which appear to be spaced differently within telomere repeats relative to bulk chromatin (discussed below).

INTERPHASE TELOMERE POSITIONING: THE RABL ORGANIZATION

Chromosome segregation at anaphase results in the polarization of chromosomes because sister centromeres are pulled in opposite directions and the rest of the chromosome trails behind. In some instances, the anaphase arrangement of chromosomes persists into the following interphase (Fig. 1); this is known as the Rabl organization (Dernburg et al., 1995). Observations of whole chromosomes in the Rabl configuration show that they occupy elongated territories, stretching from one end of the nucleus to the other. This is a departure from the cloud-shaped territory expected for a free-floating polymer in solution (Marko and Siggia, 1997) and suggests constraints acting either at the chromosomal level (i.e. condensation state), nuclear level ("squeezing" of chromosomes into linear shapes due to tight packing of chromatin in the nucleus), or sub-chromosomal level (physical attachment of centromeres and telomeres to opposite sides of the nuclear envelope [NE]).

The presence of the Rabl organization is known to vary greatly between species and among tissues or developmental stages of an organism. Some cells lose the Rabl organization soon after entering interphase, whereas others retain the organization through to the next mitosis. In a study of a variety of plants, genome size and chromosome length were postulated to be two possible reasons for this variation (Dong and Jiang, 1998). The Rabl organization was observed in wheat, rye, barley, and oats (*Avena sativa*), all of which have C values above 4,800 Mbp. Chromosomes of sorghum (*Sorghum bicolor*) and rice, both with genomes under 1,000 Mbp, lacked the Rabl configuration. Maize, which at 3,000 Mbp is intermediate in genome size, displayed neither entirely Rabl nor entirely random chromosome organization. Previous studies of other genera (*Allium*, *Vicia*, Arabidopsis, *Brassica*, *Solanum*, and *Pisum*) supported their model (Dong and Jiang, 1998), although it does not appear to extend to the animals, as *D. melanogaster*, a small-genome organism, displays a striking Rabl organization.

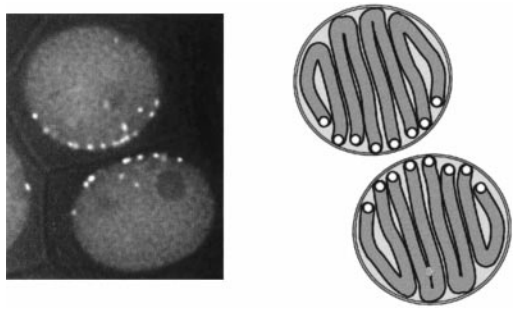

Figure 1. The Rabl organization in hexaploid wheat (*Triticum aestivum*). The chromosome configuration of anaphase is maintained throughout the next interphase. Left, Two daughter cells at interphase. The telomeres were detected using fluorescently labeled oligonucleotide probes complementary to the telomere repeat and are seen in the image as bright spots. In both nuclei the telomeres are found at the nuclear periphery facing previous division plane between the daughter cells. The chromatin, stained with 4′,6-diaminophenylindole, appears gray. A diagram of this configuration is shown at right. Telomeres are indicated by white circles at the ends of the chromosomes, which are dark gray.

One hundred twenty years after its initial observation, there are still many unanswered questions about the Rabl configuration. The Rabl organization is a direct consequence of the anaphase configuration of the chromosomes, and how it is established is not in question. More intriguing is the mechanism by which the chromosome polarization is retained through the following interphase. Is it actively maintained, e.g. through centromere and telomere interactions with opposite halves of the NE, or is it passively maintained, being the chromosome arrangement with the lowest free energy of the interphase nucleus? Is it merely a consequence of packing large genomes into a nucleus, or does it have a functional role?

HOW IS THE RABL ORGANIZATION MAINTAINED?

The Rabl organization of chromosomes in the interphase nucleus is relatively fixed over time and in the nuclear space, and the telomeres appear to provide important anchorage points. Organization of the chromosomes in the interphase nucleus may rely solely on chromosome-chromosome and chromosome-NE interactions. The nuclear lamins appear to be excellent candidates for providing chromosome attachment. The nuclear lamins are a class of intermediate filaments that form a polymer network on the inner face of the NE as well as foci throughout the nucleoplasm. Lamins can bind DNA, chromosomes, and histones in vitro (Wilson, 2000). Evidence from mice and *D. melanogaster* suggests that loss of a nuclear lamin causes chromatin to detach from the NE (Wilson, 2000).

It is unclear how the specificity for telomere-lamin interactions might arise. One possibility is suggested by evidence that histones are spaced differently in the telomeres relative to the rest of the chromatin. Telomeric nucleosomes (histones and associated DNA) are spaced 15 to 30 bp closer in telomeres than in the rest of the genome (Fajkus et al., 1995; Vershinin and Heslop-Harrison, 1998). Because histones can bind directly to the nuclear lamins, tighter nucleosome spacing in the telomeres could increase the number of histone-lamin interactions in a given length of DNA, potentially stabilizing the attachment of telomeres, though this has not been experimentally investigated.

The involvement of nuclear lamins in the maintenance of the Rabl organization is appealing because nuclear lamins are widely distributed in the eukaryotes. The dinoflagellates have a nuclear matrix immunologically related to vertebrate lamins (Minguez et al., 1994), as does the myxomycete *Physarum polycephalum* (Lang and Loidl, 1993). Plants also appear to have intermediate filament networks in the nucleus. There is strong biochemical and immunological evidence for the presence of vertebrate-type lamins in plants (Minguez and Moreno Diaz De La Espina, 1993; Wang et al., 1996), although no lamins have been cloned. It is interesting to note that the yeasts *S. cerevisiae* and *S. pombe* appear not to have nuclear lamins. Telomere organization in these fungi uses other proteins.

An intriguing interaction between HP1 chromodomain proteins and the lamin-binding receptor (LBR) may contribute to telomere attachments to the NE (Eissenberg and Elgin, 2000). Human HP1 has been shown to interact with LBR, an integral inner nuclear membrane protein. LBR also interacts with *D. melanogaster* HP1 in a yeast two-hybrid assay. *D. melanogaster* HP1 localizes to heterochromatin of interphase polytene chromosomes and to the ends of metaphase chromosomes (Eissenberg and Elgin, 2000). Swi6p from *S. pombe* is a homolog of HP1, based on its chromodomain structure. Swi6p localizes to heterochromatic regions of the chromosomes (centromeres, telomeres, and the mating type locus) and is necessary for silencing (Ekwall et al., 1995; Eissenberg and Elgin, 2000). It is unknown with which NE factors Swi6p might interact.

An alternative or supplementary means of chromosomal positioning could be the nuclear matrix, although whether such a structure exists is controversial. The nuclear matrix is a nuclear fraction left behind after chemical extraction, and is proposed to form a structural framework in the nucleus, functionally similar to the cytoskeleton in the cytoplasm. It is thought that specific chromatin regions interact with the matrix, contributing to the positioning of chromosomes in the nucleus. The major objection to the nuclear matrix is the lack of evidence for such a structure in living cells.

Components of the nuclear matrix include ribonucleoproteins, nuclear lamins, and topoisomerase II. Many of these, though originally identified in animal cells, have been shown to be present in nuclear matrix preparations from various plant cells (Yu and Moreno Diaz De La Espina, 1999). Telomeric DNA in human cells is stably associated with the nuclear matrix. In human lymphocyte nuclear matrix preparations, centromeres are extractable but telomeres are unextractable, suggesting that although both chromosomal elements are transcriptionally silent, the centromeres and telomeres differ significantly in their matrix attachment properties (Weipoltshammer et al., 1999). How telomeres are selectively anchored to the nuclear matrix is not known. It has been shown that transfected telomere (TTAGGG) repeats are not retained in nuclear matrix preparations, indicating that telomeric DNA is not sufficient for matrix attachment (de Lange, 1992).

S. cerevisiae and *S. pombe* exhibit a Rabl organization, though the polarization of chromosomes is not as obvious as in organisms with larger genomes. Recent studies have provided insight into how telomeres are clustered and positioned in the interphase nucleus of *S. cerevisiae* (Galy et al., 2000). Through a small number of DNA-protein and protein-protein

interactions, the telomeres are tethered to the nuclear pores. The telomeres and sub-telomeric regions are complexed with Ku. Ku is able to bind to nuclear pores through two bridging proteins, Mlp1 and Mlp2 (Galy et al., 2000). MLP1 and MLP2 show homology to *D. melanogaster* and human translocated promoter region (Tpr) proteins (Strambio-de-Castillia et al., 1999).

Tpr has been shown to be a component of intranuclear filaments that are attached to the nucleoplasmic face of nuclear pores in vertebrates (Cordes et al., 1997). Likewise, Mlp1p and Mlp2p form extensive filaments that project into the nucleoplasm (Strambio-de-Castillia et al., 1999). Similar filamentous projections from the nuclear pores have been described in lily (*Lilium longiflorum*) meiotic nuclei, where they were often found associated with chromatin (Holm, 1977). *D. melanogaster* Tpr appears to extend further into the nucleus. At the light microscope level, *D. melanogaster* Tpr localizes to the extrachromosomal and extranucleolar space throughout the nuclear interior and on or near nuclear pores at the nuclear periphery (Zimowska et al., 1997). Tpr/Mlp may have important functions for nuclear architecture, as a scaffolding protein for chromosome organization (a nuclear matrix), or as a "substitute" nuclear lamina in yeast.

POSSIBLE FUNCTIONS OF RABL ORGANIZATION

The Rabl configuration imposes a striking degree of order on interphase chromosomes, isolating specific chromosome regions as small, well-defined domains within the nucleus. This isolation would be important if factors necessary for maintaining certain chromatin configurations need to be sequestered. It has been strongly suggested that in wheat and many other organisms that the density of genes on the chromosome increases near the telomeres. To the extent that this is true, positioning telomeres in the nucleus is equivalent to positioning genes. Gene position in the nucleus has been found to affect expression in many systems. However, a recent study in wheat (Abranches et al., 1998) has shown that active transcription sites do not show obvious localization patterns in nuclei, but are randomly distributed. The Rabl orientation may still contribute to this pattern in a nonobvious way.

Another hint as to a possible function of the Rabl orientation comes from live imaging studies of heterochromatic foci in cell lines of muntjac (*Muntiacus muntjak*). Manders et al. (1999) demonstrated that at the G2-M transition (the entry into mitosis) when chromosomes are condensing, chromosomes do not undergo much internal reorganization (movement) to reach the structure they will have at metaphase. This would not be the case in a non-Rabl cell when individual chromosomes must rearrange from a cloud-shaped to a rod-shaped territory. Because non-Rabl cells tend to have smaller chromosomes, this indicates that the Rabl orientation may be a way of dealing with the difficult task of forming large metaphase chromosomes.

TELOMERE CLUSTERING IN MEIOSIS: THE BOUQUET

The bouquet is the clustering of chromosome ends on the NE during meiotic prophase (Fig. 2), coincident with the initiation of homologous chromosome synapsis. The bouquet has been extensively described in many species in all eukaryotic groups (Loidl, 1990; Dernburg et al., 1995; Zickler and Kleckner, 1998). There are no documented cases of plant species that lack the bouquet stage. However, both the mechanism of bouquet formation and its function in meiosis remain unknown. It has been proposed as an aid to presynaptic alignment of homologous chromosomes because it brings all chromosome ends into a common nuclear subregion and makes them all roughly codirectional.

The bouquet in mice, humans (Scherthan et al., 1996), and maize (Bass et al., 2000) appears to occur after large rearrangements of the chromosomes. In hexaploid wheat, the bouquet appears to be a tightening of the already present Rabl (Aragón-Alcaide et al., 1997; Schwarzacher, 1997). The surface area of the NE occupied by telomeres also varies between organisms, ranging from extremely tight where little intertelomere space is visible (rye and wheat) to a more loose clustering (maize and lily). It would be interesting to correlate the tightness of the bouquet with other nuclear features such as chromosome length, genome size, and presence of Rabl configuration in interphase. As of this moment, there are no rules for predicting bouquet morphology.

HOW IS THE BOUQUET FORMED?

The similarity of the bouquet to the Rabl conformation has long been noted. However, it is clear that

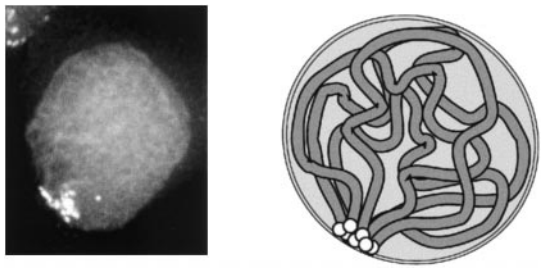

Figure 2. The bouquet arrangement in hexaploid wheat. All 84 telomeres (bright signals) in this meiotic prophase cell (left) are clustered at one spot at the nuclear periphery whereas the rest of the chromatin (gray; stained with 4′,6-diamino-phenylindole) fills the nuclear volume. Because of the tight clustering of the telomeres during the bouquet, individual telomere signals are not visible but appear as a single mass. The telomeres were detected as in Figure 1. A diagram of the bouquet is shown to the right. Telomeres are indicated by white circles; chromosomes are dark gray.

the two are not the same. Some organisms (Arabidopsis) display a bouquet but not Rabl, whereas others (*D. melanogaster*) display Rabl but no apparent bouquet. In the case of wheat, the Rabl organization of interphase is tightened to form the telomere bouquet. The Rabl organization and the bouquet show different degrees of telomere clustering. The sequence of maize bouquet formation indicates that to build on, telomere clustering does not require a preexisting Rabl organization. Maize exhibits a Rabl organization prior to the last premeiotic cell division. The Rabl organization is lost during the following interphase, and during meiotic prophase the telomeres cluster in the bouquet (Bass et al., 1997). The Rabl organization appears to utilize the centromeres in addition to the telomeres in some circumstances, whereas the bouquet appears to rely solely on the telomeres (Martínez-Pérez et al., 1999). The differences observed between the Rabl organization and the bouquet suggest that the mechanisms of telomere positioning between the two are also different.

A defining factor of the bouquet in animal cells is the spatial relationship between the centrosome (the animal microtubule organizing center [MTOC]) and the telomeres during the bouquet (Zickler and Kleckner, 1998). The telomere cluster occurs at a site on the inner nuclear membrane adjacent to the centrosome position near the outer nuclear membrane. Plants, not having a defined MTOC, are nonetheless capable of having very tight bouquets (Loidl, 1990), suggesting that the MTOC position does not define the telomere cluster position in plants. The functional relationship between centrosomes and the telomeres during the bouquet in animals has not been investigated; thus, its importance is unclear. However, a similar spatial relationship exists during the *S. pombe* bouquet, which has proved amenable to investigation.

S. pombe exhibits a prominent telomere cluster during meiotic prophase. All 12 telomeres cluster in the limited region adjacent to the spindle pole body (SPB, the fungal MTOC; Chikashige et al., 1994). The kms1 mutant of *S. pombe* (Shimanuki et al., 1997) diminishes telomere clustering during meiosis. Telomeres of kms1 mutants appear to associate with the NE at multiple sites around the nuclear periphery. Many telomere groups are associated with Sad1p antibody staining. Sad1p has been reported to be associated with the SPB throughout the cell cycle, but in the kms1 mutant, the functional SPB is still present as a single site, even though Sad1p is present at multiple sites (Shimanuki et al., 1997). Therefore, Sad1p appears to be a peripheral component of the SPB, which may be involved in attaching the telomeres to the NE.

Limited evidence exists for the functional involvement of a microtubule-related component (such as the SPB or MTOC) in bouquet formation in animals and plants. It has long been known that the antimicrotubule drug colchicine (or the related compound colcemid) leads to a failure of homologous chromosome synapsis when administered during meiosis (Loidl, 1990). Because of the proposed role of the bouquet in assisting chromosome pairing, it has been speculated that the telomere cluster may be the target of colchicine's action. However, cytoplasmic microtubules are found only rarely at the site of telomere attachment to the NE during the bouquet, according to electron microscopy analyses (Holm, 1977). The primary process disrupted by colchicine treatment remains unknown.

An important protein component of meiotic chromosomes is the synaptonemal complex (SC). The SC forms a core extending the length of each chromosome; when two homologous chromosomes synapse, their SCs become joined except at the most distal, telomeric regions. The ends of the SC (the telomeres) appear to contact the inner nuclear membrane. Specialized, thickened SC ends at telomere attachment sites have been documented in a large number of animals (Esponda and Giménez-Martín, 1972) and in a few higher plants (Holm, 1977). The thickened SC structures consistently appear to be conical in shape, the wide end of the cone being the attachment to the inner nuclear membrane. In a number of species, the inner and outer membranes appeared to have a layer of increased electron density at the telomere attachment site (Esponda and Giménez-Martín, 1972; Rasmussen, 1976; Holm, 1977). In silkworms (*Bombyx mori*), it has been suggested that the deposition of the electron-dense material onto both faces of the NE precedes the attachment of the SC ends to the NE (Rasmussen, 1976). An interesting connection between the SC and the MTOC exists in plant cells. Schmit et al. (Schmit et al., 1996) discovered that the 6c6 antibody, which localizes to the pericentriolar material in animal centrosomes, recognizes both the NE (the site of microtubule nucleation, MTOC) and the SC in *Gingko biloba* and *Funkia* sp. meiotic cells. The functional significance of this shared antigen is unclear.

A promising candidate for telomere positioning during meiosis in *S. cerevisiae* is the Ndj1 protein. Ndj1p is a meiosis-specific telomere-localized protein (Conrad et al., 1997). It has been proposed that ndj1 mutants may be defective in bouquet formation. ndj1 mutants display an increased number of Rap1p dots relative to wild type, indicative of a defect in telomere organization. In addition, ndj1 mutants exhibit delayed formation of axial elements, delayed formation of the SC, delayed completion of meiosis I, increased homolog non-disjunction, reduced sporulation and spore viability, and defective segregation of linear but not circular yeast artificial chromosomes. Recombination pathways appeared to be intact in the ndj1 mutant. The observed defects may result from the initial delay in SC installation (Conrad et al., 1997), perhaps due to the absence of a normal bouquet. No homologs of NDJ1 are currently known, so the mechanism of bouquet formation remains an open question.

POSSIBLE FUNCTIONS OF THE BOUQUET

Although the timing and universality of the bouquet immediately suggests a direct and important role in chromosome pairing, finding evidence for this hypothesis has proven difficult. It has been recognized that if homology searching is done primarily near the ends of chromosomes, then limiting the telomeres to the NE would constrain the homology search to a smaller space, making it more efficient (Loidl, 1990; Dernburg et al., 1995). Additional theories (Zickler and Kleckner, 1998) involve functions quite similar to those posited for the Rabl orientation, i.e. creating a chromosomal compartment necessary for some chromosome region-specific function. Such functions might include recombination initiation or SC formation, both of which have chromosome region-specific biases. Experiments addressing the function of the bouquet are clearly needed.

FUTURE CONTRIBUTIONS FROM PLANTS

Although many descriptive studies of telomere behavior have been carried out in plants, there is still much more that plants can contribute to our knowledge of the function and formation of Rabl organization and the bouquet. Many of the molecules thought to be important in telomere organization in the nucleus do not have cloned homologs in the plant kingdom, and thus many of the tools necessary for investigating telomere function are unavailable. With the completion of plant genome sequencing efforts over the next months and years, the necessary tools will become available for molecular functional analysis. Perhaps more important, however, are the advantages of modern molecular cytology in plants. Plants can tolerate gross disturbances of genome size and construction, which allows the study of nuclear organization based on large-scale chromosome and genome manipulations. Among other things, this approach may be able to address the relative contributions of autonomous and nonautonomous chromosome behavior. For example, one could evaluate the interphase behavior of an introgressed chromosome from a non-Rabl species in a Rabl species. In addition, ploidy manipulations (haploidization and polyploidization) could shed light on the contribution of genome size to telomere organization. Studying chromosomal derivatives, such as ring and telocentric chromosomes, would help determine sub-chromosomal requirements for order. The universality of both Rabl organization and the bouquet suggests evolutionary conservation of important functions, and an integrated molecular and cytological understanding of these questions will be forthcoming.

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LITERATURE CITED

Abranches R, Beven AF, Aragón-Alcaide L, Shaw PJ (1998) Transcription sites are not correlated with chromosome territories in wheat nuclei. J Cell Biol **143:** 5–12

Aragón-Alcaide L, Reader S, Beven A, Shaw P, Miller T, Moore G (1997) Association of homologous chromosomes during floral development. Curr Biol **7:** 905–908

Bass HW, Marshall WF, Sedat JW, Agard DA, Cande WZ (1997) Telomeres cluster de novo before the initiation of synapsis: a three-dimensional spatial analysis of telomere positions before and during meiotic prophase. J Cell Biol **137:** 5–18

Bass HW, Riera-Lizarazu O, Ananiev EV, Bordoli SJ, Rines HW, Phillips RL, Sedat JW, Agard DA, Cande WZ (2000) Evidence for the coincident initiation of homolog pairing and synapsis during the telomere-clustering (bouquet) stage of meiotic prophase. J Cell Sci **113:** 1033–1042

Bertuch A, Lundblad V (1998) Telomeres and double-strand breaks: trying to make ends meet. Trends Cell Biol **8:** 339–342

Bilaud T, Koering CE, Binet-Brasselet E, Ancelin K, Pollice A, Gasser SM, Gilson E (1996) The telobox, a Myb-related telomeric DNA binding motif found in proteins from yeast, plants and human. Nucleic Acids Res **24:** 1294–1303

Brigati C, Kurtz S, Balderes D, Vidali G, Shore D (1993) An essential yeast gene encoding a TTAGGG repeat-binding protein. Mol Cell Biol **13:** 1306–1314

Buchman AR, Lue NF, Kornberg RD (1988) Connections between transcriptional activators, silencers, and telomeres as revealed by functional analysis of a yeast DNA-binding protein. Mol Cell Biol **8:** 5086–5099

Chikashige Y, Ding D-Q, Funabiki H, Haraguchi T, Mashiko S, Yanagida M, Hiraoka Y (1994) Telomere-led premeiotic chromosome movement in fission yeast. Science **264:** 270–273

Chong L, Van Steensel B, Broccoli D, Erdjument-Bromage H, Hanish J, Tempst P, De Lange T (1995) A human telomeric protein. Science **270:** 1663–1667

Conrad MN, Dominguez AM, Dresser ME (1997) Ndj1p, a meiotic telomere protein required for normal chromosome synapsis and segregation in yeast. Science **276:** 1252–1255

Cooper JP, Nimmo ER, Allshire RC, Cech TR (1997) Regulation of telomere length and function by a Myb-domain protein in fission yeast. Nature **385:** 744–747

Cordes VC, Reidenbach S, Rackwitz H-R, Franke WW (1997) Identification of protein p270/Tpr as a constitutive component of the nuclear pore complex-attached intranuclear filaments. J Cell Biol **136:** 515–529

de Lange T (1992) Human telomeres are attached to the nuclear matrix. EMBO J **11:** 717–724

Dernburg AF, Sedat JW, Cande WZ, Bass HW (1995) Cytology of telomeres. *In* EH Blackburn, CW Greider, eds, Telomeres. Cold Spring Harbor Laboratory Press, Plainview, NY, pp 295–338

Dong F, Jiang J (1998) Non-Rabl patterns of centromere and telomere distribution in the interphase nuclei of plant cells. Chromosome Res **6:** 551–558

Eissenberg JC, Elgin SCR (2000) The HP1 protein family: getting a grip on chromatin. Curr Opin Genet Dev **10:** 204–210

Ekwall K, Javerzat J-P, Lorentz A, Schmidt H, Cranston G, Allshire R (1995) The chromodomain protein Swi6: a key component at fission yeast centromeres. Science **269:** 1429–1431

Esponda P, Giménez-Martín G (1972) The attachment of the synaptonemal complex to the nuclear envelope: an ultrastructural and cytochemical analysis. Chromosoma **38:** 405–17

Fajkus J, Kovarik A, Kralovics R, Bezdek M (1995) Organization of telomeric and subtelomeric chromatin in the higher plant *Nicotiana tabacum*. Mol Gen Genet **247:** 633–638

Galy V, Olivo-Marin J-C, Scherthan H, Doye V, Rascalou N, Nehrbass U (2000) Nuclear pore complexes in the organization of silent telomeric chromatin. Nature **403:** 108–112

Hennig W (1999) Heterochromatin. Chromosoma **108:** 1–9

Holm PB (1977) Three-dimensional reconstruction of chromosome pairing during the zygotene stage of meiosis in *Lilium longiflorum* (Thunb.). Carlsberg Res Commun **42:** 103–151

Kyrion G, Liu K, Liu C, Lustig AJ (1993) RAP1 and telomere structure regulate telomere position effects in *Saccharomyces cerevisiae*. Genes Dev **7:** 1146–1159

Lang S, Loidl P (1993) Identification of proteins immunologically related to vertebrate lamins in the nuclear matrix of the myxomycete *Physarum polycephalum*. Eur J Cell Biol **61:** 177–183

Li BB, Oestreich S, de Lange T (2000) Identification of human Rap1: implications for telomere evolution. Cell **101:** 471–483

Loidl J (1990) The initiation of meiotic chromosome pairing: the cytological view. Genome **33:** 759–778

Manders EMM, Kimura H, Cook PR (1999) Direct imaging of DNA in living cells reveals the dynamics of chromosome formation. J Cell Biol **144:** 813–821

Marko JF, Siggia ED (1997) Polymer models of meiotic and mitotic chromosomes. Mol Biol Cell **8:** 2217–2231

Martínez-Pérez E, Shaw P, Reader S, Aragón-Alcaide L, Miller T, Moore G (1999) Homologous chromosome pairing in wheat. J Cell Sci **112:** 1761–1769

Minguez A, Franca S, Moreno Diaz De La Espina S (1994) Dinoflagellates have a eukaryotic nuclear matrix with lamin-like proteins and topoisomerase II. J Cell Sci **107:** 2861–2873

Minguez A, Moreno Diaz De La Espina S (1993) Immunological characterization of lamins in the nuclear matrix of onion cells. J Cell Sci **106:** 431–439

Pryde FE, Gorham HC, Louis EJ (1997) Chromosome ends: all the same under their caps. Curr Opin Genet Dev **7:** 822–828

Rasmussen SW (1976) The meotic prophase in *Bombyx mori* females analyzed by three dimensional reconstructions of synaptonemal complexes. Chromosoma **54:** 245–293

Richards EJ, Vongs A, Walsh M, Yang J, Chang S (1993) Substructure of telomere repeat arrays. *In* J S Heslop-Harrison, RB Flavell, The Chromosome. BIOS Scientific Publishers Limited, Oxford, pp 103–114

Scherthan H, Weich S, Schwegler H, Heyting C, Haerle M, Cremer T (1996) Centromere and telomere movements during early meiotic prophase of mouse and man are associated with the onset of chromosome pairing. J Cell Biol **134:** 1109–1125

Schmit A-C, Endle M-C, Lambert A-M (1996) The perinuclear microtubule-organizing center and the synaptonemal complex of high plants share a common antigen: its putative transfer and role in meiotic chromosomal ordering. Chromosoma **104:** 405–413

Schwarzacher T (1997) Three stages of meiotic homologous chromosome pairing in wheat: cognition, alignment and synapsis. Sex Plant Reprod **10:** 324–331

Shimanuki M, Miki F, Ding DQ, Chikashige Y, Hiraoka Y, Horio T, Niwa O (1997) A novel fission yeast gene, kms1+, is required for the formation of meiotic prophase-specific nuclear architecture. Mol Gen Genet **254:** 238–249

Strambio-de-Castillia C, Blobel G, Rout MP (1999) Proteins connecting the nuclear pore complex with the nuclear interior. J Cell Biol **144:** 839–855

van Steensel B, De Lange T (1997) Control of telomere length by the human telomeric protein TRF1. Nature **385:** 740–743

van Steensel B, Smogorzewska A, De Lange T (1998) TRF2 protects human telomeres from end-to-end fusions. Cell **92:** 401–413

Vershinin AV, Heslop-Harrison JS (1998) Comparative analysis of the nucleosomal structure of rye, wheat and their relatives. Plant Mol Biol **36:** 149–161

Wang J, Yang C, Zhai ZH (1996) The nuclear lamina in male generative cells of *Ginkgo biloba*. Sex Plant Reprod **9:** 238–242

Weipoltshammer K, Schoefer C, Almeder M, Philimonenko VV, Frei K, Wachtler F, Hozak P (1999) Intranuclear anchoring of repetitive DNA sequences: centromeres, telomeres, and ribosomal DNA. J Cell Biol **147:** 1409–1418

Wilson K (2000) The nuclear envelope, muscular dystrophy and gene expression. Trends Cell Biol **10:** 125–129

Yu W, Moreno Diaz De La Espina S (1999) The plant nucleoskeleton: ultrastructural organization and identification of NuMA homologues in the nuclear matrix and mitotic spindle of plant cells. Exp Cell Res **246:** 516–526

Zentgraf U (1995) Telomere-binding proteins of *Arabidopsis thaliana*. Plant Mol Biol **27:** 467–475

Zickler D, Kleckner N (1998) The leptotene-zygotene transition of meiosis. *In* Annual Review of Genetics, Vol. 32. pp 619–697

Zimowska G, Aris JP, Paddy MR (1997) A *Drosophila* Tpr protein homolog is localized both in the extrachromosomal channel network and to nuclear pore complexes. J Cell Sci **110:** 927–944