Nuclear Organization and Chromosome Segregation

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INTRODUCTION

The genetic information underlying plant form and function is contained within the cell nucleus in the form of complex, dynamic DNA-protein structures—the chromosomes. The faithful transmission of these structures during mitosis and meiosis requires extensive chromosome reorganization and the formation of a unique, transient structure, the spindle, which serves to segregate chromosomes to daughter cells. Recent studies indicate that chromosomes can undergo dynamic structural changes and movement within the nuclei of nondividing cells as well.

The biology of the nucleus, its contents and functions, is a complex, rapidly evolving topic (Lamond and Earnshaw, 1998). This review focuses on chromosome organization within the nucleus and during cell division. Communication between the nucleus and the cytoplasm, as mediated by nuclear pores, recently has been reviewed by Heese-Peck and Raikhel (1998). For more information on nuclear envelope structure and formation, see the review by Gant and Wilson (1997).

CHROMATIN ORGANIZATION WITHIN THE NUCLEUS

The interphase nucleus is compartmentalized, with the most conspicuous compartment being the nucleolus, the site of ribosomal RNA synthesis and ribosome assembly (reviewed in Shaw and Jordan, 1995). The remainder of the nucleus is filled with chromatin and the machinery required for its function, including the transcription and DNA replication machinery. When visualized by indirect immunofluorescence, many nuclear components, such as the ribonucleoprotein complexes involved in removing introns from nuclear mRNA precursors, localize not only as discrete bodies but also diffusely throughout the nucleoplasm (Lamond and Earnshaw, 1998). Individual chromosomes, however, cannot be discriminated within the mass of chromatin by conventional DNA staining techniques. Only with the recent development of fluorescence in situ hybridization (FISH) technology have individual chromosomes been discernible as nonoverlapping cloudlike structures (Lichter et al., 1988).

Euchromatin

By visualizing sites of BrUTP incorporation in rye nuclei, Abranches et al. (1998) have shown that there are multiple sites of transcription throughout a given chromosome cloud. The transcribed chromatin regions are associated with euchromatin (diffuse chromatin), whereas the heterochromatin (condensed chromatin) is not transcribed. Furthermore, transcriptionally active euchromatin is hypersensitive to nucleases (see Paul and Ferl, 1998a, and references therein), and it has been found to contain acetylated histones (reviewed in Pollard and Peterson, 1998).

This latter observation has opened up an exciting area of current research with the discovery that some transcription factors regulate gene expression by remodeling local chromatin structure via histone acetylation (reviewed in Gregory and Horz, 1998). Transcriptional activators, such as Gcn5 from yeast, have been shown to exhibit histone acetylase activity (Candau et al., 1997), whereas some transcriptional repressors either directly deacetylate histones (Taunton et al., 1996) or function by recruiting histone deacetylases to their sites of action (Brehm et al., 1998). Recently, a link has been demonstrated between methylation-dependent gene silencing in animals and histone deacetylation.

In plants and animals, hypermethylation of CpG sites is a common feature of both silenced genes and heterochromatin (Richards, 1997). The mammalian MeCP2 protein binds to DNA methylated at CpG sites and recruits a transcriptional repressor complex containing histone deacetylases (reviewed in Razin, 1998). Whether or not a similar situation exists in plants during epigenetic or homology-dependent gene silencing remains to be determined. Nevertheless, these studies indicate that the local structure of chromatin can be reorganized to affect gene expression directly.

Heterochromatin

Heterochromatin is typically composed of repetitive DNA sequences, as in the case of centromeres and telomeres. In addition, many plants contain constitutive heterochromatin at other chromosomal sites. For example, the heterochromatic “knobs” of maize chromosomes are primarily composed of thousands of copies of a 180-bp repeat (Ananiev et al., 1998). Despite the fact that this repeat sequence does
not encode a gene product, maize knobs are not inert and in fact exert multiple effects on meiotic recombination (Rhoades, 1978).

Plant centromeres are large and complex, on the order of 1 to 10 megabases (reviewed in Richards and Dawe, 1998). Sequence analysis of centromere-associated DNAs has identified a number of repeat sequences, several of which appear to be retroelements (Richards and Dawe, 1998). In an attempt to define the functional unit of the plant centromere, Kaszas and Birchler (1998) analyzed meiotic and mitotic transmission of deletion derivatives of the maize B chromosome. They discovered that the centromere could be broken up into small but still functional units, thereby confirming at the molecular level McClintock’s early prediction that the centromere is composed of repeated units (McClintock, 1932).

The heterochromatic nature of telomeres, the specialized ends of chromosomes, may be due in part to the numerous repetitions of the canonical repeat sequence found in plant telomeres, (TTTAGGG), variants of which are found in most eukaryotes. The discovery of this DNA sequence to the ends of leading DNA strands occurs through the reverse transcriptase activity of the telomerase complex resolves the conceptual problem as to how lagging DNA strands of replicating chromosomes would be completely extended (reviewed in McKnight et al., 1997). In maize, the loss of telomeres by chromosome breakage results in “sticky chromosome ends” that can fuse together to yield unusual chromosome structures, such as rings or dicentric chromosomes (the breakage-fusion-bridge cycle). However, in certain tissues these “sticky ends” can heal to form new stable telomeres (McClintock, 1951). Telomere repair presumably involves the de novo formation of new telomere sequences at the ends of the broken chromosomes.

**Higher Order Chromatin Structure Required for Cell Division**

Before cell division, the chromatin clouds are repackaged into small, discrete structures that can be readily distributed by the spindle. This extensive repackaging involves not only chromatin condensation but also the disentangling of sister chromatids by topoisomerase II as well as the formation of the kinetochore (i.e., the microtubule attachment site at the centromere). In meiotic prophase, homologous chromosomes furthermore must pair and recombine so as to yield bivalents.

It is not known how many levels of packaging there are in nuclear chromatin or in metaphase chromosomes. At the lowest level, chromatin becomes organized into a 10-nm fiber, a “beads-on-a-string” structure composed of nucleosome arrays. The next order of structure is the 30-nm fiber that, according to the “solenoid” model, arises from the helical coiling of the 10-nm fiber (reviewed in Koshland and Strunnikov, 1996). However, Horowitz et al. (1994) present electron tomographic data supporting an alternative model for the 30-nm fiber as a zigzag ribbon. The next level of chromatin packaging results in a 200-nm fiber that is thought to consist of loops of the 30-nm fiber emanating from a nuclear matrix. Consistent with this model is Paul and Ferl’s (1998a) analysis of interphase chromatin after treatment with either DNase I or inhibitors of topoisomerase II. Their results suggest that specific chromatin loops on the order of 25 kb for Arabidopsis and 45 kb for maize exist in the nucleus. The observation of distinctive DNA domains that can be probed by specific genes might reflect matrix attachment regions, that is, specific chromosomal sequences that attach to the nuclear matrix (Avramova et al., 1995).

Metaphase chromosome structure is built upon those layers of chromosome organization that are described above (Iwano et al., 1997). Chromosome condensation proteins of the SMC/XCAP family (reviewed in Hirano, 1998) and histone H3 phosphorylation (Hendzel et al., 1997) are important in the formation of the metaphase chromosome. SMC proteins initially were identified genetically in yeast as being required for the correct segregation of mini chromosomes. Vertebrate SMC homologs, XCAP proteins (Xenopus chromosome-associated protein), form large multisubunit complexes, which, in the presence of ATP, are able to condense chromatin into chromosome-like structures in Xenopus oocyte extracts. It will be interesting to learn where in the chromosome condensation hierarchy these proteins act.

The relationship between histone H3 phosphorylation and mitotic chromosome condensation is not as well understood (Hendzel et al., 1997). Phosphorylation in mammalian prophase nuclei occurs initially at a few discrete sites corresponding to centromeric regions. Later, as chromosome compaction nears completion, whole chromosomes become highly phosphorylated. Interestingly, this pattern of phosphorylation is similar to the pattern of barley chromosome condensation during mitotic prophase (Martin et al., 1996), in which condensation initiates in the centromeric regions followed by a wave of condensation proceeding toward the telomeres.

**CHROMOSOME BEHAVIOR DURING MEIOTIC PROPHASE**

The accurate segregation of chromosomes at the first division of meiosis requires that homologous chromosomes recombine with each other, forming bivalents. Failure to undergo recombination results in the inaccurate segregation of chromosomes at metaphase I. The processes of pairing and crossing-over of homologous chromosomes occur in the extensive prophase of meiosis. Meiotic prophase has been divided into five stages based on the dramatic changes in chromosome morphology that occur: leptotene, zygote, pachytene, diploteine, and diakinesis (reviewed in Dawe, 1998). In the first stage, leptotene, the diffuse clouds of chromatin are organized into long thin fibers as the protein-
aceous axial element of the synaptonemal complex (SC) assembles onto the chromosomes. During zygotene, chromosomes begin to synapse (i.e., tightly associate along their length) as the central element of the SC is installed between the axial elements already assembled onto homologous chromosomes. During pachytene, the SC is fully formed, and meiotic recombination between homologs is completed. In diplotene, the SC disassembles, and chiasmata, the cytological manifestations of crossing-over, become visible. Finally, in diakinesis, the chromosomes undergo a final stage of chromosome condensation just before metaphase.

Telomere Clustering at the Nuclear Envelope

Before chromosomes synapse during zygotene, the nucleus itself becomes highly polarized by the formation of the telomere “bouquet,” first observed at the turn of the century (reviewed in Dernburg et al., 1995). Figure 1 depicts such a bouquet, in which the ends of the chromosomes cluster together on the inner surface of the nuclear envelope. Although the bouquet has been observed in organisms throughout the plant, animal, and fungal kingdoms, its actual role is open to debate. The close relationship between telomere clustering and chromosome synapsis has led to the suggestion that the bouquet may help to facilitate pairing and synapsis. Consistent with this potential role is the general observation that synapsis typically is initiated near the telomeres (Burnham et al., 1972). One possibility is that by orienting the ends of the chromosomes, homologous regions of the chromosomes are vectorially aligned within a specific region of the nucleus. In addition, the clustering may serve to confine homologous sequences to a small volume of the nucleus so as to promote synapsis. Identification and analysis of telomere bouquet mutants would help to resolve these questions.

In maize (Bass et al., 1997) and in mice (Scherthan et al., 1996), the telomere bouquet forms de novo at the beginning of zygotene. The kinetics of telomere attachment in these organisms are consistent with a model in which the telomeres attach to the nuclear envelope at random and then “skate” around the inner surface of the nuclear envelope until they approach each other.

An obvious question, then, is what force mediates the polarized movement of telomeres? In algae and in animals, the centrosome is found directly outside the nuclear envelope adjacent to the telomere cluster. Furthermore, in both plants and animals, treatment of premeiotic or leptotene meiocytes with the drug colchicine, a microtubule depolymerizing agent, inhibits synapsis of homologous chromosomes.

These observations have led to the suggestion that cytoplasmic microtubules, interacting with components in the nuclear envelope, may drive telomere clustering (see Dawe, 1998). However, higher plants do not have centrosome microtubule organizing centers (MTOCs) as do animals. Furthermore, other microtubule depolymerizing drugs, such as amiprophos methyl and vinblastine, do not inhibit telomere clustering in rye, thereby suggesting that bouquet formation in plants is not dependent on cytoplasmic microtubules (C. Cowan and W.Z. Cande, unpublished data). Whether a novel form of tubulin or some other colchicine-sensitive protein is involved in telomere clustering remains to be determined.

Chromosome Pairing and Synapsis

In most organisms, including maize (Dawe et al., 1994), oats (H.W. Bass and W.Z. Cande, unpublished data), humans, and mice (Scherthan et al., 1998), homologous chromosomes become associated with each other during zygotene. In wheat, Aragon-Alcaide et al. (1997) observed the premeiotic association of chromosome pairs in both tapetal and pollen mother cells.

Perhaps more important than when homologous chromosomes associate during meiosis, a major unresolved issue is how they identify each other. The axial elements of the SC do not appear to contain the information for distinguishing homology because they will synapse randomly in a haploid organism undergoing meiosis (Loidl et al., 1991; Santos et al., 1994). Thus, the general consensus is that the homology

**Figure 1.** The Bouquet Stage of Meiotic Prophase.

Three-dimensional, maximum intensity projection of a quarter of a maize meiocyte nucleus at zygotene. Chromosomes are stained with DAPI (red) and by FISH probes for telomeres (green). The telomere bouquet is demarcated. Bar = 5 μm. Figure courtesy of Hank Bass (Florida State University, Tallahassee).
search is DNA based, so that pairing occurs despite the presence of chromosome inversions, translocations, and alien chromosomes. Indeed, in a number of species, chromosome homology search and synopsis are closely coupled both temporally and spatially during zygotene. Electron microscopy has revealed the presence of large numbers of small electron-dense spheres associated with synapsing chromosomes during this stage (Hobolth, 1981). These structures are called “early recombination nodules” and were predicted well over a decade ago to function in the chromosome homology search and to contain recombination enzymes (Carpenter, 1987). Anderson et al. (1997) have confirmed this latter prediction in fly by demonstrating via immunoelectron microscopy that early recombination nodules do indeed contain the recombination protein Rad51 and its meiotic variant, Dmc (see below).

**Meiotic Recombination**

Upon completion of chromosome pairing and synapsis, the cells have entered the pachytene stage. During pachytene, meiotic recombination is completed, leading to the exchange of chromosome arms, an event that physically ties homologs together (Padmore et al., 1991). At the same time, electron-dense nodules, larger and fewer in number than the zygotene nodules, are observed between the paired lateral elements of the SC (on unpaired chromosomes, lateral elements are referred to as axial elements). These “late recombination nodules” (Carpenter, 1987) have been directly correlated with meiotic recombination (Maguire and Riess, 1994).

Although it is not known when recombination is initiated, molecular genetic studies in yeast (Keeney et al., 1997), Drosophila (McKim and Hayashi-Hagihara, 1998), and Caenorhabditis elegans (Demerg et al., 1998) indicate that meiotic recombination is initiated by double-stranded DNA breaks formed by the product of the Spo11 gene, a distant homolog of topoisomerase II. The double-stranded break serves as a substrate for Rad51-mediated homologous recombination. Rad51 and Dmc are themselves homologs of the bacterial RecA protein, and both are required for meiotic recombination in yeast (Bishop et al., 1992; Shinohara et al., 1992). Like RecA, Rad51 is capable of polymerizing onto single-stranded DNA and initiating DNA strand invasion into homologous duplex DNAs (Baumann et al., 1996). Rad51 and Dmc thus may perform the strand invasion events associated not only with recombination itself but also with the homology search.

Rad51 homologs have been identified in plants, and it is likely that fundamental aspects of the meiotic recombination pathway are conserved (Doutriaux et al., 1998). However, unlike yeast, which have many recombinations per chromosome, plants typically have only one to two per chromosome (Sherman and Stack, 1995). In any event, after the chromosomes have recombined, the SC is no longer necessary and is disassembled during diplotene. Simultaneously, or as a consequence of the dissolution of the SC, the chromosomes undergo further condensation, which continues into metaphase I.

**CHROMOSOME SEGREGATION BY THE SPINDLE**

Once the chromosomes are fully condensed, they can be segregated to the daughter cells. A special structure, the spindle, is responsible for the sorting of sister chromatids and for generating the forces that lead to their separation at mitosis and meiosis (reviewed in Baskin and Cande, 1990; Rieder and Salmon, 1998). Spindles are bipolar structures, and the microtubules that comprise their framework are organized in two sets that overlap in the spindle midzone. Within each half spindle, almost all the microtubules are of the same polarity, and the resulting bipolar symmetry of the spindle is essential for chromosome segregation.

The plus (fast-growing) ends of microtubules from one side of the spindle are either interdigitated in the middle of the spindle with the microtubule plus ends from the other half of the spindle or they are attached to chromosomes at the kinetochores. During mitosis and metaphase I of meiosis, the kinetochores of sister chromatids face opposite spindle poles (reviewed in Dawe, 1998). During metaphase I of meiosis, the sister kinetochores face the same spindle pole, whereas the kinetochores of the paired homologs face opposite poles.

The minus (slow-growing) ends of the microtubules of each half of the spindle are embedded in the spindle poles or MTOC. In higher plant cells, the MTOC is an amorphous structure, whereas in animal somatic cells the MTOC is derived from the centrosome, a structure composed of two centrioles surrounded by a proteinaceous meshwork. Nevertheless, homologous components of the animal centrosome, such as γ-tubulin and centrin, have been found in plant cells (reviewed in Vaughan and Harper, 1998). However, γ-tubulin is associated with all plant microtubule arrays and is distributed throughout the spindle rather than just at the spindle poles in animal cells (Oishi and Palevitz, 1996). Mazia (1984) has proposed that a single, flexible centrosome would spread throughout the plant cell, respond to cell cycle-specific signals, and nucleate microtubules at specific times and specific sites. An alternative view is that plants do not have a centrosome-like structure and that the spindle poles are formed de novo during spindle assembly (Smimova and Bajer, 1994, 1998).

**Plant Spindle Assembly Occurs without a Centrosome**

There are special problems in analyzing spindle assembly in plant cells because an obvious interphase centrosome complex is lacking. In the somatic plant cell during G2, cortical
microtubule arrays are rearranged in the cortex at the future division plane to form a preprophase band of microtubules. At the same time, the number of microtubules associated with the nuclear envelope increases severalfold (Vaughn and Harper, 1998). Because it has been demonstrated in vitro that the nuclear envelope surface can nucleate microtubules (Stoppin et al., 1994), it is likely that the minus ends of these microtubules are all associated with the nuclear envelope. As seen in Figures 2 and 3A, nuclear envelope-associated microtubules become rearranged as a bipolar array during mitotic prophase.

Although the details of the process are unclear, prophase spindle formation requires the preexisting, nuclear envelope-associated radial microtubule network to reorient by 180°. The minus ends of these microtubules must be pointed outward toward the cell periphery, and the plus ends must cluster in the middle of the cell around the nuclear envelope as overlapping antiparallel microtubule arrays. After nuclear envelope breakdown, the microtubule plus ends invade the nucleoplasm to engage the chromosomes at their kinetochores (reviewed in Rieder and Salmon, 1998). During prometaphase, chromosomes move into and out of the spindle midzone, until proper bipolar connections are made.

In cells that have them, centrosomes play a critical role in establishing the bipolar microtubule prophase array. In such cells, the prophase centrosome splits in two, and as the two halves separate, they drag the microtubules with them. The free plus ends of the microtubules emanating from the two separating centrosome halves then interdigitate to generate an antiparallel array (reviewed in Vernos and Karsenti, 1995).

This model, however, does not work well for plant cells, even if we accept Mazia’s (1984) view that plants have flexible centrosomes that give rise to the spindle poles. In any case, there are now examples of spindle formation in acen- triolar somatic and meiotic animal cells. Observations of spindle formation in Drosophila oocytes, for example, reveal that bipolar microtubule arrays organize around the chromatin

Figure 2. The Pathway of Spindle Assembly in Mitotic and Meiotic Plant Cells.

(A) to (D) The distribution of microtubules in somatic spindles, as viewed by using the immunogold-enhanced silver technique in Haemanthus endosperm cells at early prophase (A), midprophase (B), late prophase (C), and prometaphase (D). Bar in (A) = 10 μm. (A) to (D) courtesy of Andrew Bajer and adapted from Smirnova and Bajer (1994, 1998).

(E) to (H) The distribution of microtubules in maize meiocytes. Single optical sections taken by confocal laser scanning microscopy of a meiocyte in diakinesis (E), early prometaphase (F), metaphase I (G), and anaphase I (H). The chromosomes, stained with propidium iodide, are shown in red, and the microtubules, stained with a monoclonal antibody against tubulin, are shown in green. Bar in (E) = 10 μm. (E) to (H) adapted from Chan and Cande (1998).
mass, or karyosome, after nuclear envelope breakdown (McKim and Hawley, 1995). A similar phenomenon has been described in vitro by using Xenopus oocyte extracts and beads coated with DNA (Heald et al., 1996). In these systems too, chromatin acts as a nucleating factor for organizing bipolar microtubule arrays.

The establishment of spindle bipolarity further requires kinesin-related proteins and spindle pole components, which are recruited to the microtubule minus ends by cytoplasmic dynein. Kinetochore do not appear to be necessary in this regard because the spindle forms even in the absence of centromeric DNA. Similar scenarios may exist in plant cells, although the timing of the reorganization of cytoplasmic microtubule arrays relative to nuclear envelope breakdown may differ between mitotic versus meiotic plant cells.

The Mitotic Spindle Assembly Pathway

The most complete description of the formation of somatic plant spindles comes from the studies of Haemanthus endosperm (Smirnova and Bajer, 1994, 1998; see also Figures 2 and 3A). In this system, the radial network of microtubules surrounding the prophase nucleus is broken up into numerous smaller bundles, many of which form fan-shaped arrays, or microtubule converging centers (MTCCs). The MTCCs...
probably consist of bundled parallel microtubules whose minus ends converge. The prophase spindle then forms as the convergent ends of multiple MTCCs aggregate, and the divergent ends grow and eventually overlap with each other.

At this stage, shown in Figure 3A, the spindle may be multipolar, but the essential bipolarity of the spindle is established as the nuclear envelope breaks down and chromosomes interact with the diverging microtubule plus ends of the MTCCs so as to effect kinetochore attachment. The “fir tree-like” kinetochore microtubule organization, seen in a variety of plant cells (Palevitz, 1988; Figure 3A), likely develops as microtubules from multiple MTCCs interact with each other and are captured by the kinetochore. Significantly, MTCCs can form spindelike structures in Haemanthus cytoplasts (i.e., in the absence of a nucleus), demonstrating that neither the nuclear envelope nor the kinetochore is required for spindle formation (Smirnova and Bajer, 1998).

The mechanism of reorientation of MTCCs during mitotic prophase is unknown, but it may be similar to that observed in other acentriolar cells. As evidenced by higher rates of turnover, plant interphase microtubule arrays are much more dynamic than are animal interphase arrays (Hush et al., 1994). New microtubules, moreover, may be polymerizing near the plant nuclear envelope with opposite polarities relative to the attached microtubules. Alternatively, the MTCCs may form as microtubules, be released from the nuclear envelope surface, and reorganize to form parallel arrays.

With the aid of minus end-directed microtubule motors, such as the calmodulin-dependent, kinesin-related protein found at spindle poles (Bowser and Reddy, 1997), pole material is recruited to the minus ends of microtubules forming the convergent focus of the MTCCs. Newly formed MTCCs would interact with the remaining microtubules of opposite polarity still associated with the nuclear envelope, and, with the aid of lateral interactions between parallel microtubules, MTCCs would then aggregate into two or more caps. After nuclear envelope breakdown, capture of the plus ends of microtubules by kinetochores would lead to further aggregation of MTCCs to form a typical barrel-shaped somatic plant spindle (Figure 3A).

**The Meiotic Spindle Assembly Pathway**

Spindle formation may be less complex in plant meiocytes. Pollen mother cells lack interphase cortical microtubule arrays and the prophase band, but they do have extensive cytoplasmic networks of microtubules, which persist through diakinesis and appear, in part, to be associated with the nuclear envelope. However, prophase spindles have not been found in meiotic plant cells (reviewed in Chan and Cande, 1998). In maize meiocytes after nuclear envelope breakdown, for example, the chromosomes are surrounded by microtubules, either redistributed previously from the cytoplasmic network or nucleated by the chromatin (Figures 2 and 3B). During prometaphase, these microtubules are rapidly organized into a bipolar array, presumably with the aid of similar classes of motors, as described for Xenopus spindle assembly (Vernos and Karsenti, 1995). The spindles become longer over time and eventually reach the plasma membrane. The poles become more focused as the chromosomes attach to the spindle and form a metaphase plate (see Figure 3B).

Although some converging microtubules are seen in the developing spindle, conspicuous MTCCs, like those in mitotic cells, are not found. Moreover, the bilateral kinetochore symmetry of bivalent chromosomes at metaphase I is not required for the establishment of a bipolar spindle inasmuch as bipolar spindles form in desynaptic mutants with univalents, that is, with unpaired chromosomes that have only one functional kinetochore. This observation suggests that chromatin per se can stabilize or nucleate microtubule arrays and, in conjunction with microtubule motors, generate a bipolar antiparallel array of microtubules (Chan and Cande, 1998).

**Chromosome Movement on the Spindle**

The spindle is a highly redundant system involving many different motors. These motors play critical roles in regulating microtubule dynamics and organizing the microtubules into a bipolar array. In addition, they generate the forces that are required for the proper attachment of chromosomes to the spindle during prometaphase and that drive the chromosomes toward the spindle poles during anaphase A and the spindle poles farther apart during anaphase B. Regardless of how the forces are generated, they can thus be divided into two classes: forces that are applied directly to the chromosome, usually at the kinetochore, and that lead to chromosome movement at prometaphase and to anaphase A, and forces applied to the half spindles leading to spindle elongation.

Chromosome movement toward or away from the spindle pole is generated by a complex set of forces, some due to microtubule disassembly at the kinetochore or at the spindle pole (microtubule flux), and others generated by motors attached to the kinetochore or chromatin (reviewed in Rieder and Salmon, 1998). Coordinated microtubule depolymerization during anaphase is required for poleward chromosome movement, and in vitro studies demonstrate that microtubule depolymerization can generate sufficient force to move attached chromosomes toward an anchored microtubule minus end (Lombillo et al., 1995). During prometaphase, chromosome congression to the spindle midzone is accompanied by tubulin subunit addition at one kinetochore and loss from the corresponding sister kinetochore. Microinjection of low levels of calcium (to 1 μM) into Tradescantia stamen hairs during anaphase leads to partial depolymerization of kinetochore microtubules and accelerates poleward chromosome movement (Zhang et al., 1992). Both plus end- and minus end-directed microtubule motors are associated
with the kinetochore, including cytoplasmic dynein, and kinesin-related proteins (reviewed in Rieder and Salmon, 1998), and these motors could be involved in either prometaphase or metaphase chromosome movements. At least two kinesin-related proteins, NOD and chromokinesin/Xklp1, bind directly to chromosome arms in animal cells, and homologs of these proteins could be involved in plant spindle formation and in establishing proper spindle/chromosome connections (McKim and Hawley, 1995). Chromosome fragments generated by microsurgery so as to lack kinetochores move poleward in prometaphase Haemanthus spindles, but they move toward the spindle midzone in anaphase spindles. However, in animal cells, such chromosome fragments always move toward the spindle equator during prometaphase and anaphase I and II. Unlike kinetochores, which associate with microtubules end-on, knobs associate with microtubules via lateral interactions and move faster than the kinetochores. These knobs, or neocentromeres, represent one component of a complex meiotic drive system. It is therefore possible to use this system to identify chromosome-associated motors by selecting for mutants defective in neocentromere-driven chromosome movement (reviewed in Yu et al., 1997).

A classic example of non-kinetochore-based chromosome movement is provided by neocentromeric activity in maize during meiosis. In the presence of Abnormal chromosome 10, the heterochromatic DNA domains known as knobs (see above) attach to the spindle and move poleward during prometaphase and anaphase I and II. Unlike kinetochores, which associate with microtubules end-on, knobs associate with microtubules via lateral interactions and move faster than the kinetochores. These knobs, or neocentromeres, represent one component of a complex meiotic drive system. It is therefore possible to use this system to identify chromosome-associated motors by selecting for mutants defective in neocentromere-driven chromosome movement (reviewed in Yu et al., 1997).

The kinetochore is not only a platform for motors and a site for linking microtubules to the chromosome, it is also a site for regulation of the transition of metaphase to anaphase. Laser surgery and microinjection experiments in animal cells demonstrate that weakly attached kinetochores result in the delayed onset of anaphase until proper chromosome orientation and attachment to the spindle is achieved (Nicklas, 1997). Microinjection of protein kinase and phosphatase inhibitors into dividing Tradescantia stamen hairs at different times during prometaphase further demonstrates that progression into anaphase requires several waves of protein kinase activity and at least one dephosphorylation reaction (Wolniak and Larsen, 1995). The kinetochore-associated checkpoint could be a part of this cascade.

**Spindle Elongation**

The separation of spindle poles during anaphase involves pulling forces from outside the spindle and pushing forces within the spindle midzone. The pulling forces are generated by astral microtubules interacting with minus end-directed motors, such as dynein in the cell cortex. The pushing forces are generated in the zone of microtubule overlap at the spindle midzone by plus end-directed motors interacting with the sliding antiparallel microtubule arrays. Plants may be more dependent than animal or fungal cells on pushing forces because they lack extensive astral microtubule arrays (Baskin and Cande, 1990). Indeed, addition of ATP to isolated diatom spindles demonstrates that mechanical interactions in the zone of microtubule overlap can drive anaphase B, even in the absence of tubulin polymerization. This movement is blocked by antibodies against DSK1, a kinesin-related protein, which is located in the spindle midzone (Wein et al., 1996). In any case, the successful completion of anaphase is immediately followed by dissolution of the spindle, usually accompanied by the formation of a new antiparallel microtubule array, the phragmoplast, which is responsible for cytokinesis (reviewed in Fowler and Quatrano, 1997).

**Polarized Nuclear Organization After Anaphase**

The polarization of chromosomes during anaphase, with the centromeres leading and the telomeres following, is usually retained at telophase, when the nuclear envelope reforms around the decondensing chromosomes. This organization of the chromosomes, shown in Figure 4, is termed Rabl in honor of the investigator who first described it (Rabl, 1885). Surprisingly, the Rabl orientation of chromosomes can persist long after cell division is complete. Stack and Clark (1974) observed persistent Rabl orientation in onion root tip cells by using Giemsa staining to visualize telomeric heterochromatin.

More recently, Abranches et al. (1998) and Dong and Jiang (1998) have detected persistent Rabl orientation of chromosomes in interphase nuclei from a number of different grasses by using centromere and telomere FISH. Dong and Jiang (1998) observed Rabl in monocots with large genomes but not in those with small genomes, such as rice. They speculated that the persistence of Rabl might be merely a consequence of the constraints of chromatin diffusion in nuclei of organisms with large chromosomes or large genomes.

Alternatively, Rabl orientation may persist due to specific associations of telomeres and centromeres with the nuclear envelope, as suggested by Stack and Clark (1974). Indeed, Ashley (1979) has described the persistent association of centromeres and telomeres with the nuclear periphery in diploid Ornithogalum cells, and both telomere–telomere and centromere–centromere associations between nonhomologous chromosomes are detectable in these nuclei. Nonhomologous centromere pairing also is observed in premeiotic lily nuclei (Suzuki et al., 1997). More studies are needed to explore how universal these observations are and to develop a framework to understand the role of the higher order organization of chromosomes within the nucleus. Nevertheless, it
is apparent that chromosomes and their subdomains, such as centromeres and telomeres, localize and behave in a highly prescribed manner within the nucleus.

OVERVIEW AND FUTURE DIRECTIONS

The studies mentioned in this review lead us to speculate that processes involved in chromosome segregation, such as condensation and movement, have to some degree been adopted by interphase nuclei for the regulation of interphase functions. Recent evidence indicates that chromosome condensation, once discussed only in the context of cell division, also is used by cells to modulate gene expression. The active movement of the chromosomes during meiotic telomere clustering is reminiscent of the active movement of chromosomes on the spindle. Finally, the persistent Rabl organization observed in somatic nuclei can be viewed as a direct manifestation of chromosome segregation. It is an open question as to whether this organization and the association of centromeres and telomeres with the nuclear envelope affect gene expression. Little is known about the rearrangements of chromosomes in somatic nuclei and their importance in the regulation of nuclear function. Although controversial, it has been suggested that homologous pairing of chromosomes during the somatic cell cycle may play an important role in such poorly understood epigenetic phenomena as paramutation and cosuppression.

These speculations require that the functions assigned to the nuclear envelope be reexamined and perhaps broadened in scope. The nuclear envelope, and in particular the nuclear pores, has been shown to be involved in regulating nucleocytoplasmic interactions, especially the export of ribonucleoproteins out of the nucleus. However, the nuclear envelope also is clearly involved in some aspects of cytoplasmic organization. For example, in higher plant cells that lack a well-defined MTOC, the nuclear envelope appears to play a critical role in mitotic spindle assembly during prophase. In particular, it may act to recruit components needed to establish a bipolar microtubule array and a functional spindle pole. Within the nucleus, chromatin interactions with the nuclear envelope may play several important roles, such as in chromosome pairing during meiosis, somatic Rabl chromosome organization, and in the regulation of gene expression. It is also possible that the domain structure of the interphase nucleus is established by specific chromatin-nuclear envelope associations. If chromosomal domain organization is important in nuclear function, such associations would have profound consequences for the fate of the cell.

ACKNOWLEDGMENTS

We thank Rebecca Heald, Andrew Bajer, Jiming Jiang, Peter Shaw, and members of the Cande laboratory for helpful comments. We also...
thank the National Institutes of Health for their support of this re-
search. (Grant Nos. NIHGM23238 and GM48547).

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Nuclear Organization and Chromosome Segregation
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DOI 10.1105/tpc.11.4.523

This information is current as of June 27, 2017