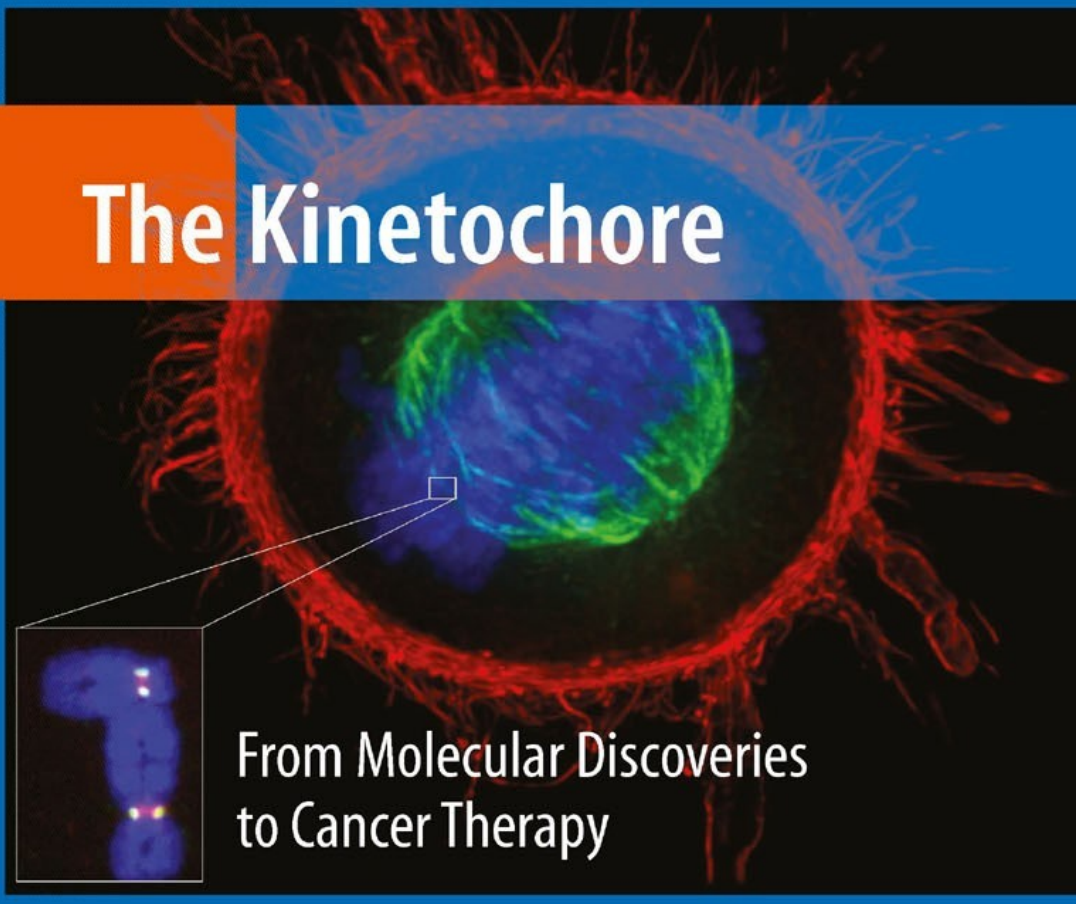
Peter De Wulf
William C. Earnshaw
Editors

The Kinetochore



From Molecular Discoveries to Cancer Therapy

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Preface

The accurate segregation of replicated chromosomes (sister chromatids) during cell division guarantees a correct number of chromosomes in subsequent generations of cells. Importantly, errors made during this process lead to aneuploid progeny with abnormal chromosome numbers, which can either cause genetic diseases, or, in the case of somatic cells, cause diseases such as cancer. For example, virtually all solid tumors known to date are aneuploid, suggesting that chromosome missegregation underlies or contributes to the initiation and/or progression of cancer.

Kinetochores are highly conserved multi-protein structures that form on the centromeric regions of sister chromatid pairs. Kinetochores orchestrate sister chromatid segregation and ensure that cellular ploidy is maintained. Following the identification of the first three kinetochore proteins in 1985 by one of us, 80–100 proteins (depending on the species) have now been localized to centromeres. These proteins act either as structural kinetochore or centromere components, or as regulators of centromere establishment, and kinetochore formation or activity. Arguably, the kinetochore is one of the most dynamic and complex protein structures known to date.

Recent years have witnessed an outpouring of studies on kinetochore components and centromeres. During the last five years alone, a yearly average of 200 and 500 papers cite the kinetochore and centromere, respectively (Pubmed). This research avalanche has resulted in an almost unmanageable amount of data. Unfortunately, since the publication of his outstanding book by Andy Choo over a decade ago (K. Choo, *The Centromere*, Oxford University Press, New York, 1997), so much has been discovered and written that the non-expert is once again overloaded and bewildered. We therefore decided to create an up-to-date reference that provides a firm basis for understanding the past, current, and also future research on kinetochores and centromeres. To do this, we decided to bring together leading researchers in kinetochore and (neo)centromere biology to share their past experiences during development of the field, to summarize the current state of the art, and to offer hypotheses and predictions that will set the framework for future research.

Chapter 1 gives an historical account of how kinetochore proteins and centromeric regions were discovered. Chapter 2 details the chromosome segregation process and the players involved in it. Chapters 3–5 discuss the chromosomal regions onto which kinetochores assemble (Chapter 3: centromeres, Chapter 4: neocentromeres, Chapter 5: artificial centromeres). Chapter 6 summarizes the composition, formation, and organization of kinetochores, while Chapter 7 reconstructs how kinetochores and centromeres developed during evolution. Chapter 8 discusses the mitotic spindle with which kinetochores interact and within which they segregate into the daughter cells. Chapter 9 describes how kinetochores establish firm contact with and bi-orient on the spindle. Chapter 10 details essential enzyme activities that regulate kinetochore assembly and function. Chapter 11 describes how the proof-reading mitotic checkpoint ensures that incorrect attachments of kinetochores to spindle microtubules are detected and corrected prior to sister chromatid segregation at anaphase. Chapter 12 explains how certain kinetochore complexes (most notably the chromosomal passenger complex) relocalize to the spindle midzone at anaphase onset, thereby regulating sister chromatid segregation and triggering cytokinesis. Chapter 13 concentrates on the roles of kinetochores and centromere-bound cohesin in meiosis. Chapter 14 describes the ongoing efforts of mapping mutations in genes encoding kinetochore proteins and measuring kinetochore protein expression levels in tumor tissues. Last, but surely not least, Chapter 15 outlines how kinetochore proteins and their regulators can be turned into targets of anti-mitotic anti-cancer drugs.

We hope that with this book we have created a useful reference that will benefit experienced researchers in the field and provide an inspiration for those younger aspiring scientists and students who may wish to understand how kinetochores and centromeres orchestrate the fascinating processes of chromosome segregation that form a crucial underpinning for the continuation of life.

We would like to express our gratitude to the panel of international experts who donated their valuable time to help us review the contents of this book:

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We also thank those of our consultants who preferred to remain anonymous.

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Chapter 1
Centromeres and Kinetochores: An Historical Perspective

Kerry S. Bloom

1.1 Identification of Yeast Centromere DNA

As a preface of tribute to Centromere and Kinetochore function, it is interesting to reflect upon the discovery of chromosomes in the late 1880s when chromosomes were named (HWG von Waldeyer, 1888) and their function in heredity proposed by Boveri and Sutton's "Chromosome Theory of Inheritance" to almost 100 years later when the elements of chromosome propagation, namely centromere, telomere, and origins of replication were clearly identified. One can only imagine that the excitement in the field in the early 1880s was matched by the bold proposal that chromosomes were the unit of inheritance by Thomas Hunt Morgan in 1915 and contained the hereditary material. The DNA was discovered by Freidrich Meischer in 1869. It is noteworthy that it took almost 30 years after the determination of the double helical DNA structure, in 1953, to identify the sequence elements of chromosome structure. Identifying genes was child's play in comparison. The centromere does not encode protein, and therefore could not simply be cloned by complementing auxotrophic mutations. Mutations in centromeres should result in the loss of an entire chromosome; there is nothing conditional about that, and even if one managed to introduce a centromere into another site on a chromosome, Barbara McClintock showed us that this would trigger a breakage fusion bridge cycle that is catastrophic to the cell (McClintock, 1939). From the genetic perspective, the centromere is readily identified; it is the genetic locus that exhibits first division segregation in organisms with ordered or linear tetrads. The centromere is the primary constriction of condensed mitotic chromosome and provided a reference point for construction of genetic maps.

From a cytological perspective, the centromere is readily identified as the site of kinetochore assembly. The first description of the specialized disc-shaped kinetochore, a proteinaceous structure found at the periphery of the centromere

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came from electron micrographs of fixed specimens (Brinkley and Stubblefield, 1966; Jokelainen, 1967). These studies revealed alternating electron-dense, -lucent, and -dense layers. A recent review of these papers along with a reproduction of the early drawings can be found in Rieder (2005).

To appreciate the cloning of the first centromere in 1980 by Louise Clarke and John Carbon (UCSB; Clarke and Carbon, 1980), we have to step back in time to realize the difficulty and daring required to undertake this project. While the tools for cloning were available (e.g., restriction enzymes, ligase, plasmid vectors for growth in bacteria, etc.), there were no shuttle vectors for plasmid amplification in eukaryotes, no PCR, no genome sequences and no software for DNA data analysis. The only personal computers available at the time were available from Radio Shack or the Apple IIes, introduced in 1980, prior to the introduction of IBM PCs in 1981. Needless to say, there were none in the beautific campus nestled between mountains and the beach on the Pacific coast.

To embark on the isolation of the centromere one had to assume that the chromosome was a single linear DNA duplex. What was the evidence for this bold premise? Chromosomes were visible in stained preparations, but the definitive separation of linear chromosomal sized DNA molecules by gel electrophoresis was not performed until 1984 (Schwartz and Cantor, 1984). The assumption was based upon kinetics of deoxyribonuclease cleavage (Gall, 1963) and nucleic acid reassociation kinetics (Britten and Kohne, 1968; Wetmur and Davidson, 1968). The rate of nucleic acid hybridization allowed these investigators to determine genome size of various organisms and therefore the amount of DNA/chromosome. Bacterial genomes were in the range of several million base pairs and in the case of *Escherichia coli*, were contained in a single circular molecule. If eukaryotic chromosomes were also linear, then one should be able to isolate genes on either side of the centromere (its position defined by the patterns of first or second-division in meiosis) and walk through the centromere.

Chromosome walking was based on a technique known as overlap hybridization. Clarke and Carbon were the first to construct a library of *E. coli* chromosomal DNA fragments by shearing the genome into small fragments and cloning these into the Col E1 plasmid vector (Clarke and Carbon, 1976). One of the insights in this paper was the number of colonies that had to be isolated to ensure that the cloned fragments covered the entirety of the genome. They needed the data from nucleic acid reassociation kinetics to know the size of the genome and estimate how many clones would ensure a greater than 99% probability that the entire genome would be represented in the collection. The cloning of DNA libraries was a cottage industry in the early 1980s with the *Saccharomyces cerevisiae* one of the first eukaryotic libraries to be constructed (Chinault and Carbon, 1979; Nasmyth and Reed, 1980). The tools were now in hand, namely DNA reassociation and clone libraries.

To start this bold adventure, Louise Clarke and John Carbon isolated genes on either side of the centromere on yeast chromosome III (*LEU2* and *PGK1*). LEU2 was isolated by complementation of an auxotrophic mutant in *E. coli*

(*leuB6*; Chinault and Carbon, 1979) and PGK1 by an innovative immunological detection system for protein expression from bacterial colonies (Hitzeman et al., 1980). The walk entailed radiolabeling the DNA encoding *LEU2*, and using it as a hybridization probe to find *E. coli* colonies that contain some or all of the *LEU2* fragment. The strategy depends upon the fact that the library was made by A/T tailing with randomly sheared fragments of the genomic DNA, and thus on average, different colonies will contain differing pieces of the same gene. As one can see, this strategy is blind to direction and thus the walk in one direction is half the rate of walking (in this case, toward and away from the centromere). Clones containing overlapping fragments are then identified, picked, and DNA amplified by shuttling back to *E. coli*. Yeast DNA inserts were radiolabeled and used in a second round of overlap hybridization. Yeoman's work indeed and the bulk of the work were performed by A. Craig Chinault, a talented postdoctoral fellow at UCSB (Chinault and Carbon, 1979). One of the first landmarks discovered in this walk was the retrotransposon (yeast TY2; Kingsman et al., 1981). Transposable elements had just been discovered in yeast (Cameron et al., 1979). There are about 30 Ty1 elements dispersed in the genome, and over 100 of the "delta" sequences repeated at the termini of TY elements. Repeated DNA is the bane of the overlap hybridization strategists. Once a repeated region is encountered, many colonies "light up," and there is little hope that one can "walk across" the repeat with the tools in hand.

As often the case with science, serendipity interceded. A new faculty member, Dr. Steven Reed arrived at UCSB, hailing from Lee Hartwell's laboratory. Dr. Hartwell was busy determining the logic circuitry of the cell cycle, and several ambitious students were busy cloning a number of these cell division cycle mutants (aka *cdc* mutants; Hartwell et al., 1970). One of these, *CDC10*, was very closely linked to the centromere on chromosome III. Louise Clarke isolated a clone complementing the temperature-sensitive *cdc10* mutation. This clone contained an 8 kb fragment that overlapped with clones in the laboratory from the *LEU2* region. The hunt was on. *CDC10* is so close to the centromere that it was hard from genetic crossing-over data to distinguish whether it was on the side of *LEU2* or the other side. It was possible that this clone contained the elusive centromere.

Now we have to bear in mind that there was no complementation, or other routine assay for centromere function. It was not a simple step from *cdc10* to the centromere. It is necessary to take another step back to understand and appreciate the isolation of the first centromere. Transformation into yeast was just being developed (Hinnen et al., 1978; timeline Table 1.1), and opened the door for gene identification. One could readily use hybrid plasmids (yeast and *E. coli*) to complement yeast auxotrophic mutations. The major laboratories each had their favorite gene (Botstein- *URA3*; Fink- *HIS3*, *LEU2*; Davis – *TRP1*, Carbon – *ARG4*, *PGK1*), and rapidly shuttled them into the respective auxotrophic yeast mutants. The problems for Clarke and Carbon were that transformation was inefficient and depended upon genomic integration. This is incompatible with centromere function, as integration of a second centromere will

Table 1.1 Timeline of historic achievements in chromosome structural elements

1966 Trilaminar structure of the Kinetochore (Brinkely and Stubblefield, 1966; Jokelainen, 1967)
1976 *E. coli* library (Clarke and Carbon, 1976)
1978 Yeast transformation (Hinnen et al., 1978)
1979 Yeast origin of replication (ARS; Stinchcomb et al., 1979)
1980 Isolation of centromere DNA (Clarke and Carbon, 1980)
1980 Identification of autoantibody to centromere proteins (Moroi et al., 1980)
1981 Fragment-mediated transformation (Orr-Weaver et al., 1981)
1981 Direct selection for centromeres (Hsiao and Carbon, 1981)
1982 Sequence of centromere DNA (Fitzgerald-Hayes et al., 1982)
1982 Isolation of yeast telomeres (Szostak and Blackburn, 1982)
1982 Chromatin structure of a yeast centromere (Bloom and Carbon, 1982)
1983 Genetic substitutions (Clarke and Carbon, 1983)
1983 Pedigree analysis of chromosome segregation, Construction of artificial chromosomes (Murray and Szostak, 1983b and 1985)
1985 First identification of centromere proteins (Earnshaw and Rothfield, 1985)
1986 *S. pombe* centromere (Clarke et al., 1986)
1987 First cloning of a centromere protein, CENP-B (Earnshaw et al., 1987)
1991 Identification of yeast centromere DNA binding proteins (Lechner and Carbon, 1991)
1992 Complete sequence of chromosome III (Oliver et al., 1992)
1995 DIC microscopy to visualize yeast chromosome movements (Yeh et al., 1995)
1995 GFP fusions of cytoskeletal components in yeast (Kahana et al., 1995; Doyle and Botstein, 1996; Fleig et al., 1996; Carminati and Stearns, 1997; Shaw et al., 1997a, b).

create dicentric chromosomes, which were known to be unstable (McClintock, 1939, 1941, 1942). The next hurdle in the field was to solve the efficiency problem. It turned out that high frequency transformation depended on providing an origin of replication to the transforming plasmid (ARS, autonomously replicating sequence). This was first accomplished in Ron Davis's laboratory (Stinchcomb et al., 1979; Struhl et al., 1979). The *TRP1* gene was very closely linked to an ARS element (*TRP1-ARS1* on a 1.4 kb *EcoRI* fragment) and when introduced into yeast gave high frequency transformation. Shortly thereafter, there followed a breakthrough from the world of recombination, where R. Rothstein and colleagues realized that linearizing transforming DNA fragments enhanced the frequency of homologous recombination in mitosis >1000-fold (Orr-Weaver et al., 1981). These were very heady times for the field, indeed. The world of "gene therapy" was opening before our very eyes.

Meanwhile, Clarke and Carbon were busy introducing various fragments from the plasmid that complemented a temperature-sensitive *cdc10* mutant into replicating vectors. The purpose was to efficiently transform yeast. Here is where persistence and the adage, "chance favors a prepared mind" (L. Pasteur) are relevant. The game at the time was identifying genes. Prior to the discovery of ARS elements, clones that complemented metabolic defects all involved plasmids that had integrated into the genomes, and all transformed cells had

the same phenotype i.e., they were protrophic for the mutation in question. However Stinchcomb et al. (1979) noted that unlike the "low frequency" transformants, all the transformants resulting from these autonomously replicating plasmids were unstable. When the cells were grown in the absence of genetic selection for the complementing plasmid, the plasmids were lost from the population. In retrospect, we understand this because the plasmids lacked a centromere, and were not actively partitioned to the daughter cells. However in 1979, this was perplexing to students and postdoctoral fellows trying to learn yeast molecular biology.

Unlike the students and fellows in the laboratory, Clarke and Carbon were undaunted, and realized that the centromere should provide an active partitioning function. When cells containing the plasmid that complemented a *cdc10* mutant were grown in the absence of genetic selection, the plasmids were not lost from the population. Furthermore, the plasmids exhibited classical Mendelian segregation in meiosis (Clarke and Carbon, 1980). The criterion of first division segregation in meiosis was met, and Clarke and Carbon had unequivocally identified the first centromere. The game was afoot to isolate the remaining 15 centromeres and identify the DNA sequence that conferred centromere function.

Only a few laboratories had the expertise for DNA sequencing in the early 1980s. Two methods for sequencing DNA appeared on the scene, chain termination (F. Sanger) and chemical sequencing (base-specific chemical cleavage, A. Maxam and W. Gilbert). J. Carbon realized that sequencing would be key to understanding centromere function and sought a postdoctoral fellow, Molly Fitzgerald-Hayes with expertise in the methodology. Between DNA sequencing and continuing to transform yeast with plasmids containing successively smaller and smaller pieces of the centromere, Molly, Louise, and John discovered that the entire centromere was encoded on a piece of DNA approximately 120 bp in length. The characteristics of this fragment were several conserved sequence elements, denoted as centromere DNA element I, II, III, and VI. CDEIII is 25 bp and partially palindromic. A single base change of CDEIII could completely compromise centromere segregation function (McGrew et al., 1986). CDEII is 76 bp and >90%AT and CDEI is 8 bp.

On the other front of identifying the remaining centromeres, the pace quickened. Both the Carbon and Davis laboratories quickly realized that the genetic selection of loss of plasmids under "no selection" was a powerful strategy. Hsiao and Carbon (Hsiao and Carbon, 1981) introduced a yeast DNA library into a strain. Once transformants were isolated, they grew the cells in the absence of selection for the complementing gene on the plasmid. This would have been heretical just 2 years back. However, after many rounds of non-selective growth, they plated cells on selective media for the complementing plasmid, and quickly found cells that contained autonomously replicating plasmids. Thus in one transformation, Hsiao and Carbon directly isolated several additional centromeres. A few years later, Phil Hieter and colleagues from the

Davis laboratory developed a colony color assay that allowed them to isolate 11 centromeres in one genetic screen (Hieter et al., 1985b).

The centromeres from all 16 chromosomes in budding yeast have similar CDEI, II, and III sequence motifs. Moreover, Clarke and Carbon demonstrated that centromere DNA sequences from different chromosomes are interchangeable (Clarke and Carbon, 1983). There is no chromosome specificity for centromere DNA sequence, nor is there positional specificity within the chromosome. This result had important implications for chromosome pairing in meiosis and pointed to sites outside the centromere as important for this function. The centromere confers genetic stability in a variety of topologies (i.e., linear chromosomes, plasmids) or sequence contexts (Lambie and Roeder, 1986). When the author was a postdoctoral fellow in the Carbon laboratory one of the models for centromere function invoked a tRNA like adaptor molecule built at each centromere to ensure accurate segregation. I am sure this reflected John's thinking from the days of the genetic code, not many years prior to 1980. This model has remained in the bowels of UCSB, and was quickly dispelled by the genomic substitutions of centromeres in different chromosomes.

The isolation of centromeres and origins of replication only shortly preceded the cloning of the first yeast telomere. In a very elegant cloning strategy, Elizabeth Blackburn and Jack Szostack took a linear DNA fragment with the telomere from Tetrahymena on one end, and yeast fragments that could function in yeast as stable linear chromosomes were selected. In this way, they cloned and characterized the first yeast telomere (Szostak and Blackburn, 1982). This laid the foundation for creating the first artificial chromosome (Clarke and Carbon, 1980; Murray and Szostak, 1983a) and serves as a paradigm to the present day for linear artificial chromosomes that function with high fidelity in mammalian cells. The four elements of chromosome structure are the gene, centromere, telomere, and origin of replication. Remarkably, we can construct an entire chromosome no bigger that a few kilobase pairs in budding yeast.

One of the last mysteries of chromosome segregation (in terms of genetic segregation and not the mechanism of motility) was tackled by a young graduate student, Andrew Murray in Jack Szostak's laboratory. Andrew was perplexed by the asymmetry of partitioning of acentric plasmids. To follow the segregation of these acentric plasmids, Andrew performed a pedigree analysis of cells and their plasmids in yeast (Murray and Szostak, 1983b), and discovered that ARS plasmids remained predominantly in the mother cell (Fig. 1.1). The centromere provided an active partitioning function, and overrides the default asymmetric pattern of segregation. The simplest model that the acentric plasmids were biased toward the mother due to catenation of DNA strands during replication was disproven by Koshland and Hartwell (Koshland and Hartwell, 1987). We still do not know why the default pathway leads to accumulation of acentric plasmids in mother cells.

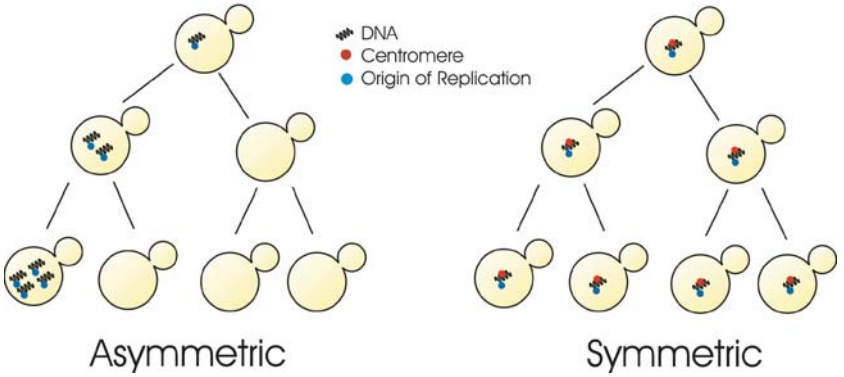

Fig. 1.1 Pedigree analysis of chromosome segregation in budding yeast. The DNA, original or replication (*red*), and centromere (*blue*) are indicated. (*See* Color Insert)

This rapid advance from cloning the first centromere to identification, sequence analysis and genomic substitution opened the door for the next generation of questions.

1.2 Point Versus Regional Centromeres

Louise Clarke next initiated studies on centromeres of the fission yeast, *S. pombe*. Much to everyone's surprise, Louise, together with the Yanagida laboratory in a simultaneous quest across the ocean discovered a considerably more complex centromere DNA sequence (Chikashige et al., 1989; Clarke et al., 1986). The complexity of the centromere sequence as well as the different nomenclatures adopted by each laboratory gave students of centromere severe headaches at the time. While several of the same strategies were applicable in *S. pombe*, and facilitated progress toward centromere identification, the initial studies revealed a highly complex array of repeated sequences and a considerably larger centromere. The centromeres in *S. pombe* were on the order of 50–100 kb (Baum et al., 1994), as opposed to 125 bp in *S. cerevisiae*. The major difference in sequence organization of centromeres from budding to fission yeast led Pluta and Earnshaw (Pluta et al., 1995) to distinguish point centromeres (*S. cerevisiae* type) from regional centromeres (*S. pombe* type).

Since that time, centromeres have been identified in a variety of organisms including yeast *Candida albicans* (Sanyal et al., 2004), bread mold *Neurospora crassa* (Centola and Carbon, 1994), plants, *Arabidopsis thaliana* (Copenhaver et al., 1999), flies *Drosophila melanogaster* (Sun et al., 2003), and humans *Homo sapiens* (Schueler et al., 2001).

The difficulty in cloning and identifying centromeres from organisms with regional centromeres is 2-fold. One is the sheer size of the centromere, in

humans the centromere region can be greater than 5 Mb, and two is the lack of a reliable artificial chromosome segregation assay. The most striking characteristic of human centromeres is the abundance of tandem repeats of simple sequence DNA. The most abundant repeat, alphoid satellite (α-satellite) was identified by Maio (1971). The monomeric repeat length of 171 bp was very provocative in light of the fact that nucleosomes were just being described. The relationship between the 171 bp α-satellite and the nucleosome core – linker (146 bp + 25 bp) – was the early evidence for a sequence code in nucleosome positioning. The arrangement of these repeats however is extremely complex (Waye et al., 1987; Willard, 1991). There are hierarchical arrangements of dimer, trimers, and pentamers of (α-satellite) that in turn are organized in higher-order arrays. With the advent of complete genome sequencing, we now have a very good understanding of the human centromere (Schueler et al., 2001), and as such there has been considerable progress on the construction and use of human artificial minichromosomes (Basu and Willard, 2006; Ren et al., 2006; Suzuki et al., 2006; Tsuduki et al., 2006; see the chapter by Masumoto in this book).

1.3 Conditional Centromeres, Conditional ARS

The major developments in yeast that led to its prominence as a genetic model system included the ease of isolating a variety of auxotrophic mutations and temperature-sensitive mutations. Conditional mutants provide the opportunity to maintain cell populations with defects in essential genes. Conditionally mutant gene products are typically altered in one or more amino acid, and render the protein defective under sub-optimal growth conditions. It is equally advantageous to have a conditional centromere, and thereby conditionally regulate individual chromosome segregation. Conditional mutants in kinetochore protein result in loss of the entire chromosome set, and not useful for studying one chromosome. Hill and Bloom (1987) discovered that strong transcriptional promoter adjacent to the centromere can inactivate centromere function, producing a conditional centromere. Similarly, a transcriptional promoter is conditionally disruptive for origin of replication function (Snyder et al., 1988). Very recently, it has been possible to produce a conditional centromere in a human artificial chromosome (HAC), by targeting histone modification activities into the kinetochore DNA array (Nakano et al., 2008). Interestingly, both induction of open chromatin or closed heterochromatin can inactivate the kinetochore.

1.4 Epigenetic Specification of Centromere Function

An epigenetic phenomenon is one in which the heritable phenotype is conferred by something in addition to the DNA genotype. This phenomenon was originally identified in Drosophila as variegated eye color by Hermann Muller in 1938. The genetic control of variegated phenotypes was dissected in yeast by

examining gene expression of telomere-linked genes (Gottschling et al., 1990). Through the use of the colony color sectoring assay, similar to that described above (see Hieter et al., 1985a) Gottschling and coworkers demonstrated variegation in gene expression. This variegation was found in centromere-linked genes adjacent to the *S. pombe* centromere (Allshire et al., 1994). Later that same year, an epigenetic feature regulating centromere function was discovered by Steiner and Clarke in their attempts to clone the minimal segregation unit from *S. pombe* centromeres (Steiner and Clarke, 1994). Much to their surprise, they found that nonfunctional centromeres on small circular minichromosomes could be converted to functional centromeres on the same chromosome. Interestingly, Earnshaw and Migeon first noted the differential segregation capacity of two centromeres on a dicentric chromosome by the presence or absence of staining with autoimmune sera that recognizes centromere proteins (CENPs; Earnshaw and Migeon, 1985) and proposed that there must be some "alteration of the chromatin conformation at the second centromere, preventing binding of the CENP species or sequestering them in an internal region of the chromosome where they are inaccessible for binding to antibodies in vitro or microtubules in vivo." There is now evidence for epigenetic phenomena in centromere function in *S. cerevisiae* (Mythreye and Bloom, 2003), *C. albicans* (Mishra et al., 2007), *S. pombe* (Folco et al., 2008), and humans (Morris and Moazed, 2007).

1.5 Centromere Proteins

Well before the yeast geneticists or biochemists identified centromere DNA binding and/or kinetochore proteins, the cytogeneticists were well on their way in this endeavor. The discovery of centromere-specific proteins came from clinical studies on patients with progressive systemic sclerosis. In particular, patients with symptoms of **c**alcinosis, **R**aynaud's phenomena, **e**sophageal dysmotility, **s**celodactyly, telangiectasia (known as CREST), contain anticentromere antibodies (Earnshaw and Rothfield, 1985; Moroi et al., 1980). The use of CREST antisera led to the first identification of CENPs in any species (Earnshaw and Rothfield, 1985), now including CENP-A through –U (reviewed in (Maiato et al., 2004)). CENP-A is a 17 kd protein that was subsequently shown to a histone H3 variant (Sullivan et al., 1994), conserved from fungi to mammals. Interestingly, the early studies revealed that CENP-A was associated with the inner domain of the kinetochore in mammalian cells (Warburton et al., 1997), and worms (Moore et al., 1999), indicative of DNA in the outermost region of the kinetochore. The distribution of this histone variant in the inner plate led to early attempts to map the path of kinetochore DNA. These studies utilized electron spectroscopy for the distribution of phosphorous (Rattner and Bazett-Jones, 1988, 1989). Unfortunately, electron spectroscopy does not distinguish phosphorylation of protein versus phosphate in the DNA backbone and evidence for DNA fibers in the kinetochore outer plate could not be confirmed (Cooke et al., 1993).

Once the centromere DNA sequence had been discovered in fungal system, the next step for the yeast geneticists was to identify centromere DNA binding proteins. Again, it is important to consider the state of scientific progress at the time. The knowledge of DNA-binding proteins was influenced by studies on operons in lambda phage and *E. coli* that taught us about high-affinity sequence-specific binding proteins and in particular motifs like helix-loop-helix. The nucleosome structure of chromatin was only recently published (Kornberg and Thomas, 1974; Olins and Olins, 1974), much less accepted in the literature (for recent historical account, see Olins and Olins, 2003). With this in mind, the early studies in the Carbon laboratory involved isolating biochemical quantities of protein and reconstitution with CEN DNA. Many a postdoctoral fellow worked diligently at this project. The breakthrough in biochemical isolation of the DNA binding complex came when J. Lechner and Carbon realized that a chaperone function (in the first experiments provided artifactually by casein) was required to facilitate sequence-specific binding of the complex to centromere DNA. They published the isolation of a 240 kd complex, denoted CBF3 for the three proteins in the complex (Lechner and Carbon, 1991). Two years later, the gene for the large subunit (110 kd) of the complex was identified simultaneously by Jiang et al. (Jiang et al., 1993) and Goh and Kilmartin (Goh and Kilmartin, 1993). Jiang et al. (1993) subjected the protein complex to tryptic digests, sequenced the peptides and synthesized degenerate oligonucleotides to screen DNA libraries. Goh and Kilmartin had been studying nuclear division cell mutants (*ndc10*) in the spirit of Hartwell's high successful cell division cycle mutant screen. Goh and Kilmartin (1993) isolated mutants that failed to segregate entire chromosome sets based on cytological screening of nuclear division. Thus almost 13 years after the identification of centromere DNA did we have the first gene for a bona fide centromere binding protein in yeast, and only 6 years after the first centromere protein, CENP-B was cloned (Earnshaw et al., 1987). It is safe to say that we do not yet have the structure of the Centromere binding factor (CBF3), and do not fully understand how this protein complex recognizes the centromere DNA.

Once the gene was in hand, the identification of genes encoding the other members of the complex was forthcoming. The breakthrough in this effort came from a genetic screen performed several years earlier that took advantage of the colony sectoring assay developed by Hieter and Koshland (Hieter et al., 1985a; Koshland et al., 1985). Spencer et al. (1990) isolated many of the kinetochore components in their screen for defects in chromosome transmission fidelity (*ctf*). The functional characterization of genes from this inspired screen continues to this day.

1.6 Organization of Centromere in Chromatin

The DNA sequence of the centromere has revealed remarkably little about its function, beyond providing a binding site in the case of budding yeast for a core centromere DNA-binding factor 3 (CBF3). To gain insights into centromere

function, determining the organization of these sequences in chromatin was the next logical step. The first analysis of centromere chromatin structure was performed in the Carbon laboratory (Bloom et al., 1984). Bloom and Carbon found that the centromere was organized into a unique structure, protected from nuclease, and slightly larger than a canonical nucleosome (centromere 220–250 bp vs. nucleosome 160 bp). Bloom went on to demonstrate that the centromere was indeed built upon a core of histone proteins containing histone H2B and H4 (Saunders et al., 1990). This led to the idea that the nonhistone binding proteins likely bind a centromere DNA-histone substrate. Later it was found that one of the centromere specific proteins (Cse4) was indeed a histone H3 variant as well as a component of the yeast kinetochore (Meluh et al., 1998). Cse4 is conserved throughout phylogeny (aka CENP-A) and present in centromeres from yeast to human. There is renewed attention to the question of the centromeric histone as a novel protein; Scm3 (Camahort et al., 2007; Mizuguchi et al., 2007; Stoler et al., 2007) has recently been discovered to reside at the budding yeast centromere.

1.7 Centromeres in Living Cells

The power of yeast as a pioneer model system included its ease of genetics, molecular manipulation of the genome, construction of yeast artificial chromosomes (YACs) and an early entry in the queue for genome sequence (chromosome III sequence, Oliver et al., 1992). However, one limitation of genetics as a method to map protein circuitry and function is the lack of mechanistic insight into a given function. The earliest reflection on the demand for mechanism can be traced to 1861, where Brucke wrote in the minutes of the meeting of the mathematical-scientific Classe of the imperial Academy of Sciences, Die Elementarorganismen, that cell histologists address mechanism (living cells, apart from which molekularstructur of the organic compounds they contain, still another and in other wise complicated structural attribute, the name organization designates) see Thompson, 1917. The definition of mechanism from the late nineteenth to early twentieth century is instructive “From a physical point of view, we understand by a ‘mechanism’ whatsoever checks or controls, and guides into determinate paths, the workings of energy: in other word, whatsoever leads in the degradation of energy to its manifestation in some form of work, at a stage short of that ultimate degradation which lapses in uniformly diffused heat” (Thompson, 1917).

An important step toward understanding centromere function was to visualize its form as it exists in living cells, in mitosis in particular. The first live cell analysis of yeast was performed by Koning (Koning et al., 1993) through the use of fluorescent lipophilic dyes to stain internal membranes. The challenge with yeast for differential interference contrast (DIC) microscopy was the high refractive index of the cell wall. Tim Stearns (Stanford U) had been

experimenting with using gelatin and other agents to match the refractive index of the cell wall and enable live cell analysis. Yeh et al. (1995) took advantage of this to determine the morphological changes and kinetics of nuclear migration and spindle elongation as cells progress through anaphase. This work showed us that cytoplasmic dynein was not a kinetochore protein, but rather contributed to spindle orientation toward the bud neck in mitosis, and secondly that there are specific kinetic phases of anaphase, which later were shown to be under the control of specific plus end-directed motor proteins (Straight et al., 1998). Shortly thereafter, several groups fused their favorite cytoskeletal proteins (actin, tubulin, spindle components) to GFP to visualize microtubule and spindle dynamics in live cells (Carminati and Stearns, 1997; Doyle and Botstein, 1996; Fleig et al., 1996; Kahana et al., 1995; Shaw et al., 1997a b). As often with breakthrough experiments, even more interesting features can be seen in retrospect. One of the most amazing aspects of budding yeast centromere function was staring us in the face in Kahana et al. (1995), namely that sister kinetochores are separated in metaphase. Why didn't we see this in 1995? There were several reasons, one was that the Nuf2 protein fused to GFP was thought to be a spindle pole component (Osborne et al., 1994). This was by virtue of a two-hybrid interaction with the nucleoporin Nup1, and secondly by its "co-localization" with spindle poles (Figs. 1.2 and 1.3). The idea that kinetochores were separated was far from anyone's mind. Second was that initial attempts to label centromeres used markers inserted at *LEU2*, 23 kb from the centromere. Straight et al. (1996) inserted 256 copies of the lac operator from *E. coli* into yeast and visualized the chromosome containing these sequences with lac repressor fused to GFP. The *LEU2* was the infamous centromere-linked gene used in chromosome walking strategy to identify the centromere (7.6 cm from CEN on chromosome III). This visualization strategy for yeast centromeres was breakthrough work, and revealed for the first time a bonafide anaphase A chromosome to pole movement, as discovered almost 20 years earlier through the use of DIC microscopy (Inoue and Ritter, 1978). From a physical perspective however 23 kb is 7.6 μm of B-form DNA, quite away from the centromere, and quite distant in an organism requiring only 125 bp for chromosome segregation.

These early papers in live cell microscopy opened the door for quantitative analysis of dynamic processes, and brought an important technical advance toward our quest for mechanism. As additional laboratories brought their questions and expertise to these problems, it became apparent that sister centromeres were indeed separated in mitosis, prior to anaphase (Goshima and Yanagida, 2000; He et al., 2000; Pearson et al., 2001; Tanaka et al., 2000). Using closely linked lac operators to the centromere (within a kilobase pair or so) it was shown that tension across the centromere results in separation of sister centromeres and proximal chromatin prior to the onset of anaphase. There is a bit of controversy still associated with this view. Goshima and Yanagida addressed the issue directly in a very nice quantitative study (Goshima and Yanagida, 2001). Several additional lines of evidence support

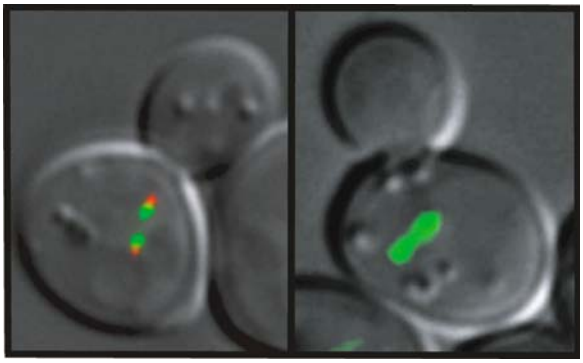

Fig. 1.2 Centromere DNA, spindle poles and microtubules in mitosis. (*left*) Centromere (CEN) DNA visualized with lac-repressor-GFP bound to lac operator integrated 1 kb from CEN3 on chromosome III (*green*) and spindle poles (*red*). Sister centromeres are separated in mitosis (*right*) Microtubules in the mitotic spindle visualized with tubulin-GFP. The spindle is thicker at the ends versus the middle. Sixteen kinetochore microtubules originate from each spindle pole and extend approximately a quarter the length of the spindle. (*See* Color Insert)

this view. One is the distribution of kinetochore markers, such as Nuf2 (Kahana et al., 1995). Nuf2 and other kinetochore proteins are visualized as two separated spots in mitosis. Furthermore, the variance of fluorescence in the two kinetochore spots (Cse4, Joglekar et al., 2006) is very low, indicative of the fact that the number of kinetochores in each of the separated spots is the same. Finally, upon anaphase, the fluorescence in separated kinetochore clusters segregate to opposite poles, as determined by fluorescence recovery after photobleaching (Molk and Bloom, unpublished).

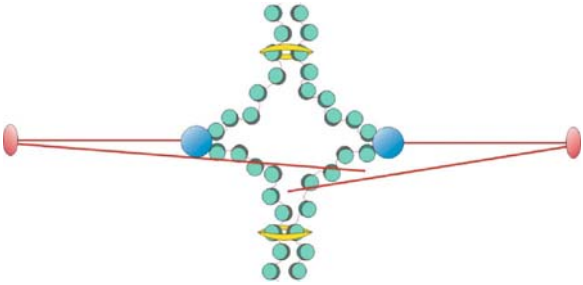

Fig. 1.3 Organization of a mitotic chromosome. Chromosome arms are closely apposed and held together via cohesin (*yellow rings*). Sister kinetochores (in *blue*) are attached to kinetochore microtubules (*red*) and the pericentric chromatin is stretched toward the spindle poles. There are 16 chromosomes in yeast, and 16 kinetochore microtubules in each spindle half. Cohesin between sister chromatids provides a mechanism to resist microtubule pulling forces and generate tension at centromeres. The function of cohesin in pericentric chromatin is not well understood. (*See* Color Insert)

This observation of clustered centromeres is consistent with electron microscopy of the yeast spindle (Peterson and Ris, 1976). Peterson and Ris found kinetochore microtubules to be discontinuous in the spindle with approximately 16 microtubules emanating from each spindle pole. However, the kinetochore microtubules are fairly short and only span roughly 25% of the spindle length. There is a gap between the two ends of the microtubules, remarkably similar to the distance between separated centromeres as seen by fluorescence microscopy 25 years later. Thus the EM and fluorescence inform us that kinetochore microtubules extend about ¼ the length of the bipolar spindle, and that they are organized in parallel arrays relative to the central interpolar microtubules centromeres, at kinetochore microtubule plus ends, and are under tension and span the distance between kinetochore microtubules emanating from each spindle pole. Based on the position of the lac operator markers, centromere proximal DNA is elongated by an average distance of 0.16 μm per kb prior to anaphase onset. Naked B-form DNA is 0.34 μm per kb and a 7-fold nucleosome compaction predicts that 1 kb of mitotic chromatin covers a distance of 0.05 μm. These results indicate that the level of DNA compaction at the centromere and surrounding chromatin is 3-fold less than that of nucleosomal DNA. Upon anaphase onset, this stretching of DNA is further amplified due to the spindle forces generated during spindle elongation.

1.8 What is the Minimal Chromosome Segregation Unit?

The question raised by the small size of CEN DNA in budding yeast is whether the knowledge gleaned from *S. cerevisiae* is instructive for understanding larger eukaryotic centromeres. The minimal centromere in *S. pombe* is 40–60 kb, and of the order of megabase pairs in mammalian cells. While 125 bp of CEN DNA is the minimal size required to build a kinetochore, there may be considerably more DNA (i.e., pericentric chromatin) recruited to the spindle during chromosome segregation. Evidence for this idea comes from the distribution of cohesin along the chromosome. The physical linkage between replicated sister chromatids is the mechanism for generating tension during mitotic metaphase. This linkage is mediated by a multisubunit complex, cohesin, composed of two members of the SMC (structural maintenance of chromosomes) family of ATPases, Smc1 and Smc3, and two non-SMC subunits, Mcd1/Scc1 and Scc3 (Huang et al., 2005; Nasmyth and Haering, 2005). It has been assumed that cohesin promotes association between sister chromatids (intermolecular linkage), and that is the basis for tension when sister chromatids are oriented to opposite spindle pole bodies. The Scc1 subunit disappears from chromosomes when sisters separate at the metaphase/anaphase transition. Scc1 is cleaved by separase upon anaphase onset. The key experiment demonstrating that loss of cohesin is sufficient for sister chromatid separation was artificial cleavage of a modified form of Scc1 by a foreign protease (TEV, Tobacco Etch Virus;

Uhlmann et al., 2000). Activation of TEV protease promotes sister chromatid separation in budding yeast cells when arrested in metaphase. The discovery of cohesin dispelled the view that sister chromatids might be held via intercatenation of sister DNAs that was resolved at anaphase due to microtubule pulling forces (Murray and Szostak, 1985).

Genome-wide chromatin immunoprecipitation (ChIP) in budding yeast has revealed the predominant sites of cohesin binding (Blat and Kleckner, 1999; Weber et al., 2004). Most notable is the finding that cohesin is enriched ~3-fold in a 20–50 kb domain flanking the centromere, relative to the concentration of cohesin on chromosome arms. Although the location of cohesin along the length of the yeast chromosome has been established, little is known about how the concentration of cohesin within pericentric chromatin contributes to the fidelity of chromosome segregation, or whether the cohesin in the pericentric region is indicative for a role of a larger chromosomal domain in kinetochore function. It has recently been demonstrated that cohesin is organized into a cylindrical array encompassing the mitotic spindle in budding yeast (Yeh et al., 2008).

1.9 Future Questions

We do not know the nature of chromatin platform on which the centromere is built. Unlike the microtubule, where it appears that the yeast Dam1 kinetochore complex encircles the plus end of the microtubule in a way that is permissive for tubulin addition and loss, there is very little understanding about the molecular structure of the chromatin platform. We have recently proposed that the core centromere (120 bp DNA wrapped around a Cse4 containing nucleosome) and flanking chromatin adopts a cruciform configuration in metaphase (Bloom et al., 2006; Yeh et al., 2008). Whether this or another structure represents the chromatin platform remains to be seen. However it is extremely likely that interesting features of the organization of pericentric chromatin will be forthcoming in future endeavors.

References

Allshire, R.C., J.P. Javerzat, N.J. Redhead, and G. Cranston. 1994. Position effect variegation at fission yeast centromeres. *Cell*. 76:157–69.

Basu, J., and H.F. Willard. 2006. Human artificial chromosomes: potential applications and clinical considerations. *Pediatr Clin North Am*. 53:843–53, viii.

Baum, M., V.K. Ngan, and L. Clarke. 1994. The centromeric K-type repeat and the central core are together sufficient to establish a functional Schizosaccharomyces pombe centromere. *Mol Biol Cell*. 5:747–61.

Blat, Y., and N. Kleckner. 1999. Cohesins bind to preferential sites along yeast chromosome III, with differential regulation along arms versus the centric region. *Cell*. 98:249–59.

Bloom, K., E. Amaya, and E. Yeh. 1984. Centromeric DNA structure in yeast. *Mol Biol Cytoskeleton*. 175–84.
Bloom, K., S. Sharma, and N.V. Dokholyan. 2006. The path of DNA in the kinetochore. *Curr Biol*. 16:R276–8.
Bloom, K.S., and J. Carbon. 1982. Yeast centromere DNA is in a unique and highly ordered structure in chromosomes and small circular minichromosomes. *Cell*. 29:305–17.
Brinkley, B.R., and E. Stubblefield. 1966. The fine structure of the kinetochore of a mammalian cell in vitro. *Chromosoma*. 19:28–43.
Britten, R.J., and D.E. Kohne. 1968. Repeated sequences in DNA. Hundreds of thousands of copies of DNA sequences have been incorporated into the genomes of higher organisms. *Science*. 161:529–40.
Camahort, R., B. Li, L. Florens, S.K. Swanson, M.P. Washburn, and J.L. Gerton. 2007. Scm3 is essential to recruit the histone h3 variant cse4 to centromeres and to maintain a functional kinetochore. *Mol Cell*. 26:853–65.
Cameron, J.R., E.Y. Loh, and R.W. Davis. 1979. Evidence for transposition of dispersed repetitive DNA families in yeast. *Cell*. 16:739–51.
Carminati, J.L., and T. Stearns. 1997. Microtubules orient the mitotic spindle in yeast through dynein-dependent interactions with the cell cortex. *J Cell Biol*. 138:629–41.
Centola, M., and J. Carbon. 1994. Cloning and characterization of centromeric DNA from Neurospora crassa. *Mol Cell Biol*. 14:1510–9.
Chikashige, Y., N. Kinoshita, Y. Nakaseko, T. Matsumoto, S. Murakami, O. Niwa, and M. Yanagida. 1989. Composite motifs and repeat symmetry in S. pombe centromeres: direct analysis by integration of NotI restriction sites. *Cell*. 57:739–51.
Chinault, A.C., and J. Carbon. 1979. Overlap hybridization screening: isolation and characterization of overlapping DNA fragments surrounding the leu2 gene on yeast chromosome III. *Gene*. 5:111–26.
Clarke, L., H. Amstutz, B. Fishel, and J. Carbon. 1986. Analysis of centromeric DNA in the fission yeast Schizosaccharomyces pombe. *Proc Natl Acad Sci USA*. 83:8253–7.
Clarke, L., and J. Carbon. 1976. A colony bank containing synthetic Col El hybrid plasmids representative of the entire E. coli genome. *Cell*. 9:91–9.
Clarke, L., and J. Carbon. 1980. Isolation of a yeast centromere and construction of functional small circular chromosomes. *Nature*. 287:504–9.
Clarke, L., and J. Carbon. 1983. Genomic substitutions of centromeres in Saccharomyces cerevisiae. *Nature*. 305:23–8.
Cooke, C.A., D.P. Bazett-Jones, W.C. Earnshaw, and J.B. Rattner. 1993. Mapping DNA within the mammalian kinetochore. *J Cell Biol*. 120:1083–91.
Copenhaver, G.P., K. Nickel, T. Kuromori, M.I. Benito, S. Kaul, X. Lin, M. Bevan, G. Murphy, B. Harris, L.D. Parnell, W.R. McCombie, R.A. Martienssen, M. Marra, and D. Preuss. 1999. Genetic definition and sequence analysis of Arabidopsis centromeres. *Science*. 286:2468–74.
Doyle, T., and D. Botstein. 1996. Movement of yeast cortical actin cytoskeleton visualized in vivo. *Proc Natl Acad Sci USA*. 93:3886–91.
Earnshaw, W.C., and B.R. Migeon. 1985. Three related centromere proteins are absent from the inactive centromere of a stable isodicentric chromosome. *Chromosoma*. 92:290–6.
Earnshaw, W.C., and N. Rothfield. 1985. Identification of a family of human centromere proteins using autoimmune sera from patients with scleroderma. *Chromosoma*. 91:313–21.
Earnshaw, W.C., K.F. Sullivan, P.S. Machlin, C.A. Cooke, D.A. Kaiser, T.D. Pollard, N.F. Rothfield, and D.W. Cleveland. 1987. Molecular cloning of cDNA for CENP-B, the major human centromere autoantigen. *J Cell Biol*. 104:817–29.
Fitzgerald-Hayes, M., L. Clarke, and J. Carbon. 1982. Nucleotide sequence comparisons and functional analysis of yeast centromere DNAs. *Cell*. 29:235–44.
Fleig, U., M. Sen-Gupta, and J.H. Hegemann. 1996. Fission yeast mal2+ is required for chromosome segregation. *Mol Cell Biol*. 16:6169–77.

Folco, H.D., A.L. Pidoux, T. Urano, and R.C. Allshire. 2008. Heterochromatin and RNAi are required to establish CENP-A chromatin at centromeres. *Science*. 319:94–7.
Gall, J.G. 1963. Kinetics of deoxyribonuclease action on chromosomes. *Nature*. 198:36–8.
Goh, P.Y., and J.V. Kilmartin. 1993. NDC10: a gene involved in chromosome segregation in Saccharomyces cerevisiae. *J Cell Biol*. 121:503–12.
Goshima, G., and M. Yanagida. 2000. Establishing biorientation occurs with precocious separation of the sister kinetochores, but not the arms, in the early spindle of budding yeast. *Cell*. 100:619–33.
Goshima, G., and M. Yanagida. 2001. Time course analysis of precocious separation of sister centromeres in budding yeast: continuously separated or frequently reassociated? *Genes Cells*. 6:765–73.
Gottschling, D.E., O.M. Aparicio, B.L. Billington, and V.A. Zakian. 1990. Position effect at S. cerevisiae telomeres: reversible repression of Pol II transcription. *Cell*. 63:751–62.
Hartwell, L.H., J. Culotti, and B. Reid. 1970. Genetic control of the cell-division cycle in yeast. I. Detection of mutants. *Proc Natl Acad Sci USA*. 66:352–9.
He, X., S. Asthana, and P.K. Sorger. 2000. Transient sister chromatid separation and elastic deformation of chromosomes during mitosis in budding yeast. *Cell*. 101:763–75.
Hieter, P., C. Mann, M. Snyder, and R.W. Davis. 1985a. Mitotic stability of yeast chromosomes: a colony color assay that measures nondisjunction and chromosome loss. *Cell*. 40:381–92.
Hieter, P., D. Pridmore, J.H. Hegemann, M. Thomas, R.W. Davis, and P. Philippsen. 1985b. Functional selection and analysis of yeast centromeric DNA. *Cell*. 42:913–21.
Hill, A., and K. Bloom. 1987. Genetic manipulation of centromere function. *Mol Cell Biol*. 7:2397–405.
Hinnen, A., J.B. Hicks, and G.R. Fink. 1978. Transformation of yeast. *Proc Natl Acad Sci USA*. 75:1929–33.
Hitzeman, R.A., L. Clarke, and J. Carbon. 1980. Isolation and characterization of the yeast 3-phosphoglycerokinase gene (PGK) by an immunological screening technique. *J Biol Chem*. 255:12073–80.
Hsiao, C.L., and J. Carbon. 1981. Direct selection procedure for the isolation of functional centromeric DNA. *Proc Natl Acad Sci USA*. 78:3760–4.
Huang, C.E., M. Milutinovich, and D. Koshland. 2005. Rings, bracelet or snaps: fashionable alternatives for Smc complexes. *Philos Trans R Soc Lond B Biol Sci*. 360:537–42.
Inoue, S., and H. Ritter, Jr. 1978. Mitosis in Barbulanympha. II. Dynamics of a two-stage anaphase, nuclear morphogenesis, and cytokinesis. *J Cell Biol*. 77:655–84.
Jiang, W., J. Lechner, and J. Carbon. 1993. Isolation and characterization of a gene (CBF2) specifying a protein component of the budding yeast kinetochore. *J Cell Biol*. 121:513–9.
Joglekar, A.P., D.C. Bouck, J.N. Molk, K.S. Bloom, and E.D. Salmon. 2006. Molecular architecture of a kinetochore-microtubule attachment site. *Nat Cell Biol*. 8:581–5.
Jokelainen, P.T. 1967. The ultrastructure and spatial organization of the metaphase kinetochore in mitotic rat cells. *J Ultrastruct Res*. 19:19–44.
Kahana, J.A., B.J. Schnapp, and P.A. Silver. 1995. Kinetics of spindle pole body separation in budding yeast. *Proc Natl Acad Sci USA*. 92:9707–11.
Kingsman, A.J., R.L. Gimlich, L. Clarke, A.C. Chinault, and J. Carbon. 1981. Sequence variation in dispersed repetitive sequences in Saccharomyces cerevisiae. *J Mol Biol*. 145:619–32.
Koning, A.J., P.Y. Lum, J.M. Williams, and R. Wright. 1993. DiOC6 staining reveals organelle structure and dynamics in living yeast cells. *Cell Motil Cytoskeleton*. 25:111–28.
Kornberg, R.D., and J.O. Thomas. 1974. Chromatin structure; oligomers of the histones. *Science*. 184:865–8.
Koshland, D., and L.H. Hartwell. 1987. The structure of sister minichromosome DNA before anaphase in Saccharomyces cerevisiae. *Science*. 238:1713–6.
Koshland, D., J.C. Kent, and L.H. Hartwell. 1985. Genetic analysis of the mitotic transmission of minichromosomes. *Cell*. 40:393–403.

Lambie, E.J., and G.S. Roeder. 1986. Repression of meiotic crossing over by a centromere (CEN3) in Saccharomyces cerevisiae. *Genetics*. 114:769–89.
Lechner, J., and J. Carbon. 1991. A 240 kd multisubunit protein complex, CBF3, is a major component of the budding yeast centromere. *Cell*. 64:717–25.
Maiato, H., J. DeLuca, E.D. Salmon, and W.C. Earnshaw. 2004. The dynamic kinetochore-microtubule interface. *J Cell Sci*. 117:5461–77.
Maio, J.J. 1971. DNA strand reassociation and polyribonucleotide binding in the African green monkey, Cercopithecus aethiops. *J Mol Biol*. 56:579–95.
McClintock, B. 1939. The Behavior in Successive Nuclear Divisions of a Chromosome Broken at Meiosis. *Proc Natl Acad Sci USA*. 25:405–16.
McClintock, B. 1941. The Stability of Broken Ends of Chromosomes in Zea Mays. *Genetics*. 26:234–82.
McClintock, B. 1942. The Fusion of Broken Ends of Chromosomes Following Nuclear Fusion. *Proc Natl Acad Sci U S A*. 28:458–63.
McGrew, J., B. Diehl, and M. Fitzgerald-Hayes. 1986. Single base-pair mutations in centromere element III cause aberrant chromosome segregation in Saccharomyces cerevisiae. *Mol Cell Biol*. 6:530–8.
Meluh, P.B., P. Yang, L. Glowczewski, D. Koshland, and M.M. Smith. 1998. Cse4p is a component of the core centromere of Saccharomyces cerevisiae. *Cell*. 94:607–13.
Mishra, P.K., M. Baum, and J. Carbon. 2007. Centromere size and position in Candida albicans are evolutionarily conserved independent of DNA sequence heterogeneity. *Mol Genet Genomics*.
Mizuguchi, G., H. Xiao, J. Wisniewski, M.M. Smith, and C. Wu. 2007. Nonhistone Scm3 and histones CenH3-H4 assemble the core of centromere-specific nucleosomes. *Cell*. 129:1153–64.
Moore, L.L., M. Morrison, and M.B. Roth. 1999. HCP-1, a protein involved in chromosome segregation, is localized to the centromere of mitotic chromosomes in Caenorhabditis elegans. *J Cell Biol*. 147:471–80.
Moroi, Y., C. Peebles, M.J. Fritzler, J. Steigerwald, and E.M. Tan. 1980. Autoantibody to centromere (kinetochore) in scleroderma sera. *Proc Natl Acad Sci USA*. 77:1627–31.
Morris, C.A., and D. Moazed. 2007. Centromere assembly and propagation. *Cell*. 128:647–50.
Murray, A.W., and J.W. Szostak. 1983a. Construction of artificial chromosomes in yeast. *Nature*. 305:189–93.
Murray, A.W., and J.W. Szostak. 1983b. Pedigree analysis of plasmid segregation in yeast. *Cell*. 34:961–70.
Murray, A.W., and J.W. Szostak. 1985. Chromosome segregation in mitosis and meiosis. *Annu Rev Cell Biol*. 1:289–315.
Mythreye, K., and K.S. Bloom. 2003. Differential kinetochore protein requirements for establishment versus propagation of centromere activity in Saccharomyces cerevisiae. *J Cell Biol*. 160:833–43.
Nakano, M., S. Cardinale, V.N. Noskov, R. Gassmann, P. Vagnarelli, S. Kandels-Lewis, V. Larionov, W.C. Earnshaw, and H. Masumoto. 2008. Inactivation of a human kinetochore by specific targeting of chromatin modifiers. *Developmental Cell*. 14:507–522.
Nasmyth, K., and C.H. Haering. 2005. The structure and function of SMC and kleisin complexes. *Annu Rev Biochem*. 74:595–648.
Nasmyth, K.A., and S.I. Reed. 1980. Isolation of genes by complementation in yeast: molecular cloning of a cell-cycle gene. *Proc Natl Acad Sci USA*. 77:2119–23.
Olins, A.L., and D.E. Olins. 1974. Spheroid chromatin units (v bodies). *Science*. 183:330–2.
Olins, D.E., and A.L. Olins. 2003. Chromatin history: our view from the bridge. *Nat Rev Mol Cell Biol*. 4:809–14.
Oliver, S.G., Q.J. van der Aart, M.L. Agostoni-Carbone, M. Aigle, L. Alberghina, D. Alexandraki, G. Antoine, R. Anwar, J.P. Ballesta, P. Benit, and et al. 1992. The complete DNA sequence of yeast chromosome III. *Nature*. 357:38–46.