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Building mitotic chromosomes

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Mitotic chromosomes are the iconic structures into which the genome is packaged to ensure its accurate segregation during mitosis. Although they have appeared on countless journal cover illustrations, there remains no consensus on how the chromatin fiber is packaged during mitosis. In fact, work in recent years has both added to existing controversies and sparked new ones. By contrast, there has been very significant progress in determining the protein composition of isolated mitotic chromosomes. Here, we discuss recent studies of chromosome organization and provide an in depth description of the latest proteomics studies, which have at last provided us with a definitive proteome for vertebrate chromosomes.

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Chromosome anatomy and formation

Mitotic chromosome structure has fascinated cell biologists since the 19th century, but we still know relatively little about the composition and topology of chromosomes. We know even less about the activities responsible for the remarkable structural transformation that occurs when the chromatin of the interphase nucleus adopts the characteristic ‘X-shaped’ morphology as metazoan cells enter mitosis.

Mitotic chromosomes have four structural/functional domains: centromeres, telomeres, the periphery, and arm chromatin (Figure 1a–d). Each domain has a characteristic protein composition. The centromere and its associated kinetochore together comprise an elaborate structure, with over 120 constituents described to date. They bind spindle microtubules and direct chromosome segregation in mitosis [1,2]. The protein composition of telomeres is relatively simpler [3,4]. Telomeres play an

essential role in protecting chromosome ends and preventing chromosome fusion events. The chromosome periphery (perichromosomal layer) may act like a skin protecting the chromosome surface [5–8]. Its components are enriched in ribosomal and nucleolar proteins [9–11]. Many may simply be ‘hitchhikers’—proteins that bind to chromosomes in the cytoplasm following nuclear envelope breakdown and serve no essential function during mitosis. Others appear to function during chromosome segregation, as discussed below.

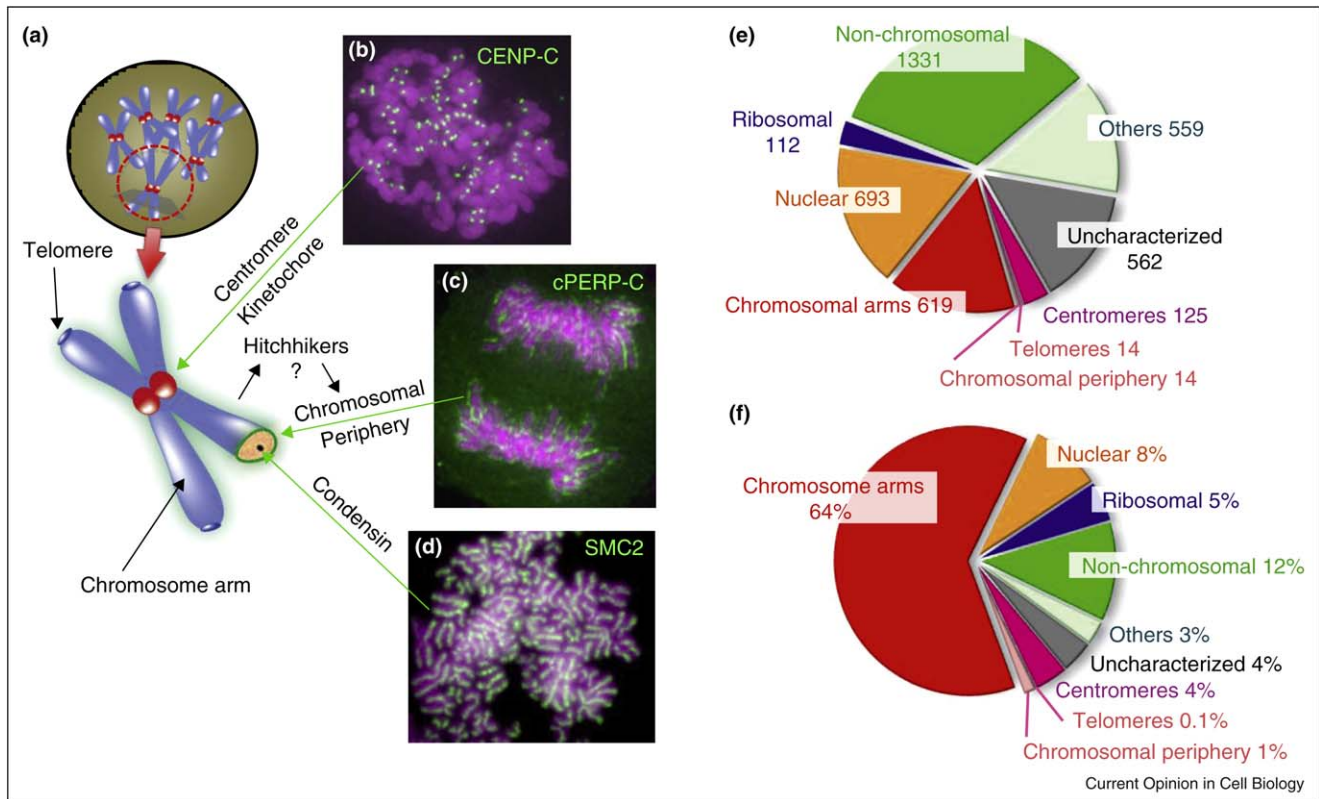
Experiments by Hirano and co-workers identified the condensin complex as a factor essential for mitotic chromosome formation in cell-free *Xenopus* egg extracts [12]. This complex is distributed along the axial region of the chromosome arms (Figure 1d). Key condensin components are the SMC proteins [13], which have roles in many types of chromosome transactions. Condensin, cohesin, and SMC proteins are reviewed regularly [14–17].

Condensin is required for successful completion of mitosis, but not for mitotic chromosome formation *in vivo* [18–21]. Condensin is important for the timing of chromosome condensation [19,20], the elastic properties of chromosomes [22] and centromeres [23], the segregation of rDNA in yeast [24,25], dosage compensation in *C. elegans* [26], and chromosome integrity during anaphase [20,21]. However, something else must be the driving factor behind mitotic chromosome formation. Dephosphorylation of a target termed RCA (regulator of chromosome architecture) by the Repo-Man:PP1 (protein phosphatase 1) holoenzyme correlates with a dramatic loss of chromosome organization during anaphase in cells lacking condensin [27].

The molecular identity of RCA has yet to be determined. RCA could be a specific non-histone protein, or a combination of histone post-translational modifications. A recent study identified H3T3^{ph}K4^{me}2R8^{me}2 (termed the PMM mark) [28] as specific for mitotic chromosomes. The PMM mark is not essential for mitotic chromosome formation, but could form part of a more complex histone modification pattern that promotes mitotic chromosome formation. Ultimately, the identity of RCA and mechanism of condensin action in mitotic chromosome formation remain mysterious.

Another protein previously linked with mitotic chromosome formation is DNA topoisomerase II (topo II) [29–31], one of the most abundant non-histone proteins of mitotic chromosomes [32^{••}]. However, RNAi and genetic

Figure 1



Functional subdomains in mitotic chromosomes (a), include, (b) Centromeres, Telomeres, (c) the Chromosome periphery and (d) chromosome arms. (e) The 9 classes of proteins found in chromosomes. (f) Estimated percentages of total chromosomal protein mass in the major classes of proteins [32**].

knockouts subsequently revealed that topo II is dispensable for mitotic chromosome formation [33–35]. A recent *in vitro* biophysical analysis has suggested that DNA entanglements have a role in determining the physical properties of mitotic chromosome arms [36**] (for review see [37]). Thus, topo II could have an important influence on the behavior of chromosomes as they respond to forces within the mitotic spindle (see also [38,39]).

Chromosome topology

Much effort has been spent in trying to confirm or refute a visionary model proposed by Laemmli—that mitotic chromosomes consist of chromatin loops constrained by interactions with a scaffolding of non-histone proteins [40,41]. Enthusiasm for a non-histone scaffold has waxed and waned over the years [42,43]. Microscopy studies have tended to support the role of some sort of axial determinants of mitotic chromosome structure [44–47]. However, *in vitro* studies suggest that if chromatin loops are constrained by proteins, those loops must be relatively small [48]. Furthermore, more recent examination of isolated chromosomes has suggested that order is minimal within the chromosome, with chromatin folding in the paired sister chromatids showing little, if any reproduc-

bility [49*]. This contrasts markedly with previous studies showing that chromosome arms can adopt a helical conformation with mirror symmetry [45,50].

There has been scant progress in recent years in understanding the higher order packing of chromatin in mitotic chromosomes. Since the first proposal of the solenoid model of nucleosome packing [51], it has been generally assumed that mitotic chromosomes consist of a hierarchy of higher order packaged chromatin fibers. Indeed, detailed analysis of budding yeast chromatin compaction *in vivo* suggested that most of the chromatin has a level of compaction consistent with the solenoid model [52]. By contrast, a recent electron cryomicroscopy study failed to find any evidence for 30 nm chromatin fibers in isolated mitotic chromosomes [53**]. Those authors suggested that the chromatin is so tightly packed that interactions between nucleosomes of adjacent fibers compete with those between nucleosomes on the same fibers [53**]. This could destabilize the solenoid, creating a densely packed amorphous mass of nucleosomes referred to as a ‘polymer melt’. An earlier EM tomography study looking at chromosomes assembled *in vitro* in *Xenopus* egg extracts had also failed to observe promi-

nent 30 nm fibers, instead visualizing nucleosomes clustered into a network of 30–40 nm domains [54]. There is no doubt that the 30 nm solenoid exists *in vitro*, but its role *in vivo* may continue to be debated over the coming years (reviewed in [55]).

Because chromosomal substructures fall into a ‘resolution gap’ between the electron microscope and conventional light microscopes, technological advances have a significant impact on our understanding of chromosome structure. In one recent study, coherent x-ray diffraction was used to examine isolated chromosomes [56]. In this pioneering study, the chromosomes appeared to have a denser axial region, contrasting with the results from electron cryomicroscopy [53]. A second approach that is just beginning to be applied to mitotic chromosomes is super-resolution light microscopy, an area in which remarkable technical advances have been made in recent years. PALM (photoactivation localization microscopy) has recently been used to analyze the kinetochore, mapping the distribution of CENP-A relative to other inner kinetochore proteins at 37 nm resolution [57]. If this or a related technology can in the future be applied to ‘native’ chromosomes, it may finally enable the path of fiber folding to be traced in intact chromosomes.

It is clear that mitotic chromosomes continue to offer mysteries and challenges, even at the most basic levels of their structure.

Chromosome composition

Isolated mitotic chromosomes are roughly 2:1 protein to nucleic acid on a mass basis [58,59]. About half of this protein is histone, but the remainder is often lumped together under the not-very informative term ‘non-histone proteins’. In recent years, significant strides have been made in the identification and characterization of these non-histone proteins.

Purification of mitotic chromosomes is not straightforward, as many cytoplasmic proteins bind tightly to the highly charged chromosomes after nuclear envelope breakdown. These proteins cannot be separated from the chromosomes without harsh chemical extractions, so it can be extremely difficult to define what is and is not a *bona fide* chromosomal protein. This issue, which we term the ‘hitchhiker problem’ [32] has been addressed by the Fukui lab [60], but a solution remains elusive because conventional fractionation procedures such as centrifugation cannot separate chromosomes from contaminants that adhere to them physically (Figure 1a).

The first two proteomic analyses of mitotic chromosomes [61,62] tried to avoid the ‘hitchhiker problem’ by characterizing chromosome scaffolds produced by digesting isolated chromosomes with micrococcal nuclease and extracting >90% of the proteins with 2 M NaCl [63].

The first report identified 62 proteins, including a novel protein of the chromosome periphery, NGB/CRFG, but was bedeviled by the presence of numerous mitochondrial contaminants [61]. A follow-up project identified 79 proteins in chromosome scaffolds [62], including the novel proteins—Borealin [64] and CENP-V [65] as well as two other proteins of the chromosome periphery. Another study characterized proteins from *Xenopus* egg extract that bound to added sperm chromatin [66]. This report did not give a lengthy description of the entire proteome identified, but instead focused on characterization of the novel kinetochore protein Bod1.

A particularly thorough set of studies of the mitotic chromosome proteome has been carried out by the Fukui laboratory [9,60,67]. They identified ~250 proteins in isolated mitotic chromosomes, ~100 of which are likely to be specific chromosomal proteins. Their subsequent work has focused on functional analysis of several proteins found at the chromosome periphery: nucleophosmin, nucleolin and regulator of ribosome synthesis 1 (RRS1). Surprisingly, all three were found to be necessary for timely and efficient alignment of the chromosomes during prometaphase [68–70]. The underlying mechanisms are unknown.

The analysis of centromeres and telomeres by proteomics has been a particular challenge, as they are differentiated regions of the single long chromosomal DNA molecule, rather than independent structures in their own right. A particularly elegant solution was taken to the isolation of telomeres. This involved the use of DNA hybridization to fish out the TTAGGG sequences that characterize human telomeres, a procedure that the authors termed PICh (proteomics of isolated chromatin segments) [71]. That study found 98 proteins common to telomeres from telomerase positive and ALT cell lines (which maintain telomeres by recombination rather than telomerase activity). These included the components of the shelterin complex, known to be involved in chromosome end protection and maintenance [3,4]. The study also found a similar number of proteins specific to each class of telomeres [71]. One surprise was the finding of several orphan receptors associated with ALT telomeres, which the authors proposed might have a role in promoting telomere association with PML bodies [71].

Proteomic characterization of kinetochores has involved affinity purification of proteins that associate with kinetochore components such as CENP-A [72–74] or CENP-S [75]. One recent study reporting the isolation of entire budding yeast minichromosomes led to the discovery of a PP1 regulatory subunit, Fin1, associated with the kinetochore [76]. Fin1 is involved with regulation of the spindle checkpoint. Kinetochores have been extensively reviewed elsewhere [1,2], so these studies will not be discussed further here.

Multi-classifier combinatorial proteomics (MCCP) of mitotic chromosomes

One recent study used a procedure developed by the Laemmli lab [77] to isolate mitotic chromosomes from chicken DT40 cells for proteomic analysis, capitalizing on quantitative proteomics software developed by the Mann lab [78^{*}]. This work yielded a list of ~4000 proteins (Figure 1e). Known and predicted chromosomal proteins comprised 72% of the total protein mass present (Figure 1f), indicating that the purification procedure was quite successful. Of the ~4000 proteins, >550 were previously uncharacterized.

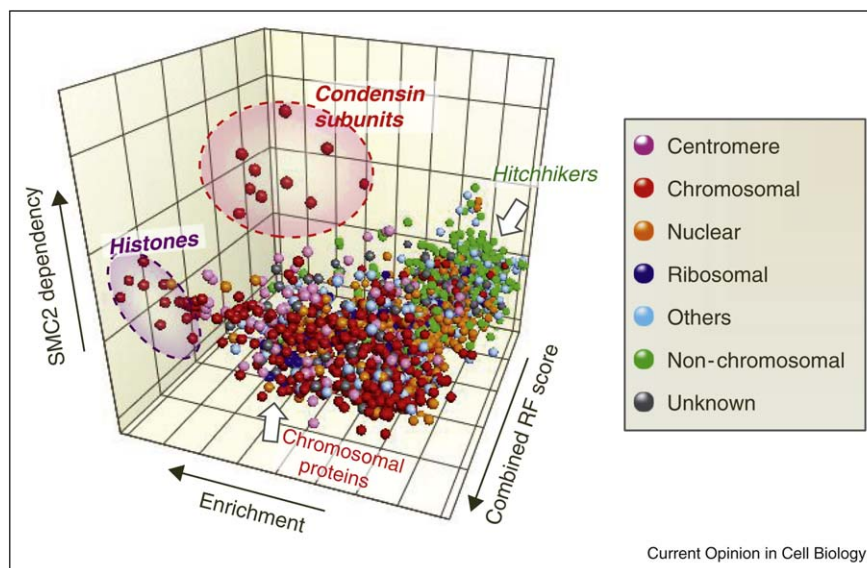
One attempt to solve the ‘hitchhiker problem’ described above was to apply stable isotope labeling by amino acids in cell culture (SILAC) [79] to chromosomes subjected to a variety of different analytical procedures. This technique accurately compares protein ratios between samples by determining ratios of individual peptides distinguished by ¹³C/¹⁵N and ¹²C/¹⁴N, using cultures grown in heavy and light medium, respectively. SILAC was used to determine the percentage of each protein in isolated chromosomes relative to an equal mass of cytosol and to measure the ability of cytosolic proteins to bind stably to isolated chromosomes. SILAC was also combined with genetic ablation of key proteins to look at dependency relationships governing the chromosomal association of various proteins and protein complexes. The data set generated from each such experiment was the ratio of heavy-to-light peptides for each protein. This quantitatively reflected the distribution of each protein in

the samples being compared, and enabled the proteome to be sorted as a ranked list. Each sorted list was termed a classifier.

This analysis was initially unsatisfying, as no classifier could reliably distinguish chromosomal from non-chromosomal proteins. This problem was solved by combining the classifiers. Since each classifier is simply a list of values, it can be used to define the axis of a graph. Using the classifiers mentioned above, one could plot for all proteins in the data set their enrichment in chromosomes versus their ability to exchange onto chromosomes versus their dependency on a protein such as condensin subunit SMC2 (a related plot is shown in Figure 2). Plotting parameters that seem to be independent of one another in this way yielded powerful insights.

The following example shows how this analysis can work. In a three-dimensional plot such as that of Figure 2, one can use the *k*-nearest neighbor algorithm (*k*-NN—a type of machine learning [80]) to ask for every uncharacterized protein in the three-dimensional space which of its *k* nearest neighbors is known to be chromosomal. This generates a list in which uncharacterized proteins are ranked according to the quality of their neighborhood. Varying *k* enables one to alter the effective ‘resolution’ of the analysis (for example identifying proteins likely to be centromeric rather than simply chromosomal). As a further step, the neighborhood values for each individual experiment and the original data can be input into another machine learning algorithm such as Random

Figure 2



Combining classifiers in 3-dimensions increases specificity. In this case the axes plot Enrichment (ratio of each protein in chromosomes versus that in an equal protein mass of cytoplasm) versus SMC2 dependency (amount of each protein in wild type chromosomes divided by its amount in chromosomes from SMC2-depleted cells) versus the Combined random forest score (calculated by combining all proteomic classifiers with nearest neighbor analysis and quantitative bioinformatic analysis of protein domains) [32^{**}]. Core histones and condensin subunits cluster in the analysis.

Forest analysis [81], which can be trained to separate chromosomal from non-chromosomal proteins using proteins of known behavior. The efficiency of this approach was tested by tagging 50 novel/uncharacterized proteins with GFP and observing their localization in mitosis (12 novel centromere proteins; 7 novel periphery proteins, 11 novel bulk chromatin proteins). Of the 50 tagged proteins 44 (88%) localized in mitosis as predicted. This enabled the prediction that among the ~550 uncharacterized proteins of the chromosome proteome, 97 new centromere-associated proteins; 46 new chromosome periphery proteins, and 90 new bulk chromatin proteins remain to be identified [32**].

The MCCP approach also enables the experimenter to reveal subtle relationships between characterized and uncharacterized proteins. The experimental framework underpinning this, cluster-heatmap analysis, has been used for many years to analyze microarray data and compare samples generated from different cell types or cell types exposed to differing experimental conditions. What has been recently realized is that this analysis need not be limited to microarray data. In fact, any combination of quantitative data can be used. To date, classifiers used in cluster-heatmap analysis have included phenotypic profiling of cell cycle defects [82,83*], SILAC ratios from proteomic experiments [32**,84,85], quantitative analysis of protein interaction data [86] and quantitative analysis of protein localization data [87*]. One powerful outcome of this analysis is that it can allow the prediction of protein function for proteins whose primary sequence is uninformative [32**].

When combined with genetics, the MCCP approach also allows one to study and even identify protein complexes in their 'native environment' by analyzing the entire mitotic chromosome fraction without requiring that protein complexes be available in soluble form. In a demonstration of this approach, genetic ablation of Ska3 was found to result in the loss of the Ska, APC/C, and RanBP2/RanGAP1 complexes from chromosomes, and all subunits of all complexes behaved in an identical manner [32**]. Thus this approach can be used to deduce the composition of functional protein complexes and dependency relationships between them without the need for biochemical fractionation.

Towards a molecular model of the chromosome

Now that comprehensive lists of proteins are available, development of the next generation of models for the molecular organization of chromosomes will require two further advances: (1) a way to determine the copy numbers of all of the various chromosomal constituents and (2) a method to map protein-protein contacts between all chromosomal proteins. The first of these is now becoming a reality. Starting with the budding yeast, where there is

known to be a single Cse4/CENP-A-positive nucleosome in the kinetochore of each of the 16 chromosomes, it has been possible to use GFP-tagged proteins and quantitative fluorescence methods to determine the copy numbers for a number of kinetochore components [88]. This analysis was extended to *S. pombe* [89], and most recently to the kinetochores of chicken DT40 cells [90*]. This analysis is quite laborious, but recent improvements in the analysis of proteomic data have permitted initial estimation of copy numbers for all kinetochore proteins in DT40 cells [32**]. These show a remarkable agreement with the values from fluorescence measurements, and when the method is further developed, rigorous quantitation of all protein components of mitotic chromosomes will be possible.

Mapping all protein interactions within entire chromosomes sounds far fetched, but is approaching the realm of possibility. This can in principle be done by protein-protein cross-linking followed by proteomic identification of all cross-linked peptides. The method has been successfully applied to the outer kinetochore-associated Ndc80 complex [91], and more recently to the considerably larger complex of RNA polymerase II holoenzyme bound to the initiation factor TIF [92*]. Significant technical advances are required before this could be applied to an entire mitotic chromosome. Nonetheless, it now appears possible that within the next few years, the molecular architecture of mitotic chromosomes will be understood at a previously unimagined level of detail.

Note added in proof

Since this review was written a second study has been published using super-resolution microscopy to study mitotic chromosome structure under conditions of minimal disruption. This study reports that *Drosophila* embryo mitotic chromatin is largely composed of ~70 nm fibers. Relating observations made under super-resolution conditions to conventional images will be a challenge for the future [93].

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Maiato H, Deluca J, Salmon ED, Earnshaw WC: **The dynamic kinetochore-microtubule interface.** *J Cell Sci* 2004, **117**:5461-5477.
2. Cheeseman IM, Desai A: **Molecular architecture of the kinetochore-microtubule interface.** *Nat Rev Mol Cell Biol* 2008, **9**:33-46.

3. de Lange T: **Shelterin: the protein complex that shapes and safeguards human telomeres.** *Genes Dev* 2005, **19**:2100-2110.
4. Palm W, de Lange T: **How shelterin protects mammalian telomeres.** *Annu Rev Genet* 2008, **42**:301-334.
5. Sharp LW: **Structure of large somatic chromosomes.** *Botanical Gazette* 1929, **88**:349-382.
6. Schrader F: *Mitosis. The Movements of Chromosomes in Cell Division.* edn 1. New York: Columbia University Press; 1944.
7. McKeon FD, Tuffanelli DL, Kobayashi S, Kirschner MW: **The redistribution of a conserved nuclear envelope protein during the cell cycle suggests a pathway for chromosome condensation.** *Cell* 1984, **36**:83-92.
8. Chaly N, Bladon T, Setterfield G, Little JE, Kaplan JG, Brown DL: **Changes in distribution of nuclear matrix antigens during the mitotic cell cycle.** *J Cell Biol* 1984, **99**:661-671.
9. Uchiyama S, Kobayashi S, Takata H, Ishihara T, Hori N, Higashi T, Hayashihara K, Sone T, Higo D, Nirasawa T *et al.*: **Proteome analysis of human metaphase chromosomes.** *J Biol Chem* 2005, **280**:16994-17004.
10. Hernandez-Verdun D, Gautier T: **The chromosome periphery during mitosis.** *Bioessays* 1994, **16**:179-185.
11. Van Hooser AA, Yuh P, Heald R: **The perichromosomal layer.** *Chromosoma* 2005, **114**:377-388.
12. Hirano T: **Condensins: organizing and segregating the genome.** *Curr Biol* 2005, **15**:R265-R275.
13. Larionov VL, Karpova TS, Kouprina NY, Jouravleva GA: **A mutant of *Saccharomyces cerevisiae* with impaired maintenance of centromeric plasmids.** *Curr Genet* 1985, **10**:15-20.
14. Hirano T: **At the heart of the chromosome: SMC proteins in action.** *Nat Rev Mol Cell Biol* 2006, **7**:311-322.
15. Hudson DF, Marshall KM, Earnshaw WC: **Condensin: architect of mitotic chromosomes.** *Chromosome Res* 2009, **17**:131-144.
16. Wood AJ, Severson AF, Meyer BJ: **Condensin and cohesin complexity: the expanding repertoire of functions.** *Nat Rev Genet* 2010, **11**:391-404.
17. Shintomi K, Hirano T: **Sister chromatid resolution: a cohesin releasing network and beyond.** *Chromosoma* 2010, in press.
18. Hagstrom KA, Holmes VF, Cozzarelli NR, Meyer BJ: ***C. elegans* condensin promotes mitotic chromosome architecture, centromere organization, and sister chromatid segregation during mitosis and meiosis.** *Genes Dev* 2002, **16**:729-742.
19. Kaitna S, Pasierbek P, Jantsch M, Loidl J, Glotzer M: **The Aurora B kinase AIR-2 regulates kinetochores during mitosis and is required for separation of homologous chromosomes during meiosis.** *Curr Biol* 2002, **12**:798-812.
20. Hudson DF, Vagnarelli P, Gassmann R, Earnshaw WC: **Condensin is required for nonhistone protein assembly and structural integrity of vertebrate mitotic chromosomes.** *Dev Cell* 2003, **5**:323-336.
21. Coelho PA, Queiroz-Machado J, Sunkel CE: **Condensin-dependent localisation of topoisomerase II to an axial chromosomal structure is required for sister chromatid resolution during mitosis.** *J Cell Sci* 2003, **116**:4763-4776.
22. Almagro S, Riveline D, Hirano T, Houchmandzadeh B, Dimitrov S: **The mitotic chromosome is an assembly of rigid elastic axes organized by structural maintenance of chromosomes (SMC) proteins and surrounded by a soft chromatin envelope.** *J Biol Chem* 2004, **279**:5118-5126.
23. Ribeiro SA, Gatlin JC, Dong Y, Joglekar A, Cameron L, Hudson DF, Farr CJ, McEwen BF, Salmon ED, Earnshaw WC *et al.*: **Condensin regulates the stiffness of vertebrate centromeres.** *Mol Biol Cell* 2009, **20**:2371-2380.
24. D'Amours D, Stegmeier F, Amon A: **Cdc14 and condensin control the dissolution of cohesin-independent chromosome linkages at repeated DNA.** *Cell* 2004, **117**:455-469.
25. Machin F, Paschos K, Jarmuz A, Torres-Rosell J, Pade C, Aragon L: **Condensin regulates rDNA silencing by modulating nucleolar Sir2p.** *Curr Biol* 2004, **14**:125-130.
26. Csankovszki G, Collette K, Spahl K, Carey J, Snyder M, Petty E, Patel U, Tabuchi T, Liu H, McLeod I *et al.*: **Three distinct condensin complexes control *C. elegans* chromosome dynamics.** *Curr Biol* 2009, **19**:9-19.
27. Vagnarelli P, Hudson DF, Ribeiro SA, Trinkle-Mulcahy L, Spence JM, Lai F, Farr CJ, Lamond AI, Earnshaw WC: **Condensin and Repo-Man-PP1 co-operate in the regulation of chromosome architecture during mitosis.** *Nat Cell Biol* 2006, **8**:1133-1142.
28. Markaki Y, Christogianni A, Politou AS, Georgatos SD: **Phosphorylation of histone H3 at Thr3 is part of a combinatorial pattern that marks and configures mitotic chromatin.** *J Cell Sci* 2009, **122**:2809-2819.
29. Uemura T, Ohkura H, Adachi Y, Morino K, Shiozaki K, Yanagida M: **DNA topoisomerase II is required for condensation and separation of mitotic chromosomes in *S. pombe*.** *Cell* 1987, **50**:917-925.
30. Earnshaw WC, Halligan B, Cooke CA, Heck MMS, Liu LF: **Topoisomerase II is a structural component of mitotic chromosome scaffolds.** *J Cell Biol* 1985, **100**:1706-1715.
31. Gasser SM, Laroche T, Falquet J, Boy de la Tour E, Laemmli UK: **Metaphase chromosome structure. Involvement of topoisomerase II.** *J Mol Biol* 1986, **188**:613-629.
32. Ohta S, Bukowski-Wills J-C, Sanchez-Pulido L, de Lima Alves F, Wood L, Chen Z, Platani M, Fischer L, Hudson DF, Ponting CP, *et al.*: **The protein composition of mitotic chromosomes determined using multi-classifier combinatorial proteomics.** *Cell* 2010, in press.
- Major study combining biochemical, genetic, and cell biological analysis of the mitotic chromosome proteome. The study reports the development of a new approach using machine learning to integrate data from the various disciplines.
33. Chang CJ, Goulding S, Earnshaw WC, Carmena M: **RNAi analysis reveals an unexpected role for topoisomerase II in chromosome arm congression to a metaphase plate.** *J Cell Sci* 2003, **116**:4715-4726.
34. Carpenter AJ, Porter AC: **Construction, characterization, and complementation of a conditional-lethal DNA topoisomerase II α mutant human cell line.** *Mol Biol Cell* 2004, **15**:5700-5711.
35. Spence JM, Phua HH, Mills W, Carpenter AJ, Porter AC, Farr CJ: **Depletion of topoisomerase II α leads to shortening of the metaphase interkinetochore distance and abnormal persistence of PICH-coated anaphase threads.** *J Cell Sci* 2007, **120**:3952-3964.
36. Kawamura R, Pope LH, Christensen MO, Sun M, Terekhova K, Boege F, Mielke C, Andersen AH, Marko JF: **Mitotic chromosomes are constrained by topoisomerase II-sensitive DNA entanglements.** *J Cell Biol* 2010, **188**:653-663.
- Elegant study using a combination of *in vitro* micromanipulation with enzyme treatments to examine the role of topo II in determining the structural properties of isolated mitotic chromosomes.
37. Marko JF: **Micromechanical studies of mitotic chromosomes.** *Chromosome Res* 2008, **16**:469-497.
38. Vagnarelli P, Morrison C, Dodson H, Sonoda E, Takeda S, Earnshaw WC: **Analysis of Scc1-deficient cells defines a key metaphase role of vertebrate cohesin in linking sister kinetochores.** *EMBO Rep* 2004, **5**:167-171.
39. Dewar H, Tanaka K, Nasmyth K, Tanaka TU: **Tension between two kinetochores suffices for their bi-orientation on the mitotic spindle.** *Nature* 2004, **428**:93-97.
40. Adolph KW, Cheng SM, Laemmli UK: **Role of nonhistone proteins in metaphase chromosome structure.** *Cell* 1977, **12**:805-816.
41. Marsden MPF, Laemmli UK: **Metaphase chromosome structure: evidence for a radial loop model.** *Cell* 1979, **17**:849-858.

42. Belmont AS: **Mitotic chromosome scaffold structure: New approaches to an old controversy.** *Proc Natl Acad Sci USA* 2002, **99**:15855-15857.
43. Belmont AS: **Mitotic chromosome structure and condensation.** *Curr Opin Cell Biol* 2006, **18**:632-638.
44. Earnshaw WC, Heck MMS: **Localization of topoisomerase II in mitotic chromosomes.** *J Cell Biol* 1985, **100**:1716-1725.
45. Maeshima K, Laemmli UK: **A two-step scaffolding model for mitotic chromosome assembly.** *Dev Cell* 2003, **4**:467-480.
46. Kireeva N, Lakonishok M, Kireev I, Hirano T, Belmont AS: **Visualization of early chromosome condensation: a hierarchical folding, axial glue model of chromosome structure.** *J Cell Biol* 2004, **166**:775-785.
47. Maeshima K, Eltsov M, Laemmli UK: **Chromosome structure: improved immunolabeling for electron microscopy.** *Chromosoma* 2005, **114**:365-375.
48. Poirier MG, Marko JF: **Mitotic chromosomes are chromatin networks without a mechanically contiguous protein scaffold.** *Proc Natl Acad Sci USA* 2002, **99**:15393-15397.
49. Strukov YG, Belmont AS: **Mitotic chromosome structure: reproducibility of folding and symmetry between sister chromatids.** *Biophys J* 2009, **96**:1617-1628.
- This careful study using the lac operator system to fluorescently tag chromosomal spots finds no evidence for higher order reproducible structure within mitotic chromosomes.
50. Boy de la Tour E, Laemmli UK: **The metaphase scaffold is helically folded: sister chromatids have predominantly opposite helical handedness.** *Cell* 1988, **55**:937-944.
51. Finch JT, Klug A: **Solenoidal model for superstructure in chromatin.** *Proc Natl Acad Sci USA* 1976, **73**:1897-1901.
52. Bystricky K, Heun P, Gehlen L, Langowski J, Gasser SM: **Long-range compaction and flexibility of interphase chromatin in budding yeast analyzed by high-resolution imaging techniques.** *Proc Natl Acad Sci USA* 2004, **101**:16495-16500.
53. Eltsov M, MacLellan KM, Maeshima K, Frangakis AS, Dubochet J: **Analysis of cryo-electron microscopy images does not support the existence of 30-nm chromatin fibers in mitotic chromosomes in situ.** *Proc Natl Acad Sci USA* 2008, **105**:19732-19737.
- Controversial study using electron cryomicroscopy to examine isolated mitotic chromosomes that fails to find evidence for an axial structure or for 30 nm chromatin fibers. The devil is in the details here. Did the chromosome isolation procedure have an effect on the internal chromatin structure?
54. König P, Braunfeld MB, Sedat JW, Agard DA: **The three-dimensional structure of in vitro reconstituted *Xenopus laevis* chromosomes by EM tomography.** *Chromosoma* 2007, **116**:349-372.
55. Maeshima K, Hihara S, Eltsov M: **Chromatin structure: does the 30-nm fibre exist in vivo?** *Curr Opin Cell Biol* 2010, **22**:291-297.
56. Nishino Y, Takahashi Y, Imamoto N, Ishikawa T, Maeshima K: **Three-dimensional visualization of a human chromosome using coherent X-ray diffraction.** *Phys Rev Lett* 2009, **102**:018101.
- Innovative study using a new approach to study mitotic chromosome structure. Biological insight are still limited, but watch this space for further developments!
57. Ribeiro SA, Vagnarelli P, Dong Y, Hori T, McEwen BF, Fukagawa T, Flors C, Earnshaw WC: **A super-resolution map of the vertebrate kinetochore.** *Proc Natl Acad Sci USA* 2010, **107**:10484-10489.
- The first use of super-resolution microscopy to study mitotic chromosome (in this case kinetochore) organization yielded information leading to a new model for kinetochore topology.
58. Salzman NP, Moore DE, Mendelsohn J: **Isolation and characterization of human metaphase chromosomes.** *Proc Natl Acad Sci USA* 1966, **56**:1449-1456.
59. Wray W, Stubblefield E: **A new method for the rapid isolation of chromosomes, mitotic apparatus, or nuclei from mammalian fibroblasts at near neutral pH.** *Exp Cell Res* 1970, **59**:469-478.
60. Takata H, Uchiyama S, Nakamura N, Nakashima S, Kobayashi S, Sone T, Kimura S, Lahmers S, Granzier H, Labeit S *et al.*: **A comparative proteome analysis of human metaphase chromosomes isolated from two different cell lines reveals a set of conserved chromosome-associated proteins.** *Genes Cells* 2007, **12**:269-284.
- The Fukui lab has conducted a number of studies of mitotic chromosome proteomics. We recommend this study, as it is a nice example of comparison of chromosomes isolated from different cell lines.
61. Adams SE, Earnshaw W.C RR: **Proteomic analysis of human metaphase chromosomes reveals topoisomerase II alpha as an Aurora B substrate.** *Nucleic Acids Res* 2002, **30**:5318-5327.
62. Gassmann R, Henzing AJ, Earnshaw WC: **Novel components of human mitotic chromosomes identified by proteomic analysis of the chromosome scaffold fraction.** *Chromosoma* 2005, **113**:385-397.
63. Laemmli UK, Cheng SM, Adolph KW, Paulson JR, Brown JA, Baumbach WR: **Metaphase chromosome structure: the role of nonhistone proteins.** *Cold Spring Harbor Sym Quant Biol* 1978, **43**:351-360.
64. Gassmann R, Carvalho A, Henzing AJ, Ruchaud S, Hudson DF, Honda R, Nigg EA, Gerloff DL, Earnshaw WC: **Borealin: a novel chromosomal passenger required for stability of the bipolar mitotic spindle.** *J Cell Biol* 2004, **166**:179-191.
65. Tadeu AM, Ribeiro S, Johnston J, Goldberg I, Gerloff D, Earnshaw WC: **CENP-V is required for centromere organization, chromosome alignment and cytokinesis.** *EMBO J* 2008, **27**:2510-2522.
66. Porter IM, McClelland SE, Khoudoli GA, Hunter CJ, Andersen JS, McAinsh AD, Blow JJ, Swedlow JR: **Bod1, a novel kinetochore protein required for chromosome biorientation.** *J Cell Biol* 2007, **179**:187-197.
- This study took a different approach, focusing on proteins that assembled onto to sperm chromatin added to *Xenopus* mitotic egg extracts. Hirano first identified condensin using a similar strategy.
67. Fukui K: **Structural analyses of chromosomes and their constituent proteins.** *Cytogenet Genome Res* 2009, **124**:215-227.
68. Amin MA, Matsunaga S, Uchiyama S, Fukui K: **Nucleophosmin is required for chromosome congression, proper mitotic spindle formation, and kinetochore-microtubule attachment in HeLa cells.** *FEBS Lett* 2008, **582**:3839-3844.
69. Ma N, Matsunaga S, Takata H, Ono-Maniwa R, Uchiyama S, Fukui K: **Nucleolin functions in nucleolus formation and chromosome congression.** *J Cell Sci* 2007, **120**:2091-2105.
70. Gambe AE, Matsunaga S, Takata H, Ono-Maniwa R, Baba A, Uchiyama S, Fukui K: **A nucleolar protein RRS1 contributes to chromosome congression.** *FEBS Lett* 2009, **583**:1951-1956.
71. Dejardin J, Kingston RE: **Purification of proteins associated with specific genomic loci.** *Cell* 2009, **136**:175-186.
- Elegant and technically demanding study describing the isolation of telomeres from mammalian cells based on fishing for the underlying DNA sequences using DNA hybridization. Over 100 proteins of the telomere chromatin are described.
72. Obuse C, Iwasaki O, Kiyomitsu T, Goshima G, Toyoda Y, Yanagida M: **A conserved Mis12 centromere complex is linked to heterochromatic HP1 and outer kinetochore protein Zwint-1.** *Nat Cell Biol* 2004, **6**:1135-1141.
73. Okada M, Cheeseman IM, Hori T, Okawa K, McLeod IX, Yates JR, Desai A, Fukagawa T: **The CENP-H-I complex is required for the efficient incorporation of newly synthesized CENP-A into centromeres.** *Nat Cell Biol* 2006, **8**:446-457.
74. Foltz DR, Jansen LE, Black BE, Bailey AO, Yates JR, Cleveland DW: **The human CENP-A centromeric nucleosome-associated complex.** *Nat Cell Biol* 2006, **8**:458-469.
75. Amano M, Suzuki A, Hori T, Backer C, Okawa K, Cheeseman IM, Fukagawa T: **The CENP-S complex is essential for the stable assembly of outer kinetochore structure.** *J Cell Biol* 2009, **186**:173-182.

76. Akiyoshi B, Nelson CR, Ranish JA, Biggins S: **Quantitative proteomic analysis of purified yeast kinetochores identifies a PP1 regulatory subunit.** *Genes Dev* 2009, **23**:2887-2899.
The first isolation of kinetochores (as opposed to other proteomics studies all of which fished out particular kinetochore proteins together with proteins bound to them). This hinged on the isolation of a small minichromosome from budding yeast.
77. Lewis CD, Laemmli UK: **Higher order metaphase chromosome structure: evidence for metalloprotein interactions.** *Cell* 1982, **29**:171-181.
78. Cox J, Mann M: **MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification.** *Nat Biotechnol* 2008, **26**:1367-1372.
MaxQuant is a key resource for quantitative proteomics. This software forms the basis for many proteomic studies published since and has been instrumental to determine the protein composition of mitotic chromosomes.
79. Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, Pandey A, Mann M: **Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics.** *Mol Cell Proteomics* 2002, **1**:376-386.
80. Cover TM, Hart PE: **Nearest Neighbor Pattern Classification. IEEE Transactions on Information Theory, IT-13(1):21-27, January 1967.** In *Pattern Recognition*. Edited by Fu KS. Chinese University Press; 1980.
81. Breiman L: **Random forests.** *Machine Learn* 2001, **45**:1.
82. Kittler R, Pelletier L, Heninger AK, Slabicki M, Theis M, Miroslaw L, Poser I, Lawo S, Grabner H, Kozak K *et al.*: **Genome-scale RNAi profiling of cell division in human tissue culture cells.** *Nat Cell Biol* 2007, **9**:1401-1412.
83. Neumann B, Walter T, Heriche JK, Bulkescher J, Erfle H, Conrad C, Rogers P, Poser I, Held M, Liebel U *et al.*: **Phenotypic profiling of the human genome by time-lapse microscopy reveals cell division genes.** *Nature* 2010, **464**:721-727.
This large consortium study used cluster-heatmap analysis to analyze a large scale RNAi screen of human genes involved in mitotic progression.
84. Andersen JS, Lam YW, Leung AK, Ong SE, Lyon CE, Lamond AI, Mann M: **Nucleolar proteome dynamics.** *Nature* 2005, **433**:77-83.
85. Boisvert FM, Lam YW, Lamont D, Lamond AI: **A quantitative proteomics analysis of subcellular proteome localization and changes induced by DNA damage.** *Mol Cell Proteomics* 2010, **9**:457-470.
86. Hubner NC, Bird AW, Cox J, Splettsjoesser B, Bandilla P, Poser I, Hyman A, Mann M: **Quantitative proteomics combined with BAC TransgeneOmics reveals in vivo protein interactions.** *J Cell Biol* 2010, **189**:739-754.
87. Hutchins JR, Toyoda Y, Hegemann B, Poser I, Heriche JK, Sykora MM, Augsburg M, Hudecz O, Buschhorn BA, Bulkescher J *et al.*: **Systematic analysis of human protein complexes identifies chromosome segregation proteins.** *Science* 2010, **328**:593-599.
This large consortium study used cluster-heatmap analysis to analyze protein localization data obtained following use of recombineering to label large numbers of proteins involved in mitotic progression.
88. Joglekar AP, Bouck DC, Molk JN, Bloom KS, Salmon ED: **Molecular architecture of a kinetochore-microtubule attachment site.** *Nat Cell Biol* 2006, **8**:581-585.
89. Joglekar AP, Bouck D, Finley K, Liu X, Wan Y, Berman J, He X, Salmon ED, Bloom KS: **Molecular architecture of the kinetochore-microtubule attachment site is conserved between point and regional centromeres.** *J Cell Biol* 2008, **181**:587-594.
90. Johnston K, Joglekar A, Hori T, Suzuki A, Fukagawa T, Salmon ED: **Vertebrate kinetochore protein architecture: protein copy number.** *J Cell Biol* 2010, **189**:937-943.
The first attempt to calculate copy numbers for a number of components of the vertebrate kinetochore based on fluorescence intensity of GFP-labeled proteins compared to labeled strains of budding yeast.
91. Maiolica A, Cittaro D, Borsotti D, Sennels L, Ciferri C, Tarricone C, Musacchio A, Rappsilber J: **Structural analysis of multiprotein complexes by cross-linking, mass spectrometry, and database searching.** *Mol Cell Proteomics* 2007, **6**:2200-2211.
92. Chen ZA, Jawhari A, Fischer L, Buchen C, Tahir S, Kaminski T, Rasmussen M, Lariviere L, Bukowski-Wills JC, Nilges M *et al.*: **Architecture of the RNA polymerase II-TFIIF complex revealed by cross-linking and mass spectrometry.** *EMBO J* 2010, **29**:717-726.
This is the largest protein complex to date to have a detailed topology revealed by chemical cross-linking followed by use of mass spectrometry to identify the cross-link sites.
93. Matsuda A, Shao L, Boulanger J, Kervrann C, Carlton PM, Kner P, Agard D, Sedat JW: **Condensed mitotic chromosome structure at nanometer resolution using PALM and EGFP- histones.** *PLoS ONE* 2010, **5**:e12768.