CONDENSIN AND COHESIN: MORE THAN CHROMOSOME COMPACTOR AND GLUE

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Two related protein complexes, cohesin and condensin, are essential for separating identical copies of the genome into daughter cells during cell division. Cohesin glues replicated sister chromatids together until they split at anaphase, whereas condensin reorganizes chromosomes into their highly compact mitotic structure. Unexpectedly, mutations in the subunits of these complexes have been uncovered in genetic screens that target completely different processes. Exciting new evidence is emerging that cohesin and condensin influence crucial processes during interphase, and unforeseen aspects of mitosis. Each complex can perform several roles, and individual subunits can associate with different sets of proteins to achieve diverse functions, including the regulation of gene expression, DNA repair, cell-cycle checkpoints and centromere organization.

Every time a cell divides, it must accurately distribute one copy of each duplicated chromosome to its daughter cells. To accomplish that task, chromosomes radically change their shape and undergo distinctive movements. Replicated chromosomes are glued together, compacted, untangled and then pulled to opposite sides of the cell (BOX 1). These events must be coordinated and executed with precision to avoid aneuploidy, which is a condition of inappropriate chromosome number that is often associated with birth defects and cancer. In the past decade, the discovery of two multiprotein complexes, cohesin and condensin, has provided important insights into the molecular mechanisms of chromosome segregation. Although mitotic chromosome segregation might seem unrelated to interphase functions, such as gene regulation and DNA repair, it is becoming increasingly clear that cohesin and condensin subunits influence all of these chromosomal processes.

Cytological observations of mitotic chromosome dynamics originally indicated the existence of factors that condense chromatids or hold sister chromatids together. In an exciting convergence of results, the recovery of mutants that were defective in these processes, and the biochemical identification of mitotic chromosome-associated factors, led to the discovery of condensin and cohesin. At their cores, condensin and cohesin contain chromosomal ATPases of the structural maintenance of chromosomes (SMC) protein family[1–3], but the two complexes are structurally and functionally distinct.

Although cohesin and condensin were named for their roles in sister-chromatid cohesion and condensation during mitosis, genetic analysis has shown a wide range of other chromosomal processes that rely on cohesin and condensin subunits. Screens that were designed to understand processes as diverse as sex-specific gene regulation, nerve-cell development, transposon-enhancer blocking and DNA repair, have identified mutations in these genes and have expanded our view of their functions. The effects of mutations in cohesin and condensin subunits are now being tested in specific functional assays to further explore their roles in gene regulation, DNA repair, cell-cycle checkpoints and centromere structure.

It is not yet known if a single molecular mechanism underlies these different functions, or whether a single complex can act through several mechanisms. For example, a common change in chromosome structure might influence all these diverse processes.

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Alternatively, subunits of each complex could perform different functions depending on their chromosomal context, cell-cycle-dependent regulation and protein partners. Because individual cohesin or condensin subunits can interact with different protein partners to form complexes with different roles, the phenotypic consequences of mutating an individual subunit might reflect not only the function of that complex, but also other complexes that contain that subunit.

Several excellent articles have covered the canonical roles of these SMC complexes in cohesion and condensation, and should be consulted for a thorough review[4–6]. After providing a brief overview, we focus here on the non-canonical roles of cohesin and condensin, discussing their influence on gene regulation, DNA repair and centromere function. We also highlight how insights into these roles have been gained by investigating individual subunit mutations in organisms that are amenable to genetic and cytological analysis. These studies provide a greater appreciation of the versatility of cohesin and condensin, and promise that insights into many cellular functions will be gained by examining these important chromosomal proteins.

Cohesin and condensin — the canonical role

Cohesin is considered to be part of the glue between sister chromatids, which is laid down at DNA replication so that sister chromatids are handled as a pair and subsequently segregated away from each other (BOXES 2,3). Mutations in cohesin subunits cause precocious sister-chromatid separation[7–10], and mutations that prevent cohesin from being removed inhibit chromosome separation[11–13]. Once chromosomes are properly aligned in the mitotic spindle, cohesin is cleaved to allow sister chromatids to segregate into the two daughter cells (BOX 2). Cohesin is also essential for meiosis, and its chromosomal location clearly parallels the differential cohesion events of meiosis I and II. During meiosis I, in which homologues pair, cohesin localizes all along the chromosome arms. It is released along the arms when homologues segregate at the end of meiosis I, but remains at the centromeres until the end of meiosis II to keep the sister chromatids paired until their segregation (BOX 2).

Condensin is required for the substantial reorganization of chromosome structure as chromosomes compact during mitosis (BOX 4). Condensin components were identified in screens for chromosome-segregation mutants in yeast[14], and biochemically purified from

Box 1 | **Chromosome events of mitosis**

Preparing for mitosis: replication and establishment of cohesion
Preparations for mitotic chromosome segregation begin in the S phase of the cell cycle, as each chromosome is copied to generate two identical sister chromatids and cohesion is established between them. Cohesion is thought to be mediated both by DNA connections (topological catenations) that are generated during DNA replication and by a proteinaceous linkage (probably the cohesin complex) that hold sister chromatids together until anaphase.

Prophase
After replication and cohesin loading, chromosomes are in an extended configuration and appear as an amorphous mass in the nucleus. As cells enter prophase, chromosomes begin the process of condensation, undergoing a marked change in structure that continues until they are fully compacted at metaphase. During prophase condensation, the sister chromatids begin to dissociate along their arms and organize along their individual axes — a process that is called resolution. In many organisms this can be viewed cytologically as the transition from indistinguishable chromosomes to two rod-shaped arms that are attached at the centromere.

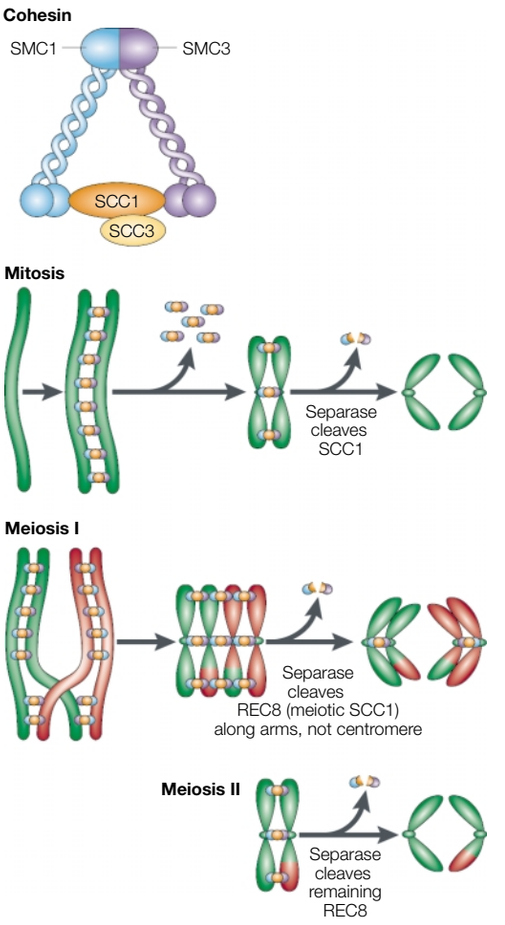
Prometaphase
Prometaphase is defined as the stage during which the nuclear envelope breaks down. A specialized chromosome region, the centromere, assembles a proteinaceous structure — the kinetochore — that mediates attachment to the microtubule spindle. Sister chromatids are arranged so that each centromere interacts with microtubules from only one pole, a phenomenon that is called bi-orientation.

Metaphase
Chromosomes become fully condensed by metaphase. Condensation provides mechanical strength and reduces volume so that chromosomes can withstand spindle forces as they are pulled to opposite sides of the cell. At metaphase, the chromosomes are completely aligned at the centre of the cell, along what is called the metaphase plate. A spindle-checkpoint mechanism monitors chromosome–spindle attachment and delays anaphase until the chromosomes are attached and under tension[76].

Anaphase
At anaphase, the sister chromatids separate in a coordinated burst of movement. Once the spindle checkpoint is relieved, the anaphase-promoting complex (APC) triggers the proteolysis of target proteins and chromosome segregation follows[83]. One target of the APC is a protein called securin that prevents the protease separase from cleaving a cohesin subunit until the commitment is made to anaphase[84]. This mechanism ensures that the cohesin glue is not dissolved until the cell is ready for division.

Telophase
In telophase, the newly segregated chromosomes decondense; subsequently, cytokinesis divides the cytoplasm.

Box 2 | **Cohesin**



Cohesin is a four-member protein complex that is required to hold together the sister chromatids of newly replicated chromosomes. Cohesin contains a heterodimer of the SMC proteins SMC1 (blue) and SMC3 (purple), which is associated with the non-SMC proteins SCC1 (orange) and SCC3 (yellow).

Cohesin proteins were identified by two *Saccharomyces cerevisiae* laboratories in genetic screens that were designed to identify the 'glue' that is required for sister-chromatid attachment[7,8]. Both screens correctly presumed that mutational disruption of the glue would cause precocious sister-chromatid separation in metaphase-arrested cells. Cytological proof of sister-chromatid separation defects relied on clever approaches to mark a single locus on each sister chromatid. Similar proteins have now been identified as members of a cohesin complex in several organisms[10,75,85–88] (TABLE 1).

Cohesin has the cell-cycle-dependent chromatin-localization pattern that would be predicted for a cohesion factor. In yeast, cohesin loads onto chromatids at replication, and remains bound until its abrupt dissociation at anaphase, when cohesion must be released[7,8,89,90]. In higher organisms, the bulk of cohesin dissociates at prophase, although a smaller amount persists at centromeres and dissociates at anaphase[85,91]. This prophase release of cohesin is necessary for sister-chromatid resolution[92]. If cohesin is not removed during anaphase, sister chromatids fail to separate[11–13]. Chromatin immunoprecipitation and immunofluorescence experiments show cohesin along chromosome arms and concentrated at the centromere, which is a site of tight cohesion, as would be expected for a complex that connects sister chromatids[40,42,64,93,94].

The localization pattern of cohesin during meiosis reveals the most about its role in cohesion. During meiosis I when homologues are paired, cohesin localizes along chromosome arms, but when sister chromatids are paired during meiosis II, cohesin is only found between centromeres[88,90,95–97]. Most organisms contain a meiosis-specific cohesin complex, with a meiosis-specific SSC1 variant called REC8 in place of SCC1 (TABLE 1). Meiotic chromatids that lack REC8 cannot maintain cohesion. Reciprocally, meiotic chromatids on which cohesin inappropriately persists cannot lose cohesion and fail to separate at meiosis I and meiosis II.

Dissociation of cohesin at anaphase is triggered by the proteolytic cleavage of its SCC1 or REC8 subunit by a protease called separase[84,98]. Cleavage of SCC1 is necessary for sister-chromatid separation, and is an irreversible entry into anaphase. Separase is inhibited by a protein called securin until the anaphase-promoting complex (APC) UBIQUITYLATES and destroys securin[83]. The APC, in turn, is unable to perform its role until the spindle checkpoint has been cleared. These and other mechanisms ensure that cohesin remains between sister chromatids until the cell is ready to enter anaphase.

condensed mitotic chromosomes in *Xenopus* egg extracts[15,16]. In yeast, the mutational inactivation of condensin subunits increased the average distance between two loci on a mitotic chromosome arm[17–20]. In *Xenopus*, sperm DNA added to a mitotic egg extract assembled into condensed mitotic chromosomes, and depletion of condensin disrupted condensation[15,16]. So, condensin was proposed to drive mitotic chromosome condensation (BOXES 3,4). Condensin seems to be more than a simple chromosome compactor, however, and is crucial for resolving connections between sister chromatids. In a *Drosophila* condensin subunit mutant called *gluon*, sister chromatids are 'fuzzy' and fail to resolve into two distinct rods at prophase, yet compaction along the longitudinal axis seems normal at metaphase[21]. Similarly, depletion of condensin components in *Caenorhabditis elegans* causes stringy rather than rod-shaped chromosomes at prometaphase, yet a high degree of compaction still occurs by metaphase[22]. So, condensin might have a more complex role in chromosome organization, which is necessary for sister-chromatid resolution. Consistent with this idea, sister chromatids in condensin mutants remain connected by chromatin bridges as they pull apart at anaphase during mitosis[14,21–24] and meiosis II (REF. 22).

UBIQUITYLATION
The covalent addition of the small protein ubiquitin to another protein. Ubiquitin conjugation generally targets proteins for degradation by proteases.

Cohesin and condensin in gene regulation

Several mechanisms ensure that genes are expressed at the proper time, place and level. Control mechanisms

Table 1 | **SMC protein complexes**

	Saccharomyces cerevisiae	*Schizosaccharomyces pombe*	*Caenorhabditis elegans*	*Drosophila melanogaster*	*Xenopus laevis*	*Homo sapiens*
Cohesin						
SMC1	Smc1	Psm1	HIM-1	SMC1	SMC1	SMC1α
SMC3	Smc3	Psm3	SMC-3	SMC3	SMC3	SMC3
SCC1	Scc1/Mcd1	Rad21	SCC-1/COH-2	Rad21	RAD21	RAD21
SCC3	Scc3	Psc3	SCC-3	SA	SA1, SA2	SA1, SA2
REC8 (meiosis-specific SCC1)	Rec8	Rec8	REC-8	–	–	REC8
Condensin						
SMC2	Smc2	Cut14	MIX-1 (MIX-1)*	SMC2‡	CAP-E	CAP-E
SMC4	Smc4	Cut3	SMC-4 (DPY-27)*	SMC4/gluon	CAP-C	CAP-C
CAP-D2	Ycs4	Cnd1	HCP-6‡ (DPY-28)*	CG1911‡	CAP-D2/Eg7	CAP-D2/CNAP1
CAP-G	Ycs5/Ycg1	Cnd3	–	CG17054‡	CAP-G	CAP-G
CAP-H	Brn1	Cnd2	(DPY-26)*	Barren	CAP-H	CAP-H
DNA repair						
SMC5	Smc5 (YOL034w)	Spr18	C27A2.1‡	CG3248‡	SMC5‡ (AB103030.1)	SMC5
SMC6	Smc6/Rhc18	Rad18	C23H4.6‡	CG5524‡	SMC6‡ (AB103031.1)	SMC6
			F54D5.14‡			
NSE1	Nse1	–	–	–	–	NSE1‡

*_Caenorhabditis elegans_ contains a second condensin-like complex that functions in X-chromosome dosage compensation. ‡Proteins that have been identified by homology, but have not yet been shown to be members of the complex.

that modulate gene output can operate globally at a chromosome-wide level, regionally over a subchromosomal domain or locally on an individual gene. *Cis*-regulatory DNA elements, such as enhancers, silencers and insulators, mediate these levels of gene regulation by recruiting *trans*-acting factors that influence transcription. Recent studies have shown that either cohesin or condensin contributes to each of these levels of regulation, in part by influencing enhancers, silencers and insulators.

Global and local gene repression. X-chromosome dosage compensation is a regulatory process that alters gene expression along an entire chromosome. This form of regulation evolved in organisms that use chromosomal mechanisms to determine sex (XX/XY or XX/XO) and ensures equivalent levels of X-linked gene products in males and females, despite the difference in X-chromosome number. Animals have solved this problem in different ways: human females completely inactivate one X, male flies hypertranscribe their single X and hermaphrodite nematodes partially downregulate both X chromosomes[25]. In these known cases, dosage-compensation factors associate specifically with the X chromosome of one sex to superimpose chromosome-wide gene regulation on the unique spatial and temporal regulation of each gene.

Dosage-compensation factors in *C. elegans* resemble condensin subunits, and form a complex that binds hermaphrodite X chromosomes and brings about a reduction in gene expression. Worm dosage-compensation genes were discovered through a genetic analysis that showed a regulatory hierarchy that controls sex determination and dosage compensation. Both processes respond to the primary sex-determining signal: the ratio of X chromosomes to autosomes[25] (FIG. 1). An X-chromosome counting mechanism translates the twofold differences in this ratio into the on/off response of the sexual-switch XO lethal gene (*xol-1*). When it is active, *xol-1* specifies male fate, whereas when it is inactive, it specifies both hermaphrodite sex determination and dosage compensation by allowing the expression of the *sdc* genes[25] (FIG. 1). Two types of genetic screen were used to identify dosage-compensation genes. First, mutations in dosage-compensation genes were recognized by the XX-specific lethality and dumpy (short and fat) phenotypes that are associated with inappropriate overexpression of X-linked genes. Second, mutations in dosage-compensation genes rescued the XO lethality of a *xol-1* mutant by relieving the inappropriate halving of gene expression from the single X.

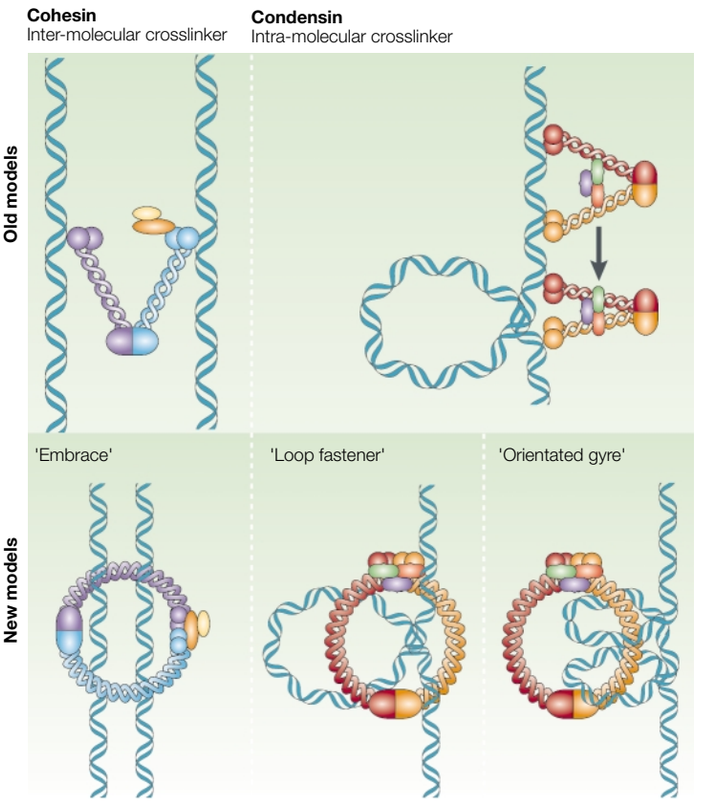
The molecular similarity of dosage-compensation genes to condensin genes was initially surprising, as it was the first indication that condensin might function outside the context of mitosis. *mix-1* and *dpy-27* encode SMC2 and SMC4 homologues, respectively[26,27], and DPY-26 and DPY-28 have limited homology to the non-SMC subunits CAP-H and CAP-D2, respectively[28,29] (TABLE 1). These dosage-compensation proteins form a complex that resembles condensin, but this complex does not seem to affect mitotic chromosomes. Instead, this condensin-like complex assembles onto hermaphrodite X chromosomes to downregulate X-linked gene expression.

Although the dosage-compensation complex is specialized for regulating gene expression, phenotypic analysis indicates that some of its subunits have other chromosomal functions. Mutations in *dpy-26* and *dpy-28*, for example, cause defects in meiotic chromosome segregation[30,31]. Mutations in *mix-1*

cause mitotic chromosome segregation defects that are lethal to both sexes[26]. These *mix-1* phenotypes are reminiscent of condensin defects, but are not shared by other subunits of the dosage-compensation complex, which has prompted a search for further MIX-1-interacting proteins.

Biochemical and genomic approaches showed that MIX-1 (SMC2) partners with the SMC4 variant DPY-27 for its role in dosage compensation, but with SMC-4 for its role in mitosis, in a complex that seems to be the true mitotic condensin of *C. elegans*[22] (FIG. 1). So, MIX-1 is shared between two condensin complexes, one that functions in mitosis and another that regulates dosage compensation. The dosage-compensation complex is probably a recent evolutionary adaptation that arose to fine-tune X-chromosome gene expression. As dosage compensation evolved, proteins such as MIX-1 were apparently recruited for this new role and shared between the mitotic and the dosage-compensation complexes. Others, such as DPY-27, might have arisen from

Box 3 | **Mechanism of cohesin and condensin action**



The molecular mechanisms by which cohesin attaches sister chromatids and condensin reconfigures chromosome structure remain mysterious, but recent studies have provided new insight into their modes of action. Condensin binds DNA and has ATPase activity. When incubated with relaxed circular DNA in the presence of a type I topoisomerase, condensin causes ATP-dependent POSITIVE SUPERCOILING**[22,99,100]. In the presence of a type II topoisomerase, condensin converts nicked circular DNA into positive knots[99]. These results led to the model that condensin introduces global writhe into the DNA, promoting condensation by stabilizing large positively supercoiled DNA loops. Conversely, cohesin catenates nicked circular DNA when incubated with topoisomerase II, and causes DNA–protein aggregates in gel-shift experiments[101]. So, condensin, which compresses one sister into itself, has intramolecular activites, whereas cohesin, which adheres two different sister chromatids, has intermolecular activites. Electron microscopy (EM) shows that the arms of condensin are close together, whereas those of cohesin are spread apart in a 'V' shape[102]. These activities and structures support the model that condensin acts as an intramolecular crosslinker by grabbing sites on a single DNA strand and bringing them together, whereas cohesin acts as an intermolecular crosslinker by grabbing and holding two different sister chromatids.**

New microscopic views of cohesin and condensin have inspired a revised view of their mechanism of action. ATOMIC-FORCE MICROSCOPY **shows that the SMCs of condensin form a globular head onto which the non-SMCs assemble, and a coiled tail the end of which touches DNA[103]. These results suggest a 'loop fastener' model in which the condensin hinge binds one region of DNA, then non-SMC proteins mediate an ATP-dependent opening and closing of the SMC 'V' to enclose a loop of DNA. Another study using electron spectroscopic imaging of condensin suggests an 'orientated gyre' model rather than a 'global writhe' model, because a single condensin molecule seems to introduce two stacked supercoils into closed plasmid DNA[104]. On the basis of this finding, it has been proposed that an ATP-hydrolysis cycle changes the conformation of condensin and allows it to trap two orientated positive supercoils in its coiled-coil arms[105]. A related 'embrace model' has been proposed for cohesin. EM and interaction data indicate that SMC1 and SMC3 are linked at one end by hinge interactions and at the other by interaction with SCC1 (REF. 106). Cohesin was, therefore, proposed to form a large loop that encircles both sister chromatids, fastened by SCC1 at one end. Whereas earlier models envisioned cohesin grabbing sister chromatids, this model indicates that the arms of cohesin might hold two sister chromatids in an 'embrace' until proteolytic cleavage of SCC1 disrupts the cohesin loop[106].**

PARALOGUES
Homologous genes in the same organism that have evolved from a gene duplication and a subsequent divergence of function.

POSITIVE SUPERCOILING
Twisting of the DNA about its own axis. Twisting opposite to the direction of the double-helix turns produces negative supercoils, whereas twisting in the same direction produces positive supercoils.

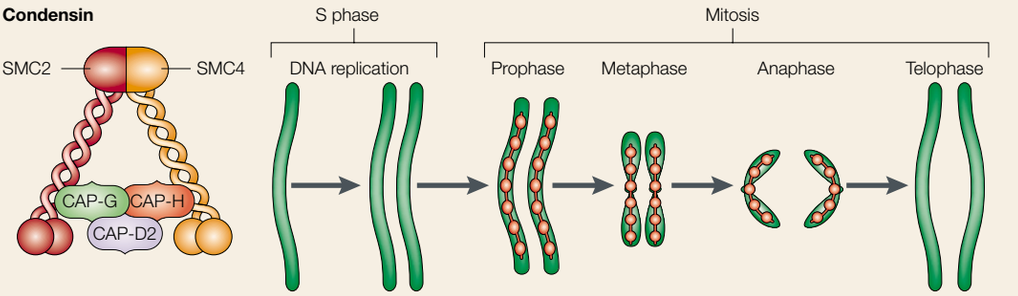
ATOMIC-FORCE MICROSCOPY
A method that maps submicroscopic surfaces to give information about their nature at the atomic level. A mechanical probe with a sharp tip is tracked over the surface of interest, and the deflection of the cantilevered tip is measured.

duplication and divergence of the *smc-4* gene to create a PARALOGUE that is specialized for dosage compensation. This example shows that an entire condensin-like complex can be used for functions other than mitosis, and that a single condensin subunit can participate in two complexes with different functions. Several examples now exist for such diversity in condensin and cohesin subunit function — a recurring theme throughout this review.

The dosage-compensation complex is directed onto the X chromosomes of hermaphrodites by specific targeting proteins. These X-targeting proteins, SDC-2 and SDC-3, are required for both sex determination and dosage compensation. The SDCs form a subcomplex that associates with the condensin-like subunits and is crucial for X recognition and assembly. As well as targeting SDC and dosage-compensation proteins to the X chromosome, SDC-2 and SDC-3 promote hermaphrodite sexual development by targeting these proteins to the male-determining autosomal gene *her-1*, which becomes repressed 20-fold (REF. 32) (FIG. 1). So, the condensin-like dosage-compensation complex (along with the associated SDC proteins) is versatile enough to cause both a twofold chromosome-wide repression and a 20-fold gene-specific repression.

Although the mechanism of X-chromosome repression is unknown, the involvement of condensin-related proteins indicates that changes in chromatin structure might contribute to X regulation. A slight condensation of each X might, ultimately, reduce gene expression. Chromosome condensation during mitosis is correlated with gene inactivation and the displacement of RNA polymerase II and certain transcription factors[33]. It will

Box 4 | **Condensin**



Condensin is a five-member protein complex that is required for chromosome organization and segregation. It contains a heterodimer of SMC proteins (SMC2, red; SMC4, orange) and three associated non-SMC proteins (CAP-D2, CAP-G and CAP-H). In most organisms, condensin (red) only associates with chromosomes at times of the cell cycle when they are condensed (prophase to anaphase).

Our understanding of condensin comes from both biochemical and genetic studies. Chicken SCII, later determined to be SMC2, was identified as one of the proteins that remained after chromosomes were stripped down to an insoluble 'chromosome scaffold', on which looped chromatin domains are thought to be organized[107]. The complete known complex, containing CAP-E (SMC2), CAP-C (SMC4), CAP-D2, CAP-G and CAP-H, was identified by a sedimentation method for purifying Xenopus mitotic chromosome associated proteins (CAPs) from egg extracts[15,16]. CAP-D2 was also identified independently[108]. The five-member protein complex was named condensin because sperm chromosomes introduced into an egg extract that was depleted of any subunit formed a diffuse mass rather than a condensed structure.

Genetic studies also uncovered condensin subunits. Mutations in *Schizosaccharomyces pombe cut3* (*SMC2*) and *cut14* (*SMC4*) cause a cell untimely torn ('cut') phenotype when the division septum cuts through unsegregated chromosomes at the cell centre[14]. These mutants were proposed to affect chromosome condensation rather than the separation of centromeres on sister chromatids, because the distance between two fluorescent *in situ* hybridization (FISH) probes on a chromosome arm increased in the mutants, but centromere probes separated normally[14]. The three *S. pombe* non-SMC condensin subunits were later biochemically identified and shown to associate with the SMC proteins[24]. In *Saccharomyces cerevisiae*, condensin subunit mutations were created and shown by a similar FISH assay to have defects in chromosome segregation and condensation[17,18].

In *Drosophila* and *Caenorhabditis elegans*, condensin subunits were identified fortuitously in genetic screens for unrelated processes, and were later shown to affect mitotic chromosome morphology and segregation[21,23,26]. Observations of chromosomes in these condensin subunit mutants indicated that condensin might have an important role in resolving sister chromatids at prophase, but might not be the only metaphase condensation factor. Mutation of a *Drosophila* condensin subunit caused wider less-distinct prophase sister chromatids, yet these chromatids had shortened along their longitudinal axis by metaphase[21]. Similarly, depletion of *C. elegans* condensin components caused wispy instead of rigid prophase chromosomes that nevertheless align into a relatively compact metaphase plate[22,109]. A common and notable phenotype of condensin mutants in many organisms is the failure to completely separate connections between sister chromatids as they pull apart at anaphase during mitosis[14,21–24]. A similar anaphase segregation defect has also been observed during meiosis II (REF. 22).

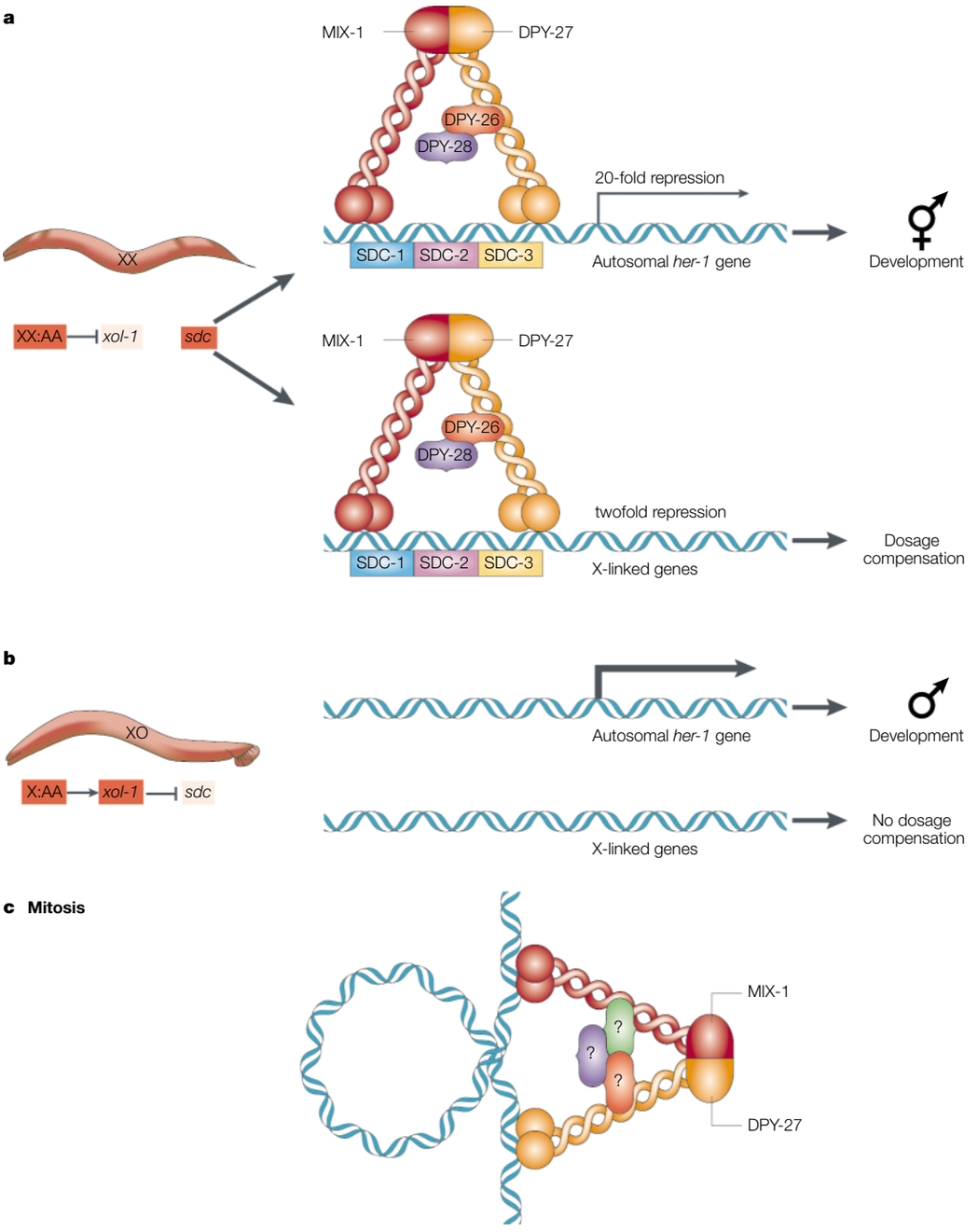


Figure 1 | **A condensin-like complex mediates gene-specific and chromosome-wide repression in *Caenorhabditis elegans*.** **a** | Sex determination and X-chromosome dosage compensation are triggered in response to the ratio of X chromosomes to sets of autosomes (A). In XX animals, the XX:AA ratio causes repression of the sexual-switch gene *xol-1*, which allows activity of the *sdc* genes. The SDC proteins SDC-1, SDC-2 and SDC-3 recruit the condensin-like dosage-compensation complex to the autosomal male-determining gene *her-1* and all along the X chromosomes. Binding of SDC and dosage-compensation proteins leads to the 20-fold repression of *her-1*, promoting hermaphrodite development, and to the twofold repression of X-linked genes, thereby equalizing X-linked gene expression between XX and XO animals during dosage compensation. The dosage-compensation complex contains the condensin subunit homologues MIX-1 (SMC2), DPY-27 (SMC4), DPY-26 (CAP-H) and DPY-28 (CAP-D2). **b** | In XO animals, the X:AA ratio leads to the activation of *xol-1*. High levels of *xol-1* repress the *sdc* genes, and the SDC and dosage-compensation proteins are not recruited to *her-1* or the X chromosome. Full expression of *her-1* follows, which promotes male development. Full expression of X-linked genes occurs from the single male X chromosome. **c** | As well as the dosage-compensation complex that regulates gene expression, *C. elegans* contains a second condensin complex with conserved functions in mitotic chromosome structure and segregation. MIX-1 (SMC2) is found in both complexes. Although it interacts with the SMC4 homologue DPY-27 in the dosage-compensation complex, it interacts with a different SMC4 homologue, called SMC-4, in the mitotic complex. Other members of the mitotic complex are not yet known.

GENE CONVERSION
A specific type of recombination, which results in non-reciprocal genetic exchange, in which the sequence of one DNA strand is used to alter the sequence of the other.

be interesting to learn how the dosage-compensation complex represses gene activity, and why the level of this repression differs so greatly between the X chromosome and the *her-1* locus.

Gene regulation in subchromosomal domains. Chromosomes are thought to be organized into domains — units of chromosome packaging and coordinated gene regulation. In some cases, domains are bordered by special insulator elements that prevent the spread of regulatory influences or actual chromatin structure into neighbouring regions34. Insulators help restrict the activity of enhancers and silencers, which are elements that would otherwise be capable of contacting heterologous promoters over long distances.

One of the best-studied domains is the silent mating-type locus in budding yeast. *Saccharomyces cerevisiae* has two mating types, *a* and α, which are determined by whether *a*- or α-genes are expressed from the active MAT (mating type) locus (FIG. 2). Genes that encode the opposite mating type exist in two different silent domains — HML (homothallic left) and HMR (homothallic right). Mating-type switching occurs by intrachromosomal GENE CONVERSION between the expressed genes at the MAT locus and the alternative genes at one of two silent loci. These loci are repressed by the E and I silencers, which are essential and important DNA sequences that recruit silencing proteins to modify histones and organize a domain of repressive chromatin structure (FIG. 2). The silent domain is bordered by two insulator elements that prevent the spread of repressive chromatin into the neighbouring regions[35]. Reporter genes that are placed within the confines of these insulators are silenced.

An unexpected link between cohesin and the ability to establish silencing over the mating-type domain has recently been uncovered. Previous evidence had indicated that the establishment of silencing requires passage through the S phase of the cell cycle, but not DNA replication itself [36,37]. A new study now shows that passage through mitosis is also required to establish silencing, and indicates that the cleavage of cohesin (or at least its Scc1/Mcd1 subunit) is necessary to establish this silencing[38]. Silencing proteins were found to localize to the mating-type locus at G2/M, yet despite their presence robust silencing was not established until cells progressed further through the cell cycle. In the absence of Scc1/Mcd1, however, the silencing proteins present at the locus during G2/M could fully repress transcription. Conversely, expression of an uncleavable mutant form of Scc1/Mcd1 prevented silencing[38]. So, it seems that Scc1/Mcd1 cleavage at the metaphase-to-anaphase transition is necessary to establish transcriptional silencing over the yeast mating-type domain. Also, chromatin immunoprecipitation experiments have localized Scc1/Mcd1 to the mating-type locus and to other silent heterochromatic domains in both *S. cerevisiae* and *Schizosaccharomyces pombe*, such as telomeres, centromeres and ribosomal DNA (rDNA) loci[39–41]. It is not yet clear whether Scc1/Mcd1 affects the establishment of silencing at these other loci.

Cohesin might also contribute to gene regulation by influencing the ability of insulators to separate regions of active and inactive chromatin. Chromatin immunoprecipitation experiments showed that sites of Scc1/Mcd1 association along an *S. cerevisiae* chromosome are mainly AT-rich intergenic regions with a periodicity of ~13 kb, similar to the proposed size of chromatin domains[42]. The idea that cohesin localization might correspond to domain boundaries prompted a subsequent study that showed the localization of Scc1/Mcd1 to several regions that border repressed and active chromatin[39]. These regions included the borders between copies of the repeated rDNA genes, the HMR insulators but not the intervening silent domain, and the boundary between active chromatin and a silent subtelomeric domain. These results are interesting in light of genetic evidence that certain mutant alleles of *SMC1* and *SMC3* disrupt activity of the HMR insulators and allow repressive chromatin to spread outside the domain[43] (FIG. 2). It will be important to examine whether cohesin is crucial for insulator function at the boundaries of other chromosomal domains.

A connection between condensin and silencing at the *S. cerevisiae* mating-type locus has also been reported. This finding relies on the fact that inappropriate derepression of a silent locus allows cells to express both *a*- and α-genes, and the cells fail to arrest or form projections in response to mating pheromone. Using this assay for desilencing, it was shown that mutations in *YCS4*, which encodes a CAP-D2 homologue, prevented arrest in response to α-factor[44]. An allele of *SMC4*, but not of *SMC2*, also had this effect. These results imply that condensin subunits help repress the silent mating-type locus (FIG. 2). However, derepression of a reporter gene in the silent locus was not observed, perhaps because only weak alleles could be used. The finding that

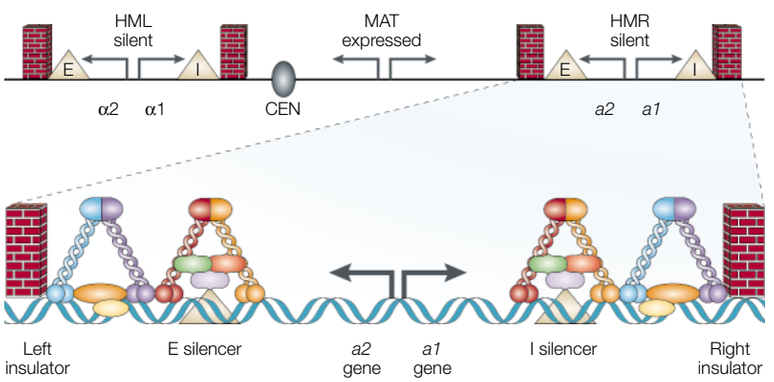


Figure 2 | **Cohesin and condensin might influence silencing and insulation at the yeast mating-type locus.** *Saccharomyces cerevisiae* exists as an *a*- or α-mating type, and switches mating type by intrachromosomal gene conversion between the active MAT (mating type) locus and one of two silent loci — HML (homothallic left) and HMR (homothallic right). The silent loci each contain two genes (arrows), surrounded by two DNA-silencer elements that keep them inactive (E and I, yellow triangles) and bracketed by insulator elements (brick walls). Cohesin (blue complex) seems to prevent the establishment of silencing at HMR, whereas condensin subunits (red complex) might be required for silencing. Cohesin also localizes to the insulators and might assist them in preventing the spread of silencing into neighbouring regions. Although cohesin localization to the locus has been shown by chromatin immunoprecipitation, it is not known whether condensin is associated with HMR.

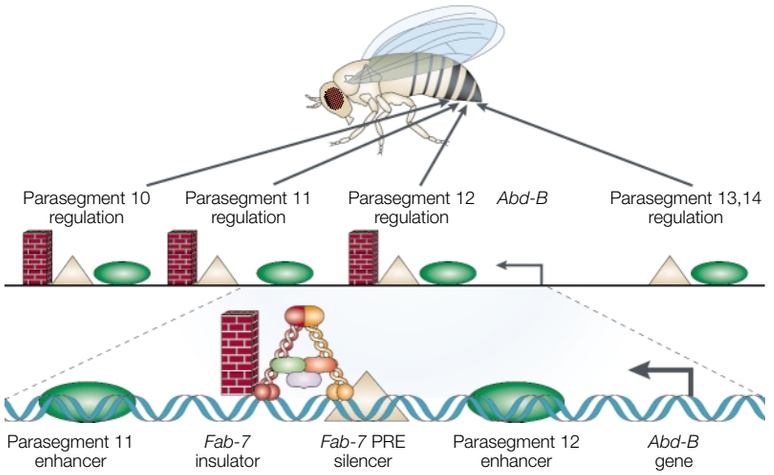


Figure 3 | **Condensin might influence silencing or insulation at elements that regulate fly body-segment identity.** Segment-specific regulatory domains control *Abdominal-B* (*Abd-B*) expression (arrow) to determine body-segment identity in *Drosophila*. Each domain contains enhancers (green ovals) and silencers (yellow triangles) and is bordered by insulator elements (brick walls). The *Fab-7* insulator prevents cross-regulation between the PARASEGMENT 11 and 12 domains. An adjacent silencer, the *Fab-7* Polycomb response element (PRE), binds Polycomb-silencing proteins to maintain the inactivity of the parasegment 12 domain in appropriate segments. A condensin subunit was shown to localize to many PRE silencers by chromatin immunoprecipitation. Mutations in this subunit enhance the segment-transformation phenotype that is caused by deleting the *Fab-7* insulator and PRE, which suggests a potential role in insulation or silencing.

some, but not all, condensin subunit mutations show desilencing raises the possibility that condensin subunits associate with a complex other than condensin to mediate this process.

Maintaining repression of a regulatory region. Condensin studies in *Drosophila* began with a genetic screen for mutations that affected the development of the peripheral nervous system. The *barren* (*barr*) gene, which was later recognized to encode a homologue of the condensin subunit CAP-H, was identified by a mutation that causes embryonic lethality and defects in neuronal cell division[23]. Although *barr* phenotypes are most easily recognized in the nervous system, *barr* mutations affect general embryonic cell division and result in aberrant anaphase chromosome segregation[23]. Mutations in *barr* have been used to determine whether condensin has a role in regulating gene expression through subchromosomal domains.

barr influences a well-studied example of domain organization and gene regulation, the *Drosophila Abdominal-B* (*Abd-B*) gene. *Abd-B* specifies body-segment identity in the posterior of the fly, and is regulated by a series of segment-specific regulatory domains. Each domain is a collection of enhancers and silencers that are surrounded by insulators (FIG. 3). Transient embryonic-patterning proteins establish whether each domain is active or inactive in a particular body segment, then a separate set of proteins maintains this activity state. Domain inactivity is maintained by Polycomb-group proteins — factors that alter chromatin structure to provide a 'cellular memory' of repression[45]. Polycomb-group proteins act through specific DNA regulatory elements in each domain, called Polycomb response elements (PREs). For example, the regulatory domains that influence *Abd-B* expression in parasegments 11 and 12 both include a PRE silencer and are separated by an insulator called *Fab-7* (FIG. 3). In mutant flies that carry a *Fab-7* deletion, these segments change their identity because regulatory influences can inappropriately spread between the domains[46–49].

The suggestion has been made that condensin helps Polycomb-group proteins maintain gene repression over the *Abd-B* gene regulatory domains[50]. Mutations in *barr* behave, in some respects, similarly to mutations in Polycomb-group genes. Both relieve PRE-induced silencing of a reporter transgene and enhance the phenotype of a *Fab-7* insulator deletion[50]. Likewise, mutations in some Polycomb-group genes act similarly to *barr* mutations, causing incomplete chromosome segregation at anaphase. Moreover, Barren and Polycomb-group proteins co-localize at PRE-silencer elements, according to chromatin immunoprecipitation studies[50] (FIG. 3). These findings raise the possibility that Barren might work with Polycomb proteins to maintain gene inactivation by altering the chromatin structure of *Abd-B* regulatory domains. This possibility is intriguing, but further tests are necessary. First, it is important to distinguish whether condensin has a role at PREs, insulator elements or both, as both classes of elements are often located in close proximity and the genetic effect on the *Fab-7* deletion does not differentiate between effects on either element. Second, other available tests for Polycomb-group function, such as the ability of mutants to derepress *Abd-B* reporter genes or to enhance Polycomb-group mutant phenotypes, should be applied. Third, other condensin subunit mutations should be tested to determine whether this activity reflects condensin function or a specific role for Barren.

Regulating enhancer–promoter communication. It has been proposed that the mechanism used by cohesin to attach two different sister chromatids *in trans* might also be used *in cis* on the same sister chromatid to help enhancers contact promoters over long distances[51]. This idea stems from the identification of a cohesin regulatory-factor homologue in a genetic screen for mutations that affect the ability of an enhancer to overcome an insulator.

A *Drosophila* transposon called *gypsy* functions as an insulator to block enhancer–promoter communication, and *gypsy* insertions cause loss-of-function phenotypes when located between an enhancer and a promoter[34]. For example, at *cut*, which is a gene that is important for wing development, the insertion of *gypsy* between a wing enhancer and the *cut* promoter partially blocks their interaction (FIG. 4). The resulting failure to properly activate *cut* in wing-margin cells produces a 'cut wing' phenotype. A genetic screen for mutations that exacerbate the *gypsy*-induced cut-wing phenotype identified *Nipped-B*, which is a gene with similarity to the cohesin regulatory factor Scc2 (REF. 51). It was, therefore, suggested that *Nipped-B*

PARASEGMENT
In *Drosophila* development, body-patterning genes are expressed in parasegmental units that are out of register with morphologically visible segments. A parasegment contains the posterior portion of one segment and the anterior section of the next segment.

TOPOISOMERASE II
An ATP-dependent enzyme that creates transient breaks in both strands of the DNA sugar-phosphate backbone, then passes one strand through the other and reseals the break. Such enzymes can remove or create supercoils in duplex DNA.

SYNTHETIC LETHALITY
A phenomenon that refers to lethality that is caused by the combination of two mutations, neither of which causes lethality by itself. Synthetic lethality of double mutants can indicate that two genes might function in related processes.

DNA LIGASE III
Ligases are enzymes that seal nicks in one strand of double-stranded DNA by creating an ester bond between adjacent 3′OH and 5′PO_4 ends on the same strand. DNA ligase III acts in DNA repair to heal single-stranded DNA breaks.

DNA POLYMERASE-ε
Polymerases are enzymes that synthesize new DNA strands using a DNA template. DNA polymerase-ε acts in DNA repair when nucleotides have been excised, and also associates with the replication fork as a lagging-strand polymerase.

normally facilitates long-range enhancer–promoter communication, and when mutated, reduces the ability of the enhancer to overcome the *gypsy* insulator[51] (FIG. 4). In support of this idea, *Nipped-B* mutations also enhance the phenotype caused by a *gypsy* insertion between a distant enhancer and promoter at another locus. Also, *Nipped-B* mutations cause a weak cut-wing phenotype even in the absence of *gypsy*, which confirms a normal role in gene regulation at the *cut* locus. To determine whether the effect of *Nipped-B* on gene regulation results from a disruption of cohesin activity, mutations in cohesin subunits should be analysed. The speculation that similar factors adhere sister chromatids, and adhere enhancers to their promoters along a single sister chromatid, is an attractive idea that awaits further testing.

Together, these examples illustrate how genetic screens that had been designed to understand developmental processes as varied as X-chromosome dosage compensation, nerve-cell formation and wing patterning, have exposed gene regulatory functions for condensin and cohesin subunits. Once identified, these mutations allowed the continued exploration of further gene regulatory functions.

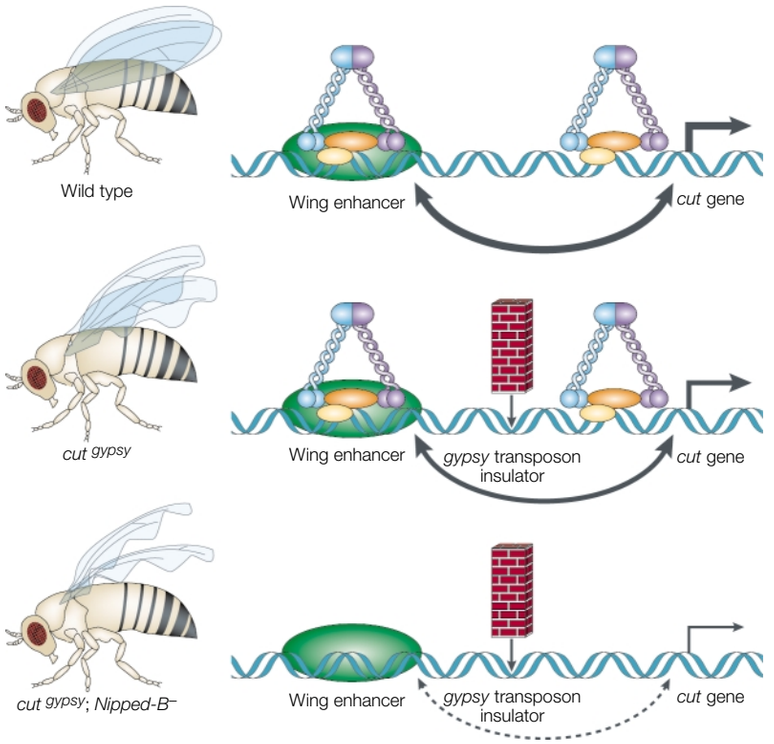


Figure 4 | **A cohesin regulatory protein might affect enhancer–promoter communication in *Drosophila*.** A wing enhancer (green oval) regulates the *Drosophila cut* gene promoter (straight arrow) from a distance of 85 kb, to promote normal wing development (top). A transposon called *gypsy* contains an insulator element (brick wall) and partially blocks this enhancer–promoter interaction when inserted between them, causing a 'cut wing' phenotype (middle). This *gypsy*-induced wing defect is even more severe when *Nipped-B*, a gene encoding a homologue of the cohesin regulatory protein Scc2, is mutated (bottom). This finding supports the model that *Nipped-B* (and perhaps cohesin, blue complexes) normally facilitates long-range enhancer–promoter communication (curved arrows). Cohesin localization to this locus has not been proven, but is shown to illustrate the model.

Recombination and repair

As well as cohesin and condensin, eukaryotes contain a third SMC complex that specializes in DNA repair. Recent evidence indicates that all three SMC complexes might help cells cope with DNA damage. Cohesin and condensin subunits have been implicated both in the actual repair of DNA damage and in a DNA-damage-checkpoint pathway that senses damage and delays replication until the damage is fixed.

The multiprotein SMC complex that specializes in the repair of DNA damage includes an SMC5–SMC6 heterodimer[52–54]. Long before it was recognized to encode the SMC6 protein, *rad18* was identified in *S. pombe* in a screen for radiation-sensitive mutations[55]. This repair function of Rad18 was later shown to be distinct from the standard nucleotide-excision repair pathway, and analysis of *rad18* mutants showed further roles in mitotic growth and the DNA-damage-checkpoint response[52,56]. Also, mutations that affect Rad18 and TOPOISOMERASE II show SYNTHETIC LETHALITY, which indicates that this SMC complex might influence chromosome organization[56].

The first indication that cohesin subunits were involved in DNA repair was the discovery that mammalian SMC1 and SMC3 are part of a biochemically purified recombinational repair complex, RC-1 (REF. 57). Unlike mitotic cohesin, this complex does not contain SCC1 and SCC3, but instead contains DNA LIGASE III and DNA POLYMERASE-ε, and promotes the repair of DNA gaps and deletions. Genetic studies also connected cohesin subunits to repair. *SCC1/MCD1* was first identified in *S. pombe* as *rad21*, a gene that is involved in double-strand break repair[58]. This function is conserved, as deletion of the chicken *SCC1* or mutation of *S. cerevisiae SCC1/MCD1* or *SMC1* hinders double-strand break repair and reduces sister-chromatid exchange[59,60]. Mutations in *S. cerevisiae SMC1*, *SMC3*, *SCC3* and *PDS5* are hypersensitive to damage by γ-irradiation, and cohesin must be present in S phase for efficient repair of double-strand breaks[60]. It is likely that cohesin facilitates repair by promoting cohesion between sisters, so ensuring that the correct sequence is used as a donor template for repair[3].

As well as DNA repair, the cohesin subunit Smc1 participates in a signal-transduction pathway that elicits a checkpoint response to DNA damage. The Atm kinase, which is mutant in the human disorder ataxia-telangiectasia, is activated in response to irradiation and phosphorylates important cell-cycle targets. In response to radiation, two serines of Smc1 are phosphorylated by Atm, and this modification is essential for the S-phase DNA-damage checkpoint[61,62] (FIG. 5). Biochemical studies indicate that both Smc1 and Smc3 might associate in a complex that contains DNA-damage-response proteins such as Atm, Nbs1, the Bloom syndrome factor Blm and the breast cancer tumour suppressor Brca1 (REF. 61) (FIG. 5). The mechanism by which phosphorylated Smc1 helps prevent DNA synthesis in response to damage is unknown.

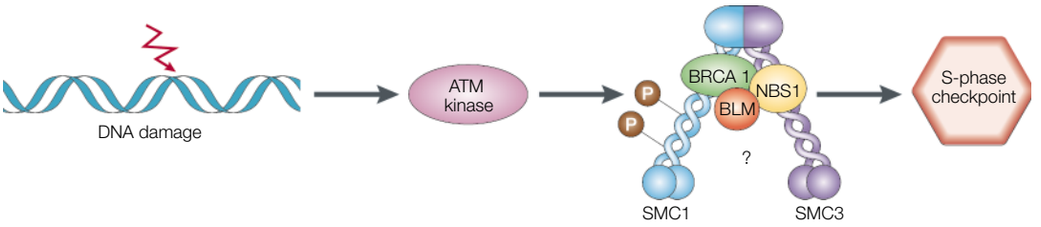


Figure 5 | **Cohesin subunits participate in a DNA-damage-checkpoint response pathway.** DNA damage produced by irradiation (red arrow) activates the ATM kinase, which phosphorylates (P) the cohesin subunit SMC1. SMC1 is found in a complex distinct from cohesin, and some evidence indicates that this complex might also contain SMC3 and the DNA-damage-response proteins NBS1, BLM and BRCA1. Phosphorylation of SMC1 is essential for eliciting the S-phase DNA-damage checkpoint that will delay DNA replication until damage is repaired. Although not illustrated here, a condensin subunit has also been implicated in the ATM-checkpoint pathway.

A condensin subunit is also required for DNA repair and for the DNA-damage checkpoint. In *S. pombe*, mutations in the gene that encodes the non-SMC protein Cnd2 (CAP-H), but not in genes that encode the SMC proteins Cut14 (SMC2) and Cut3 (SMC4), result in hypersensitivity to DNA-damaging agents[63]. Mutations in *cnd2* cause accumulation of damage such as THYMINE DIMERS, and in combination with mutations in excision-repair genes they cause increased sensitivity to irradiation. Although only Cnd2 seems to be required for repair, all subunits of the condensin complex seem to influence the DNA-damage checkpoint. Indicative of this role, mutation of any gene that encodes a condensin subunit fails to activate the DNA-damage-checkpoint kinase Cds1. Also, *cnd2* mutations cause a cell-cycle delay that is relieved when combined with mutations in the checkpoint regulators *crb2* (*BRCA1*) and the *rad3* (*ATM*) kinase[63]. So, condensin, as well as cohesin, might have a role during interphase as part of the ATM-checkpoint pathway.

The mechanisms by which these complexes or their subunits promote repair and checkpoint function are not yet clear. Several models have been suggested[3]. One possibility is that cohesin and condensin organize chromosome structure, even during interphase, in a way that facilitates recombinational repair. Alternatively, certain subunits might interact with DNA recombination and repair proteins to form distinct complexes. These studies again illustrate that careful analysis of cohesin and condensin subunit mutations has the potential to reveal new roles for cohesin and condensin.

Cohesin and condensin at the centromere

Cohesin at the centromere. The centromere is where cohesin performs its best-described role: adhering sister chromatids until anaphase. The centromere is a chromosomal region with a distinct chromatin structure that contains modified histones and a unique histone H3 variant called CENP-A. A proteinaceous structure called the kinetochore assembles onto this site and mediates microtubule attachment. As well as forming the microtubule-attachment site, the centromere is also an important site of sister-chromatid cohesion. New studies of cohesin mutants indicate that the cohesin complex is not simply a structural bridge that links sister centromeres. Instead, cohesin might have a broader impact on centromere function during mitosis, which influences the orientation of centromeres towards the poles and their attachment to spindle microtubules.

Interplay of cohesin and centromere proteins. Centromere chromatin proteins help recruit cohesin to the centromere. Chromatin immunoprecipitation experiments in budding yeast indicate that Cse4(CENP-A) is required to localize cohesin components to the centromere[64]. When CENP-A is directed to ectopic sites along human chromosome arms by overexpression, it recruits the cohesin subunit SMC1 as well as kinetochore proteins[65]. At the centromere in *S. pombe*, histone H3 is methylated at lysine 9 (REF. 66). This modification is required to recruit the heterochromatin protein Swi6(HP1), which in turn is required for cohesin association with centromeres, but not arms[40,41] (FIG. 6a). However, cohesin does not seem to be required to localize centromeric chromatin proteins to the centromere. Cohesin mutations, for example, do not disrupt Swi6 (HP1) binding[40,41] or Cse4 (CENP-A) binding[67].

Cohesin also requires kinetochore proteins for its proper localization. The budding yeast kinetochore protein Ndc80/Hec1 interacts with Smc1 in a two-hybrid assay and its overexpression suppresses the lethality of mutations in *SMC1* (REF. 68). Chromatin immunoprecipitation experiments also indicate that the budding yeast kinetochore proteins Ndc10 and Mif2 (CENP-C) are required to localize cohesin components to the centromere[64]. So, centromere and kinetochore proteins are instrumental in recruiting cohesin to this chromosomal location.

Sister chromatid bi-orientation and tension. Accurate chromosome segregation requires chromosome 'bi-orientation' in which each kinetochore on a pair of attached sister chromatids faces outwards and presents a microtubule-binding face towards only one spindle pole. This arrangement ensures that a pair of kinetochores attaches to two different poles (bi-polar attachment) rather than to the same pole (mono-polar attachment) (FIG. 6b). Proper segregation also requires tension between kinetochores as the microtubule spindle aligns the chromosomes. In the absence of proper attachment and tension, the spindle

THYMINE DIMERS
A pair of abnormal covalently bonded adjacent thymine residues in DNA that are caused by DNA damage.

SPINDLE MIDZONE
A region of overlapping microtubules at the centre of the spindle-microtubule apparatus. The midzone forms when chromosomes segregate, and is required for proper spindle bi-polarity and elongation, chromosome movement and cytokinesis.

checkpoint engages and delays anaphase until the problem is corrected.

Cohesin is crucial for bi-orientation. In both yeast and vertebrate *SCC1* mutants, monopolar spindle attachment is observed[59,69]. Bi-orientation in budding yeast has been visualized by the transient separation and reassociation of green fluorescent protein (GFP)-marked centromeres. A mutation in the cohesin subunit gene *SCC1* disrupts this transient centromere splitting[69,70]. One possibility is that cohesin not only glues sister chromatids together, but might also impose a 'back-to-back' structural organization so that kinetochores are more likely to be captured by microtubules from opposite poles.

Cohesin might also promote bi-orientation and tension by assisting the mitotic kinase Aurora B. This kinase, along with INCENP (inner centromere protein) and BIR1(Survivin), forms a complex that is essential for chromosome segregation. These proteins are known as 'chromosome passengers' because they 'ride' metaphase chromosomes until anaphase, when they redistribute to the SPINDLE MIDZONE. Mutations in budding yeast *IPL1*(Aurora B) were shown to cause mono-polar attachment that occurs preferentially with the spindle pole inherited from the previous division. The same preference was observed for mutations in *SCC1* or in genes that encode the Dam/Duo kinetochore protein complex[71,72]. It was, therefore, proposed that sister chromatids initially associate with microtubules from the old spindle-pole body, and in the absence of Ipl1 cannot break this association to allow connection to the other spindle pole. According to this model, Ipl1 is part of the mechanism to correct mono-polar attachment by destabilizing microtubule-kinetochore interactions until chromatids re-orientate, form bi-polar attachments and establish tension[72]. Ipl1 is also required for spindle-checkpoint activation by sister chromatids that are not under tension[73]. Ipl1 is, therefore, proposed to be a 'tensiometer' that promotes bi-orientation by monitoring tension at the kinetochore.

Cohesin could be important for the tension-sensing and attachment-correcting properties of Ipl1 in several ways that are not mutually exclusive. As cohesin adheres sister chromatids it might orientate them so that the kinetochores are more likely to be captured by microtubules from opposite poles. Alternatively, the tension that cohesin generates by gluing sister chromatids together might stabilize kinetochore–microtubule interactions and be sensed by Ipl1. Finally, cohesin might simply be required to localize the passenger complex that contains Ipl1. Mutations in *S. pombe rad21* (*SCC1*), deletion of chicken *SCC1* and depletion of *Drosophila* Rad21(Scc1) all disrupt the localization of passenger proteins[59,74,75].

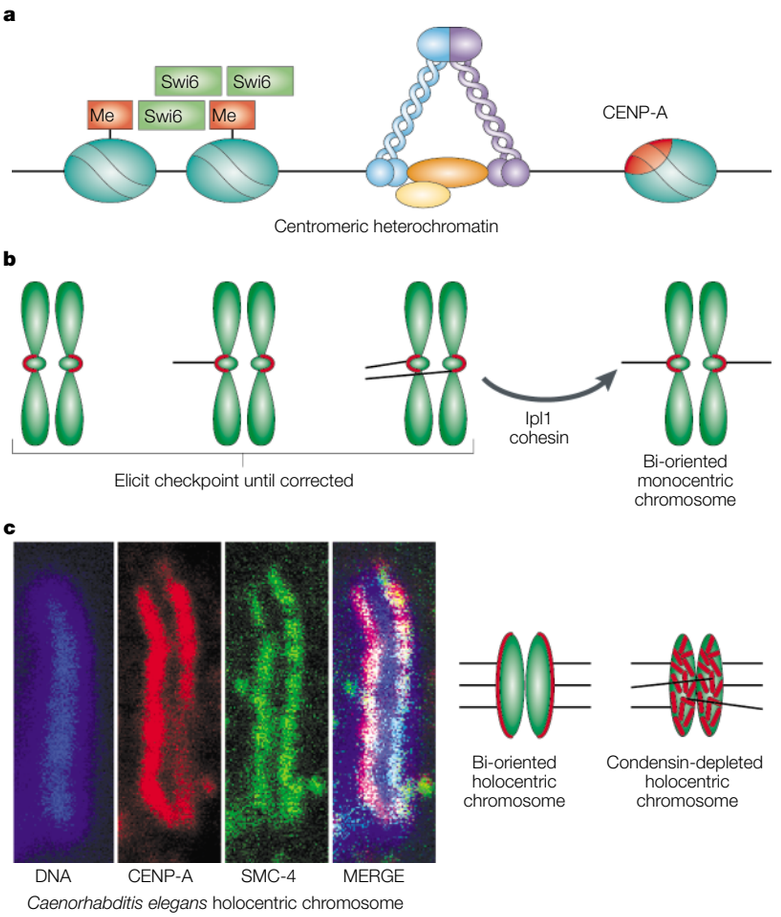


Figure 6 | **Cohesin and condensin at the centromere. a** | Centromere proteins recruit cohesin. Chromatin at the centromere in *Schizosaccharomyces pombe* contains nucleosomes (turquoise ovals) and histone H3, which is methylated (Me) on lysine 9 (red flag). This modification, which is characteristic of heterochromatin, recruits the heterochromatin protein Swi6 (HP1) (green box) to this site. Swi6 (HP1), in turn, is required to recruit cohesin (blue complex) to centromeric heterochromatin, where it performs important functions. In many organisms, the histone H3 variant CENP-A (red), which is found in centromeric nucleosomes, is also required to recruit cohesin. **b** | Cohesin is important for bi-polar spindle attachment. At mitosis, paired sister chromatids (green) assemble kinetochores (red) onto their centromeres (green circle) to mediate attachment to microtubules (lines). A spindle-checkpoint mechanism delays sister-chromatid separation if chromosomes are improperly attached to microtubules. Cohesin is required for bi-polar microtubule attachment, and mutations in cohesin subunits trigger the spindle checkpoint. A budding yeast kinase, Ipl1, is required for cohesin mutants to trigger the checkpoint. Ipl1 has been proposed to correct mono-polar attachment by destabilizing inappropriate microtubule–kinetochore interactions, or to sense tension between properly attached sister chromatids. **c** | Condensin might organize centromere structure. *Caenorhabditis elegans* condensin (SMC-4, green) co-localizes with centromeric proteins (CENP-A, red) on mitotic chromosomes (DNA, blue). *C. elegans* chromosomes (drawn in green) are holocentric, meaning that the centromere and kinetochore (red) extend along the length of the chromosome, rather than forming a single site as for the monocentric chromosomes drawn in part **b**. Depletion of condensin subunits disrupts centromere organization (right), and can result in lagging chromosomes at anaphase.

Cohesin and the spindle checkpoint. Mutations in cohesin subunits trigger the spindle checkpoint[67]. This checkpoint monitors proper kinetochore–spindle attachment, delaying anaphase if sister chromatids are unattached, attached only at one kinetochore, or both attached to the same pole (FIG. 6b). How proper attachment is signalled is not clear, but the checkpoint is thought to detect a lack of tension at the kinetochore or unattached kinetochores[76]. The finding that cohesin subunit mutations elicit this checkpoint response extends the evidence that cohesin influences bi-orientation and/or tension. Mutations in *S. pombe mis4* (*SCC2*) and *rad21*(*SCC1*) genes cause mitotic delay and hypersensitivity to spindle poisons such as

thiabendazole. This mitotic delay seems to result from engaging the spindle checkpoint, as the combination of these mutations and checkpoint-gene mutations prevents delay[67]. It will be important to determine the generality of these findings. Although it has been suggested that depletion of cohesin from human cells impairs *in vitro* SPINDLE-ASTER assembly[77], the effect of cohesin depletion on spindle assembly has not yet been addressed in the well-characterized *Xenopus* egg-extract system. In budding yeast, mutations in genes that encode the cohesin loading factor Ctf7/Eco1 (REF. 78) or the replication protein Ctf18 (REF. 79) disrupt cohesion and trigger the spindle checkpoint. However, the exact relationship among the cohesin complex, the localization of passenger proteins and the spindle checkpoint has not yet been determined.

Condensin and the centromere. Recent evidence from *C. elegans* indicates that condensin might influence centromere organization. Most model organisms have monocentric chromosomes, that is, the centromere is a single discrete region (FIG. 6b). *C. elegans*, however, has holocentric chromosomes — the centromere extends along the length of the chromosome (FIG. 6c). *C. elegans* condensin components co-localize with centromeric proteins, such as CENP-A, along the chromosome length[22] (FIG. 6c). Mutation or depletion of a condensin subunit disrupts centromere bi-orientation, and centromere proteins fail to show their normal restricted orientation towards the spindle poles[22]. Moreover, mutation of the *C. elegans* CAP-D2 homologue causes lagging anaphase chromosomes that seem to result from attachment of each sister chromatid to both poles[80]. So, the organization imposed by condensin might help build and orientate the centromere on these holocentric chromosomes.

Whether condensin organizes centromeres on monocentric chromosomes is unknown, but circumstantial evidence indicates that it might. Chromatin immunoprecipitation experiments in *S. pombe* have shown that condensin localizes to the centromere[63]. In *S. cerevisiae*, chromosomes in *brn1* (*CAP-H*) mutants are displaced from the spindle, perhaps owing to defective kinetochore–spindle attachment[19,20]. In *Xenopus* egg extracts, immunodepletion of condensin causes disorganized localization of a kinetochore protein, which indicates a role in kinetochore morphology[81].

Perspectives and future directions

Genetic analysis of cohesin and condensin has provided a better understanding of how chromosomes adhere, compact, resolve and separate during cell division. Concerted investigation of cohesin and condensin mutants, using an expanded set of functional assays, has also shown new roles for these proteins in a wide range of chromosomal processes throughout the cell cycle, including gene silencing and insulator function, DNA-damage sensing and repair, and centromere orientation and function. Also, genetic screens that were designed to understand processes as diverse as X-gene regulation, enhancer–promoter communication, nerve-cell proliferation and DNA repair have unexpectedly identified cohesin and condensin mutations.

One concept that emerges from these studies is that individual subunits of cohesin and condensin might associate with different sets of proteins to perform different functions. For example, SMC1 and SMC3 associate with distinct sets of proteins in the cohesin complex, the RC-1 recombination repair complex and the ATM DNA-damage-response complex. Similarly in *C. elegans,* MIX-1(SMC2) is a component of both mitotic condensin and a specialized condensin-like complex that is central to X-chromosome gene regulation. Several protein partners and roles should be kept in mind as the functions of cohesin and condensin subunits are investigated. Mutant phenotypes could represent the action of a single complex in more than one process, or the participation of a single subunit in different complexes with different functions. Although chromosome segregation is probably the ancestral role of these complexes, certain subunits might have been recruited during evolution for more specialized functions such as coordinating the cell cycle or fine-tuning gene expression.

It remains to be seen whether these seemingly unrelated activities primarily result from the influence that condensin and cohesin exert on chromosome structure. Cohesin and condensin subunits impact several loci that are organized into silent chromatin domains, such as the centromere, the rDNA repeats, the yeast silent mating-type loci and the fly *Abd-B* regulatory elements. Even on interphase chromosomes, cohesin and condensin might define domains of chromatin folding that impact processes such as gene regulation. In one model, cohesin forms the boundaries of chromatin-loop domains that are formed by condensin[82]. This model stems from the periodicity of cohesin localization sites and the requirement for cohesin in re-establishing condensation after temperature-sensitive condensin mutants are returned to a permissive temperature[82]. Further studies will be necessary to understand whether cohesin and condensin influence these domains by directing higher levels of chromosome structure.

The finding that cohesin and condensin participate in a growing assortment of chromosome processes provides the perspective that new roles for these proteins will probably be discovered. Even before such new roles might be detected, evaluating the evolutionary conservation of the known non-canonical functions will help elucidate the biological context and possible mechanisms of these roles. Moreover, in cases in which a single subunit is known to function outside of the classical condensin and cohesin activities, the question remains as to whether the observed phenotype reflects the role of a subunit in these defined complexes or in another complex. Many fascinating questions remain to be answered; studying these two crucial protein complexes should continue to provide important insights into chromosome biology.

SPINDLE ASTER
A star-shaped cluster of microtubules that emanate towards the cell cortex from the microtubule-organizing centres at the poles of the spindle. Astral microtubules help position the mitotic apparatus.

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Online links

DATABASES
The following terms in this article are linked online to:
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cnd2 | *cut3* | *cut14* | *mis4* | *rad18* | *rad21*
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