Mechanisms for Chromosome and Plasmid Segregation

Santanu Kumar Ghosh, Sujata Hajra, Andrew Paek, and Makkuni Jayaram

Section of Molecular Genetics and Microbiology, University of Texas at Austin, Austin, Texas 78712-0612; email: ghoshsk@mail.utexas.edu, hajras@mail.utexas.edu, apaek@email.arizona.edu, jayaram@icmb.utexas.edu

Annu. Rev. Biochem.
2006. 75:211–41

First published online as a Review in Advance on February 22, 2006

The *Annual Review of Biochemistry* is online at biochem.annualreviews.org

doi: 10.1146/annurev.biochem.75.101304.124037

0066-4154/06/0707-0211$20.00

Key Words

genome partitioning, cohesion, condensing, *par* loci, Rep proteins, *STB*

Abstract

The fundamental problems in duplicating and transmitting genetic information posed by the geometric and topological features of DNA, combined with its large size, are qualitatively similar for prokaryotic and eukaryotic chromosomes. The evolutionary solutions to these problems reveal common themes. However, depending on differences in their organization, ploidy, and copy number, chromosomes and plasmids display distinct segregation strategies as well. In bacteria, chromosome duplication, likely mediated by a stationary replication factory, is accompanied by rapid, directed migration of the daughter duplexes with assistance from DNA-compacting and perhaps translocating proteins. The segregation of unit-copy or low-copy bacterial plasmids is also regulated spatially and temporally by their respective partitioning systems. Eukaryotic chromosomes utilize variations of a basic pairing and unpairing mechanism for faithful segregation during mitosis and meiosis. Rather surprisingly, the yeast plasmid 2-micron circle also resorts to a similar scheme for equal partitioning during mitosis.

Contents

INTRODUCTION

The faithful duplication and transmission of genetic information to daughter cells is a fundamental attribute of life. Yet, the process is fraught with spatial, topological, mechanical, and mechanistic challenges. Genomes present in multiple copies, i.e., certain bacterial plasmids, for example, can propagate stably by random segregation. They may maintain their steady-state copy number by appropriate replication control. Single-copy (or low-copy) genomes such as bacterial chromosomes and certain bacterial plasmids have to utilize active partitioning mechanisms to prevent missegregation. The task of faithful genome segregation is further magnified in bacteria and viruses with segmented genomes and in haploid eukaryotes. Diploid eukaryotes have the additional task, during mitosis, of

guarding against a daughter cell receiving a pair of sister chromatids instead of a pair of chromosome homologues. Conversely, during meiosis, they must hold chromosome sisters together, segregate homologues during the first division, and subsequently segregate sisters during the second division.

In this review, our goal is to summarize the logic of evolutionary solutions to some of the problems outlined above. We rely primarily on *Escherichia coli* and *Saccharomyces cerevisiae* (the budding yeast) as model systems to illustrate general principles and unifying themes. Occasionally, we turn to other systems as well, especially when they harbor specialized mechanisms. Supplemental movies of plasmid and chromosome segregation and protein oscillations related to bacterial cell division are available. (Follow the Supplemental Material link from the Annual Reviews home page at **http://www.annualreviews.org**.)

CHROMOSOME SEGREGATION IN BACTERIA

The once held view that the bacterial nucleoid is an amorphous entity, perhaps the "last refuge of entropy," is no longer valid (1). The emerging picture reveals the nucleoid as a well-organized moiety within which chromosome replication and partitioning are carried out with remarkable spatial and temporal regulation. Some of the essential ingredients for accomplishing this feat are (*a*) the localization of replication to one or a small number of fixed sites, (*b*) the force from replication that directs daughter chromosomes away from each other, (*c*) the condensation of replicated regions of the chromosome at defined cell positions, and (*d*) the action of localized molecular machines to insure chromosome separation and movement away from the division septum.

Orientation and Dynamics of the Bacterial Nucleoid

The bacterial nucleoid has a finite orientation, with well-defined cellular locations for the replication origin and terminus (2, 3). Under slow-growth conditions, the origin (*oriC*) and terminus (*terC*) of the circular chromosome in a newborn *E. coli* cell are located at the nucleoid borders proximal to the old and new cell poles, respectively (4). They migrate to the mid-cell region, as if in preparation for the start of bidirectional DNA replication (**Figure 1*a***) (5, 6). The duplicated *oriC*s move away from each other toward opposite poles. After completion of replication, chromosome segregation and cell division, the termini will be situated close to the new poles. Under fast-growth conditions, when the generation time is equal to or less than the replication time, the *E. coli* cell responds by increasing the frequency of *oriC* firing. Chromosome dynamics are also adjusted accordingly (**Figure 1*b***). When cells divide every 20 min, a newborn will contain four *oriC*s. Of these, the two localized near the poles continue to more or less hold their position during cell growth. The other two move toward each other as replication proceeds and reach the mid-zone in the predivision cell. At this stage, the duplicated termini occupy the one-fourth and three-fourths positions or the mid-zones of the would-be daughter cells. After division, the choreography of coordinated replication and DNA movement repeats itself.

***oriC*:** replication origin of bacterial chromosome

***terC*:** replication terminus of bacterial chromosome

The Driving Force for DNA Segregation: A Replication Factory?

What provides the motive force for the directed chromosome movement? The earlier notion that the duplicated DNA molecules are attached to the membrane and move passively as the cell elongates (7) has been largely discounted by contrary evidence. The measured rate of chromosome movement in *Bacillus subtilis*, for example, is much faster than that of cell elongation: 0.17 μm min^{-1} versus 0.011 to 0.025 μm min^{-1} (8). In principle, an abundant and powerful cellular motor such as RNA polymerase could drive DNA translocation, provided the transcription machinery is constrained within the cell.

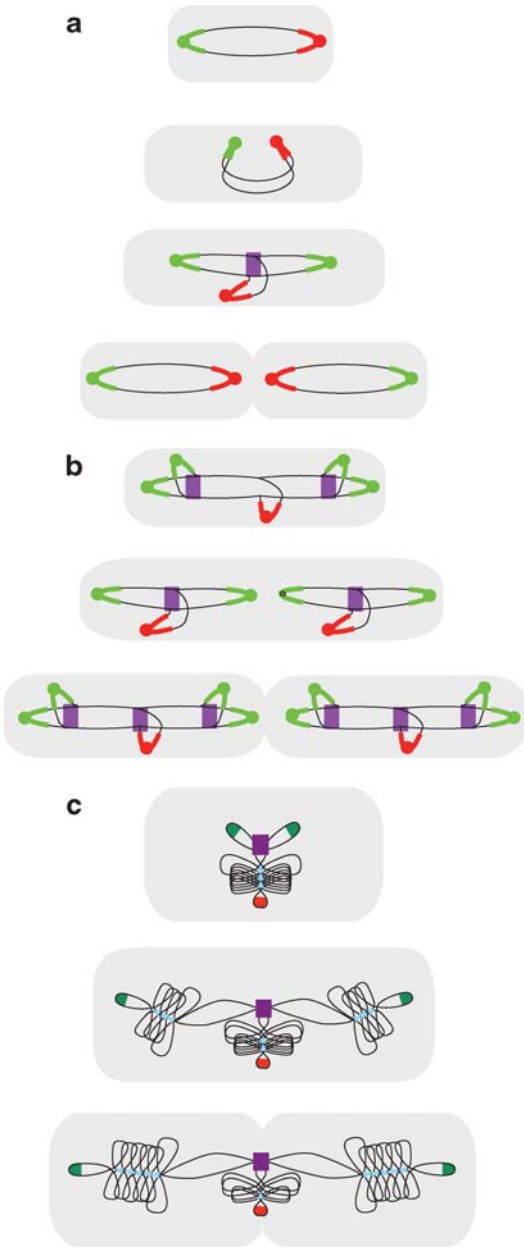

Figure 1

Dynamics of chromosome replication in *E. coli*. The movements of the replication origin (*green*) and terminus (*red*) in slow- (*a*) and fast- (*b*) growing *E. coli* cells are schematically shown. The squares (*purple*) indicate the replication factories. In the "extrusion and capture" model for chromosome segregation (*c*), the nascent duplexes emerging from the replication factory are ejected in opposite directions. The origins are captured near cell poles, and the rest of the DNA is condensed into the nucleoid. The diagram is adapted from Draper & Gober (5) with permission.

Consistent with this idea, an inhibitor of RNA polymerase prevents the separation of newly replicated duplex regions near the replication origin in *B. subtilis* (9). The biased orientation of transcription units away from the origin can impart the proper directionality to the segregation force.

Using similar reasoning, chromosome segregation may be promoted by physically constraining the replication machinery itself. A stationary replisome (also called a replication factory) located at the mid-cell position may function as a DNA pump, ingesting the parental duplex and extruding the daughter duplexes (**Figure 1*c***). In the "extrusion-capture" model (10), the spooling effect produced by the replisome pushes the newly replicated molecules away from each other to be captured and positioned by an anchoring mechanism (**Figure 1*c***). This positional information may be provided by the replication origin itself or sequences in its proximity in conjunction with a cognate protein or proteins. The pushing force generated by the replisome may be complemented and reinforced by the pulling force provided by proteins that condense DNA (discussed below). However, further assistance from a motor protein such as FtsK of *E. coli* (11, 12) is required to dispatch the nascent DNA emerging from the replisome toward the cell pole before it collapses into a random coil.

B. subtilis sporulation provides another example of active DNA transfer in a vectorial fashion. The RacA protein, which binds to short repeated sequences within a centromere-like region spanning the origin, is responsible for capturing sister chromosomes and anchoring them to opposite ends of the cell (13). During asymmetric patterning into a large mother cell and a smaller prespore cell, the sporulation septum traps only about 30% of the chromosome, spanning the origin and centromere, in the latter compartment (14, 15). The rest is pumped into the prespore by the SpoIIIE motor protein, a Maxwell's demon acting at the septum. The ATPase activity of SpoIIIE, stimulated by double-stranded

DNA, permits it to track along DNA (or to translocate DNA if the protein is stationary) (16). SpoIIIE is synthesized during vegetative phase as well and appears to enhance the fidelity of chromosome segregation by sweeping away any DNA trapped at the site of septum closure.

Cellular Addresses for Chromosomal Loci

Perhaps the most striking case of replication-coupled and ordered distribution of nascent duplexes in "future" daughter cells came from a sophisticated analysis carried out recently in *Caulobacter crescentus* by Viollier et al. (17). Among a set of 112 chromosomal loci examined by fluorescence tagging, each one had a specific subcellular address, and all were arrayed in a linear order along the long axis of the cell. Genes closest to the origin localized near the origin-proximal cell pole, those farthest from it localized to the terminus-proximal cell pole, and the rest lined up in between in a follow-the-leader fashion (**Figure 2**). Furthermore, time-lapse microscopy revealed that, as the origin and a set of 10 selected loci in the origin-proximal half of the chromosome were duplicated, the nascent DNA segments corresponding to each of them moved in a chronological order to their final destinations. Because replication was bidirectional, chromosome order in a resting cell and chromosome dynamics in a dividing cell were directly correlated to the temporal order in which each locus was duplicated. The spatial specification of loci likely reflected an intrinsic structure of the chromosome itself (1) or, perhaps, one conferred on it by an underlying subcellular foundation.

The organizational unit of bacterial chromosomes, the topological domain, is likely of the order of 10 kbp (18, 19). Given the resolution limit of the Viollier et al. (17) assays, the colinearity of loci in *Caulobacter* may be less perfect than meets the eye; deviations from linearity within domains would not have been revealed (1). Nevertheless, the almost obsessive neatness with which the *Caulobacter* cell lays down its DNA (which is a thousand times longer than itself) suggests strong evolutionary selection for this pattern of genome organization. Perhaps this arrangement is important not only for ordered replication but also for spatial and temporal control of gene expression. Let entropy find its last refuge elsewhere; certainly, not in the nucleoid!

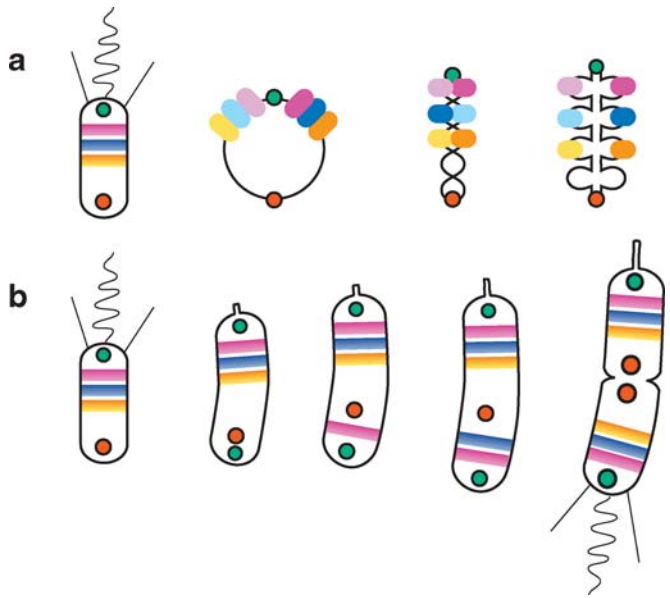

Figure 2

Ordered chromosome organization and segregation in *C. crescentus*. (*a*) The replication origin (*green*), the terminus (*red*) and the loci in between (shown in different colors) have a linear order within the *Caulobacter* nucleoid. This order could derive from a plectonemic or side-by-side configuration of the chromosome (1, 17). (*b*) The migration of the origin and the rest of the chromosome during replication also follows the same linear order.

Do Nascent Duplexes Stay Associated During Replication?

Whether sister duplexes part their ways immediately or stay together for a while during bacterial chromosome replication has been an issue of debate. The notion of cohesion came about with the observations of Sunako et al. (20) on the segregation timings of the origin, the terminus, and several loci in between,

Cohesion: the pairing of sister chromatids by the cohesin complex

Cohesin: the multiprotein complex that mediates chromosome pairing

marked by fluorescence in situ hybridization (FISH). In synchronously replicating *E. coli* chromosomes, sister copies of loci in the *oriC*-proximal half of the chromosome appear to stay together until late in replication and separate from each other at about the same time as those of loci near the terminus. The process is reminiscent of cohesion of sister chromatids followed by their separation in eukaryotes. Obviously, this segregation pattern challenges the idea of a stationary replication factory and the mechanism of DNA extrusion and capture. A subsequent experiment combining flow cytometry and fluorescence microscopy seems to indicate that little time elapses before duplicated copies of the original, marked by green fluorescent protein, separate from each other (21), thus apparently contradicting the cohesion model.

Bates & Kleckner (22) revisited the cohesion problem using a novel and highly efficient method for cell synchronization. They found that the *oriC* and *terC* sequences behave as functionally independent domains from the rest of the *E. coli* chromosome with respect to segregation. The replicated *oriC* sequences did not immediately separate. Furthermore, even after the *oriC*s split, other sister loci remained colocalized (cohesed) until a large portion of the chromosome had been replicated. They then separated in a coordinated single-step event, presumably triggered by a concerted loss of cohesion, and moved apart to form a bi-lobed nucleoid. This en masse separation led to a reorganization of *oriC* and *terC* sequences. The net result of these dynamics was to establish the *ori*-out/*ter*-in conformation that signified the twofold symmetry of chromosome segregation (**Figure 3**; also **Figure 1**).

Because *E. coli* does not possess a molecular equivalent of the eukaryotic cohesin complex to hold sisters together, the pairing is likely mediated through catenane links. Although the sister cohesion mode of segregation does not absolutely exclude a stationary replication factory, the data from Bates & Kleckner (22) argue against a long-lived factory.

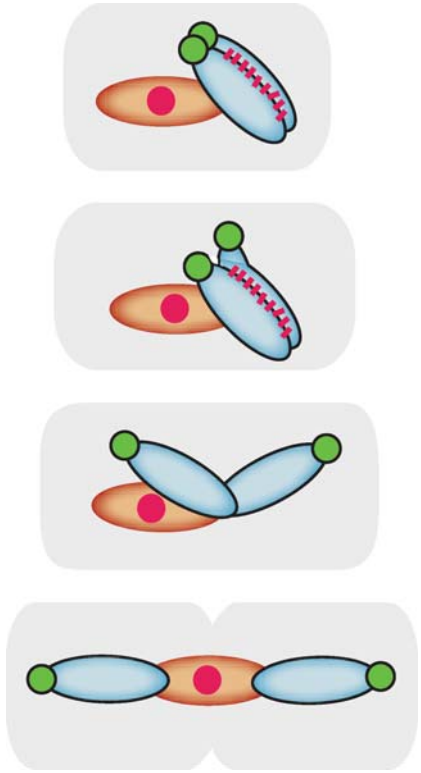

Figure 3

Cohesion between sister duplexes during chromosome replication and segregation in *E. coli*. In this highly schematized representation of the nucleoid, replication origin (*green circles*) and the terminus (*red circles*) are shown. Duplicated and unduplicated portions of the chromosome are colored blue and red, respectively. The sequential steps in chromosome segregation involve splitting of the paired origins, subsequent unpairing of sisters, and reorientation of replicated and unreplicated regions of the chromosome (22).

CHROMOSOME SEGREGATION IN EUKARYOTES

The principles that govern the mechanics of chromosome segregation in eukaryotes and prokaryotes are quite different. The primary infrastructure for moving eukaryotic chromosomes is the tubulin-based cytoskeletal apparatus, the spindle. Attachment of individual chromosomes to the spindle is mediated by microtubules connected to the kinetochore, an elaborate protein assembly organized

at the centromere (23). A multisubunit protein complex called cohesin holds replicated sister chromatids together, so that their kinetochores can be attached to the spindle in the opposite orientations during mitotic chromosome segregation (24, 25). Furthermore, a surveillance mechanism ensures that wrong connections are undone and indeed corrected (26, 27). (The spindle-kinetochore attachments are managed quite differently during meiosis.) To avoid the entanglement and self-guillotining of segregating chromosomes, catenane linkages resulting from replication are resolved by topoisomerase II (28), and each chromatid is kept away from its sister by DNA condensation promoted by the condensin complex (29). Finally, the disassembly of cohesin by proteolytic cleavage of one of its subunits causes chromosome sisters to separate and be pulled away toward opposite cell poles (30).

Remembering Replication and Counting Chromosomes

The pairing of sister chromatids by the cohesin complex serves several useful purposes during eukaryotic chromosome segregation. It provides the cell with a memory of replication events. It permits a pair of sister chromatids to be distinguished from the corresponding pair of homologue sisters. And it is an efficient mechanism for counting the products of replication in a binary fashion. The time interval between the duplication (S) and segregation (M) phases of the eukaryotic cell cycle offers the opportunity for checkpoint mechanisms to postpone the commitment to chromosome segregation until DNA damages have been repaired, replication events completed, and spindle integrity and bi-oriented spindle attachment of sister chromosomes verified (**Figure 4**). These checkpoints either block entry into mitosis by stopping S-phase progression, arresting cells in G2, or prevent the onset of anaphase by arresting them in metaphase (31–33).

The logic underlying the complex events of eukaryotic mitosis is simple, yet highly effective: Keep the sister duplexes formed by replication together and give the cell enough time to complete quality control tests before they are allowed to split asunder. A general analogy, albeit a bit contrived, is made to the solution devised by two blind shoppers who realize that the socks they purchased have all been placed in one shopping bag (34). (Of course, by a curious and convenient coincidence, the number of pairs and the different colors they bought happen to be identical, so the bag contains two pairs of a given color.) Using a pair of scissors, they cut the label holding together each pair, and distribute the individual socks into two separate bags. At the end, each man's bag would contain a full set of socks and no mismatched ones. Indeed, the pairing and unpairing strategy greatly simplifies the equal segregation challenge.

Condensin: the multiprotein complex that compacts chromosomes

Molecular Basis for Chromosome Cohesion

The cohesin complex in the budding yeast is composed of four primary subunits: Smc1p, Smc3p, Scc1p/Mcd1p, and Scc3p (**Figure 5**). In addition, a fifth protein, Pds5p, may associate with the complex after its assembly on chromosomes and is required only for the maintenance of sister chromatid cohesion (34). The Smc subunits belong to the SMC (structural maintenance of chromosomes) family of proteins that are conserved in bacteria, archaea, and eukaryotes and that play important roles in chromosome condensation, cohesion, and repair (35, 36). The functional equivalents of the non-Smc subunits of cohesin are distributed throughout eukaryotes. Mitotic and meiotic cohesins may differ in their non-Smc components. For example, the meiosis-specific counterpart of Scc1p in *S. cerevisiae* is Rec8p; the corresponding mitotic and meiotic proteins in *Schizosaccharomyces pombe* are Rad21p and Rec8p, respectively. In mammals, there exists a meiotic version of Smc1 termed Smc1-beta.

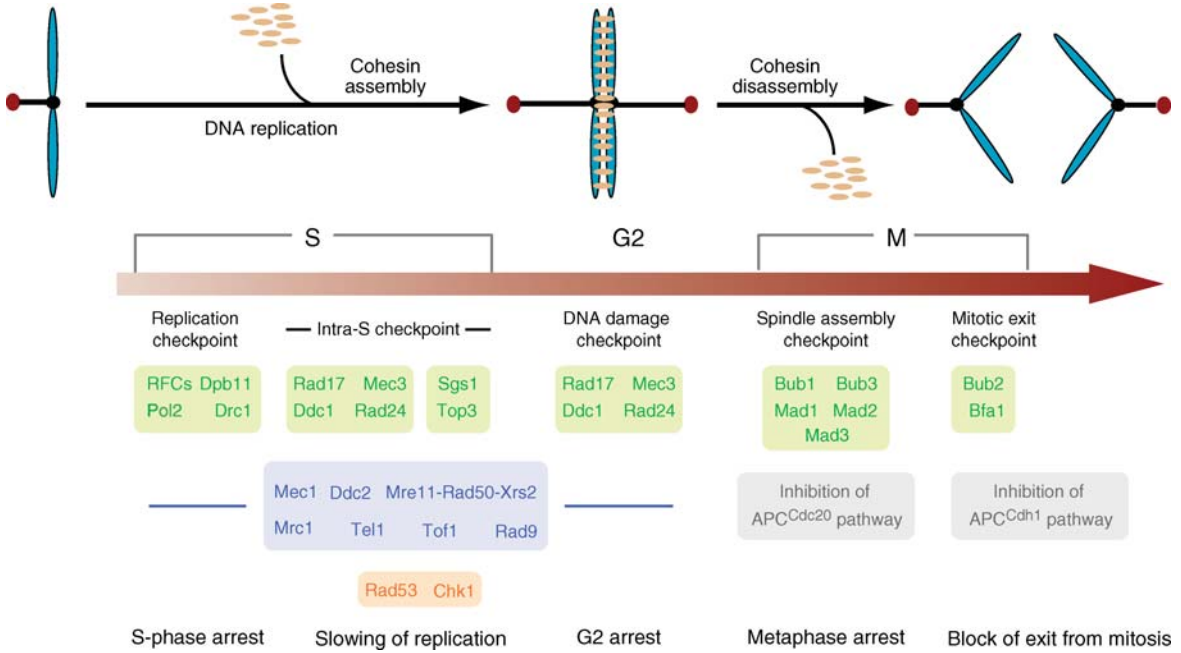

Figure 4

Cell cycle checkpoints and mitotic chromosome segregation in *S. cerevisiae*. Chromosomes duplicated during S phase are paired with their sisters by the cohesin complex and remain so until the disassembly of cohesin during anaphase. Sister kinetochores are attached to the spindle in a bipolar fashion. Following cohesin disassembly, the pulling force exerted by the microtubules dispatches the separated sisters toward opposite cell poles. Checkpoints operating during the cell cycle along with the responsible protein factors are indicated (RFC, replication factor C). The S, G2, and M checkpoints take advantage of the interval between chromosome duplication and anaphase to ensure that replication is complete, DNA damages are repaired, the spindle is functional, and sister chromatids are bi-oriented before permitting chromosome segregation to proceed.

The Smc1 and Smc3 proteins are intramolecularly folded through their long coiled-coil arms via a hinge region to form V shaped molecules (**Figure 5**), thus bringing their N- and C-terminal globular domains into juxtaposition (37). As a result, the Walker A motif from the N-terminal domain and the Walker B motif from the C-terminal domain are united to form an ATP-binding domain, which is structurally related to the nucleotide-binding domain of the ABC (ATP-binding cassette) family of proteins (38). The Scc1 and Scc3 subunits are likely associated with the globular heads and may form a closed protein ring (39). It is possible that sister chromatids are held captive topologically within this ring (24). Consistent with this topological restraining mechanism, release of sister-to-sister cohesion can be effected not only by the cleavage of Scc1 (as is the norm during the cell cycle) but also by artificial cleavage of the coiled-coil region of Smc3p (40). Although the notion of sister chromatids being held together within the embrace of the cohesin ring is quite appealing, the proof for it is not absolute.

Centromeres are strong cohesin-binding sites, with additional sites distributed at approximately 10 kb intervals along the budding yeast chromosomes (41). Yeast kinetochores serve as enhancers to promote cohesion assembly at pericentric regions that cover tens

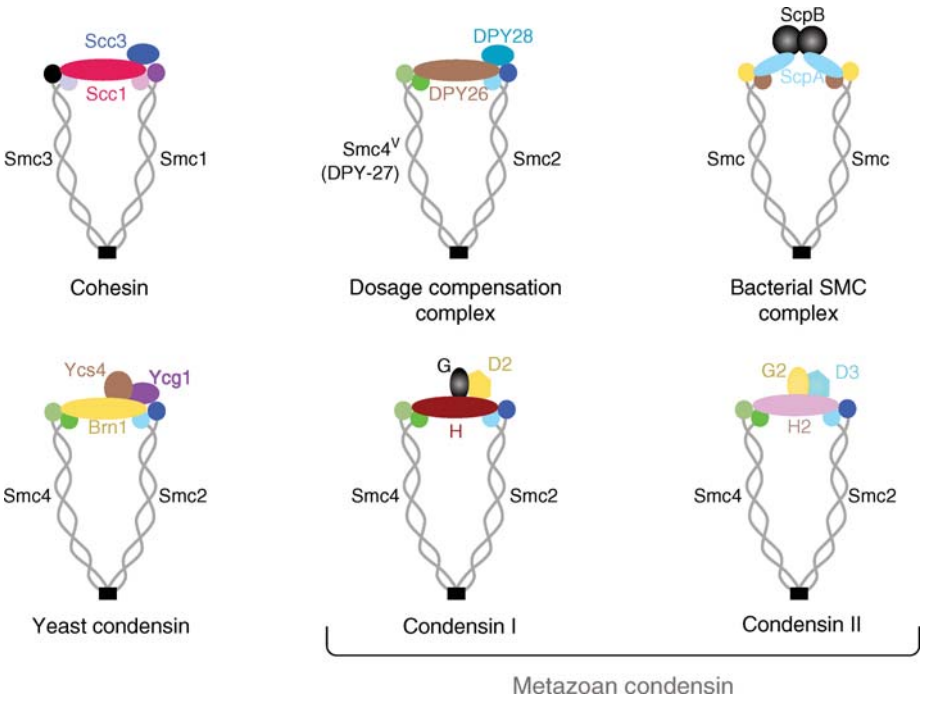

Figure 5

Architecture of the cohesin, condensin, and related complexes. The general structures of cohesin, condensin, and related complexes (*Drosophila* dosage compensation and bacterial SMC complexes) are schematically shown. Their common V-shaped backbone is provided by the hinge and coiled-coil regions of the SMC proteins, whose globular head domains associate with regulatory subunits. In the metazoan condensins, the condensin-associated proteins are CAP-H (or H2), CAP-G (or G2), and CAP-D2 (or D3).

of kilobases (42). Chromosomal cohesin loading is dependent on a separate complex consisting of the Scc2 and Scc4 proteins (43). The establishment of cohesion, but not its maintenance, is dependent on the Ctf7 protein (44). Although the cohesin complex may associate with chromosomal loci in late G1 phase, cohesion appears to occur concomitantly with the passage of replication forks. An advancing fork is believed to pause at a cohesin-binding site and exchange the resident polymerase with polymerase σ as a prerequisite for establishment of cohesion (45). This step appears to require participation of the processivity clamp proliferating cell nuclear antigen and an alternate form of the clamp loader replication factor C. Cohesin loaded at chromosomal sites may translocate to more permanent locations that represent sites of convergent transcription (46). This unexpected flexibility is suggestive of a protein ring that can slide along DNA when pushed by a protein machine.

In the budding yeast, once cohesion between sister chromatids has been established, it lasts until the onset of anaphase when the cysteine protease Esp1 (separase), released from its sequestered state in association with securin, cleaves Scc1p at two target sites (47). The timely cleavage of Scc1p is controlled, at least in part, through its phosphorylation by the polo-like kinase Cdc5p (48). This straightforward "cut and separate" mechanism may be an oversimplified picture. Additional regulatory steps must operate because deletion of the securin gene (*pds1*Δ), together with

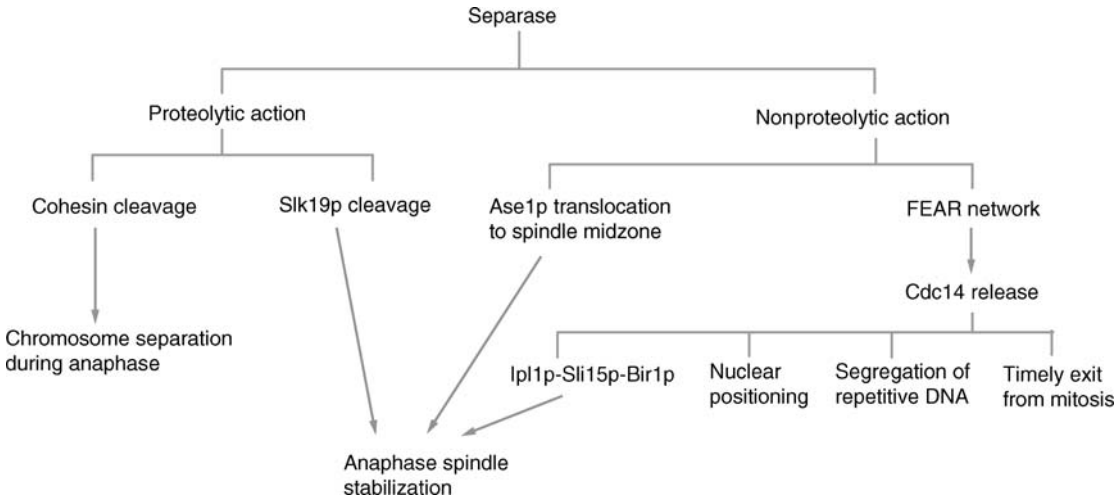

Figure 6

Multiple functions of separase. In addition to cleaving the Scc1p/Mcd1p subunit of the cohesin complex (and Slk19p of the FEAR complex), separase is involved in a number of steps that ensure the orderly progression of M phase and the exit from mitosis. Nearly all of these functions are mediated through the Cdc14 phosphatase.

overexpression of Cdc5p, does not advance the timing of Scc1p cleavage significantly. In *Xenopus* egg extracts, separase is phosphorylated by the cyclin-dependent kinase Cdk1; a dephosphorylation step, in addition to the destruction of securin, is likely required for separase activation (49). Unlike the budding yeast, higher eukaryotes remove cohesin in two distinct waves (50, 51). The first occurs during prophase and causes the dissociation of cohesin bound to chromosome arms. The process is independent of protease cleavage but is dependent on phosphorylation by the polo and aurora kinases. The second occurs in anaphase and primarily removes centromere-bound cohesin by proteolysis. Perhaps protective molecule(s) present at the centromere can locally block the action of the prophase pathway.

Separase: Not Just a Protease

Separase function in chromosome segregation goes beyond its proteolytic role in the cleavage of Scc1p (**Figure 6**). In a protease-independent manner, separase functions as part of the FEAR (fourteen early anaphase release) network to release Cdc14 phosphatase from the nucleolus, where it is anchored by Net1p (52). Although chromosome segregation at a global level can be achieved by cohesin disassembly alone, repeated DNA in budding yeast (rDNA and telomeres) fail to segregate when the FEAR pathway and release of Cdc14p are blocked (53, 54). This cohesin-independent cohesion of rDNA is resolved in a step (which requires condensin) that probably does not involve DNA compaction per se. Although this last-to-be-broken bonding between sisters is likely mediated by catenane links, and they are disjoined by topo II (54), the issue is as yet unsettled (53). In addition to facilitating the "last act" of sister chromatid segregation, separase contributes toward stabilizing and orienting the spindle, and it helps set the stage for exit from mitosis by counteracting Cdk1p activity in multiple ways (54–56) (**Figure 6**).

Pairing and Unpairing During Meiosis

The basic mechanism of pairing and unpairing has to be suitably modified spatially and

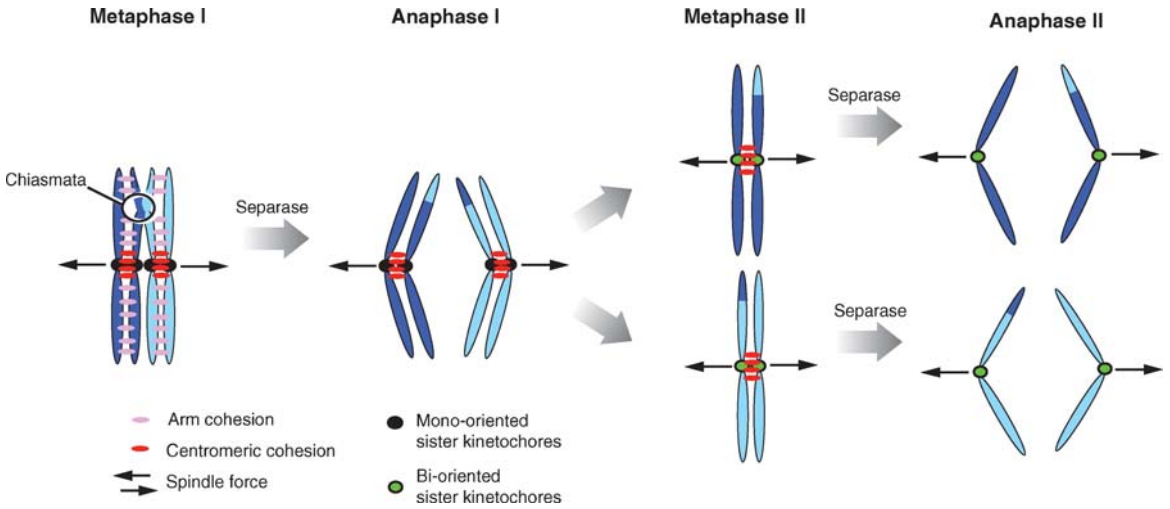

Figure 7

Chromosome pairing and unpairing in meiosis. During meiosis I, sisters become paired by cohesin, and DNA exchange between homologues takes place. Unlike in mitosis, sister kinetochores attach to the spindle in monopolar fashion; attachment of the homologue kinetochores is bipolar. Following resolution of crossovers and cohesin disassembly along the arms (but not at centromeres), the homologues segregate. During meiosis II, sister kinetochores bi-orient on the spindle, and cohesin removal at the centromeres triggers their segregation.

temporally to meet the demands of meiotic chromosome segregation. In order to produce haploid gametes, the meiotic cell must perform two rounds of segregation following a single round of chromosome duplication (**Figure 7**). During meiosis I, sisters must stay together, and homologues that have undergone recombination events must segregate. This is accomplished by preserving kinetochore cohesion between sisters and ensuring their monopolar (syntellic) attachment to the spindle. Arm cohesion, by contrast, is destroyed coincidently with the resolution of crossovers or chiasmata. In budding yeast, Spo13p, a meiosis-specific centromere-associated protein, facilitates the recruitment of the monopolin complex, consisting of monopolin (Mam1p) and the nucleolar proteins Csm1p and Lrs4p, that is essential for monopolar attachment and segregation of homologues (57, 58). During meiosis II, the rules of mitosis, bi-orientation of sister kinetochores and dissolution of kinetochore cohesion, come into play to segregate sisters.

Because the same enzyme, separase, appears to be responsible for cohesin cleavage during meiosis I and meiosis II in worms, yeasts, and mice (59–62), an important question is how the differentiation between centromere cohesion and arm cohesion is accomplished in the two cases. In principle, the existence of a meiosis I-specific protector of centromeric cohesin could take care of the problem. The search for the protector in the fission yeast has identified shugoshin (Sgo1p), guardian angel in Japanese, which localizes to the centromere in a Bub1 kinase-dependent manner and shields cohesin's Rec8p from cleavage by separase (63, 64). In the absence of Sgo1p, centromeric cohesion cannot be sustained during meiosis I, and sister chromosomes segregate randomly. The Sgo1p of the budding yeast is not only required for proper meiosis I, but also plays a role in sister chromatid segregation during meiosis II (and mitosis as well) (65). The fission yeast has a paralogue Sgo2p that is required for faithful chromosome segregation during

mitosis (63). Sgo2p is also expressed during both meiotic divisions, and *sgo2* mutants display high incidence of precocious equational division (segregation of sisters). Perhaps Sgo2p contributes to monopolar orientation as well as protection of centromeric cohesion during meiosis I. How does it then function during meiosis II (and mitosis), during which segregation demands bipolar orientation and loss of centromeric cohesion? The activity of Sgo2p may be regulated differentially to perform different roles under the two distinct contexts. The same argument may apply to Sgo1p in budding yeast, which does not have the Sgo2p counterpart and acts during mitosis, mieosis I, and meiosis II. Shugoshins have been identified in fungi, plants, flies, and vertebrates (63, 64) by virtue of conserved architectural motifs—an N-terminal putative coiled-coil, a central region rich in charged and hydroxylated residues, and a C-terminal basic motif—rather than by high amino acid sequence conservation. Perhaps these proteins have evolved by convergence or have coopted similar peptide modules to perform mitotic and meiotic functions in their specific biological contexts. It will not be surprising if, in vertebrate mitosis, a shugoshin-like protein mediates the retention of cohesin at centromeres during the prophase removal of arm-bound cohesin.

CONDENSINS AND CHROMOSOME SEGREGATION

The condensin complex is architecturally quite similar to the cohesin complex (**Figure 5**) but plays a very different role in chromosome segregation. Two Smc proteins, Smc2p and Smc4p in the budding yeast, bent at their hinge regions provide the V-shaped structural frame of condensin. The coiled-coil arms of the V end in twin-lobed globular ATPase heads, with which additional subunits interact to perhaps form a protein ring, are analogous to how ring closure has been proposed to take place for the cohesin complex (29, 66). Vertebrate cells have two condensin complexes, each with a distinct set of regulatory subunits. Condensin II acts during prophase, in the early step of chromosome compaction. Condensin I engages a chromosome after breakdown of the nuclear envelope and cooperates with condensin II to organize highly condensed and fully resolved metaphase chromosomes. By contrast, there is only one condensin complex, corresponding to condensin I, in the budding yeast. The relatively small sizes of individual yeast chromosomes as well as the lack of nuclear membrane breakdown during fungal mitosis may account for this evolutionary parsimony. However, the correlation between genome size and the presence or absence of condensin II is not perfect. For example, all condensin II genes are present in the unicellular red algae *Cyanidioschyzon merolae*, whose genome size is comparable to that of the budding yeast (29). Although condensin I is conserved from yeast to humans, the nematodes *Caenorhabditis elegans* and *Caenorhabditis briggsae* are exceptions to this rule. Perhaps their holocentric chromosome structure, with multiple centromeres arrayed along the entire length of chromosomes, may override the requirement for condensin I (67). Or, these nematodes may have adapted and retooled a primordial condensin I to perform dosage compensation that equalizes the expression of X-linked genes in the two sexes (68). Despite the similarity in their subunit composition, condensin and cohesin display distinct arm conformations with characteristic hinge angles when examined by electron microscopy (39). This structural difference may be important in their functional specializations.

Although the spatial and temporal controls of condensin action during the cell cycle may differ widely among organisms from the budding yeast to vertebrates, they are nevertheless directed toward the common goal of organizing and maintaining metaphase chromosome architecture that is conducive for unimpeded segregation. Condensation of the rDNA array in the budding yeast can be divided into two

distinct cell cycle–regulated pathways (69). The first one spanning G2 to M is a multistep process in which an early "random coil" state of condensation matures by "alignment" and "resolution" into a high-order state of condensation in a cohesin-dependent manner (**Figure 8**). It has been suggested that cohesin bound at its cognate sites along a chromosome may provide boundary marks or anchoring points that assist condensin to assemble a uniform array of condensed DNA domains. The second one is a post-metaphase process that is independent of cohesin but requires the Ipl1 (aurora) kinase, one of whose targets is the condensin subunit Ycg1 (69). The reason for orchestrating chromosome condensation in two separate steps is not immediately obvious. Perhaps the second phase of condensation, after cohesin has already been disassembled from chromosomes, may provide an additional safeguard against sister chromatid bridging and/or the trapping of chromosome laggards in the plane of cytokinesis.

Condensin's chromosome compacting role is central to chromosome segregation during meiosis as well. In the budding yeast, condensin subunits localize along the axial core of pachytene chromosomes (70). In condensin mutants, Zip1p, a component of the central element, is improperly localized, leading to defective assembly of the synaptonemal complex. Furthermore, lack of condensin function adversely affects the processing of meiotic double-strand breaks, pairing of homologues, resolution of recombination-mediated homologue linkages during meiosis I, and segregation of sister chromatids during meiosis II. The requirement for condensin during meiosis I and II has been demonstrated in *Arabidopsis* and *C. elegans* as well (71, 72). In contrast to the budding yeast situation, the association of condensin with chromosomes and the process of chromosome restructuring in the worm occur only after exit from pachytene. Additionally, the non-Smc components of the dosage compensation complex are required for meiotic chromosome segregation (73), but they do not seem to play a role in mitotic chromosome segregation.

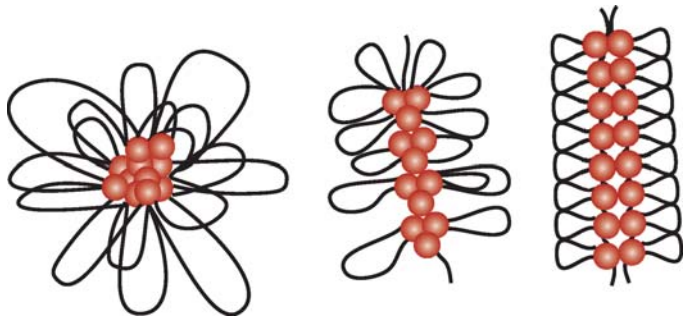

Figure 8

The stepwise condensation of budding yeast rDNA locus in the G2-M cell cycle window. The early intermediate in rDNA condensation by the condensin complex is a random coil (*left*), which undergoes partial alignment (*center*) before proceeding to the highly organized state of condensation (*right*). The process is dependent on the cohesin complex, which binds at centromeres and along chromosome arms with fairly uniform periodicity. The cohesin-bound sites may thus provide anchoring points for arranging uniform loops of condensed DNA. The diagram is adapted from Lavoie et al. (69).

Chromosome Condensation and Bacterial DNA Segregation

DNA condensation plays an important role in prokaryotic chromosome segregation as well (74, 75). Unlike eukaryotes, bacteria usually possess a single SMC protein or a distantly related, although functionally equivalent, protein such as MukB, the active core of the MukBEF complex of *E. coli*. Mutations in *smc* of *B. subtilis* or *C. crescentus* or in *mukB* of *E. coli* result in poorly condensed chromosomes that segregate abnormally (76–78). The segregation defect caused by the lack of *mukB* function can be suppressed by increasing the negative superhelical density of the chromosome, implying collaborative roles for plectonemic supercoiling and DNA condensation in promoting bacterial chromosome segregation (79, 80). In the extrusion-capture model for replication and segregation, the MukB protein may pull a newly replicated duplex poleward by supercoiling it into a more condensed form. The observation that *mukB* mutants are hypersensitive to inhibitors of gyrase

(81) strengthens the argument that DNA supercoiling by the two proteins acts additively in condensing daughter duplexes away from each other.

Two auxiliary subunits of the SMC complex, ScpA and ScpB, have been discovered in archaea and gram-positive bacteria (82, 83). ScpA shows similarity to the Scc1 subunit of eukaryotic cohesin (84), and its binding to the SMC head domains is stabilized by ScpB (85). In *B. subtilis*, *scpA* and *smc* mutations produce nearly identical phenotypes, including high-frequency anucleate cells and chromosome guillotining. The SMC complexes in archaea and bacteria thus share architectural and functional similarities with eukaryotic condensins.

Mechanism of DNA Compaction by Condensin

In vitro experiments have yielded important clues on plausible modes of condensin action, although the precise mechanism of DNA compaction is not fully understood (29, 86). In the presence of ATP and condensin, DNA knots formed by topoisomerase II are chiral, with exclusively + crossings (**Figure 9*a***). This result fits a model in which condensin stabilizes global positive writhe in DNA. Recent data using atomic force microscopy suggest a loop fastener mechanism for condensin action (**Figure 9*b***; middle). Here, the hinge region of the Smc proteins bind to one region of DNA, and in an ATP-dependent opening and closing reaction, the non-Smc subunits clamp the V to enclose a DNA loop. Electron spectroscopic imaging favors an "oriented gyre" (**Figure 9*b***; right) because two stacked supercoils appear to be captured by a single condensin molecule. A single DNA molecule assay using magnetic tweezers demonstrates highly reversible and dynamic compaction of DNA by condensin I. The reaction requires ATP hydrolysis and may proceed by a looping mechanism.

Stable binding of double-stranded DNA by bacterial Smc dimers involves the engagement of their catalytic head domains through two bound ATP molecules (87, 88). This DNA association may occur either through an intramolecular "embrace" mechanism or an intermolecular "hand-in-hand" interaction between adjacent Smc dimers (**Figure 9*c***). The non-SMC subunits ScpA and ScpB may exert either negative or positive regulation on DNA association, depending on whether they stabilize ring closure before or after the DNA has been engaged by the Smc dimer.

Roles for Condensin and Cohesin not Directly Related to Chromosome Segregation

Aside from their critical role in chromosome segregation, condensin and cohesin complexes participate in control of gene expression globally in a chromosome-wide manner, regionally within chromosomal domains, and locally for individual genes (86). Examples include transcriptional control by the condensin-like dosage compensation complex (see **Figure 5**) during hermaphrodite (XX) development in nematodes, repression of the silent mating cassettes *HML* and *HMR* in the budding yeast and regulation of the *Drosophila Abd-B* gene that specifies body segment identity in the posterior of the fly. Cohesin and related complexes such as the Smc5-Smc6 complex are also crucial for promoting repair of DNA damages (89–91). The Smc5p and Smc6p functions are required in the budding yeast for the proper segregation of repeated loci, rDNA, and telomeres. Presumably, they assist resolution of the X-shaped recombination intermediates formed during sister chromatid exchange (92). The meiotic recombination protein Rad50p, which forms part of the MRX (Mre11p, Rad50p, Xrs2p) double-strand break repair and telomere maintenance complex in the budding yeast (MRN complex in humans; N = Nbs1), is a structural relative of SMC proteins, displaying the globular ATPase head domains and two coiled-coil

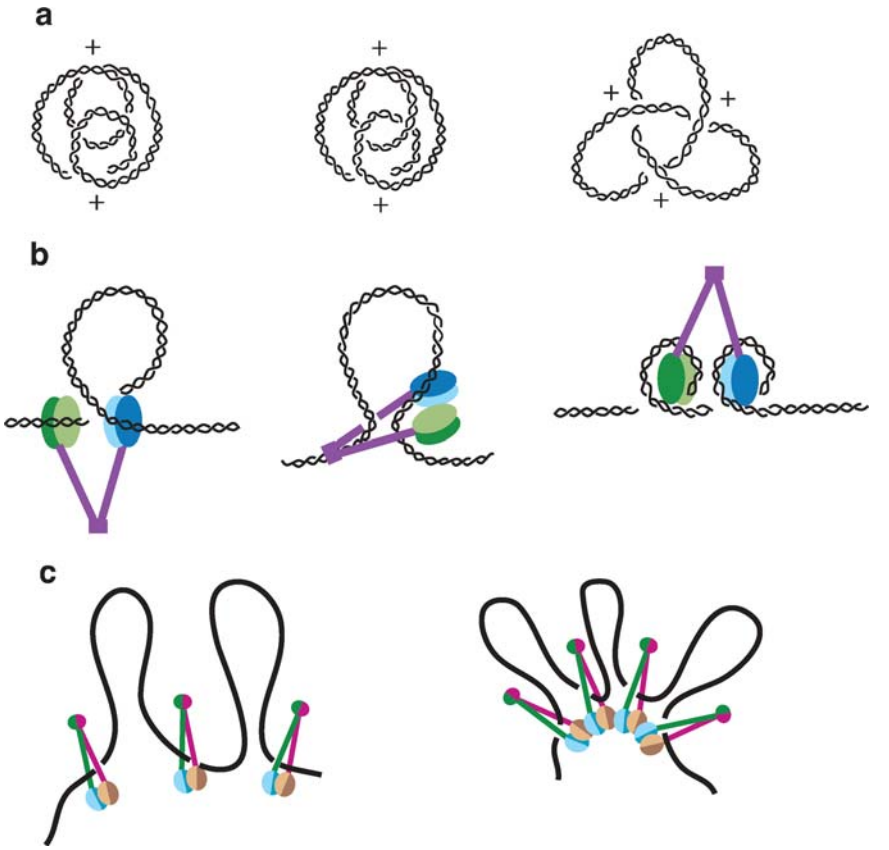

Figure 9

Plausible mechanisms of DNA compaction by condensin. (*a*) Knots generated by topoisomerase II in presence of condensin and ATP have exclusive + crossings, suggesting that condensin induces global positive writhe in DNA (*b*, *left*). Strand passage by topoiosmerase II between two overlying positively writhed DNA loops will generate a (+) trefoil (a simple knot with three crossings) as shown in panel *a*. (*b*) Other plausible modes of DNA condensation include a loop fastener mechanism (*middle*) or a twin-looped oriented gyre mechanism (*right*). (*c*) Engagement of the catalytic sites by two bound ATP molecules stabilizes the closed configuration of the bacterial SMC dimer and promotes stable encircling of DNA by the coiled-coil arms. Catalytic site engagement may occur by an intramolecular "embrace" mechanism (*left*) or an intermolecular "hand-in-hand" mechanism (*right*).

regions connected by a "zinc-hook" hinge motif (93).

The condensin and cohesin complexes are more than just chromosome compactors and glue, respectively, devoted to chromosome segregation alone (86). By combining pairs of SMC or SMC-like proteins with alternative sets of regulatory components, evolution has formulated solutions to multiple problems related to organization, integrity, accessibility, and transmission of genomes.

SEGREGATION OF BACTERIAL PLASMIDS

Depending on copy number, stable propagation of a bacterial plasmid can be accomplished via random segregation or active partitioning. Provided the steady-state copy number is reasonably high, and there is no barrier to free diffusion, random segregation will suffice. The probability of a plasmid-free cell arising at any given cell division will be

par locus: partitioning locus of bacterial plasmids

Par proteins: partitioning proteins of bacterial plasmids

quite low. Furthermore, control mechanisms operating at the replication level can correct for any deviation from the normal copy number because of unequal segregation. By contrast, unit-copy or low-copy number plasmids must rely on an active partitioning system to avoid missegregation. Certain plasmids also possess an intricate postsegregational killing mechanism to eliminate competition from plasmid-free cells formed as a result of missegregation events. The "addiction module" harbored by such plasmids codes for a long-lived toxin and a short-lived antidote (94–96). Cells that lose the plasmid are condemned to death as they inherit the toxin without the benefit of the antidote. Whereas the stable toxins are normally protein moieties, the unstable antidotes may be either proteins or small antisense RNA molecules that prevent translation of the toxin mRNAs.

Partitioning Systems of Low-Copy Plasmids

Unit-copy or low-copy bacterial plasmids such as P1, F, and R1 encode partitioning genes (*par*) whose protein products act in conjunction with a centromere-like locus to facilitate their faithful segregation (97, 98). To avoid possible confusion by different nomenclatures used in differing systems, we present a simplified picture of their unifying features in **Figure 10*a***. In general, the *par* locus specifies two *trans*-acting proteins encoded within an operon and a *cis*-acting "centromere-like" element. The polypeptide products of the upstream and downstream *par* genes are generalized here as ParA and ParB, respectively. The centromere *parC* (*sopC* in F, *parS* in P1, and *parC* in R1) contains iterons, multiple copies of a sequence element that is characteristic of each individual plasmid. ParA (SopA in F, ParA in P1, and ParM in R1) is an ATPase that can associate with the centromere, usually assisted by its partner ParB (SopB in F, ParB in P1, and ParR in R1). ParB normally binds directly to *parC* to form a "prepartitioning" complex that then recruits ParA to complete the partitioning complex. The location of *parC* with respect to the operon is variable. For example, *parC* may harbor the promoter to the operon or may reside distal to it and downstream of the protein-coding sequences. Some of the *par* loci are characterized by multiple direct repeats upstream and downstream of the *par* genes, and the two sets may independently or cooperatively serve the centromere-like function. An extensive search of sequence databases using partitioning ATPases of well-studied plasmid systems has revealed novel organization patterns for plasmid partitioning loci (99). On the basis of these additional data, plasmid partitioning systems have been divided into five representative classes. Nevertheless, all of these systems reflect variations of a common theme.

The ParA ATPases can be divided into two groups, the Walker type and the actin-like ATPases (100, 101). Four of the five partitioning classes (that include P1 and F) harbor ATPases that display Walker A, Walker B, and A′ motifs, typifying nucleotide-Mg^{2+} binding, plus a fourth motif whose function is not known. The ATPases of the other partitioning group (that includes R1) contain ATP-binding motifs characteristic of the actin-like ATPase family. There is strong evidence that the *par* loci are under strict autoregulation, presumably because the correct stoichiometry of the Par proteins is important for partitioning. The ParA protein of P1 (and SopA of F) can associate with operator sequences in the *par* promoter region using an N-terminal DNA-binding domain to downregulate transcription; the extent of transcriptional repression is augmented by the ParB (or SopB) protein acting as a corepressor (102–105). In the case of F, maximal repression also requires the centromere, suggesting a role for DNA looping in this process (106). In vitro studies indicate that P1 ParA interacts with the centromere-ParB partition complex in the ATP-associated form and binds to the *par* promoter more strongly in the ADP-associated form (107). Thus, the dual function of ParA, as

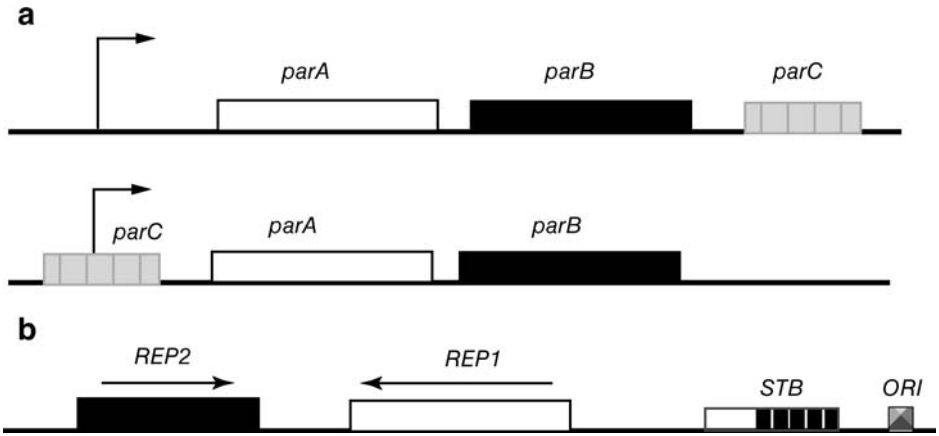

Figure 10

Organization of partitioning loci in bacterial and yeast plasmids. (*a*) In this simplified representation of bacterial *par* loci, *parA* and *parB* are the genes coding for the partitioning proteins, and *parC* is the centromere-like locus consisting of iterated sequence elements. ParA proteins are ATPases that harbor typical Walker motifs or belong to the actin-like family of ATPases. The ParB proteins in general bind to *parC* and recruit ParA to the partitioning complex. The bent arrows indicate the site of initiation and direction of transcription. (*b*) The yeast plasmid partitioning system also consists of two plasmid-coded proteins Rep1p and Rep2p and the *cis*-acting locus *STB*. There is no functional correspondence between the partitioning proteins of the bacterial and yeast plasmids. The *STB* element can be functionally divided into two subloci. The one proximal to the replication origin (*ORI*) contains approximately six iterations of a consensus 65-bp repeat element. The distal-*STB* region is important in maintaining the active configuration of *STB* and contains the termination site for two transcripts directed toward the origin.

a partitioning agent or as a transcription regulator, appears to be subject to allosteric regulation by the bound form of the nucleotide ligand. In the R1 plasmid, the ParM protein (equivalent to ParA ATPase) does not appear to participate in regulating the *par* operon, leaving this function entirely to the ParR protein (equivalent to ParB) (108).

Mechanisms of Plasmid Partitioning

Our current understanding of how the partitioning proteins assist plasmid segregation is primarily phenomenological. In general, the partitioning complex, formed by association of the Par proteins with the centromere, specifies plasmid localization within the bacterial cell and provides an important spatial determinant for proper segregation (99). The identities of host factors involved in bacterial plasmid segregation have remained elusive. Although the *E. coli* integration host factor protein promotes the assembly of the P1 partitioning apparatus by increasing the affinity of ParB for its centromere, it is not essential for the partitioning process (109). The P1 and F plasmids are normally present at the center of a newborn cell and stay there until they are replicated (110, 111). The duplicated copies then quickly move to the one-fourth and three-fourths cell positions (112), which mark the midpoints of the future daughter cells. An occasional second round of replication prior to cell division can result in a daughter cell containing two copies of the plasmid. Time-lapse fluorescence microscopy has provided a more refined and better resolved perspective of P1 localization and dynamics in live *E. coli* cells (113). A focus containing one or more plasmid molecules is captured at the cell center just prior to replication, and foci of nascent plasmid molecules are ejected bidirectionally along the long axis of the cell before cell division (**Figure 11*a***). Plasmid

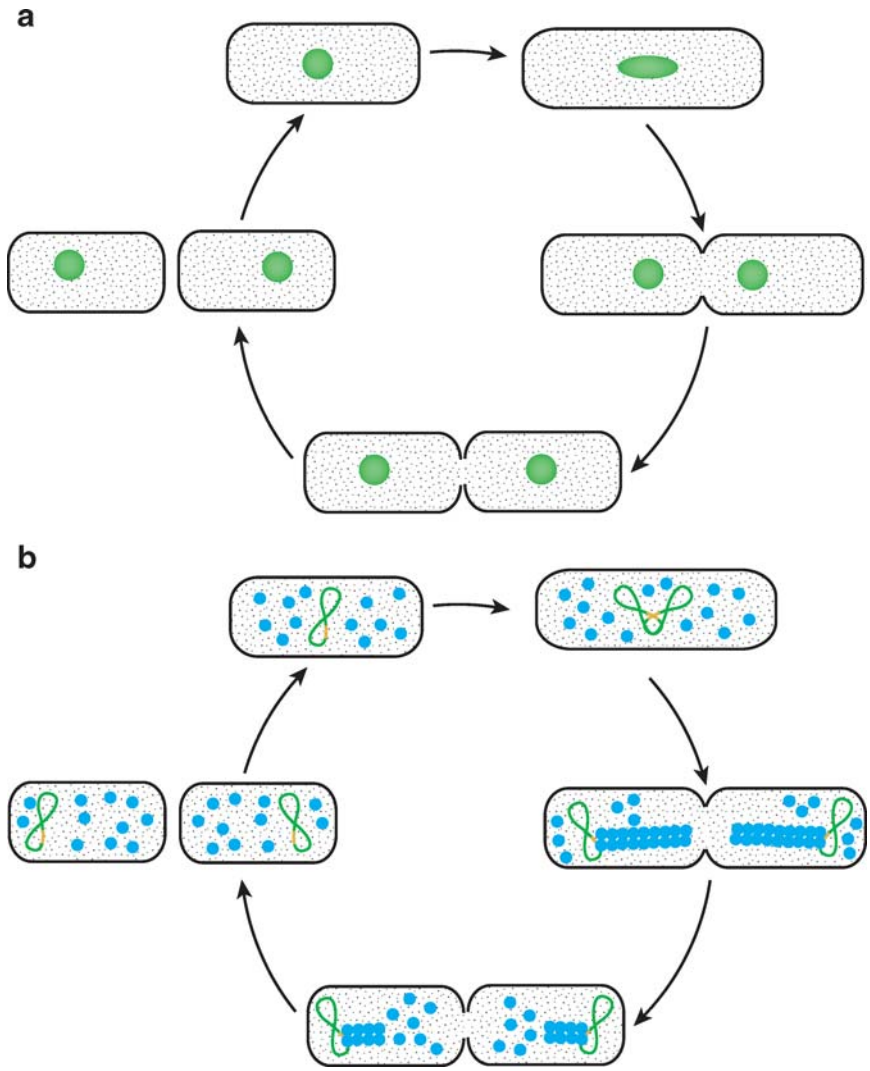

Figure 11

Segregation of P1 and R1 plasmids in *E. coli*. (*a*) The focus of P1 plasmid, which is free to diffuse in a newborn cell, localizes to the mid-cell position just before replication. The foci of duplicated plasmid molecules are extruded in opposite directions. (*b*) The R1 plasmid (*green*) has a pole-proximal location in the newborn cell. The plasmid translocates to the cell center for replication. ParM (*blue*)-ATP polymerization into filaments at the ParR-bound *parC* loci (*gold*) transports the replicated plasmid molecules toward opposite cell poles. The filament depolymerizes, starting at the tail end, to generate ParM-ADP. The figure is patterned after diagrams from Li & Austin (113) and Gerdes and coworkers (122).

copies are free to move, associate, and dissociate in the newborn daughter cell before they are captured at the cell center to start a new replication and segregation cycle. At the mid-cell position (or the one-fourth or three-fourths positions of a cell about to divide), there does not appear to be a single "partitioning center" to which plasmids harboring distinct partitioning systems are tethered. Pairs of compatible plasmids such as F and RK2 do

reside close to each other but are not necessarily colocalized (114). They also differ in their segregation timings. Although the importance of accurate plasmid localization in association with the partitioning complex for efficient segregation is well documented (111, 115), the subsequent mechanisms remain obscure.

An analysis of in vivo plasmid topology, along with a related set of cell biological observations, suggests that P1 or F plasmid molecules are physically restrained within the partitioning complex or at their cellular tethering site and may need to be released from this state prior to segregation (105, 115, 116). ParB/SopB is likely responsible for plasmid pairing or grouping through its association with the centromere sequence; perhaps it is up to ParA/SopA to actively unpair/ungroup the replicated molecules. Consistent with this notion, certain types of mutations in ParA/SopA cause "worse than random" segregation, as if the copy number has been lowered because the plasmids have been "glued" together (117, 118). Furthermore, as revealed by time-lapse assays, ParB is required for tethering the P1 plasmid focus at mid-cell, whereas the ParA ATPase is essential for the active longitudinal (and bidirectional) ejection of foci after replication (119).

The most well-understood bacterial plasmid segregation system, with respect to mechanism, is that harbored by the R1 plasmid (120). The plasmid shows a dynamic pattern of localization within the *E. coli* cell. Although it is positioned near the cell pole in a newborn cell, it moves to the central position during cell growth (for duplication by the replisome), and the daughter plasmids move back to their pole-proximal location (121). The foci formed by the ParA ATPase of R1 (the ParM protein) are coincident with the plasmid and show identical dynamics. The actin-like ParM protein can oligomerize into filaments in vivo and in vitro (122). Filament formation in vitro is dependent on ATP and Mg^{2+}, whereas filament disassembly requires ATP hydrolysis. The ParB protein of R1 (ParR) bound to the centromere *parC* can mediate the pairing of two plasmid molecules in vitro (123). The ParR-*parC* complex is essential for ParM polymerization in vivo and for filament formation in vitro at low ParM concentrations. Furthermore, in vivo the plasmids are located at the distal tips of the ParM filament, as it extends from the cell center toward the poles (124). These findings provide the basis for the R1 segregation pathway described below and illustrated in **Figure 11*b***.

Following duplication of R1, the daughter plasmids are held together at *parC* by the bound ParR. The ParR-*parC* complex provides the nucleation site for the polymerization of ParM-ATP. As the filament grows, the plasmids are unpaired and forced apart from each other toward the cell poles. It has been suggested that the plasmid transport is mediated by a bundle of protofilaments, perhaps each one connected to one of the ten ParR-binding sites within *parC*. The filament pushes the plasmids to their native locations within would-be daughter cells. Depolymerization of the filament at the tail end would release ParM-ADP monomers into the cytoplasm. They have to be recharged by nucleotide exchange for the next round of plasmid segregation in the subsequent cell cycle.

Centromeres and Par Proteins in the Segregation of Bacterial Chromosomes

Partitioning systems of bacterial plasmids are simple in organization yet efficient in function. It is natural to wonder whether bacterial chromosomes also harbor partitioning loci that are functionally similar to their plasmid counterparts. Indeed, almost all of them do with the exception, perhaps, of certain γ-proteobacteria, including *E. coli* and *Haemophilus influenzae* (97, 125). The ParA and ParB homologues of *B. subtilis* are Soj and Spo0J, respectively, and there are eight centromere iterons (*parS*) in the origin-proximal region of the chromosome (126). Although the cellular localization patterns of Spo0J in

Rep1p, Rep2p: partitioning proteins of the yeast 2-micron plasmid

***STB*:** partitioning locus of the yeast 2-micron plasmid

***FRT*:** Flp recombination target

the presence or absence of Soj are generally consistent with those expected of a centromere system, origin localization and dynamics are not affected in the absence of Spo0J (8). However, deletion of the *spo0j* locus does result in a small but significant fraction of anucleate cells during vegetative growth (127). The role of the Soj-Spo0J-*parS* system appears to be rather subtle during mitotic chromosome segregation, whereas it is more prominent during sporulation. Genetic and cytological analyses reveal a partial functional overlap between Soj and the RacA protein in bringing *oriC* to the DivIVA protein located at the pole of the forespore (128). The *par*-system of *C. crescentus* is constituted by the ParA and ParB proteins together with the origin-proximal ParB-binding sites (*parS*). The ParAB proteins are not only important in chromosome segregation but also serve a checkpoint-like function in linking chromosome segregation to cell division and/or DNA replication (129, 130). In other systems that have been investigated, *Pseudomonas putida* and *Streptomyces coelicolor*, the Par proteins are required for proper chromosome segregation during sporulation or in cells transitioning from exponential growth to stationary phase (131, 132). Lack of Par functions appears to have no effect or only modest effects on chromosome segregation during vegetative growth, although overproduction of Par proteins leads to an increased frequency of anucleate cells in *P. putida* (131, 133).

Is a partitioning locus truly absent in *E. coli*, or is there such a novel locus that is yet to be characterized? A recent analysis, based on the assumption that the centromere sequence would be the first to migrate away from the site of replication toward the cell pole, has identified a potential centromere at 89′ on the *E. coli* chromosome, close to *oriC* at 84′ and probably a second one at 79′ (134). Interestingly, a 25-bp sequence *migS*, located at the putative centromeric region at 89′, serves as a *cis*-acting element that directs the bipolar positioning of replicated *oriC*s (135). Future work will decide whether *migS* and associated sequences (perhaps) represent an authentic chromosomal centromere and, if so, what the corresponding Par proteins are.

PLASMID SEGREGATION IN YEAST

The 2-micron plasmid, nearly ubiquitously present in *Saccharomyces* yeast strains, is a multicopy extrachromosomal element that resides in the nucleus and propagates itself stably in host cell populations (136). The plasmid does not confer any advantage to its host, nor does it impose any obvious disadvantage at its steady-state copy number of 40–60 molecules per cell. The chromosome-like stability of the plasmid (a loss rate of 10^{-5} to 10^{-4} per cell division) is conferred by the combined action of a plasmid partitioning system and a plasmid amplification system. The former ensures equal or roughly equal distribution of replicated plasmid molecules to daughter cells; the latter corrects any decrease in copy number caused by a rare missegregation event. The partitioning system consists of two plasmid-coded proteins Rep1p and Rep2p and a *cis*-acting partitioning locus *STB* (**Figure 10*b***). The amplification system consists of the plasmid-coded Flp site-specific recombinase and a pair of *FRT* (Flp recombination target) sites present on the plasmid genome in head-to-head orientation. Here, we discuss the mechanism of action of the partitioning system, and we outline the amplification process below.

It may seem paradoxical that a plasmid with as high a copy number as 40 to 60 would require a partitioning system. Random segregation should be eminently suitable for plasmid propagation especially because a copy number can be corrected by the amplification system. As it turns out, the plasmid exists as a tight-knit cluster of dynamic foci within the yeast nucleus, and these foci stay together throughout the cell cycle (137). The Rep1 and Rep2 proteins form an integral part of the plasmid cluster by their association with *STB*. The cluster, which likely includes host proteins as

well, is also the entity in segregation (137, 138), thus reducing the copy number effectively to unity. Hence, the operation of a partitioning system makes sense.

The 2-micron circle partitioning system appears to resemble bacterial plasmid partitioning systems in its general organization in that both consist of two protein components that act in conjunction with a *cis*-acting centromere-like locus (**Figure 10**). However, there are no functional similarities between the two. A number of recent experiments suggest that the Rep/*STB* system is a clever molecular device to channel components of chromosome segregation toward plasmid partitioning. The plasmid-chromosome connection in segregation has been suspected from observations indicating that the dynamics and segregation kinetics of a fluorescence-tagged reporter plasmid are remarkably similar to those of a similarly tagged chromosome (137). Furthermore, conditional mutations that affect segregation of chromosomes appear to affect the 2-micron plasmid in quite a similar manner. In the nonpermissive state, the plasmid cluster appears to missegregate in tandem with the bulk of the chromosomes. This comissegregation phenotype is lost when either of the Rep proteins is mutated or the *STB* locus is removed from the plasmid, thus identifying the partitioning system as the potential agent that couples segregation of the plasmid with that of the chromosomes.

The Cohesin Complex and Yeast Plasmid Partitioning

A breakthrough in elucidating the possible mechanism of 2-micron circle partitioning came with the discovery that the yeast cohesin complex is recruited specifically to the *STB* locus in a Rep1p-Rep2p-dependent manner (139). Cohesin associates with the plasmid during S phase in synchrony with chromosomal cohesin-binding sites, and the lifetime of this associated state is the same for the plasmid and the chromosomes. The disassembly of cohesin by Scc1p/Mcd1p cleavage during anaphase is as critical for plasmid segregation as it is for chromosome segregation. When a noncleavable version of Scc1p is overexpressed from an inducible promoter, the replicated plasmids do not split into two separate clusters, just as sister chromatids fail to separate. These findings imply that the cohesin complex plays similar functional roles during chromosome segregation and plasmid segregation. We propose that, concomitant with plasmid replication, cohesin holds sister clusters together until dissolution of the cohesin bridge annuls this union, and they are segregated in a one-to-one fashion (**Figure 12**).

The chromatin structure at the *STB* locus, which is dependent on the RSC2 chromatin remodeling complex, is important in equal plasmid segregation (140). In an *rsc2*Δ yeast strain, the 2-micron plasmid is lost at a high rate. Lack of Rsc2p blocks Rep1p association with *STB* and consequently cohesin recruitment by the plasmid (141, 142). Chromatin immunoprecipitation (ChIP) assays suggest that the functional nucleosome organization at *STB* is reset de novo during each cell cycle between the late G1 phase and early S phase. During this window, the Rep proteins transiently dissociate from *STB* and reassociate with it shortly afterwards, in time for cohesin recruitment. It is likely that the recycling of the Rep proteins, assembly of the functional chromatin architecture at *STB*, and the acquisition of cohesin by the plasmid are tightly coordinated with DNA replication.

The Mitotic Spindle Promotes Recruitment of Cohesin by the Yeast Plasmid

The yeast mitotic spindle promotes 2-micron circle segregation in quite an unconventional manner (143). Normally, the plasmid cluster has a specific nuclear address close to the spindle pole, and preparations of chromosome spreads reveal the presence of the plasmid. It is not clear whether plasmids are directly attached to the chromosomes or are anchored

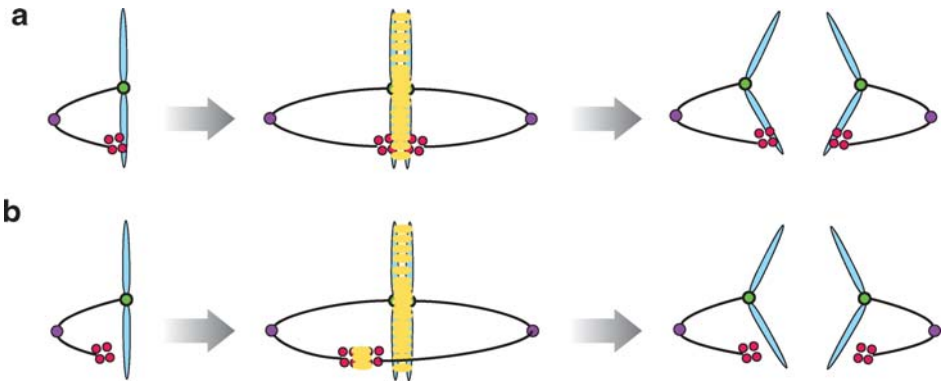

Figure 12

Models for segregation of the yeast 2-micron plasmid. (*a*) In this hitchhiking model, the plasmid cluster is tethered to a chromosome. Cohesin assembly at the *STB* locus during DNA replication keeps the duplicated clusters paired, facilitating the attachment of the second cluster to the sister chromosome. Following disassembly of cohesin, each of the two segregating sisters carries with it one plasmid cluster. (*b*) In the chromosome-independent model, plasmid segregation is still dependent on cohesin-mediated pairing and unpairing but takes place without chromosome assistance. The model does not specify how the driving force for plasmid segregation is derived. Recent findings demonstrate that precise nuclear localization of the plasmid and cohesin acquisition by it are dependent on the nuclear spindle. Perhaps the plasmid segregates in a spindle-associated fashion.

in some way to the same subcellular structures as chromosomal domains. When spindle integrity is disrupted by microtubule depolymerizing agents or by specific tubulin mutations, the plasmid cluster becomes less compact and loses its precise nuclear localization. Concomitantly, chromosome spreads fail to display the plasmid, and cohesin assembly at *STB* does not occur. A role for the mitotic spindle in cohesin acquisition is quite surprising because neither centromeric cohesion nor chromosome arm cohesion is dependent on the spindle. Plasmid molecules replicate normally in the absence of spindle, just as chromosomes do; when the spindle is allowed to reform, cohesin associates with *STB*. However, this postreplicative association is not effective in plasmid segregation, presumably because replication-dependent one-to-one pairing of sister clusters is a mandatory step in partitioning. Chromosomes, in contrast, having been cohesed in a replication-dependent manner, go on to form bipolar spindle attachment and segregate normally. It is only through spindle disassembly or through mutations of the partitioning system itself that we have been able to uncouple 2-micron circle segregation from chromosome segregation.

Models for Yeast Plasmid Segregation

Two general models under consideration for plasmid segregation in yeast are outlined in **Figure 12**. According to the hitchhiking model, the plasmid cluster is tethered to one of the chromosomes, perhaps with the assistance of the Rep proteins bound at *STB*. DNA replication and cohesin-mediated DNA bridging result in a pair of sister clusters, linked to each other as well as attached to sister chromatids. Subsequent cohesin disassociation will permit the clusters to hitchhike in opposite directions on the sister chromosomes. Stable propagation by attachment to host chromosomes has been demonstrated for bovine papilloma and Epstein-Barr viruses, whose genomes have an episomal existence (144). In the chromosome-independent model, cohesin still mediates the one-to-one segregation of the plasmid clusters but without physical linkage between plasmid and chromosome. How the unpaired

plasmid clusters move away from each other is not specified in the model. Perhaps this movement occurs in association with the spindle or with a subnuclear entity that is partitioned evenly between daughter cells.

In both bacterial plasmids and the yeast plasmid, precise cellular localization mediated through their respective partitioning systems appears to be an important spatial determinant for proper segregation. Positioning of the 2-micron circle is critically dependent on the integrity of the mitotic spindle. The role of host factors in specifying the locations of bacterial plasmids is not understood. Subsequent events in partitioning are quite different for the bacterial and yeast plasmid systems. As suggested by the example of the R1 plasmid, the *par* system appears to be functionally autonomous and mediates segregation apparently independently of the chromosome segregation machinery. The yeast plasmid, by contrast, utilizes its partitioning system to gain access to the highly efficient pathway that faithfully segregates the chromosomes of its host.

THE CYTOSKELETON, DYNAMIC PROTEIN RINGS, COILS, AND OSCILLATORS IN BACTERIAL CELL DIVISION AND CHROMOSOME SEGREGATION

The participation of cytoskeletal structures, such as a dynamic scaffolding to regulate cell shape and movement, chromosome segregation, and cell division (once thought to be an exclusive feature of the eukaryotic cell), has been unveiled in bacteria as well. All three signature elements of the eukaryotic cytoskeleton, actin, tubulin, and intermediate filaments, play important roles in cellular organization and dynamics in bacteria (145). The actin-like Mre/Mbl proteins form extended continuously moving helical filaments (which are well suited for transporting cellular components and structures and, perhaps, for promoting chromosome segregation) underneath the cell membrane. Targeted and programmed inactivation of MreB in *Caulobacter* specifically affects the poleward migration of *oriC*, with no effect on DNA replication per se or the segregation of the remainder of the chromosome (146). The role of the ParM helical filament in the segregation of the R1 plasmid was discussed above. The ring structure formed by the tubulin-like FtsZ protein to mark the bacterial division site (whose assembly and disassembly is regulated by the collaborative action of several proteins) may itself be a helix with a highly compressed pitch (147). The location of the ring is specified through a dual-protein (MinC-MinD) harmonic oscillator, set up by the MinCDE system, that sweeps from cell pole to cell pole in a helical wave (148; J. Lutkenhaus, unpublished results). Similarly, oscillations by partitioning ATPases may help localize plasmids or chromosomal replication origins within bacterial cells (124). Rod-shaped bacteria appear to have evolved a system of molecular spirals and oscillators for maintaining cell shape, mediating cell growth, and specifying important cellular landmarks.

TOPOISOMERASES, SITE-SPECIFIC RECOMBINASES AND DNA TRANSLOCASES: FAITHFUL CHROMOSOME SEGREGATION AND GENOME MAINTENANCE

Topoisomerases are indispensable in overcoming the topological barriers to replication and in completely undoing the inevitable catenane linkages between daughter duplexes. In *E. coli*, DNA gyrase is primarily responsible for removing positive supercoils ahead of the fork, whereas topo IV, a type II topoisomerase, almost exclusively resolves precatenane and catenane links between nascent duplexes (3, 149). Topo III, a type I topoisomerase, may also contribute to precatenane resolution by acting at the single-stranded DNA present at the replication fork (150).

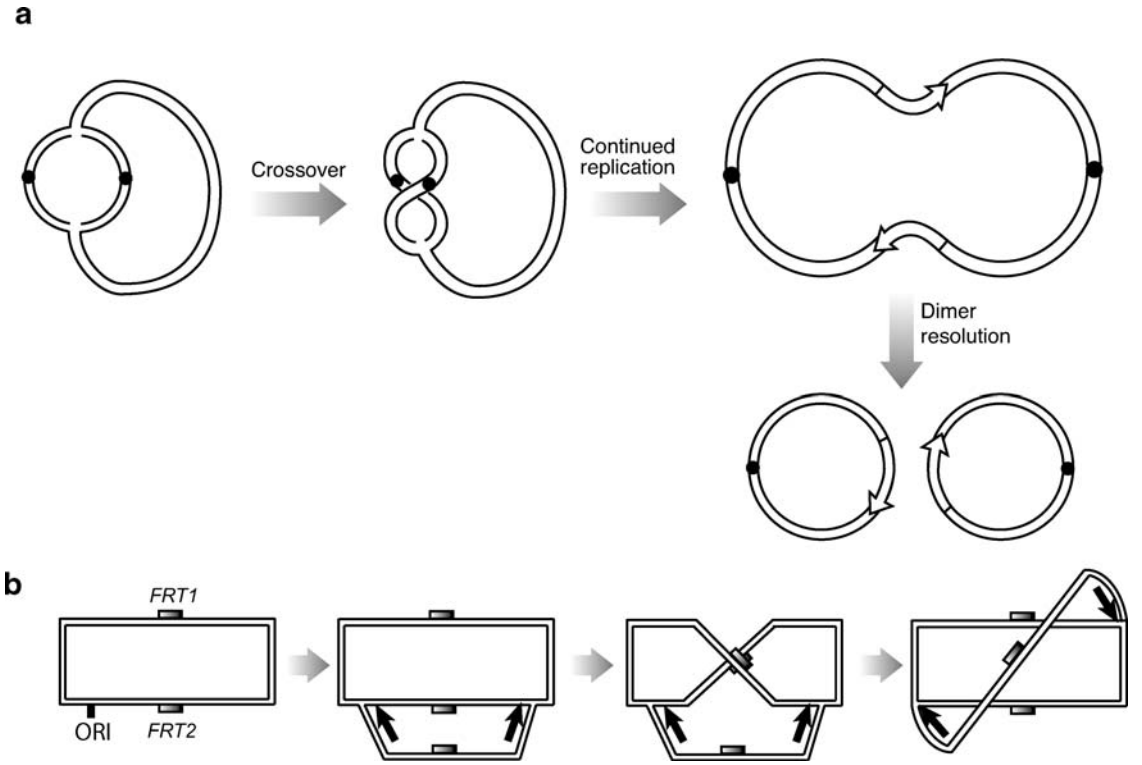

Figure 13

Site-specific recombination promotes genome segregation or copy-number maintenance. (*a*) In *E. coli*, resolution of a chromosome dimer resulting from homologous recombination is mediated by the XerC/XerD site-specific recombinase. Coordination of recombination and cell division is mediated through the FtsK motor protein that localizes at the division septum and also activates the XerD subunit of the recombinase. (*b*) Copy-number amplification of the 2-micron yeast plasmid is triggered by a replication-coupled recombination event mediated by the Flp site-specific recombinase. During bidirectional replication, the origin-proximal Flp recombination target site 2 (*FRT2*) is duplicated before the distal *FRT1*. Recombination between one of the duplicated sites and the unduplicated site inverts the direction of one of the replication forks and triggers the amplification process.

Whereas decatenation is critical in the segregation of both circular and linear chromosomes, circularity further complicates matters for a unit copy genome. A single crossover or an odd number of crossovers during homologous recombination will result in a chromosome dimer that has to be resolved into monomers (**Figure 13*a***). This reaction is mediated in *E. coli* by a tyrosine family site-specific recombinase XerC/XerD, and by its relatives in other bacteria (151). The location of the recombination target site *dif* near the replication terminus on the *E. coli* chromosome helps coordinate the recombination event with the act of cell division. A central player in this regulation is FtsK, a DNA-translocating ATPase that localizes to the division septum (11, 12, 152, 153). There are multiple roles for FtsK in bacterial chromosome segregation (152, 154): restraining *terC* regions of chromosome dimers at the mid-cell position, promoting synapsis of *dif* sites by XerC/XerD, recruiting topo IV and XerC/XerD to the division septum, activating decatenation and chromosome dimer resolution, and clearing DNA away from the constricting septum.

Site-specific recombination serves a different purpose for the yeast plasmid in the context of genome maintenance, namely, copy-number amplification. The highly asymmetric location of the plasmid replication origin with respect to the head-to-head *FRT* sites is a clever design for increasing copy number without violating the ban on more than one replication initiation event per cell cycle. As suggested by Futcher (155), an appropriately timed recombination reaction during bidirectional plasmid replication can invert one of the forks with respect to the other (**Figure 13*b***). The two unidirectional forks chasing each other around the circular template will give rise to an amplicon comprised of multiple tandem copies of the plasmid. A second recombination event may terminate amplification by restoring bidirectionality to the forks. Individual copies of the plasmid can be resolved from the amplicon by homologous recombination or by Flp-mediated site-specific recombination.

The actions of XerC/XerD and Flp, both tyrosine recombinases, have starkly different physiological consequences. The former neutralizes the threat posed by homologous recombination to equal chromosome segregation; the latter negates potential erosion in plasmid copy number caused by missegregation. Thus, two related recombination systems contribute to genome maintenance and integrity via chemically similar yet biologically distinct pathways.

ADDENDUM

We have listed below some of the features of plasmid and chromosome segregation that have come to light or been brought to our notice since submission of this chapter.

1. There have been new revelations on the role of ParA ATPases in proper positioning and segregation of bacterial plasmids (and perhaps chromosomes as well). Partitioning mechanisms based on dynamic protein filaments, first demonstrated for the R1 plasmid, appear to be more widespread. The partitioning ATPase SopA of the F plasmid polymerizes into filaments in vitro in an ATP-dependent manner and elongates at a rate consistent with plasmid separation in vivo (156). Furthermore, SopA undergoes cycles of polymerization/depolymerization inside the cell and shuttles back and forth between nucleoprotein complexes consisting of SopB associated with the plasmid partitioning locus *sopC*. Dynamic polymerization of SopA is likely the driving force for F plasmid separation.
2. Although the mechanism by which oscillations of filament forming ATPases mediate plasmid segregation is unknown, a simple model based on the outward force exerted by ParM filaments on the R plasmid has been posited (157). The assumption is that the ATPase oscillation occurs over the distance of the nucleoid, whose borders provide toeholds for the oscillating protein. According to the model, the force exerted on a single plasmid focus over an oscillation cycle will localize it to the center of the cell. Under the same force, two plasmid foci will be dispatched to quarter cell positions.
3. The structure of the partitioning protein ParB of plasmid P1 has been solved (158). The rotational freedom of the DNA binding helix-turn-helix modules of ParB about a flexible linker explains how the protein recognizes the A and B boxes despite their complex arrangements within a sharply bent *parS* locus.
4. It has been brought to our attention (J. Pogliano, personal communication) that the model for P1 plasmid segregation described here (113) may not represent the whole picture. The notion that replicated plasmid molecules

are held at cell center until just before septation/division is not consistent with time-lapse assays conducted by Gordon et al. (159).

5. Simultaneous tracking of pairs of genetic loci and divisome proteins in *E. coli* provides new insights into how DNA replication, chromosome segregation, chromosome organization, and cell division are related to each other. Observations on different loci in the replication termination region (*ter*) suggest an asymmetric pattern of segregation of leading and lagging strand templates following their duplication (160).
6. Further evidence has been provided in support of the notion that the cohesin complex topologically entraps sister chromatids by forming a ring around them (161). Circular minichromosomes isolated from yeast nuclei retain some tightly bound cohesin, and the association between DNA and protein can be relieved by cleavage of either a cohesin component by a protease or the minichromosome by a restriction enzyme.

SUMMARY POINTS

1. Within the bacterial nucleoid, chromosome replication is subjected to strict spatial and temporal controls. In *C. crescentus*, chromosomal loci display a remarkably ordered organization. The directed movement of daughter duplexes as they are formed, likely coupled with DNA compaction, recreates the original chromosome order in newborn cells.
2. Eukaryotic chromosome segregation is facilitated by two architecturally similar protein assemblies, the cohesin and condensin complexes. Cohesin is central to a sister chromatid pairing and unpairing mechanism that retains replication memory, distinguishes chromosome sisters from homologues, and accommodates equational or reductional modes of segregation. Condensin promotes ordered intramolecular compaction of DNA and prevents chromosome entanglement during segregation.
3. Single-copy or low-copy bacterial plasmids harbor partitioning proteins and partitioning loci that function collaboratively to impose spatial and temporal controls, which ensure efficient and faithful plasmid segregation.
4. The multicopy yeast plasmid, because of its clustered organization, is effectively a single-copy entity in segregation. The Rep-*STB* partitioning system promotes a one-to-one segregation of duplicated plasmid clusters via the cohesion-mediated pairing and unpairing strategy, which is analogous to that utilized by the yeast chromosomes.
5. Cytoskeletal structures play a central role in prokaryotic genome segregation, as illustrated by the importance of the FtsZ ring in specifying the bacterial cell division septum, the ParM filament in R1 plasmid partitioning, and the MreB filament in *Caulobacter* chromosome segregation.
6. DNA topoisomerases, translocases, and site-specific recombinases contribute to genome segregation and integrity by promoting topological unlinking, chromosome dimer resolution, and copy-number maintenance.

FUTURE ISSUES TO BE RESOLVED

1. The generality of the impeccable order in organization and segregation revealed for the *Caulobacter* genome needs to be verified in other bacterial systems.
2. With the exception of the R1 plasmid system, investigations on the partitioning of bacterial plasmids such as P1 and F call for a deliberate shift in emphasis from phenomenology to mechanism.
3. The demonstrated roles for actin-like filaments in R1 plasmid and *Caulobacter* chromosome segregation demand incisive studies on the involvement of the bacterial cytoskeleton in plasmid and chromosome segregation mechanisms in general.
4. The question whether cohesin-mediated segregation of the yeast 2-micron plasmid occurs in a chromosome-tethered fashion or not awaits resolution.
5. The advent of single-molecule studies is likely to shed new light on the role of DNA motor proteins, condensins, and cohesins in ordered, rapid, and large-scale transport of chromosomes during segregation.

ACKNOWLEDGMENTS

We thank S. Austin, D.J. Sherratt, W. Margolin, J. Pogliano, and L. Shapiro for providing the movies on bacterial chromosome/plasmid segregation. We acknowledge critical comments on sections of this chapter from D. Bates, T. Hirano, W. Margolin, J. Pogliano, and M. Thanbichler. We have been supported over the years by the National Institutes of Health, National Science Foundation, Robert F. Welch Foundation, Council for Tobacco Research, Texas Higher Education Coordinating Board, and Human Frontiers in Science Program.

LITERATURE CITED

1. Breier AM, Cozzarelli NR. 2004. *Proc. Natl. Acad. Sci. USA* 101:9175–76
2. Lau IF, Filipe SR, Soballe B, Okstad OA, Barre FX, Sherratt DJ. 2003. *Mol. Microbiol.* 49:731–43
3. Sherratt DJ. 2003. *Science* 301:780–85
4. Niki H, Hiraga S. 1998. *Genes Dev.* 12:1036–45
5. Draper GC, Gober JW. 2002. *Annu. Rev. Microbiol.* 56:567–97
6. Gordon GS, Wright A. 2000. *Annu. Rev. Microbiol.* 54:681–708
7. van Helvoort JM, Woldringh CL. 1994. *Mol. Microbiol.* 13:577–83
8. Webb CD, Graumann PL, Kahana JA, Teleman AA, Silver PA, Losick R. 1998. *Mol. Microbiol.* 28:883–92
9. Dworkin J, Losick R. 2002. *Proc. Natl. Acad. Sci. USA* 99:14089–94
10. Lemon KP, Grossman AD. 2001. *Genes Dev.* 15:2031–41
11. Pease PJ, Levy O, Cost GJ, Gore J, Ptacin JL, et al. 2005. *Science* 307:586–90
12. Saleh OA, Bigot S, Barre FX, Allemand JF. 2005. *Nat. Struct. Mol. Biol.* 12:436–40
13. Ben-Yehuda S, Fujita M, Liu XS, Gorbatyuk B, Skoko D, et al. 2005. *Mol. Cell* 17:773–82
14. Sharpe ME, Errington J. 1996. *Mol. Microbiol.* 21:501–9
15. Wu LJ, Errington J. 1997. *EMBO J.* 16:2161–69
16. Bath J, Wu LJ, Errington J, Wang JC. 2000. *Science* 290:995–97

17. Viollier PH, Thanbichler M, McGrath PT, West L, Meewan M, et al. 2004. *Proc. Natl. Acad. Sci. USA* 101:9257–62
18. Higgins NP. 1999. In *Organization of the Prokaryotic Genome*, ed. RL Charlebois, pp. 189–202. Washington, DC: ASM Press
19. Pettijohn DE. 1996. In *Escherichia coli and Salmonella*, ed. FC Neidhardt, pp. 158–66. Washington, DC: ASM Press
20. Sunako Y, Onogi T, Hiraga S. 2001. *Mol. Microbiol.* 42:1233–41
21. Li Y, Sergueev K, Austin S. 2002. *Mol. Microbiol.* 46:985–96
22. Bates D, Kleckner N. 2005. *Cell* 121:899–911
23. Hauf S, Watanabe Y. 2004. *Cell* 119:317–27
24. Nasmyth K. 2002. *Science* 297:559–65
25. Uhlmann F. 2004. *Exp. Cell Res.* 296:80–85
26. Musacchio A, Hardwick KG. 2002. *Nat. Rev. Mol. Cell Biol.* 3:731–41
27. Tanaka TU. 2002. *Curr. Opin. Cell Biol.* 14:365–71
28. Wang JC. 2002. *Nat. Rev. Mol. Cell Biol.* 3:430–40
29. Hirano T. 2005. *Curr. Biol.* 15: R265–75
30. Nasmyth K. 2005. *Cell* 120:739–46
31. Kolodner RD, Putnam CD, Myung K. 2002. *Science* 297:552–57
32. Bartek J, Lukas C, Lukas J. 2004. *Nat. Rev. Mol. Cell Biol.* 5:792–804
33. Taylor SS, Scott MI, Holland AJ. 2004. *Chromosome Res.* 12:599–616
34. Nasmyth K. 2001. *Annu. Rev. Genet.* 35:673–745
35. Hagstrom KA, Holmes VF, Cozzarelli NR, Meyer BJ. 2002. *Genes Dev.* 16:729–42
36. Jessberger R. 2003. *IUBMB Life* 55:643–52
37. Haering CH, Lowe J, Hochwagen A, Nasmyth K. 2002. *Mol. Cell* 9:773–88
38. Lowe J, Cordell SC, van den Ent F. 2001. *J. Mol. Biol.* 306:25–35
39. Anderson DE, Losada A, Erickson HP, Hirano T. 2002. *J. Cell Biol.* 156:419–24
40. Gruber S, Haering CH, Nasmyth K. 2003. *Cell* 112:765–77
41. Laloraya S, Guacci V, Koshland D. 2000. *J. Cell Biol.* 151:1047–56
42. Weber SA, Gerton JL, Polancic JE, DeRisi JL, Koshland D, Megee PC. 2004. *PLoS Biol.* 2:E260
43. Ciosk R, Shirayama M, Shevchenko A, Tanaka T, Toth A, Nasmyth K. 2000. *Mol. Cell* 5:243–54
44. Toth A, Ciosk R, Uhlmann F, Galova M, Schleiffer A, Nasmyth K. 1999. *Genes Dev.* 13:320–33
45. Carson DR, Christman MF. 2001. *Proc. Natl. Acad. Sci. USA* 98:8270–75
46. Lengronne A, Katou Y, Mori S, Yokobayashi S, Kelly GP, et al. 2004. *Nature* 430:573–78
47. Uhlmann F. 2001. *EMBO Rep.* 2:487–92
48. Alexandru G, Uhlmann F, Mechtler K, Poupart MA, Nasmyth K. 2001. *Cell* 105:459–72
49. Stemmann O, Zou H, Gerber SA, Gygi SP, Kirschner MW. 2001. *Cell* 107:715–26
50. Gimenez-Abian JF, Sumara I, Hirota T, Hauf S, Gerlich D, et al. 2004. *Curr. Biol.* 14:1187–93
51. Waizenegger IC, Hauf S, Meinke A, Peters JM. 2000. *Cell* 103:399–410
52. Stegmeier F, Visintin R, Amon A. 2002. *Cell* 108:207–20
53. D'Amours D, Stegmeier F, Amon A. 2004. *Cell* 117:455–69
54. Sullivan M, Higuchi T, Katis VL, Uhlmann F. 2004. *Cell* 117:471–82
55. Higuchi T, Uhlmann F. 2005. *Nature* 433:171–76
56. Ross KE, Cohen-Fix O. 2004. *Dev. Cell* 6:729–35
57. Katis VL, Matos J, Mori S, Shirahige K, Zachariae W, Nasmyth K. 2004. *Curr. Biol.* 14:2183–96

58. Rabitsch KP, Petronczki M, Javerzat JP, Genier S, Chwalla B, et al. 2003. *Dev. Cell* 4:535–48
59. Buonomo SB, Clyne RK, Fuchs J, Loidl J, Uhlmann F, Nasmyth K. 2000. *Cell* 103:387–98
60. Kitajima TS, Miyazaki Y, Yamamoto M, Watanabe Y. 2003. *EMBO J.* 22:5643–53
61. Siomos MF, Badrinath A, Pasierbek P, Livingstone D, White J, et al. 2001. *Curr. Biol.* 11:1825–35
62. Terret ME, Wassmann K, Waizenegger I, Maro B, Peters JM, Verlhac MH. 2003. *Curr. Biol.* 13:1797–802
63. Kitajima TS, Kawashima SA, Watanabe Y. 2004. *Nature* 427:510–17
64. Rabitsch KP, Gregan J, Schleiffer A, Javerzat JP, Eisenhaber F, Nasmyth K. 2004. *Curr. Biol.* 14:287–301
65. Katis VL, Galova M, Rabitsch KP, Gregan J, Nasmyth K. 2004. *Curr. Biol.* 14:560–72
66. Hirano T. 2004. *Cell Cycle* 3:26–28
67. Maddox PS, Oegema K, Desai A, Cheeseman IM. 2004. *Chromosome Res.* 12:641–53
68. Blackwell TK, Walker AK. 2002. *Genes Dev.* 16:769–72
69. Lavoie BD, Hogan E, Koshland D. 2004. *Genes Dev.* 18:76–87
70. Yu HG, Koshland DE. 2003. *J. Cell Biol.* 163:937–47
71. Chan RC, Severson AF, Meyer BJ. 2004. *J. Cell Biol.* 167:613–25
72. Siddiqui NU, Stronghill PE, Dengler RE, Hasenkampf CA, Riggs CD. 2003. *Development* 130:3283–95
73. Lieb JD, Capowski EE, Meneely P, Meyer BJ. 1996. *Science* 274:1732–36
74. Pogliano K, Pogliano J, Becker E. 2003. *Curr. Opin. Microbiol.* 6:586–93
75. Strunnikov A. 2006. *Plasmid*. 55:135–44
76. Britton RA, Lin DC, Grossman AD. 1998. *Genes Dev.* 12:1254–59
77. Jensen RB, Shapiro L. 1999. *Proc. Natl. Acad. Sci. USA* 96:10661–66
78. Niki H, Jaffe A, Imamura R, Ogura T, Hiraga S. 1991. *EMBO J.* 10:183–93
79. Holmes VF, Cozzarelli NR. 2000. *Proc. Natl. Acad. Sci. USA* 97:1322–24
80. Sawitzke JA, Austin S. 2000. *Proc. Natl. Acad. Sci. USA* 97:1671–76
81. Weitao T, Nordstrom K, Dasgupta S. 1999. *Mol. Microbiol.* 34:157–68
82. Mascarenhas J, Soppa J, Strunnikov AV, Graumann PL. 2002. *EMBO J.* 21:3108–18
83. Soppa J, Kobayashi K, Noirot-Gros MF, Oesterhelt D, Ehrlich SD, et al. 2002. *Mol. Microbiol.* 45:59–71
84. Schleiffer A, Kaitna S, Maurer-Stroh S, Glotzer M, Nasmyth K, Eisenhaber F. 2003. *Mol. Cell* 11:571–75
85. Volkov A, Mascarenhas J, Andrei-Selmer C, Ulrich HD, Graumann PL. 2003. *Mol. Cell. Biol.* 23:5638–50
86. Hagstrom KA, Meyer BJ. 2003. *Nat. Rev. Genet.* 4:520–34
87. Hirano M, Hirano T. 2004. *EMBO J.* 23:2664–73
88. Lammens A, Schele A, Hopfner KP. 2004. *Curr. Biol.* 14:1778–82
89. Harvey SH, Sheedy DM, Cuddihy AR, O'Connell MJ. 2004. *Mol. Cell. Biol.* 24:662–74
90. Lehman AR. 2005. *DNA Repair* 4:309–14
91. Strom L, Lindroos HB, Shirahige K, Sjogren C. 2004. *Mol. Cell* 16:1003–15
92. Torres-Rosell J, Machin F, Farmer S, Jarmuz A, Eydmann T, et al. 2005. *Nat. Cell Biol.* 7:412–19
93. Shin DS, Chahwan C, Huffman JL, Tainer JA. 2004. *DNA Repair* 3:863–73
94. Engelberg-Kulka H, Glaser G. 1999. *Annu. Rev. Microbiol.* 53:43–70
95. Gerdes K, Gultyaev AP, Franch T, Pedersen K, Mikkelsen ND. 1997. *Annu. Rev. Genet.* 31:1–31

96. Greenfield TJ, Ehli E, Kirshenmann T, Franch T, Gerdes K, Weaver KE. 2000. *Mol. Microbiol.* 37:652–60
97. Gerdes K, Moller-Jensen J, Jensen RB. 2000. *Mol. Microbiol.* 37:455–66
98. Hiraga S. 2000. *Annu. Rev. Genet.* 34:21–59
99. Funnell BE, Slavcev RA. 2004. In *Plasmid Biology*, ed. BE Funnell, PJ Phillips, pp. 81–103. Washington, DC: ASM Press
100. Bork P, Sander C, Valencia A. 1992. *Proc. Natl. Acad. Sci. USA* 89:7290–94
101. Koonin EV. 1993. *J. Mol. Biol.* 229:1165–74
102. Friedman SA, Austin SJ. 1988. *Plasmid* 19:103–12
103. Mori H, Mori Y, Ichinose C, Niki H, Ogura T, et al. 1989. *J. Biol. Chem.* 264:15535–41
104. Hayes F, Radnedge L, Davis MA, Austin SJ. 1994. *Mol. Microbiol.* 11:249–60
105. Hirano M, Mori H, Onogi T, Yamazoe M, Niki H, et al. 1998. *Mol. Gen. Genet.* 257:392–403
106. Yates P, Lane D, Biek DP. 1999. *J. Mol. Biol.* 290:627–38
107. Bouet JY, Funnell BE. 1999. *EMBO J* 18:1415–24
108. Jensen RB, Dam M, Gerdes K. 1994. *J. Mol. Biol.* 236:1299–309
109. Funnell BE. 1991. *J. Biol. Chem.* 266:14328–37
110. Gordon GS, Sitnikov D, Webb CD, Teleman A, Straight A, et al. 1997. *Cell* 90:1113–21
111. Niki H, Hiraga S. 1997. *Cell* 90:951–57
112. Onogi T, Miki T, Hiraga S. 2002. *J. Bacteriol.* 184:3142–45
113. Li Y, Austin S. 2002. *Plasmid* 48:174–78
114. Ho TQ, Zhong Z, Aung S, Pogliano J. 2002. *EMBO J.* 21:1864–72
115. Erdmann N, Petroff T, Funnell BE. 1999. *Proc. Natl. Acad. Sci. USA* 96:14905–10
116. Edgar R, Chattoraj DK, Yarmolinsky M. 2001. *Mol. Microbiol.* 42:1363–70
117. Fung E, Bouet JY, Funnell BE. 2001. *EMBO J.* 20:4901–11
118. Libante V, Thion L, Lane D. 2001. *J. Mol. Biol.* 314:387–99
119. Li Y, Dabrazhynetskaya A, Youngren B, Austin S. 2004. *Mol. Microbiol.* 53:93–102
120. Moller-Jensen J, Borch J, Dam M, Jensen RB, Roepstorff P, Gerdes K. 2003. *Mol. Cell* 12:1477–87
121. Jensen RB, Gerdes K. 1999. *EMBO J.* 18:4076–84
122. Moller-Jensen J, Jensen RB, Lowe J, Gerdes K. 2002. *EMBO J.* 21:3119–27
123. Jensen RB, Lurz R, Gerdes K. 1998. *Proc. Natl. Acad. Sci. USA* 95:8550–55
124. Gerdes K, Moller-Jensen J, Ebersbach G, Kruse T, Nordstrom K. 2004. *Cell* 116:359–66
125. Yamaichi Y, Niki H. 2000. *Proc. Natl. Acad. Sci. USA* 97:14656–61
126. Lin DC, Grossman AD. 1998. *Cell* 92:675–85
127. Ireton K, Gunther NW 4th, Grossman AD. 1994. *J. Bacteriol.* 176:5320–29
128. Wu LJ, Errington J. 2003. *Mol. Microbiol.* 49:1463–75
129. Mohl DA, Easter J Jr, Gober JW. 2001. *Mol. Microbiol.* 42:741–55
130. Mohl DA, Gober JW. 1997. *Cell* 88:675–84
131. Godfrin-Estevenon AM, Pasta F, Lane D. 2002. *Mol. Microbiol.* 43:39–49
132. Kim HJ, Calcutt MJ, Schmidt FJ, Chater KF. 2000. *J. Bacteriol.* 182:1313–20
133. Lewis RA, Bignell CR, Zeng W, Jones AC, Thomas CM. 2002. *Microbiology* 148:537–48
134. Fekete RA, Chattoraj DK. 2005. *Mol. Microbiol.* 55:175–83
135. Yamaichi Y, Niki H. 2004. *EMBO J.* 23:221–33
136. Jayaram M, Mehta S, Uzri D, Voziyanov Y, Velmurugan S. 2004. *Prog. Nucleic Acids Res. Mol. Biol.* 77:127–72
137. Velmurugan S, Yang XM, Chan CS, Dobson M, Jayaram M. 2000. *J. Cell Biol.* 149:553–66
138. Scott-Drew S, Wong CM, Murray JA. 2002. *Cell Biol. Int.* 26:393–405

139. Mehta S, Yang XM, Chan CS, Dobson MJ, Jayaram M, Velmurugan S. 2002. *J. Cell Biol.* 158:625–37
140. Wong MC, Scott-Drew SR, Hayes MJ, Howard PJ, Murray JA. 2002. *Mol. Cell. Biol.* 22:4218–29
141. Huang J, Hsu JM, Laurent BC. 2004. *Mol. Cell* 13:739–50
142. Yang XM, Mehta S, Uzri D, Jayaram M, Velmurugan S. 2004. *Mol. Cell. Biol.* 24:5290–303
143. Mehta S, Yang XM, Jayaram M, Velmurugan S. 2005. *Mol. Cell. Biol.* 25:4283–98
144. McBride AA, McPhillips MG, Oliveira JG. 2004. *Trends Microbiol.* 12:527–29
145. Michie KA, Löwe J. 2006. *Annu. Rev. Biochem.* 75:467–92
146. Gitai Z, Dye NA, Reisenauer A, Wachi M, Shapiro L. 2005. *Cell* 120:329–41
147. Margolin W. 2003. *Curr. Biol.* 13:R16–18
148. Lutkenhaus J. 2002. *Curr. Opin. Microbiol.* 5:548–52
149. Espeli O, Marians KJ. 2004. *Mol. Microbiol.* 52:925–31
150. Nurse P, Levine C, Hassing H, Marians KJ. 2003. *J. Biol. Chem.* 278:8653–60
151. Barre F-X, Sherratt DJ. 2002. In *Mobile DNA II*, ed. NL Craig, R Craigie, M Gellert, AM Lambowitz, pp. 149–61. Washington, DC: ASM Press
152. Massey TH, Aussel L, Barre FX, Sherratt DJ. 2004. *EMBO Rep.* 5:399–404
153. Weiss DS. 2004. *Mol. Microbiol.* 54:588–97
154. Espeli O, Lee C, Marians KJ. 2003. *J. Biol. Chem.* 278:44639–44
155. Futcher AB. 1986. *J. Theor. Biol.* 119:197–204
156. Lim GE, Derman AI, Pogliano J. 2005. *Proc. Natl. Acad. Sci. USA* 102:17658–63
157. Ebersbach G, Gerdes K. 2005. *Annu. Rev. Genet.* 39:453–79
158. Schumacher MA, Funnell BE. 2005. *Nature* 438:516–19
159. Gordon S, Rech J, Lane D, Wright A. 2004. *Mol. Microbiol.* 51:461–69
160. Wang X, Possoz C, Sherratt DJ. 2005. *Genes Dev.* 19:2367–77
161. Ivanov D, Nasmyth K. 2005. *Cell* 122:849–60

Annual Review
of Biochemistry

Volume 75, 2006

Contents