Closing the Ring: A New Twist to Bacterial Chromosome Condensation

Martin Thanbichler[1,*]
[1]Independent Junior Research Group Prokaryotic Cell Biology, Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Straße, D-35043 Marburg, Germany
*Correspondence: thanbichler@mpi-marburg.mpg.de
DOI 10.1016/j.cell.2009.04.055

The mechanisms underlying chromosome organization in bacteria are still shrouded in mystery. Sullivan et al. (2009) and Gruber and Errington (2009) now report that the DNA-binding protein ParB ensures proper arrangement and partitioning of chromosomal DNA in *Bacillus subtilis* by recruiting the condensin SMC to the replication origin region.

Proper segregation and packaging of DNA is a prerequisite for generating viable offspring during cell division, and bacteria and eukaryotes have evolved very different mechanisms to achieve this goal. Bacteria typically have one circular chromosome, whose replication is initiated at a single origin. Segregation of the newly synthesized sister chromosomes occurs during the course of replication and involves a mechanism similar to mitosis in which the origin regions are actively segregated (Thanbichler and Shapiro, 2006). Recent work suggests that origin segregation is mediated by type I DNA partitioning systems, which consist of the Walker ATPase ParA (Soj in *Bacillus subtilis*), the DNA-binding protein ParB (Spo0J in *B. subtilis*), and *parS* sites that are found near the chromosomal origin of replication (Ebersbach and Gerdes, 2005). Upon binding to *parS* sites, ParB spreads into the flanking regions, forming nucleoprotein filaments that encompass several kilobases of DNA. These centromere-like complexes then recruit ParA, which is thought to assemble into dynamic polymeric structures that mediate segregation. The bulk of the chromosome then follows as a consequence of DNA condensation. The reports by Sullivan et al. (2009) and Gruber and Errington (2009) in this issue integrate an important new player into this scheme. They show that Spo0J recruits the structural maintenance of chromosomes (SMC) complex to the origin regions to facilitate proper partitioning and organization of chromosomal DNA.

Although bacteria do not have histones and apparently lack higher-order chromatin structure, their chromosomes nevertheless display a conserved architecture, in which the subcellular location of individual loci directly correlates with their position on the circular chromosomal map (Viollier et al., 2004). This defined structure points to the existence of factors that help arrange chromosomal DNA within the cell. A strong candidate for such a factor is the bacterial SMC complex, based on the fact that its inactivation causes severe defects

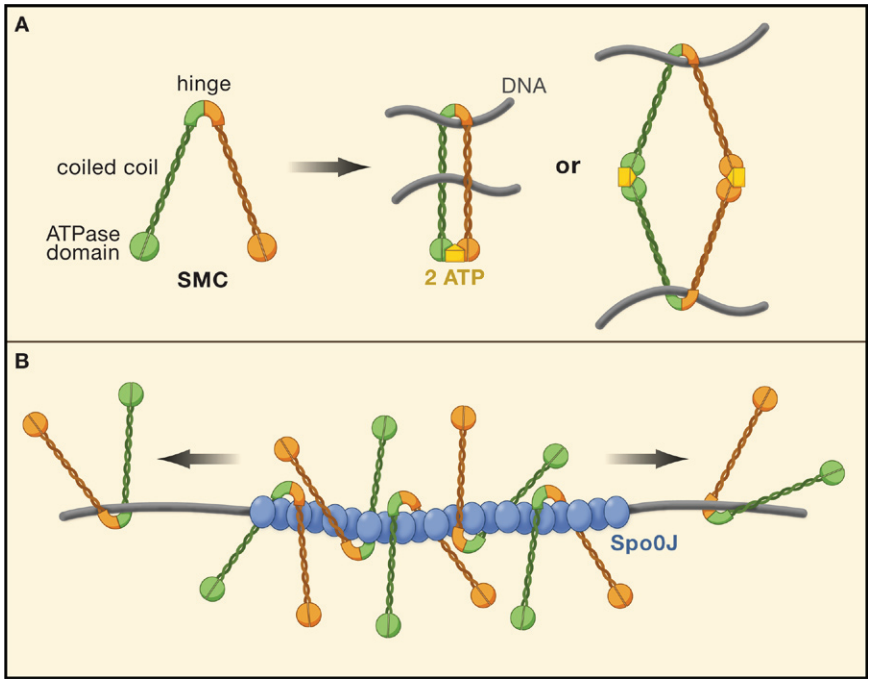

Figure 1. The Recruitment and Function of SMC
(A) Mechanism of DNA condensation by the structural maintenance of chromosomes (SMC) complex. SMC forms V-shaped dimers, whose two arms are connected by a flexible hinge. After association of the hinge region with DNA, the two head domains can interact in an ATP-dependent manner, forming a ring-like structure that embraces segments of double-stranded DNA. Engagement of the head domains from different molecules results in the formation of larger rings or more complex structures, such as rosettes and filaments (Hirano, 2006).
(B) Model for the recruitment of SMC to the replication origin region in *B. subtilis*. Spo0J interacts with origin-proximal *parS* sites and spreads into the flanking chromosomal regions, giving rise to large nucleoprotein filaments. SMC is specifically recruited to these complexes and then becomes distributed over the replication origin region by lateral diffusion (Gruber and Errington, 2009; Sullivan et al., 2009).

in DNA compaction and segregation (Britton et al., 1998). In eukaryotes, SMC proteins are involved in a variety of processes, such as sister-chromatid cohesion, mitotic DNA condensation, recombination, and X chromosome dosage compensation. Most bacteria contain a single type of SMC protein, which forms flexible, V-shaped homodimers that associate with the two regulatory factors ScpA and ScpB (Hirano, 2006). These basic units are thought to assemble into ring- or rosetta-shaped structures, acting as molecular clamps that interconnect different regions of the chromosome or stabilize them in a twisted conformation (Figure 1). Consistent with a role in DNA organization, bacterial SMC complexes localize to discrete sites within the cell (Mascarenhas et al., 2002). However, their precise function and the mechanism coordinating their condensing activity with chromosome dynamics have remained unknown.

The work from the Rudner and Errington laboratories now demonstrates that SMC is specifically targeted to the replication origin region of the *B. subtilis* chromosome, using Spo0J nucleoprotein filaments as loading zones. Both groups observe that the clusters formed by SMC and Spo0J are frequently overlapping or adjacent to each other. This pattern of SMC localization is disrupted in a *spo0J* mutant. Indicative of a direct interaction between the two proteins, SMC copurifies with Spo0J (Gruber and Errington, 2009) and in vitro SMC shows a clear binding preference for DNA that is covered with Spo0J (Sullivan et al., 2009). Using genome-wide analysis, SMC is found to be markedly enriched in the chromosomal origin region, with distinct peaks in the vicinity of *parS* sites (Gruber and Errington, 2009). Upon inactivation of Spo0J, occupancy of these hot spots is reduced to background levels, indicating a direct role for Spo0J in the recruitment of SMC. In support of this notion, mutation of individual *parS* sites weakens the association of SMC with the corresponding chromosomal region. Conversely, insertion of ectopic *parS* sites creates new binding hot spots for SMC (Gruber and Errington, 2009), accompanied by the appearance of Spo0J-SMC clusters with aberrant localization (Sullivan et al., 2009). Interestingly, Gruber and Errington (2009) reveal that SMC is enriched not only at *parS* sites but also at highly transcribed genes, such as those encoding ribosomal proteins and tRNAs. However, these interactions are largely insensitive to defects in Spo0J, indicating that they are mediated by a thus far unknown, Spo0J-independent pathway.

Why is SMC specifically targeted to the region containing the replication origin? Previous work has shown that *spo0J* mutants only display a minor defect in DNA segregation, as determined by the sporadic production of anucleate cells (Ireton et al., 1994). This finding suggests that nonspecific association of SMC with the chromosome, mediated by its intrinsic DNA-binding activity, is largely sufficient for normal growth. However, by analyzing the positioning of chromosomal loci within sporulating *B. subtilis* cells, Sullivan et al. (2009) reveal that in the absence of Spo0J, a large (~1 Mbp) region surrounding the replication origin is severely disorganized. The same effect is observed in cells that produce a nonspreading variant of Spo0J or in cells that lack all origin-proximal *parS* sites. Thus, the interaction of SMC with Spo0J complexes appears to be required for proper condensation of the replication origin region. Interestingly, a single *parS* site is sufficient to ensure wild-type chromosome structure (Sullivan et al., 2009), suggesting that SMC uses Spo0J nucleoprotein filaments as entry points to the chromosome and then spreads over the origin region by lateral diffusion. Given that Spo0J is essential for cells that express a hypomorphic derivative of SMC (Gruber and Errington, 2009), a certain minimum level of SMC activity at the origin, and thus origin organization, seems to be necessary for cell survival. Consistent with this notion, Sullivan et al. (2009) observe that transplantation of *parS* sites from the origin to the region where replication terminates results in dramatic defects in chromosome organization and segregation, as reflected by a high incidence of anucleate cells and entrapment of chromosomal DNA in the division septum.

Whereas mutation of Spo0J disrupts the organization of about one quarter of the *B. subtilis* chromosome, inactivation of Soj (ParA) only affects positioning of a discrete region located next to the replication origin (Sullivan et al., 2009). Soj is therefore likely to cooperate with Spo0J to actively partition the origin regions. Condensation of newly synthesized DNA, coordinated by the loading of SMC onto the separated origin regions, might then drive segregation of the rest of the two sister chromosomes. In addition to its role in chromosome segregation, Soj has also been implicated in replication initiation (Murray and Errington, 2008). The new work suggests that Spo0J is equally multitalented, acting at the interface of DNA replication, segregation, and condensation in *B. subtilis*. Gruber and Errington (2009) succeed in isolating mutant forms of Spo0J that are defective in either the Soj- or SMC-dependent pathway, suggesting that the different functions of Spo0J are independent of each other. It remains to be clarified whether Spo0J allows regulatory crosstalk between the molecular systems that it connects. Moreover, it will be interesting to see whether its homologs have the same dual role in other bacteria.

The work of Sullivan et al. (2009) and Gruber and Errington (2009) bring us considerably closer to understanding chromosome organization in bacteria. However, the molecular mechanisms responsible for recruitment of SMC to Spo0J nucleoprotein filaments and highly transcribed genes are still unknown. Moreover, it is unclear how SMC organizes chromosomal DNA and how its condensing activity is coordinated with DNA replication, transcription, and other processes involved in chromatin remodeling. Resolving these issues will be a challenging but highly rewarding task for the future.

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Shedding UV Light on Alternative Splicing

Matthew S. Marengo[1,2,*] and Mariano A. Garcia-Blanco[1,2,3,*]
[1]Department of Molecular Genetics and Microbiology
[2]Center for RNA Biology
[3]Department of Medicine
Duke University Medical Center, Durham, NC 27710, USA
*Correspondence: matt.marengo@duke.edu (M.S.M), garci001@mc.duke.edu (M.A.G.-B.)
DOI 10.1016/j.cell.2009.04.054

After DNA damage, cells modulate pre-messenger RNA (pre-mRNA) splicing to induce an anti- or proapoptotic response. In this issue, Muñoz et al. (2009) uncover a cotranscriptional mechanism for activating alternative pre-mRNA splicing after ultraviolet irradiation that depends unexpectedly on hyperphosphorylation of the RNA polymerase II C-terminal domain and decreased rates of transcription elongation.

In response to a genotoxic insult, normal eukaryotic cells activate the DNA-damage response, which includes programs that mediate DNA repair and apoptosis. For decades, research on the DNA-damage response has focused on signaling kinases, the targets of transcription factors, and transcriptional regulation. More recently, however, it has been shown that alternative pre-messenger RNA (pre-mRNA) splicing is also a target of the DNA-damage response (Katzenberger et al., 2006; Matsuoka et al., 2007). In this issue of *Cell*, Muñoz et al. (2009) describe a new mechanism for genotoxicity-induced alternative splicing that takes a shortcut around the DNA-damage response to target RNA polymerase II (RNAPII), the enzyme that synthesizes pre-mRNA.

Muñoz et al. show that ultraviolet (UV) radiation changes the phosphorylation state of the carboxy-terminal repeat domain (CTD) of RNAPII (Figure 1). Using single-locus imaging by fluorescence recovery after photobleaching, the authors conclude that transcription elongation is slower in irradiated cultured human cells. This change in the RNAPII elongation rate seems to affect the RNA available for cotranscriptional splicing (Goldstrohm et al., 2001). This results in alternative splicing of the *BCL-X* and *CASPASE 9* pre-mRNAs, leading to a proapoptotic response. Alternative splicing of transcripts from these genes appears to occur independently of DNA-damage response signals that are known to play cotranscriptional or posttranscriptional regulatory roles. For example, *BRCA1*, originally identified as a proto-oncogene in breast cancer, encodes a protein that acts as a critical scaffold for DNA lesion detection and repair. At a DNA lesion, BRCA1 binds directly to the BRCA1-associated RING domain protein (BARD1), a ubiquitin ligase. BARD1 in turn can block RNAPII from interacting with the mRNA polyadenylation factor CstF and also marks RNAPII for degradation (Kim et al., 2006 and references therein) (Figure 1). This could affect steady-state mRNA levels of genes undergoing transcription. However, Muñoz et al. find that neither depleting BARD proteins nor changing the polyadenylation signals on RNA transcripts modulates a UV-induced splicing event. A broadly specific pharmacological block of the DNA-damage response signaling kinases, ATM and ATR, which are known to phosphorylate splicing factors (Matsuoka et al., 2007), also had no effect. Importantly, Muñoz et al. show that the effect of UV irradiation on alternative splicing is independent of p53, a central regulator of DNA-damage response-induced gene expression. Together, these results suggest the existence of a previously unidentified signaling pathway that modulates alternative splicing in response to DNA damage.

The pleiotropic and incomplete effects of the pharmacological inhibition and the small-interfering RNA-mediated depletion of DNA-damage response proteins leave room for alternative explanations. Using a clever chemical genetics approach, however, the investigators were able to demonstrate a role for the RNAPII CTD in the modulation of alternative splicing. They poisoned the activity of endogenous RNAPII in cultured human cells with the toxin α-amanitin and introduced into the same cells α-amanitin-