Dispatches

Chromosome Organization: Original Condensins

Two new studies reveal the main actors involved in the resolution and segregation of newly replicated origins in bacteria. These results have important implications for our understanding of the mechanisms involved in precisely coordinating chromosome organization, segregation and replication.

Diego I. Cattoni, Antoine Le Gall, and Marcelo Nöllmann*

In eukaryotes and bacteria, chromosome organization and segregation need to be carefully orchestrated to ensure faithful transmission of the genetic material during cell division. The molecular mechanisms responsible for bacterial chromosome organization and segregation remain elusive, possibly because these processes are highly influenced by the action of many other processes operating on DNA that overlap in time and space (e.g., replication, transcription, or repair).

Bacterial chromosomes are condensed mainly by negative supercoiling and by histone-like, nucleoid-associated proteins (NAPs), segmented into ~10–20 kbp-sized topologically insulated supercoiled micro-domains [1], and further organized into large (500 kbp–1 Mbp), genetically insulated macrodomains that impose specific dynamics and segregation rules on the genes they carry [2]. Two reports in this issue of *Current Biology* investigate the main actors that coordinate the resolution and segregation of newly replicated origins [3,4].

Structural maintenance of chromosome (SMC) complexes are widespread from bacteria to higher eukaryotes, and may be implicated in the organization of topological and macro domains. In eukaryotes, SMC complexes play different roles depending on their protein composition: cohesins are responsible for holding sister chromatids together after replication, whereas condensins ensure faithful segregation through compaction and organization of sister chromatids at the late stages of mitosis [5]. Different cohesin and condensin sub-complexes play distinct cellular functions, such as the regulation of chromatin structure through the formation of specific long-range DNA–DNA interactions, or the regulation of transcription. Most major branches of bacteria possess canonical SMC kleisin-like complexes composed of two SMC subunits, a kleisin (ScpA) and a third factor (ScpB) (Figure 1). SMC–ScpAB complexes are essential for chromosome segregation and condensation [6,7].

Most bacteria with SMC–ScpA-like complexes also encode a partitioning system with three essential components: a centromere site (parS), a centromere-binding protein (ParB), and a Walker-type ATPase (ParA). Studies have demonstrated that SMC–ScpAB complexes localize to the origin of replication region in a manner dependent on ParB bound to parS [8,9]. These studies further suggested a link between chromosome organization and segregation by proposing that the specific localization of SMC–ScpAB may be responsible for the proper organization and segregation of the origin region. The reports by Wang *et al.* [3] and Gruber *et al.* [4] in this issue provide important new insights into the mechanism by which SMC–ScpAB/parABS form a kinetochore-like complex that specifically segregates newly replicated origins and influence the segregation of the remainder of the chromosome (Figure 1).

Pioneering studies showed that cells with SMC-null mutations display major chromosome segregation defects and are only viable at low temperatures or slow growth rates [6]. Even under these conditions, however, cells show aberrant and highly heterogeneous nuclear morphologies hampering previous attempts to determine the nature of these defects. Wang *et al.* [3] and Gruber *et al.* [4] developed new strategies to obtain conditional/degradable SMC–ScpAB alleles that allowed, for the first time, the study of the precise role of prokaryotic condensin in chromosome organization and segregation.

Cells with depleted levels of SMC–ScpAB components grow like wild-type cells in minimal medium independently of temperature but fail to grow at all in rich medium [4]. More importantly, absence of functional SMC during rapid growth leads to an inability of cells to separate and segregate newly replicated origins [3,4]. Ultimately, this defect results in abnormal segregation of the nucleoid and the guillotining of unsegregated nuclei [3].

Interestingly, this phenotype remains unchanged when growth rate is reduced by partial inhibition of translation or transcription but can be differentially relieved either by stimulating the stringent response or by depletion of the cellular pool of nucleotide precursors [4]. These results suggest that SMC–ScpAB is most needed to resolve and segregate newly replicated origins when there is a large number of firing origins and in conditions of fast replisome progression. But then what is holding replicated origins together in the absence of SMC–ScpAB?

The opening of the two DNA strands during replication leads to the production of compensatory positive supercoiling ahead of the fork (resolved by gyrase and topoisomerase I) and pre-catenanes behind (decatenated by topoisomerase IV) [10]. Thus, SMC–ScpAB may be required to recruit topoisomerase IV (topo IV) to decatenate newly replicated chromosomes before active segregation can proceed. Surprisingly, this is not the case in *Bacillus subtilis*, as origins can be resolved and segregated in the absence of functional topo IV, suggesting that SMC–ScpAB-dependent origin resolution does not require the specific recruitment and unlinking activity of topo IV [3]. In stark contrast, MukBEF, the *Escherichia coli* condensin, seems to work by an entirely different mechanism. MukBEF gets recruited to replication origins by an unknown mechanism [11], where it physically interacts with topo IV and seems to form a higher-order protein scaffold that organizes and

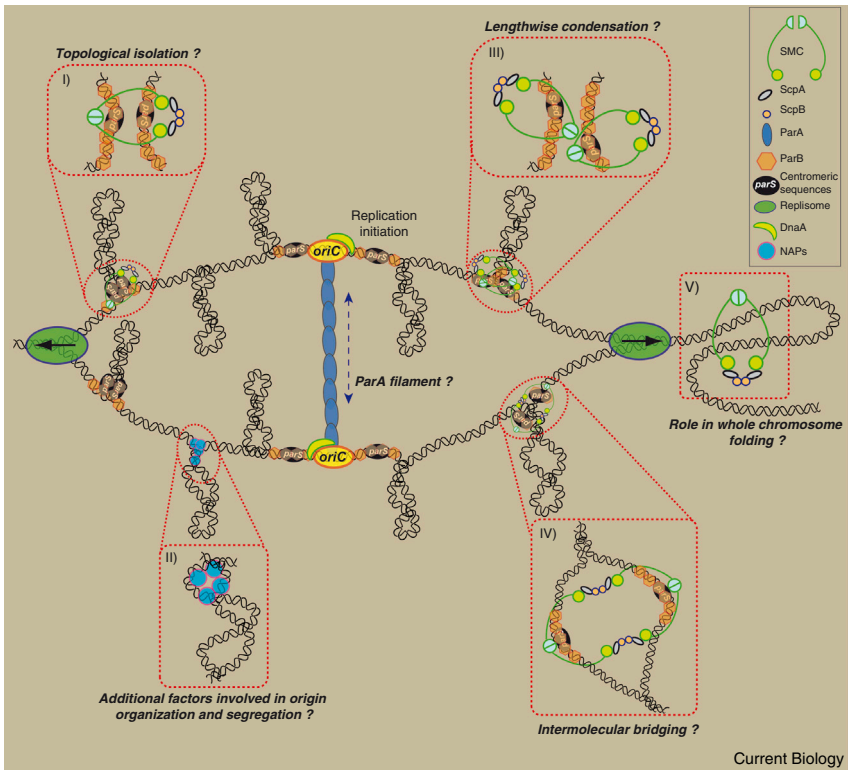

Figure 1. The main actors involved in the organization and segregation of replicated origins.
The SMC–ScpAB complex is specifically recruited to the origin of replication (oriC) by ParB, which binds specifically to centromere-like sequences (ParS sites) clustering around oriC. The ATPase activity of ParA is stimulated by ParB. ParA is required for chromosome segregation and the regulation of DNA replication initiation [18]. SMC–ScpAB/ParABS are required for the resolution of replicated origins, and may play other roles in the isolation of topological domains (panels I and IV), and the folding and segregation of the bulk of the chromosome (panels III and V). Other factors, such as nucleoid-associated proteins (NAPs), may also participate in these functions (panel II). The mechanism by which ParA may pull origins apart is not well understood and several competing models exist [20].

decatenates newly replicated DNA [12]. More studies will be required to establish whether other topoisomerases may be able to compensate for the lack of topo IV in *B. subtilis* (as was observed in *E. coli*) or whether SMC–ScpAB and MukBEF play different roles in chromosome segregation. This latter possibility would not be surprising, as MukBEF is restricted to a small subset of γ-proteobacteria, and *E. coli* does not seem to encode a parABS partition system. Overall, these results indicate that SMC–ScpAB acts by a mechanism involving the capture and the organization of newly replicated origins, facilitating the removal of pre-catenanes at high replication fork velocities [3,4]. But is this activity restricted to the origins?

Notably, in the absence of SMC–ScpAB, loci located far from the origin display several individual clusters that group together on the same side of the nucleus, whereas loci on either chromosomal arm localize on opposite sides [3]. These results indicate that, in the absence of SMC–ScpAB, DNA synthesis continues but replicated loci fail to segregate and intermingle in a left-ori-right orientation. Interestingly, these replicated (yet unsegregated) loci can be properly segregated if replication is halted [3], indicating that segregation of the bulk of the chromosome can proceed in the absence of both SMC–ScpAB and active replication. Importantly, this segregation process requires the ParA motor, suggesting a key role of the parABS partition system in chromosome segregation in the absence of condensin. Consistent with this idea, ParB is essential for origin resolution and normal nucleoid morphology in cells with low SMC levels [3,13]. Moreover, cells lacking both ParA and SMC–ScpAB are unable to resolve origins and display major defects in chromosome segregation [3,13]. Thus, the parABS system is not only required for the recruitment of SMC–ScpAB to the origin region, but seems to work together with condensin in segregating newly replicated origins, and may contribute to bulk chromosome segregation.

Previous studies suggested that bacterial chromosome segregation was driven by entropy maximization [14]. However, it is now clear that DNA segregation is not left to chance, but is rather carefully orchestrated by large and specialized ATP-fueled machineries that tightly couple DNA segregation to replication and cell division: segregation of the terminus region is coupled to septal closure by FtsK/SpoIIIE-like motors [15–17], while the synergistic action of SMC–ScpAB/ParABS complexes may be responsible for the coordination of origin resolution and segregation with the initiation of DNA replication [18]. But how is the remainder of the chromosome segregated? An interesting proposal suggests that condensin complexes may be required to segregate the bulk of the chromosome by a process involving lengthwise condensation [19]. Future research will be required to refute or validate this model and to dissect the molecular mechanisms by which large, ATP-fueled molecular complexes cooperate to organize and segregate chromosomes.

The reports by Wang *et al.* [3] and Gruber *et al.* [4] are important in that they unveil the main actors involved in the organization and segregation of replicated origins. Despite these advances, many critical questions remain unanswered: how does the separation of replicated origins lead to the segregation of the bulk of the chromosome and what factors (NAPs? supercoiling?) may be involved in this process? Do ParABS/SMC–ScpAB define a functional macro-domain? Is this organization required to regulate replication initiation? Does SMC–ScpAB play a role in the isolation of topological domains? Are ParABS/SMC–ScpAB complexes also involved in tethering replicated origins to their new quarter positions? Is this tethering important to organize or drive the segregation of the bulk of the chromosome? Resolving these questions will be challenging but will constitute a fundamental keystone to

understanding the full life cycle of bacterial chromosomes.

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Department of Single-Molecule Biophysics, Centre de Biochimie Structurale, CNRS UMR5048, INSERM U1054, Universités Montpellier I et II, 29 rue de Navacelles, 34090 Montpellier, France.
*E-mail: marcelo.nollmann@cbs.cnrs.fr

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Spatial Mapping: Graded Precision of Entorhinal Head Direction Cells

Representation of head direction in medial entorhinal cortex shows a gradient of precision, from high directional precision dorsally to low ventrally; this parallels the gradient of spatial scale in place and grid cells, and suggests that the brain constructs spatial maps of varying resolution, perhaps to serve different requirements.

Kate Jeffery

The medial temporal cortex of the brain, which includes the hippocampus and associated structures, is well known to have a specialised role in spatial cognition. This navigation system collects together information about head direction, travel distance and context, in order to construct a representation of an individual's current location and heading [1]. This representation culminates in the focal firing patterns, or 'place fields', of the hippocampal place cells, which are sometimes thought of as a map which serves both to guide navigation and to store/retrieve memories. Early studies of place cells revealed a gradient of spatial scale, with small place fields in the dorsal-most regions of hippocampus, and large fields ventrally [2,3]. More recently [4], the Moser lab discovered that an important cortical input to the place cells, the grid cells in dorso-medial entorhinal cortex (MEC), also shows spatial scaling. Grid cells produce multiple, often evenly-spaced firing fields arranged in a hexagonal close-packed array, with a characteristic orientation in a given environment, and a characteristic spacing for a given cell (or set of cells); they may provide an estimation of distance travelled — path integration — for place cells. Just as with place cells, grid spacing (scale) increases markedly from around 30 cm dorsally to some metres ventrally [5,6]. Now, as they report in this issue of *Current Biology* [7], the Moser lab has discovered that head direction cells in this same region of MEC also show a gradient of precision, with a range of tuning curves dorsally but only the broader tuning curves ventrally.

Giocomo *et al.* [7] analysed large numbers of medial entorhinal neurons in both rats and mice for directional firing preference. As Sargolini *et al.* [8] have also reported previously, head direction cells were found throughout the MEC in layers III, V and VI (not layer II). In layer III (but not V/VI), the authors observed a clear gradient, or topography, of directional precision, with sharply tuned cells being found only dorsally. The gradation in tuning was observed to be continuous, unlike that for grid cells in which the increase in scale from dorsal to ventral occurs in stepped transitions that imply a modular organisation [9]. This large sample also confirmed the absence of clustering of directional firing; directional firing preferences were distributed evenly around the 360 degrees, even though grids — the presumed major recipient of head direction information — have six-fold rotational symmetry.

The finding of a continuous gradation of directional tuning raises questions about the network interactions