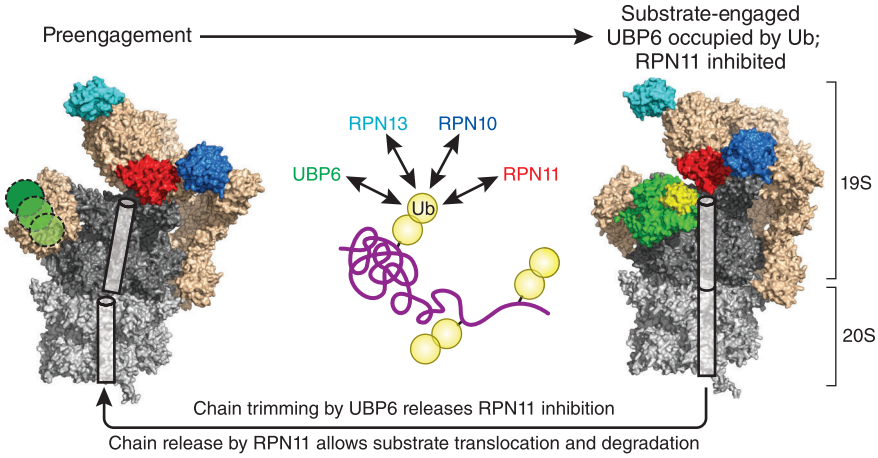

Figure 1 Deubiquitination by RPN11 and UBP6 coordinates the cycle of substrate processing. Shown are proteasome structures based on pseudoatomic models from PDB 4CR2 (left) and 5A5B (right)[2,13]. Left, before substrate engagement, the 19S RP is characterized by off-axial positioning of the AAA ATPases (dark gray) with respect to the 20S core particle (light gray). Apo-UBP6 probably occupies a range of flexible positions (indicated by green circles). Right, substrate engagement is accompanied by alignment of the translocation channel and repositioning of RPN11 (red) at its entrance. UBP6 (green) bound to ubiquitin (Ub, yellow) stabilizes this substrate-engaged conformation and inhibits RPN11 by steric hindrance. A new round of substrate engagement requires ubiquitin removal and disengagement of UBP6. Center, ubiquitins attached to substrate are subject to competing interactions with ubiquitin receptors (RPN13 and RPN10) and deubiquitinases (UBP6 and RPN11) in the proteasome. The nature of the ubiquitin signals and how they are arranged on the substrate are likely to affect the speed of degradation[16].

polyubiquitin signals associated with most substrates into simpler forms for RPN11 to process. Chain trimming by UBP6 also releases the substrate from ubiquitin receptors. As long as UBP6 is occupied by ubiquitin, the substrate will not be ready for RPN11 and final release into the proteolytic chamber. Such a mechanism would promote the efficiency of ubiquitin recycling, at a cost of slower degradation overall. An alternative (but not mutually exclusive) model is that UBP6 disassembles free polyubiquitin that has been released by RPN11 *en bloc* from the substrate; until those ubiquitins are cleared from the proteasome, the ATPases will not engage the next substrate.

Native proteasome substrates[16] have ubiquitin signals that are more diverse and more complex than those tested by Bashore *et al.*[3]. Thus, it is also possible that for a subset of substrates, UBP6 functions to 'proofread' the ubiquitin signal and to rescue poorly ubiquitinated substrates from degradation, through its noncatalytic inhibitory function. The human ortholog, USP14, has been proposed to be a therapeutic target whose inhibition may promote protein degradation in neurodegenerative diseases[9], and thus it is important to sort out the functional effects of USP14 inhibition on different proteasome substrates. It is also worth noting that in *Schizosaccharomyces pombe* and organisms that are more complex, a third, highly conserved DUB resides on the proteasome and is a constitutive subunit of the 19S RP[17]. This DUB, UCH37, is absent in budding yeast, and its later arrival during evolution may reflect a response to additional layers of substrate complexity[18]. Nonetheless, even for the 'simpler' *Saccharomyces cerevisiae* proteasome containing only UBP6 and RPN11, these recent studies offer compelling evidence that the interplay between proteasome DUBs helps to govern the timing of substrate processing.

COMPETING FINANCIAL INTERESTS
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SMC condensin: promoting cohesion of replicon arms

Frank Bürmann & Stephan Gruber

Two studies using chromosome conformation capture (3C) analyses in the Gram-positive bacterium *Bacillus subtilis* have revealed a global pattern of chromosome organization that originates from loading sites of the Smc–ScpAB complex. Loading Smc–ScpAB at a single genomic location is sufficient to promote genome-wide folding of DNA into a well-defined structure.

Ever since the first morphological description of chromosomes by Walther Flemming and others in the second half of the nineteenth century, generations of biologists have been fascinated by mitotic chromosomes' distinctive shapes and have wondered how they might form and be internally organized. Condensin, a multisubunit SMC protein complex whose activity has recently been reconstituted from purified components[1], has a key role in shaping mitotic and meiotic chromosomes[2]. The mechanistic basis for the formation of rod-shaped chromosomes, however, is still largely enigmatic.

Understanding of bacterial and eukaryotic chromosome organization has progressed markedly over the last decade, driven by technological innovations such as 3C and live-cell imaging. Two new reports provide hints regarding the underlying molecular mechanisms of bacterial chromosome

Frank Bürmann and Stephan Gruber are at the Chromosome Organization and Dynamics Research Group, Max Planck Institute of Biochemistry, Martinsried, Germany.
e-mail: sgruber@biochem.mpg.de

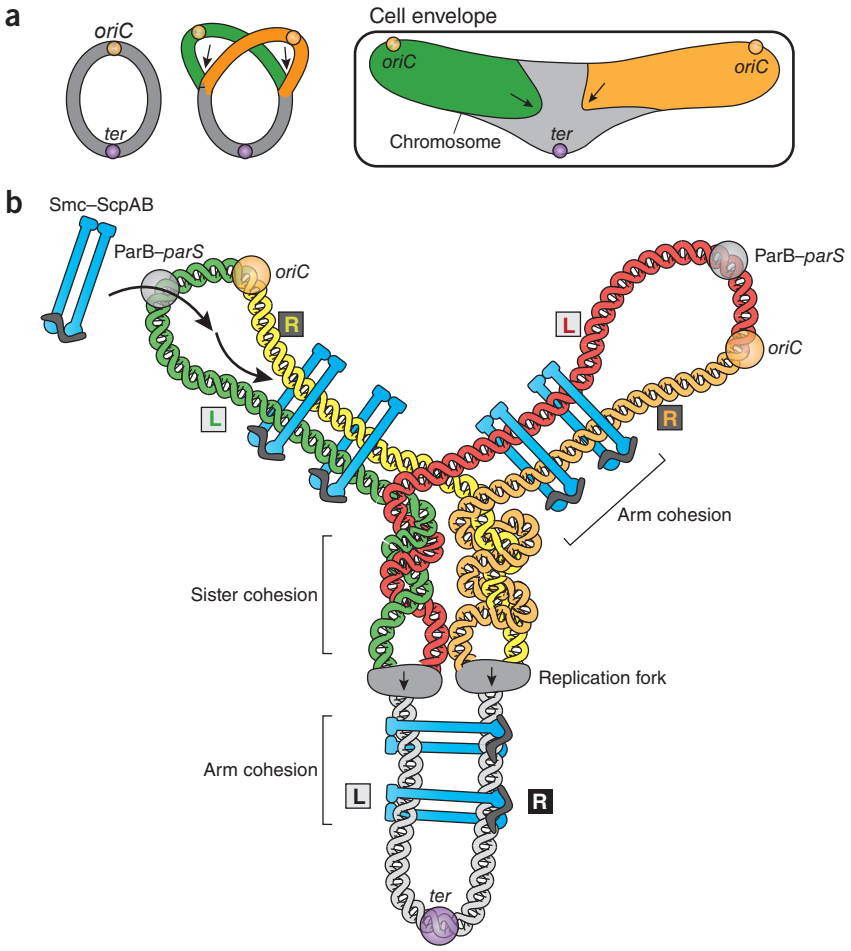

Figure 1 Organization of the replicating chromosome in *B. subtilis*. (**a**) Replicated origins segregate toward the cell poles, whereby the arms of each nascent chromosome are juxtaposed within one half of the cell. *oriC*, replication origin; *ter*, termination site. (**b**) Loading of the Smc–ScpAB complex at ParB–*parS* sites promotes juxtaposition of emerging chromosome arms. Smc–ScpAB may migrate along large DNA loops or actively extrude them, thereby promoting the separation of sister DNA molecules and the alignment of chromosome arms. Left and right arms are labeled L and R, respectively.

organization, which may well be relevant to the analogous process in eukaryotes[3,4].

In most bacteria, replicating chromosomes are organized in a longitudinal fashion, with the replication origins positioned at opposite ends of elongating chromosomes. Loci on the left and right arms of a nascent chromosome are frequently juxtaposed and arranged linearly between the polar replication origin and the more centrally located replication terminus[5–9] (**Fig. 1a**). In *Caulobacter crescentus*, two systems have been implicated in setting up the longitudinal configuration of the chromosome: ParABS and Smc–ScpAB[6,7]. ParABS is encoded in most bacterial genomes and comprises three components: ParA and ParB proteins and the *cis*-acting *parS* sites. ParB, a helix-turn-helix protein, binds the 16-bp *parS* DNA sequences located near the replication origin. Together with the ParA ATPase, the ParB–*parS* complex ensures the proper bipolar positioning of replication origins and thus promotes bacterial chromosome segregation[10].

At least in *B. subtilis* and *Streptococcus pneumoniae*, the ParB–*parS* nucleoprotein complex has another ParA-independent function: it loads the Smc–ScpAB complex onto the chromosome, near the replication origin[11–14].

3C techniques determine the relative proximity of pairs of DNA segments within a cell[15]. Using a high-throughput derivative of the 3C assay (HiC), Marbouty *et al.*[4] and Wang *et al.*[3] have confirmed the longitudinal folding of the *B. subtilis* chromosome and have dissected its genetic requirements. Mutations in ParABS or Smc–ScpAB eliminate the pronounced juxtaposition of the two replicon arms, concordantly with previous studies performed in *C. crescentus*[3,4,6,7]. Surprisingly, deletion of *parA* has little or no effect on the global organization of chromosomes, thus implying that ParB–*parS* acts in chromosome organization by loading Smc–ScpAB onto chromosomes, rather than by using the ParABS system[3]. Intriguingly, the presence of a single *parS* site is necessary and sufficient for the global folding of a chromosome, regardless of its genomic location. The position of *parS*, however, defines the apex of the chromosome, with its flanking sequences being juxtaposed by Smc–ScpAB[3,7]. Clearly, loading of Smc–ScpAB at *parS* can set up global chromosome organization in a way that is largely independent of the origin and directionality of DNA replication or the orientation of transcription units[3].

How might the pattern of chromosome folding—nucleated at *parS* sites by the loading of Smc–ScpAB—propagate to distant locations of the chromosome? Possibly, the Smc–ScpAB complex itself could translocate from *parS* toward the terminus region, thus bringing together distant loci on the two arms of the chromosome (**Fig. 1b**). Smc–ScpAB forms a highly elongated structure with an annular topology[16]; it presumably acts as a molecular DNA clamp, keeping selected pairs or groups of DNA fibers in proximity[14,17,18]. The chromatin immunoprecipitation–DNA microarray (ChIP-on-chip) profile of Smc in *B. subtilis* displays a fairly broad distribution over the bacterial chromosome, although most if not all Smc–ScpAB is loaded onto the chromosome in a ParB–*parS*–dependent manner[11,14]. Conceivably, Smc–ScpAB might capture a loop of DNA at *parS* and then move along the DNA toward the terminus by extruding flanking DNA through its ring[19] (**Fig. 1b**). Yeast cohesin—a related eukaryotic SMC complex—is indeed able to relocate from its loading site at centromeres to locations on the chromosome arms several tens of kilobases away[20]. A DNA loop–extrusion mechanism could explain how Smc–ScpAB may bring together distant DNA segments located on opposite arms of the chromosome but at the same time avoid capturing sister DNA molecules. The movement along DNA would also be helpful in detecting any linkages between sister DNAs, in the form of intertwined DNA, covalent DNA junctions or protein bridges, because those would probably impede the translocation of Smc–ScpAB toward the terminus (**Fig. 1b**).

Inactivation of Smc–ScpAB in *B. subtilis* results in the massive accumulation of sister chromosomes interlinked at the replication-origin region, especially under conditions of fast growth. The main function of the Smc complex might thus be to promote the timely disengagement of sister replication origins[21,22]. Chromosome organization might arise as an indirect but beneficial consequence of Smc–ScpAB's action in the unlinking of sister DNA molecules.

Could such a mechanism also be relevant for the formation of mitotic chromosomes? Condensin is thought to bring together intrachromosomal DNA segments. Much like Smc–ScpAB in bacteria, condensin must avoid creating linkages between sister chro-

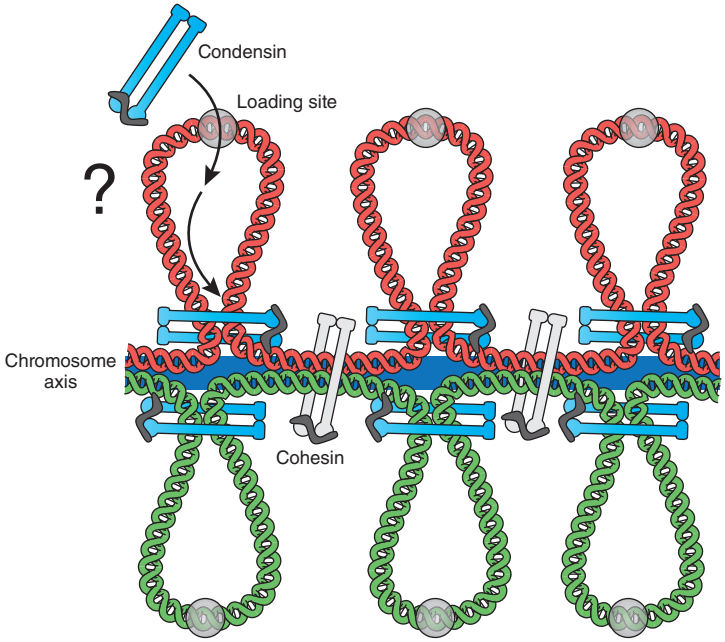

Figure 2 Putative scenario for formation of the mitotic chromosome. Condensin complexes are loaded at the future apices of DNA loops, which then emerge by DNA extrusion through the complex and determine a self-organizing chromosome axis.

matids, which would probably be detrimental to chromatid resolution in prophase and segregation in anaphase. A plausible and simple model would comprise two steps (**Fig. 2**): the initial loading of condensin onto an emerging loop of DNA and the subsequent extrusion of DNA until condensin hits the self-organizing chromosome axis[23,24]. The formation of topologically associating domains (TADs) might accordingly be brought about by the loading of cohesin or condensin onto DNA within a TAD and the extrusion of DNA until the domain boundaries are reached.

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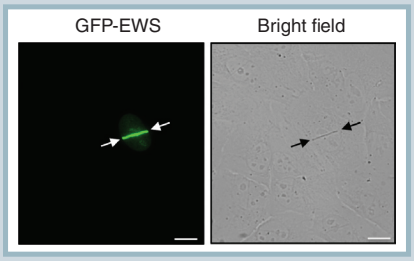
PAR and the organization of the DNA damage response



Chains of poly(ADP-ribose), or PAR, are rapidly synthesized at DNA break sites and contribute to the DNA damage response, likely by recruiting repair factors. Now Altmeyer, Lukas and coworkers add another layer of complexity: they propose that PAR acts as a molecular seed to assemble intrinsically disordered proteins into dynamic, membrane-free compartments at DNA damage sites (*Nat. Commun.* doi:10.1038/ncomms9088, published 19 August 2015).

The formation of such subcellular structures, for example the nucleolus or stress granules, was previously shown to involve specific RNA molecules and proteins containing low-complexity domains (LCDs), regions composed of repetitive motifs that are often intrinsically disordered. Assembly of these membrane-free compartments is akin to the formation of liquid droplets in an oil-water mixture—i.e., phase separation by liquid demixing.

An analysis of previous PAR-protein association data revealed an enrichment in proteins featuring LCDs. Given its chemical similarity to RNA, PAR could potentially have a similar effect in triggering phase separation. The authors test this hypothesis, focusing on three LCD-containing, PAR-associated proteins: FUS/TLS (fused in sarcoma/translocated in sarcoma), EWS (Ewing sarcoma) and TAF15 (TATA box-binding protein associated factor); these are collectively referred to as the FET proteins.

Using GFP fusions and inflicting DNA damage with laser microirradiation, the authors find that the FET proteins assemble at DNA damage sites in a PAR-dependent manner. The FET proteins have 2 types of LCDs, whose functions are dissected: the RGG-repeat module is recruited to PAR via electrostatic interactions, whereas the prion-like SYQG-rich module mediates phase separation and form assemblies at high concentrations. These structures are formed at DNA damage sites and could be directly observed in bright-field microscopy as zones showing distinct light diffraction properties (see right panel, black arrows; left panel shows fluorescence microscopy for GFP-EWS, with white arrows marking orientation of the laser). The assemblies include other LCD-containing proteins, but downstream DNA damage factors had differential access to the PAR-seeded compartments: while MDC1 could accumulate together with the FET proteins, 53BP1 was excluded.

These observations lead to a model whereby PAR, a polymer lacking sequence information, allows cells to compartmentalize specific proteins within the nucleus. By changing the physicochemical properties of the environment around chromosomal lesions, PAR-seeded transient microorganelles orchestrate, both temporally and spatially, the molecular events that engender DNA damage response and repair.

Inês Chen