FEMS Microbiology Reviews, fuaa028, 44, 2020, 725–739

doi: 10.1093/femsre/fuaa028
Advance Access Publication Date: 13 July 2020
Review Article

REVIEW ARTICLE

Compaction and control—the role of chromosome-organizing proteins in *Streptomyces*

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One sentence summary: *Streptomyces* employ distinct repertoires of proteins to organize their chromosomes throughout their complex life cycle.

Editor: Justin Nodwell

ABSTRACT

Chromosomes are dynamic entities, whose organization and structure depend on the concerted activity of DNA-binding proteins and DNA-processing enzymes. In bacteria, chromosome replication, segregation, compaction and transcription are all occurring simultaneously, and to ensure that these processes are appropriately coordinated, all bacteria employ a mix of well-conserved and species-specific proteins. Unusually, *Streptomyces* bacteria have large, linear chromosomes and life cycle stages that include multigenomic filamentous hyphae and unigenomic spores. Moreover, their prolific secondary metabolism yields a wealth of bioactive natural products. These different life cycle stages are associated with profound changes in nucleoid structure and chromosome compaction, and require distinct repertoires of architectural—and regulatory—proteins. To date, chromosome organization is best understood during *Streptomyces* sporulation, when chromosome segregation and condensation are most evident, and these processes are coordinated with synchronous rounds of cell division. Advances are, however, now being made in understanding how chromosome organization is achieved in multigenomic hyphal compartments, in defining the functional and regulatory interplay between different architectural elements, and in appreciating the transcriptional control exerted by these 'structural' proteins.

Keywords: *Streptomyces*; nucleoid-associated proteins; topoisomerase; chromosome domain; chromosome organization; chromosome topology; sporulation; regulation of gene expression; condensin

INTRODUCTION

Bacterial chromosomes are dynamic macromolecules that are subject to multilevel organization; however, the basic chromosomal unit can vary between bacteria. Most bacteria possess a single, circular chromosome. There are, however, bacteria that have linear chromosomes, as well as ones that have multiple chromosomes per cell. In all cases, these chromosomes are compacted into 'nucleoid' structures within the cytoplasm (Murphy and Zimmerman 1995; Feijoo-Siota *et al.* 2017; Dame, Rashid and Grainger 2019). While extensive compaction is essential in order to accommodate the chromosome within a cell, chromosome organization must be flexible enough to allow for simultaneous DNA replication, chromosome segregation and transcription.

Compact, dynamic nucleoids are maintained by a set of DNA-organizing proteins including those that are highly conserved (e.g. topoisomerases) (Forterre *et al.* 2007), and others that are unique to particular bacterial groups (Dame, Rashid and Grainger 2019). In the case of *Streptomyces*, these soil-dwelling, antibiotic-producing bacteria have linear chromosomes (Kirby 2011), and an unusual multicellular life cycle with stages that include multichromosomal hyphal filaments (Flärdh *et al.* 2012)

Received: 11 March 2020; **Accepted:** 9 July 2020

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and single genome spore compartments (Elliot and Flärdh 2012). Consequently, these organisms face unique challenges in terms of chromosome replication, segregation and condensation, as well as the need to coordinate these processes with the different stages of their life cycle (Flärdh and Buttner 2009; Jones and Elliot 2018). Here, we describe the chromosome properties and processes that are conserved throughout bacteria, and discuss how streptomycetes achieve dynamic chromosome organization and circumvent the challenges associated with their large (8–13 Mbp), linear chromosome and their complex life cycle. We further discuss the role of chromosome topology in controlling gene expression, stress adaptation and antibiotic production in these remarkable bacteria.

CHROMOSOME ORGANIZATION—GENERAL CHARACTERISTICS AND WELL-STUDIED EXAMPLES

Bacterial chromosomes tend to adopt a loosely twisted conformation that is dictated by macromolecular crowding (de Vries 2010), and initially, these molecules were not expected to be organized in a defined manner. Chromosome orientation was first probed using fluorescent labels that allowed for the localization of the origin (*oriC*) and terminus (*ter*); the positioning of each turned out to be both dynamic and predictable over the course of a cell cycle (Webb *et al.* 1997; Niki and Hiraga 1998; Espéli and Boccard 2006; Espeli, Mercier and Boccard 2008). Intriguingly, the specific orientations of the *oriC* and *ter* loci were found to be organism specific (Wang, Montero Llopis and Rudner 2013; Badrinarayanan, Le and Laub 2015). Many bacteria position their *oriC* and *ter* regions at opposite poles of the newborn cell (e.g. *Caulobacter cresentus*, *Vibrio cholerae*), while others have evolved a subpolar *oriC* location (e.g. *Myxococcus xanthus*, *Mycobacterium smegmatis*) (Ebersbach *et al.* 2008; Harms *et al.* 2013; David *et al.* 2014; Hołówka *et al.* 2018). Growth rate and life cycle stage can also influence *oriC* positioning. For example, in slow-growing *Escherichia coli,* the *oriC* is located at mid-cell, while in fast-growing cultures, it is located at the cell pole (Niki, Yamaichi and Hiraga 2000; Kleckner *et al.* 2014; Badrinarayanan, Le and Laub 2015). In *Bacillus subtilis,* life cycle stage impacts the configuration of the chromosome, with *oriC* shifting from a central position during vegetative growth, to a polar location at the onset of sporulation (Errington 2001; Wang, Montero Llopis and Rudner 2013; Badrinarayanan, Le and Laub 2015).

In bacteria with a polarly localized *oriC,* the onset of DNA replication is followed by the translocation of one copy of the *oriC* to the opposite pole. In contrast, in cells with subpolar or central *oriC* orientations, both *oriC* copies move toward opposite poles (Ben-Yehuda, Rudner and Losick 2003; Ebersbach *et al.* 2008; Wang, Montero Llopis and Rudner 2014). In all cases, however, the newly replicated *oriC*(s) reach their final destination (pole, subpole or mid-cell) before replication is complete. In most bacterial species (except for the γ-proteobacteria), *oriC* positioning is dictated by a chromosome segregation system comprising the ParA and ParB proteins, and the *parS* DNA sequences (Lim *et al.* 2014; Badrinarayanan, Le and Laub 2015; Kawalek *et al.* 2020). ParA is an ATPase that dimerizes and binds DNA non-specifically when in an ATP-bound configuration, while ParB is a DNA-binding protein that specifically recognizes and binds multiple *parS* sequences that flank the *oriC;* these *parS* sequences are distributed over an area encompassing anywhere from 3% (in *Helicobacter pylori*) to 20% (in *Bacillus subtilis*) of the total chromosome (Lin and Grossman 1998; Livny, Yamaichi and Waldor 2007). Upon binding these *parS* sequences, ParB is proposed to spread non-specifically along the DNA and bridge distant chromosomal regions in a CTP-dependent manner (Osorio-Valeriano *et al.* 2019; Soh *et al.* 2019; Jalal, Tran and Le 2020), ultimately forming a large nucleoprotein complex known as the segrosome (Graham *et al.* 2014; Song *et al.* 2017). Segrosomes interact with nucleoid-bound ParA, and this interaction stimulates ParA ATPase activity, triggering protein release from DNA. Thus, the presence of segrosomes results in the formation of a ParA-depleted zone, and this in turn drives ParB complexed-DNA toward higher concentrations of nearby nucleoid bound ParA (Vecchiarelli, Neuman and Mizuuchi 2014; Surovtsev, Campos and Jacobs-Wagner 2016). Notably, segregation proteins not only control the subcellular position of the *oriC* region but also organize this region in a way that facilitates its segregation.

It has been more challenging to follow the specific localization and organization of the chromosome arms (between *oriC* and *ter*); however, recent innovations in fluorescent microscopy and chromosome conformation capture technologies are revealing reproducible architectures for these regions as well (Niki, Yamaichi and Hiraga 2000; Valens *et al.* 2004; Viollier *et al.* 2004; Lioy *et al.* 2018). In *E. coli,* the chromosome arms, as well as the *oriC* and *ter* regions, are organized into well-compacted macrodomains that are separated by unstructured DNA regions (Valens *et al.* 2004). The principles governing macrodomain organization in *E. coli* are poorly understood, with the exception of the *oriC* and *ter* macrodomains, whose spatial architecture are dictated by the MaoP and MatP proteins, respectively (Mercier *et al.* 2008; Valens, Thiel and Boccard 2016). MaoP and MatP bind and bridge their cognate *maoS/matS* DNA sequences, which are distributed along ∼800 kb DNA fragments. MaoP and MatP homologs are, however, restricted to the enteric bacteria, and consequently, it is not yet clear whether such organization is limited to *E. coli* and its close relatives, or whether other bacteria have evolved analogous systems for organizing their origin and terminus regions.

In bacterial species with polar *oriC* (and *ter*) configurations, the left and right chromosomal arms remain in close proximity throughout the cell cycle. This is thought to result from long-distance arm cohesion driven by DNA condensins (Wang *et al.* 2017) that function using a loop extrusion mechanism (Marko *et al.* 2019). In most bacteria, condensin complexes are composed of SMC (structural maintenance of chromosomes), a large coiled-coil protein dimer and two partner proteins, ScpA and ScpB (segregation and condensation proteins). These SMC/ScpAB complexes are recruited to DNA through interaction with the segregation protein ParB (Gruber and Errington 2009; Sullivan, Marquis and Rudner 2009; Minnen *et al.* 2011). Thus, the ParB nucleoprotein complex is essential for not only organizing and segregating *oriC* regions but also facilitating SMC loading, which in turn impacts global chromosome compaction. Interestingly, in *E. coli* (which lacks ParAB proteins), recruitment of the SMC homolog MukB is controlled by the *ter*-associated MatP protein (Nolivos *et al.* 2016; Mäkelä and Sherratt 2020). Moreover, MukB also interacts with topoisomerase IV during *oriC* segregation (Hayama and Marians 2010; Nolivos *et al.* 2016).

Locally, bacterial chromosomes are organized into specific domains that differ in both size and spatial architecture. In *Caulobacter crescentus,* chromosome conformation capture experiments have revealed the chromosome is divided into >20 ‘chromosomal interaction domains’ (CIDs), each averaging ∼160 kb in size (Le *et al.* 2013). The boundaries of particular domains often coincide with highly expressed genes, suggesting that transcription may make a major contribution to the

integrity of each CID and their subsequent spatial organization into higher order structures similar to *E. coli* macrodomains (Badrinarayanan, Le and Laub 2015; Shen and Landick 2019). Within any given CID, the DNA is organized into plectonemically supercoiled loops called 'topological domains' or 'supercoiling domains', having an estimated average size of ~10 kb (Postow *et al.* 2004). Like the larger domains (macrodomains and CIDs), the intrinsic architecture of topological domains is maintained by nucleoid associated proteins (NAPs) and topoisomerases, as well as by the transcriptional activity of genes located within the particular domains. NAPs are a heterogeneous group of small, basic, often dimeric proteins that bind DNA with varying degrees of sequence specificity (Hardy and Cozzarelli 2005). Like eukaryotic histones, NAPs are responsible for DNA bending (HU, IHF or Fis homologs), wrapping (Lrp and Dps homologs) or local bridging of DNA fragments (H-NS-like proteins), and all are proposed to stabilize topological domains or modulate their architecture (Badrinarayanan, Le and Laub 2015; Dame, Rashid and Grainger 2019). NAPs can also profoundly affect gene transcription (Dorman 2013; Dorman *et al.* 2020). Notably, the repertoire of NAPs employed by different bacterial species can be highly variable (Table 1).

Topological domain compaction is also controlled by topoisomerases; these enzymes are responsible for adding or removing DNA supercoils through the breaking and rejoining of phosphodiester bonds. Topoisomerases are critical for removing the supercoils that result from DNA replication and transcription, and further facilitate DNA strand separation during recombination (Brochu, Breton and Drolet 2020). Two topoisomerases are essential for viability and are present in all bacteria: TopA, which removes negative supercoils from DNA, and gyrase, which introduces negative supercoils. The opposing activities of TopA and gyrase are crucial for maintaining the appropriate balance between supercoiling-driven DNA compaction, and DNA accessibility—a phenomenon known as topological homeostasis (Ahmed *et al.* 2015; Ferrandiz *et al.* 2016; Szafran *et al.* 2016). Interestingly, some nucleoid-associated proteins (e.g. the mycobacterial HU homolog HupB) can influence topoisomerase activity through their changing of DNA spatial structure, or through direct protein–protein interactions (Ghosh, Mallick and Nagaraja 2014). Nucleoid-associated proteins can also limit the diffusion or spread of DNA supercoils by establishing local barriers, ultimately leading to the generation of distinct supercoiling gradients along the bacterial chromosome (Lal *et al.* 2016; Ferrándiz *et al.* 2018). Overall, the organization of topological domains is dynamic and relies on the combined activities of DNA-binding proteins and DNA-processing enzymes.

Streptomyces spp. employ the same chromosome organization systems as many other bacteria, including the ParABS system for chromosome segregation, alongside dedicated NAPs, SMC-type condensins and topoisomerases. As in other systems, the activity of the various chromosome-organizing proteins in *Streptomyces* depends on their life cycle stage. In unicellular and unigenomic bacteria, chromosome organization is tightly coordinated with cell cycle and cell shape. Given the unusual nature of the filamentous and sporulating streptomycetes, the chromosome-organizing machineries in these bacteria must deal with multiple chromosomes in the vegetative and aerial hyphal filaments, as well as the synchronous segregation and compaction of these chromosomes during sporulation, to ensure that each spore inherits a single chromosome.

STREPTOMYCES LIFE CYCLE

The *Streptomyces* life cycle begins with a unigenomic spore (Fig. 1A). Spore germination initiates with the emergence of one or two germ tubes at sites marked by the polarity determinant DivIVA (Flärdh 2003a) (Fig. 1B). Unlike most other bacteria, *Streptomyces* do not undergo binary fission. Instead they grow filamentously via polar tip extension (Gray, Gooday and Prosser 1990), and forgo cell division except during sporulation (McCormick 2009). Extension of these initial germ tubes yields long hyphal filaments, from which periodic hyphal branches emerge. This branching, hyphal growth strategy ultimately leads to the formation of a network of filamentous vegetative cells, each of which contains multiple, largely uncondensed chromosomes (Kwak and Kendrick 1996) (Fig. 1C).

Hyphal tip growth is governed by DivIVA, which directs cell wall biosynthesis and is essential for *Streptomyces* viability (Flärdh 2003a). Beyond its role in coordinating peptidoglycan biosynthesis, DivIVA acts in concert with a number of other proteins at the hyphal tip. These tip-associated proteins are collectively referred to as the 'polarisome', and they include the intermediate filament-like protein FilP, which interacts directly with DivIVA (Fuchino *et al.* 2013; Fröjd and Flärdh 2019); and the coiled-coil protein Scy, which interacts with both DivIVA and FilP, as well as with ParA—the chromosome segregation-associated protein (Ditkowski *et al.* 2013; Holmes *et al.* 2013). The interaction between ParA and Scy couples hyphal tip growth with chromosome segregation in the growing vegetative hyphae (Kois-Ostrowska *et al.* 2016).

Branch formation is initiated when the polarisome splits, and a new polarisome complex assembles along the lateral cell wall, driving new tip growth (Hempel *et al.* 2008; Flärdh *et al.* 2012; Richards *et al.* 2012). Importantly, while the activity of DivIVA and its associated proteins are required for branch formation, continued branch growth requires the successful capture/translocation of a chromosome into the nascent branch; if a branch fails to acquire a chromosome, its elongation ceases (Kois-Ostrowska *et al.* 2016).

When nutrients are abundant, vegetative growth continues. However, when resources become scarce, or when stressful conditions are encountered, *Streptomyces* colonies embark on one of two developmental trajectories: (i) reproductive growth, involving the raising of aerial hyphae and formation of spore chains (Elliot and Flärdh 2012) or (ii) exploratory growth, involving rapid colony expansion and an ability to colonize new areas (Jones *et al.* 2017) (Fig. 1D and E).

Aerial hyphal growth, like that of the vegetative hyphae, is driven by DivIVA and its associated polarisome constituents, although branching is not observed in the aerial hyphae (Fig. 1E). The growth of aerial hyphae is closely coupled to chromosome replication and segregation (discussed below), and the cessation of growth is correlated with the onset of sporulation. Sporulation is an exquisitely choreographed event, requiring the synchronous compaction and segregation of chromosomes, such that the resulting ParB-*oriC* complexes are distributed at regularly spaced intervals along the sporogenic hyphae (Jakimowicz et al. 2005, 2007). At the same time, these multigenomic hyphae are subdivided into 10–50 unigenomic spore compartments (Fig. 1F). These processes require the upregulation of genes involved in chromosome segregation (*parAB*) (Jakimowicz *et al.* 2006), chromosome compaction (multiple nucleoid-associated proteins) and cell division (e.g. *ftsZ*) (Flärdh *et al.*

Table 1. Conservation of select proteins having roles in chromosome dynamics in phylogenetically diverse bacteria.

	E. coli	*B. subtilis*	*C. crescentus*	*M. tuberculosis*	*S. coelicolor*
Genome content	AT-rich	AT-rich	GC-rich	GC-rich	GC-rich
Gram +/–	Gram-negative	Gram-positive	Gram-negative	Gram-positive	Gram-positive
Protein:					
HU	HU (α and β subunits)	Hbsu	HCc	HupB (Rv2986)	HupA, HupS
DpsA	Dps	Dps	Dps	–	DpsA, DpsB, DpsC
Fis	Fis	–	–	–	–
IHF homologs and functional equivalents	IHF (α and β subunits)		IHF	mIHF (functional equivalent)	sIHF (functional equivalent)
H-NS and H-NS-like proteins	H-NS, StpA (H-NS paralog)	Rok (functional equivalent)	GapR[a] (functional equivalent)	Lsr2 (functional equivalent)	Lsr2 (functional equivalent)
ParA/ParB	–	Soj/Spo0J	ParAB	ParAB	ParAB
Noc	–	Noc	–	–	–
Condensin	MukB	SMC	SMC	SMC	SMC
MaoP	MaoP	–	–	–	–
MatP	MatP	–	–	–	–
TopA (TopoI)	TopA	TopA	TopA	TopA	TopA
TopB (TopoIII)	TopB	TopB	–	–	–
GyrAB (gyrase)	GyrA and GyrB	GyrBA	GyrA and GyrB	GyrBA	GyrBA
ParCE (TopoIV)	ParC and ParE	ParC and ParE	ParC and ParE	–	ParC and ParE
Ssb	Ssb	SsbA and SsbB	–	Ssb	SsbA and SsbB

[a]GapR is found in the α-proteobacteria and, like H-NS, has an affinity for AT-rich DNA (Ricci *et al.* 2016).
Gray shading: essential genes. For species with SsbA and SsbB, typically only SsbA is essential.

2000). FtsZ initiates cell division, forming an array of regularly spaced rings. The characterized streptomycete cell division machinery consists of a limited number of conserved cell division proteins, including FtsZ, FtsW and cell wall synthesizing enzymes (e.g. PBPs, FtsI); however, in contrast to unicellular bacteria, most of these proteins were dispensable for viability (although not for sporulation) (Flärdh and Buttner 2009; McCormick and Flärdh 2012). Moreover, the streptomycetes lack homologs of proteins involved in the regulation of septum positioning found in other bacteria, such as SlmA, Noc or Min (Wu and Errington 2004; Bernhardt and de Boer 2005; Lutkenhaus 2007). Instead, the positioning of FtsZ in sporulating hyphae is positively controlled by the *Streptomyces*-specific proteins SsgA and SsgB (Willemse *et al.* 2011; Jakimowicz and van Wezel 2012; McCormick and Flärdh 2012).

The mechanisms governing cell growth and chromosome dynamics during exploration are less well understood compared with vegetative and reproductive growth, and what features are conserved or required during this developmental stage remains an open question (Jones *et al.* 2017; Jones and Elliot 2018).

The onset of secondary metabolism, and the production of antibiotics and other natural products, are temporally and genetically coupled to reproductive growth (Hallam, Malpartida and Hopwood 1988). This metabolic transition does, however, tend to be spatially segregated from the reproductive cells, being confined to the vegetative hyphae. Increasingly, it appears that sporulation and secondary metabolism are both profoundly impacted by the activity of nucleoid-associated proteins, and other DNA-processing enzymes. Whether and how secondary metabolism is affected during the exploration phase of development remains to be determined.

CHROMOSOME ORGANIZATION DURING *STREPTOMYCES* VEGETATIVE GROWTH

The filamentous nature of *Streptomyces* vegetative hyphae and the polar tip/apical growth of these hyphal filaments mean that chromosome segregation and organization in these bacteria are subject to different constraints compared with most other bacteria. The large, linear nature of the *Streptomyces* chromosomes further expands the possible configurations that could be adopted by these molecules, compared with a circular chromosome of equivalent size. During vegetative growth, the multiple chromosomes are not obviously separated and remain uncondensed, occupying most of the hyphal cell volume (Kwak and Kendrick 1996). The development of fluorescence *in situ* hybridization (FISH) techniques and fluorescent reporter–operator systems (FROS) have made it possible to localize *oriC* and *ter*, and to follow the localization of segrosomes and replisomes (Yang and Losick 2001; Jakimowicz *et al.* 2005; Ruban-Ośmiałowska *et al.* 2006; Wolánski *et al.* 2011).

A distinctive feature of chromosome positioning during *Streptomyces* vegetative growth is the anchoring of the *oriC* of the apical chromosome to the growing hyphal tip. During spore germination, chromosome replication initiates before germ tube emergence. There is often more than one replisome detectable in germinating spores, suggesting that intensive chromosome replication may be required to ensure there are sufficient copies of the chromosome to populate the emerging hyphal tube(s) (Ruban-Ośmiałowska *et al.* 2006; Wolánski *et al.* 2011). The first chromosome is translocated into the growing hyphal filament once the germ tube is ~2 μm long. This chromosome then remains in close proximity to the hyphal tip throughout growth (Kois-Ostrowska *et al.* 2016). In contrast to the precise positioning of the apical chromosome, the subapical copies exhibit more flexible distribution along the length of the hyphal filament, although they too follow the extending tip (Yang and Losick 2001; Kois-Ostrowska *et al.* 2016).

Notably, in *Streptomyces* hyphae, the *oriC* of the apical chromosome is positioned at the edge of the nucleoid, giving it a configuration similar to the polarly organized chromosomes of other bacteria like *Caulobacter* and *Vibrio* (Ebersbach *et al.* 2008; David *et al.* 2014; Kois-Ostrowska *et al.* 2016). Whether the subapical chromosomes are similarly oriented is not yet known. Using

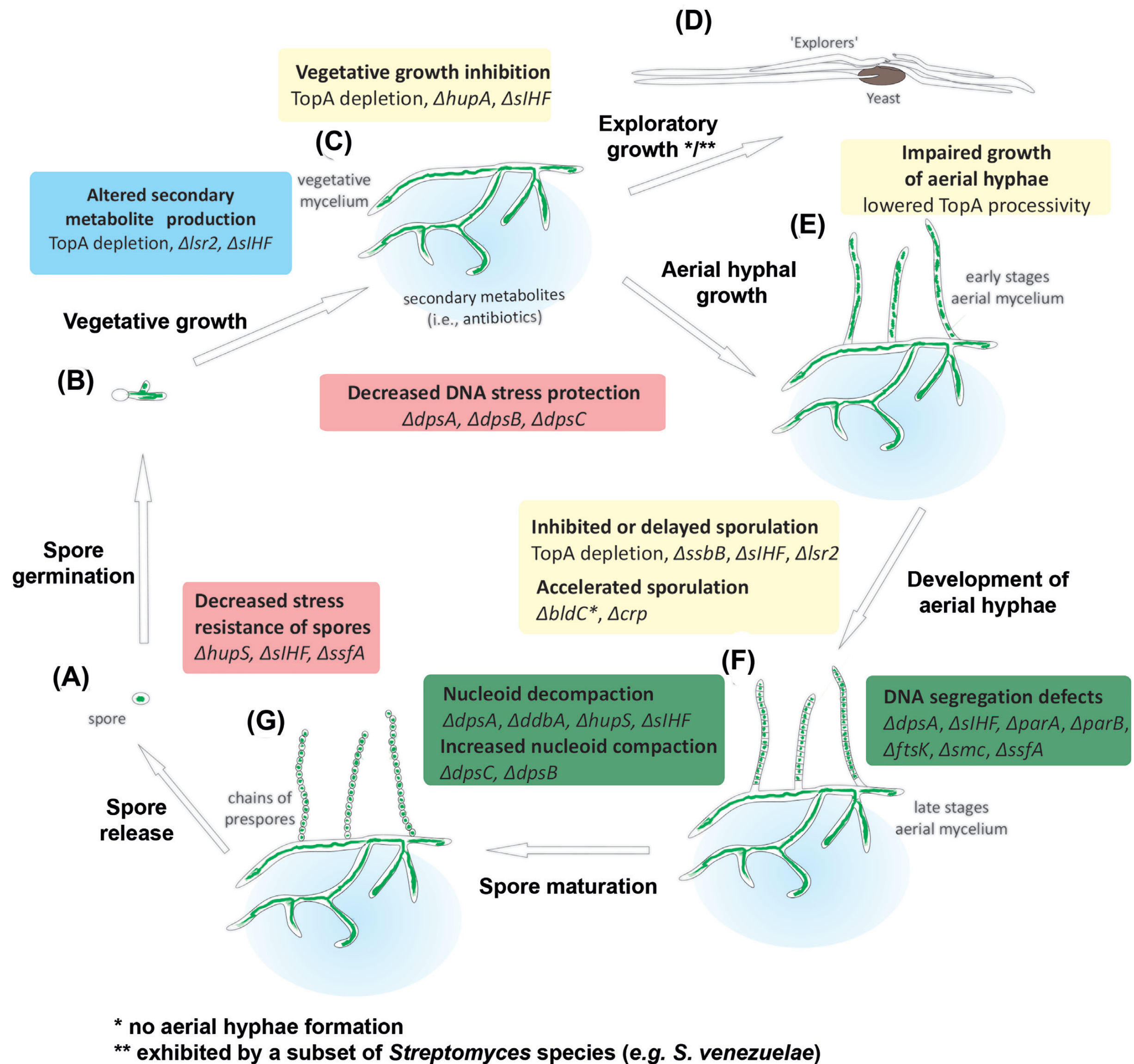

Figure 1. The role of DNA-processing proteins during the *Streptomyces* life cycle. The *Streptomyces* life cycle begins with single spore **(A)**, which germinates **(B)** and grows via tip extension and branching to form a network of vegetative hyphae **(C)**. Nutrient depletion or environmental stress induces exploratory growth **(D)** or sporulation initiated by aerial hyphal growth **(E)**. The aerial hyphae mature into chains of pre-spores **(F)** and subsequently spores **(G)** that are disseminated. The phenotypic effects of mutations in genes encoding chromosome-organizing proteins are indicated. Pink boxes: altered stress resistance. Blue box: modified secondary metabolism. Yellow boxes: modified growth. Green boxes: altered chromosome topology.

FISH, the ends of the linear chromosomes were found to co-localize with each other, but not with the *oriC* region (Yang and Losick 2001). This co-localization is assumed to be due to interaction of *ter*-bound proteins like Tap (telomere-associated protein) and/or Tpg (terminal protein gene) (Bao and Cohen 2003). Tap and Tpg maintain the integrity of the *Streptomyces* chromosomal termini, ensuring they are properly replicated by recruiting PolA (DNA polymerase) and TopA (topoisomerase) to the terminal telomeres (Bao and Cohen 2004). It is interesting to note, however, that Tap and Tpg are not universally conserved in the streptomycetes (see Table 2). Loss of Tap in *S. coelicolor* and *S. lividans* leads to DNA rearrangements, including chromosome circularization, which, surprisingly, does not visibly impair growth (Yang *et al.* 2002, 2013, 2017). *Streptomyces* genomes encode topoisomerase IV (encoded by *parC* and *parE*), which is primarily involved in the progression of replication forks and the decatenation of newly replicated DNA, and it is this decatenase activity that has the potential to support the separation of circular chromosomes, like those formed in the absence of Tap (Huang *et al.* 2013). Why *Streptomyces* chromosomes are linear remains a mystery, but this configuration presumably affords some fitness advantages over the more conventional circular orientation (Chen *et al.* 2002; Kirby 2011; Hopwood 2019).

The multigenomic nature of *Streptomyces* hyphal compartments raises the question of whether chromosome segregation is important during vegetative growth. *Streptomyces* genomes encode a typical ParABS segregation system in which the *parAB* genes are co-transcribed. Two promoters direct the expression of the *parAB* operon: one promoter is preferentially active during vegetative growth, while the second is active during aerial

Table 2. *Streptomyces* proteins that contribute to chromosome dynamics or are abundant nucleoid-associated proteins.

Protein name/*S. coelicolor* A3(2) gene number (*Streptomyces venezuelae*[a] gene number)	Predicted DNA-binding domain & sequence preference	Contribution to chromosome dynamics (experimentally determined)	Cell cycle stage when active (based on mutant phenotype or transcript/protein abundance)	Mutant effect on spore formation and resistance?	Reference
BldD/*sco1489* (*vnz_0 5285*)	Yes; specific motif	Unknown	Vegetative growth (transcription)	Accelerated sporulation (no aerial hyphae formation)	(Elliot *et al.* 1998; Tschowri *et al.* 2014)
Crp/*sco3571* (*vnz_16 460*)	Yes; specific motif	Unknown	Constitutive (protein abundance)	Accelerated sporulation	(Derouaux *et al.* 2004; Gao *et al.* 2012; Bradshaw, Saalbach and McArthur 2013)
DdbA/*sco2075* (*vnz_0 8485*)	Yes; specificity unknown	Nucleoid condensation and nucleoid structure	Sporulation (transcript levels)	Defects in chromosome compaction and spore stress resistance	(Aldridge *et al.* 2013; Gehrke *et al.* 2019)
DpsA/*sco0596* (n/a)	Yes; specificity unknown	Osmotic stress, heat shock	Sporulation (phenotype and immunoblotting); inducible in response to osmotic shock	Defects in chromosome segregation; heterogeneous spore sizes	(Facey *et al.* 2009)
DpsB/*sco5756* (n/a)	Yes; specificity unknown	Nucleoid condensation (negative effect)	Sporulation (phenotype; low level constitutive expression determined via immunoblotting)	Shorter spores with more compact chromosomes	(Facey *et al.* 2009)
DpsC/*sco1050* (*vnz_34 055*)	Yes; specificity unknown	Nucleoid condensation (negative effect)	Sporulation (phenotype; constitutive expression via immunoblotting); inducible at high temperatures	Shorter spores with more compact chromosomes	(Facey *et al.* 2009)
FtsK/*sco5750* (*vnz_26 720*)	Yes; specificity unknown	Chromosome stability	Sporulation (reporter fusions)	Chromosome segregation defects	(Wang *et al.* 2007; Dedrick, Wildschutte and McCormick 2009)
GyrBA/*sco3874–73* (*vna17955–50*)	Yes; specificity unknown	Replication and supercoiling	All (constitutive)	Predicted to be essential	n/a
HupA/*sco2950* (*vnz_13 325*)	Yes; specificity unknown	Unknown	Vegetative growth (transcription)	Unknown	(Salerno *et al.* 2009)
HupS/*sco5556* (*vnz_25 950*)	Yes; specificity unknown	Nucleoid compaction during sporulation	Sporulation (mutant phenotype and transcription)	Reduced pigmentation; reduced heat resistance	(Salerno *et al.* 2009)
Lsr2/*sco3375* (*vnz_15 890*)	Yes; AT-rich sequences	Unknown	Onset of secondary metabolism (aerial hyphae formation)	Delayed sporulation	(Gehrke *et al.* 2019)
ParA/*sco3886* (*vnz_18 010*)	Yes; non-specific binding predicted	Chromosome segregation	Vegetative (translocation into branches); sporulation (mutant phenotype and transcription)	Defects in chromosome segregation	(Jakimowicz *et al.* 2007)

Table 2. Continued

Protein name/*S. coelicolor* A3(2) gene number (*Streptomyces venezuelae*[a] gene number)	Predicted DNA-binding domain & sequence preference	Contribution to chromosome dynamics (experimentally determined)	Cell cycle stage when active (based on mutant phenotype or transcript/protein abundance)	Mutant effect on spore formation and resistance?	Reference
ParB/*sco3887* (*vnz_18 015*)	Yes; *parS* sequences	*oriC* organization and chromosome segregation	Vegetative (translocation into branches); sporulation (mutant phenotype and transcription)	Defects in chromosome segregation	(Kim *et al.* 2000; Jakimowicz *et al.* 2005)
SepG or YlmG/*sco2078* (*vnz_0 8500*)	No (effects are likely indirect)	Nucleoid organization during sporulation	Sporulation (mutant phenotype)	Few spore chains; heterogeneous spore sizes	(Zhang *et al.* 2016)
SmeA-SffA/*sco1415–16* (*vnz_0 4885–90*)	Unknown; unknown	Chromosome compaction and segregation	Sporulation	Defects in chromosome segregation; heterogeneous spore sizes; reduced heat resistance	(Ausmees *et al.* 2007)
sIHF/*sco1480* (*vnz_0 5240*)	Yes; non-specific	Nucleoid compaction during sporulation	All (constitutive)	Delayed and reduced sporulation; defects in chromosome segregation; heterogeneous spore sizes	(Swiercz *et al.* 2013)
SMC + ScpAB/*sco5577* + *sco1770–69* (*vnz_26 075* + *vnz_06875–70*)	Yes; specificity not tested	Chromosome compaction	Sporulation	Defects in chromosome segregation	(Dedrick, Wildschutte and McCormick 2009; Kois *et al.* 2009)
SsbB/*sco2683* (*vnz_12 100*)	Yes; single-stranded DNA	Chromosome segregation	Sporulation (mutant phenotype)	Defects in chromosome segregation; heterogeneous spore sizes; delayed sporulation	(Paradzik *et al.* 2013)
Tap/*sco7733* (n/a)	Yes; single-stranded telomeric DNA	Maintenance of telomeres and chromosome linearity	Unknown	Unknown	(Bao and Cohen 2003; Yang *et al.* 2017)
TopA/*sco3543* (*vnz_16 300*)	Yes; non-specific but with AT-preference	DNA relaxation and chromosome decompaction; telomere maintenance	All (constitutive)	Essential gene; no sporulation in TopA-depletion strain	(Bao and Cohen 2004; Szafran *et al.* 2013, 2014)
Tpg/*sco7734* (n/a)	Yes; non-specific (both single and double stranded DNA)	Maintenance of telomeres and chromosome linearity	Unknown	Unknown	(Bao and Cohen 2003; Yang *et al.* 2013)

[a] *Streptomyces venezuelae* strain NRRL B-65442.

hyphae formation/sporulation (Jakimowicz *et al.* 2006). ParB binds multiple *parS* sites, which flank the *oriC* and are found throughout a region spanning ~500 kb (Jakimowicz, Chater and Zakrzewska-Czerwińska 2002; Donczew *et al.* 2016). The number of ParB-binding sites in *Streptomyces* genomes exceeds that of most other bacteria. This increased concentration of *parS* sequences may be related to the relatively large size (~8–10 Mb) of the *Streptomyces* chromosome. Alternatively, it may reflect the need for greater compaction due to the linear organization of *Streptomyces* chromosomes, or it may be related to the multigenomic nature of *Streptomyces* hyphal filaments. It is worth noting that the unigenomic bacterium *Myxococcus xanthus* has similar numbers of *parS* sequences on its large (~9 Mb) circular chromosome (Harms *et al.* 2013; Iniesta 2014), suggesting that chromosome size may be the strongest driver of *parS* sequence number.

In vegetatively growing cells, all chromosomal *oriC* regions appear to be decorated with ParB (Kois-Ostrowska *et al.* 2016). Unexpectedly, ParB's binding partner, the ATPase ParA (Jakimowicz *et al.* 2007), is found predominantly at the hyphal tips, courtesy of its interaction with the Scy protein, an integral member of the tip polarisome complex (Walshaw, Gillespie and Kelemen 2010; Holmes *et al.* 2013). The polar positioning of ParA is necessary for anchoring the *oriC* of the apical chromosome to the tip: loss of ParA, or disruption of its interaction with either ParB or Scy, leads to impaired localization of the tip-associated *oriC* (Kois-Ostrowska *et al.* 2016). The interaction of ParA with polar proteins is reminiscent of the situation in *C. crescentus, V. cholera* and *Mycobacterium tuberculosis*, where ParA associates with dedicated polar tip proteins (TipN/PopZ, HpbP and DivIVA, respectively) (Ptacin et al. 2010, 2014; Ginda *et al.* 2013).

Streptomyces vegetative hyphae are characterized by the frequent initiation of branching, with continued growth of the new hyphal branch depending on the successful translocation of a chromosome into that growing branch (Kois-Ostrowska *et al.* 2016). Shortly after a new branch emerges, it is populated with a chromosome from the hyphal stem. ParA and ParB are needed to capture the chromosome at the base of the new branch, and promote its translocation into the emerging branch. The requirement for ParAB in populating new branches with chromosomes could explain their constitutive production during vegetative growth, despite no obvious need for chromosome segregation. It is worth noting, however, that mutations in *parAB* have only subtle phenotypic effects in vegetatively growing *S. coelicolor* (Kois-Ostrowska *et al.* 2016), at least under laboratory conditions. Whether these effects would have a significant fitness cost compared with wild-type strains, or during growth in the soil, remains to be determined.

Labeling of replisomes using DnaN fluorescent protein fusion has revealed that replication of the multiple chromosomes within the vegetative hyphal compartments is asynchronous (Ruban-Ośmiałowska *et al.* 2006). Moreover, the replicative activity differs between individual hyphal filaments. During replication, newly duplicated *oriC*s are bound by ParB; however, their separation efficiency depends on their position within the vegetative hyphae. While the subapical segrosomes seem to be separated passively and slowly, newly replicated *oriC*s associated with the apical chromosome are separated promptly after their duplication (Kois-Ostrowska *et al.* 2016). The efficiency of segrosome separation depends on the presence of apically localized ParA. Since ParA anchors one of the newly replicated *oriC*s at the tip, the simple act of hyphal tip extension seems to contribute to the separation of origins.

Chromosomes within the vegetative hyphae are not visibly compacted and occupy the majority of the intracellular space (Kwak and Kendrick 1996). This apparent loose, relaxed conformation needs to be maintained by DNA-organizing proteins, including topoisomerases and NAPs. *Streptomyces coelicolor* possesses a single essential DNA relaxase, named TopA, which is a highly processive enzyme (Szafran *et al.* 2013, 2014). Reducing TopA levels strongly increases chromosome negative supercoiling, inhibits growth rate, blocks sporulation and affects secondary metabolism (Szafran *et al.* 2013). Moreover, TopA depletion severely affects chromosome distribution in vegetative hyphal compartments, inhibiting the separation of ParB complexes and impeding *oriC* tip anchorage (Strzalka *et al.* 2017). TopA-depleted strains grow very slowly, and this may be due in part to the defects in chromosome distribution during vegetative growth. TopA depletion also results in pronounced transcriptional changes, including altered expression of nucleoid organizing protein-encoding genes (Szafran *et al.* 2019), suggesting that TopA activity is intertwined with that of NAPs, in maintaining appropriate chromosome topology.

Many NAPs are produced during *Streptomyces* vegetative growth (Table 2). These include the two HU homologs HupA and HupS, alongside sIHF, Dps-like proteins and Lsr2 (Facey *et al.* 2009; Salerno *et al.* 2009; Swiercz *et al.* 2013; Gehrke *et al.* 2019). Among these, HupA, Lsr2 and sIHF are the most abundant in *S. coelicolor* vegetative hyphae (Bradshaw, Saalbach and McArthur 2013; Szafran *et al.* 2019). To date, the role of HupA in *Streptomyces* growth is poorly understood, although in *S. coelicolor,* the *hupA* gene is transcribed most highly during vegetative growth (Szafran *et al.* 2019). In *E. coli,* HU proteins promote global organization of the nucleoid and facilitate chromosome segregation (Lioy *et al.* 2018). In *S. lividans,* a *hupA* deletion slows vegetative growth, but its effects on chromosome dynamics have not yet been probed (Yokoyama *et al.* 2001). Interestingly, expression of *hupA* is upregulated in response to increased negative supercoiling (Szafran *et al.* 2019). Like HupA, sIHF is produced constitutively during the *Streptomyces* life cycle, and is expressed most highly during vegetative growth, although its effects are most pronounced during reproductive growth (Bradshaw, Saalbach and McArthur 2013; Swiercz *et al.* 2013). In contrast to HupA and sIHF, loss of Lsr2 and Dps homologs did not visibly impair *S. coelicolor* vegetative growth (Fig. 1C), although *sIHF* and *dps* mutations decreased tolerance to stress conditions (Facey *et al.* 2009; Salerno *et al.* 2009; Gehrke *et al.* 2019). Similar observations have been made for *E. coli* and *M. smegmatis,* where strains with mutations in NAP-encoding genes were able to maintain their overall nucleoid architecture during growth under optimal conditions, but these mutant strains were more sensitive to stress than their wild-type parents (Dillon and Dorman 2010; Bartek *et al.* 2014; Holowka *et al.* 2017). The relatively minor growth effects associated with the loss of individual NAPs in *Streptomyces,* and other bacteria, suggest these proteins may share some level of functional redundancy.

CHROMOSOME DYNAMICS DURING AERIAL GROWTH AND SPORULATION

Streptomyces strains sporulate in response to nutrient limitation and stressful growth conditions. The vast majority of *Streptomyces* species can sporulate during growth on solid culture, but only a handful of tested species (e.g. *S. venezuelae*, *S. griseus, S. albus*) are capable of sporulating in liquid culture (Daza *et al.* 1989). Historically, the best-studied *Streptomyces* species has been *S. coelicolor,* which sporulates only during growth on solid medium. This has made it challenging to follow chromosome

dynamics in real time, and thus, most information on chromosome organization during sporulation has come from analyses of still microscopy images. Recently, S. *venezuelae* has been developed for cell biology studies, and its ability to sporulate in liquid culture is enabling the direct microscopic observation of sporogenic hyphal development and the associated chromosome dynamics (Donczew *et al*. 2016; Schlimpert, Flärdh and Buttner 2016).

Before the onset of sporulation, the aerial or sporogenic hyphae rapidly elongate. This occurs in parallel with intensive DNA replication (Ruban-Ośmiałowska *et al*. 2006), yielding up to 50 chromosomes per sporogenic hyphal compartment (Fig. 1F and G). This rapid replication requires enhanced topoisomerase activity. *Streptomyces* (and their relatives) have unusually processive TopA enzymes, and this processivity appears to be related to a series of C-terminal Lys repeats that stabilize TopA−DNA interactions (Strzałka *et al*. 2017; Szafran, Strzałka and Jakimowicz 2020). While TopA levels are constant throughout the S. *coelicolor* life cycle (Szafran *et al*. 2013), depleting TopA completely blocks sporulation; however, lowering its processivity (by deleting Lys repeats) leads to shorter aerial compartments and shorter spore chains (Szafran *et al*. 2013; Strzalka *et al*. 2017), suggesting that high processivity is critical for maximal sporulation.

As aerial hyphae elongate and chromosome replication ramps up, ParAB and FtsZ production levels begin to rise. Expression of their associated genes depends on the aerial hyphae-specific regulators WhiA and WhiB (although only *ftsZ* is a direct target of these regulators) (Flärdh *et al*. 2000; Jakimowicz *et al*. 2006; Bush *et al*. 2013, 2016). Within these aerial compartments, ParA functions to both control hyphal elongation, and promote the uniform distribution of chromosomes down the length of the hyphae (Ditkowski *et al*. 2013; Donczew *et al*. 2016).

The ParA-mediated cessation of aerial growth is accompanied by the synchronous assembly of FtsZ in a ladder-like configuration extending the length of the aerial filament, delineating future spore compartments (Donczew *et al*. 2016). At the same time, ParB–DNA nucleoprotein complexes assemble, and ParA/B activity positions the *oriC* regions of chromosomes between FtsZ rings, such that each spore compartment inherits a single chromosome. Loss of *parA* or *parB* leads to increased numbers of anucleate spores (Kim *et al*. 2000; Jakimowicz *et al*. 2007), and further impacts FtsZ localization and spore septum positioning, suggesting a tight coordination of chromosome segregation and cell division during *Streptomyces* sporulation (Jakimowicz *et al*. 2007; Donczew *et al*. 2016). In *C. crescentus* and *Rhodobacter spheroides,* chromosome segregation is coupled to cell division through the interaction of ParB with MipZ, an oscillating protein that controls septum placement (Mohl and Gober 1997; Thanbichler and Shapiro 2006; Dubarry *et al*. 2019). Although, transmission electron micrographs depicting chromosomes spanning the space between encroaching cell division septa (e.g. Flärdh 2003b) suggest that unlike many other bacteria, *Streptomyces* lack an obvious nucleoid-occlusion system, the rigorous coordination between chromosome segregation and septum positioning indicates the existence of a dedicated control mechanism. How this control ties into the positive regulation of Z-ring formation by SsgA and SsgB remains to be determined. SepG represents an intriguing candidate for such a role: it is a membrane-associated protein that does not bind DNA directly, but strongly affects sporogenic nucleoid shape and influences SsgB localization (Zhang *et al*. 2016). Moreover, *Streptomyces* employ DNA pumps like FtsK and SffA, which appear to function to clear chromosomal DNA away from the closing septum (Ausmees *et al*. 2007; Sepulveda, Vogelmann and Muth 2011). Although many of the main players involved in cell division and chromosome segregation have been identified in *Streptomyces,* how their activities are coordinated to ensure appropriate synchronization requires further study.

The uniform distribution of ParB–DNA complexes within the sporogenic hyphae is abolished in TopA-depleted strains (Szafran *et al*. 2013). This inability to effectively segregate chromosomes has been proposed to explain the inhibition of cell division and overall spore formation observed for a TopA-depleted strain. The formation of the architecturally complex segrosome is expected to create topological tension within the *oriC* region, and resolution of this would require the relaxation activity of TopA activity. This notion is supported by the observations in S. *coelicolor* that TopA is recruited to the vicinity of ParB-occupied *parS* sites, and that *parB* deletion can partially suppress the sporulation defects associated with TopA depletion (Szafran *et al*. 2013). Since bacterial TopAs are recruited to single-stranded DNA (ssDNA) (Li, Mondragón and DiGate 2001), it has been suggested that segrosome assembly may generate ssDNA. Consistent with this proposal is the discovery that the single-stranded binding protein (SsbB) is required for chromosome segregation during S. *coelicolor* sporulation: the *ssbB* null mutant exhibits delayed sporulation and forms anucleate spores (Paradzik *et al*. 2013). Whether SsbB and TopA cooperate to alleviate the topological tension resulting from ParB-driven segrosome assembly during *Streptomyces* sporulation awaits future investigation.

The maturation of sporogenic hyphae is accompanied by a global rearrangement of *Streptomyces* nucleoid architecture—from uncondensed chromosomes during the initial growth of sporogenic/aerial hyphae, to highly compacted chromosomes during cell division and DNA segregation into pre-spore compartments (Donczew *et al*. 2016). In bacteria, the chromosome compacting activity of topoisomerases is coordinated with the activity of condensins (SMC/MukBEF) and nucleoid-associated proteins (Nolivos *et al*. 2016; Lioy *et al*. 2018). Surprisingly, deleting *smc* in S. *coelicolor* had only mild effects on DNA compaction and chromosome segregation (Dedrick, Wildschutte and McCormick 2009; Kois *et al*. 2009). Instead, chromosome defects were much more pronounced when the *smc* deletion was combined with either *parB* or *ftsK* deletions (Dedrick, Wildschutte and McCormick 2009), where ParB is part of the central chromosome segregation machinery, and FtsK functions as a translocase that pumps DNA out of the closing septum (Ausmees *et al*. 2007; Bigot *et al*. 2007). This suggests that chromosome compaction and segregation are driven by proteins sharing overlapping or redundant functions.

Beyond effective segregation, chromosomes must also be compacted during sporulation in order to effectively fit into the spore compartment, and to maximize resistance to DNA damage. A number of NAP-encoding genes are specifically upregulated during sporulation (Table 2), while other constitutively expressed ones have important functions during sporulation. Among them, DpsA, HupS and DdbA are key players, at least in S. *coelicolor* (Table 2). Each of their genes is expressed at low levels during vegetative and pre-sporogenic aerial growth, with their transcript levels rising dramatically during sporulation (Facey *et al*. 2009; Salerno *et al*. 2009; Aldridge *et al*. 2013). In the case of *dpsA,* its expression could also be induced during vegetative growth in response to osmotic stress, suggesting not only a developmental function for its gene product, but also a role in responding to environmental stresses. *Streptomyces coelicolor dpsA* mutants display variation in spore size and nucleoid volume. *dpsA* deletion further results in increased numbers of spores containing two or more nucleoids, implicating DpsA in

chromosome segregation (Facey *et al.* 2009). It is worth noting, however, that DpsA is not broadly conserved in *Streptomyces* species (Table 2); whether other Dps proteins are able to substitute for it beyond *S. coelicolor* and its relatives has not yet been tested. In contrast, *ddbA* is highly conserved, and its deletion in *S. coelicolor* affects both chromosome compaction and spore resistance to various stresses, including osmotic and oxidative stress (Aldridge *et al.* 2013). Similar phenotypes were observed for a *hupS* mutant in *S. coelicolor*, which displayed reduced chromosome compaction, increased nucleoid size and decreased spore resistance to heat stress (Salerno *et al.* 2009). HupS is an HU-like protein, and like mycobacterial HupB and actinobacterial TopA (and eukaryotic histones), it possesses a C-terminal domain enriched in lysine repeats (Holowka *et al.* 2017; Szafran, Strzałka and Jakimowicz 2020); this Lys-rich repeat region is absent from the vegetative-specific HU-like protein HupA. Interestingly, an equivalent histone-like domain was also identified for DdbA within its N-terminus; truncation of DdbA at its N-terminus abolishes protein interaction with DNA (Aldridge *et al.* 2013).

Unlike DdbA, HupS and the Dps family of proteins, sIHF is an actinobacterial-specific nucleoid-associated protein that is expressed throughout growth, and functions to promote nucleoid condensation during sporulation (Swiercz *et al.* 2013; Nanji *et al.* 2019). Unlike its ortholog from *Mycobacterium* (mIHF) (Pedulla and Hatfull 1998; Odermatt *et al.* 2018), sIHF is not essential for *S. coelicolor* viability. Instead, its loss leads to reduced sporulation and aberrant nucleoid compaction (Swiercz *et al.* 2013). Unusually for a nucleoid-associated protein, sIHF functions as a monomer, and while it is only ∼100 aa in size, it has multiple DNA-binding faces. The crystal structure of sIHF revealed the presence of two DNA-binding surfaces capable of bridging two DNA duplexes, while subsequent small angle X-ray scattering structures revealed a third DNA-binding site (Nanji *et al.* 2019). *In vitro* experiments have revealed that sIHF binding to DNA can inhibit the DNA relaxation activity of TopA, and that it can act further to restrain negative supercoils (Swiercz *et al.* 2013; Nanji *et al.* 2019).

Our understanding of DNA organization during sporulation remains incomplete, as does our understanding of the interplay between the different chromosome-organizing proteins. Loss of any individual nucleoid-associated protein has a modest effect on the chromosome architecture during sporulation, suggesting that there is considerable functional redundancy shared between these proteins. The absence of individual nucleoid-associated proteins can, however, impact spore resistance to environmental stress, and can influence gene expression throughout the *Streptomyces* life cycle.

THE EFFECT OF CHROMOSOME-ORGANIZING PROTEINS ON GENE REGULATION AND CONTROL OF SECONDARY METABOLISM

In addition to playing fundamental roles in *Streptomyces* growth and development, chromosome-organizing proteins can also profoundly influence secondary metabolism, and make significant contributions to gene regulation. Recent work has revealed that Lsr2, an H-NS-like dimeric protein, functions as a major regulator of gene expression in *S. venezuelae* (Gehrke *et al.* 2019). Members of the Lsr2/H-NS family of proteins bind to AT-rich sequences and typically repress gene expression by either bridging DNA segments and trapping RNA polymerase, or polymerizing along the DNA and forming a rigid filament that is transcriptionally inactive (Dame 2005; Gordon *et al.* 2010; van der Valk *et al.* 2017). Coupling RNA-sequencing (RNA-seq) with the mapping of Lsr2-binding sites using chromatin immunoprecipitation and deep sequencing (ChIP-seq) led to the discovery that Lsr2 serves as a metabolic gatekeeper, directly repressing the expression of genes in the majority of secondary metabolic clusters in *S. venezuelae* (Gehrke *et al.* 2019). In contrast to Lsr2's repressive effects, altering chromosome topology by either inhibiting gyrase or decreasing TopA levels in vegetatively growing *S. coelicolor* led to the discovery that changes in chromosome supercoiling also function as positive regulators of gene transcription, inducing the expression of several antibiotic biosynthetic clusters, among many other genes (Szafran *et al.* 2013, 2019). Indeed, long-term TopA depletion had pleiotropic effects and led to transcriptional changes in at least 7% of *S. coelicolor* genes. sIHF also impacts antibiotic production in a growth medium-dependent manner, although how its regulatory effects are exerted is currently unclear (Yang *et al.* 2012; Swiercz *et al.* 2013). On some media types, an *sIHF* mutant exhibits less antibiotic production than its wild-type parent, while on other media types, it overproduces antibiotics. It is conceivable that altered chromosomal supercoiling contributes to this differential antibiotic production by the *sIHF* mutant (as sIHF can inhibit TopA activity *in vitro*) (Swiercz *et al.* 2013), in addition to any direct regulatory effects stemming from its DNA binding.

Increasingly, the distinction between NAPs and transcription factors is becoming less clear (Dorman *et al.* 2020), as is exemplified by two classical transcription regulators, Crp and BldC. Crp is conserved in many bacteria, and is one of the best-studied transcription factors. It is also among the most abundant DNA-binding proteins in *S. coelicolor* (Bradshaw, Saalbach and McArthur 2013), and is known to bend DNA when it binds its consensus sequence, thus remodeling the local DNA architecture; these are characteristics typically ascribed to NAPs. In *S. coelicolor*, Crp was experimentally determined to have ∼400 binding sites, and to impact the expression of similar numbers of genes (Gao *et al.* 2012). Like Lsr2, Crp appears to have a central role in governing secondary metabolism, only its effect is predominantly one of activation, in contrast to the repressive effects seen for Lsr2. More recently, the MerR-family transcription factor BldC has also been discovered to have NAP-like properties. This small regulator binds to DNA with flexible sequence specificity, and associates with DNA in a head-to-tail configuration that leads to both the formation of nucleoprotein filaments and distortion of the bound DNA (Bush *et al.* 2019). Again, the impact of BldC on DNA topology would suggest it functions as a nucleoid-associated protein. ChIP-seq experiments have revealed BldC binds to >350 sites throughout the *S. venezuelae* chromosome, while RNA-seq experiments revealed its regulatory effects to be both positive and negative. Unlike Crp and Lsr2, however, BldC has little effect on secondary metabolism, and instead has profound impacts on classical development, influencing the expression of many genes needed for growth (e.g. *divIVA*), cell division (e.g. *ftsZ*) and chromosome segregation and compaction (e.g. *hupS*, *sepG* and *sffA*) (Bush *et al.* 2019).

Manipulating the activities of Lsr2 and Crp has proven to be productive avenue for stimulating the expression and production of 'cryptic' secondary metabolites, and it is conceivable that modulating the function of other NAPs, and/or generally altering chromosome architecture or local domain structure, may yield new activation strategies for many of the uncharacterized biosynthetic clusters encoded in the genomes of *Streptomyces* spp. Proteomic analysis of the *S. coelicolor* nucleoid (Bradshaw, Saalbach and McArthur 2013) revealed many of the expected

NAPs (HupA, HupS, sIHF, Lsr2, Crp). But alongside these were many uncharacterized proteins, as well as more conventional transcription factors like BldD and AfsQ1, where BldD is a well-studied transcription factor that controls both development and secondary metabolism in various streptomycetes (Elliot *et al.* 1998; den Hengst *et al.* 2010; Wang *et al.* 2013) and AfsQ1 is a classical response regulator that has antibiotic modulatory capabilities (Ishizuka *et al.* 1992; Daniel-Ivan *et al.* 2017). It is clear that we are lacking a comprehensive understanding of the factors capable of influencing chromosome structure in the streptomycetes, and as we begin to appreciate how these different proteins function, there will be a corresponding opportunity to shed light on new levels of secondary metabolic control.

Chromosome dynamics are intimately entwined with transcriptional regulation, and it will be important to consider proteins involved in chromosome organization, when defining any regulatory cascade of interest. Reciprocally, it will be critical to account for the architectural contributions made by 'conventional' transcription factors, when investigating the factors that influence chromosome structure.

CONCLUDING REMARKS

It is an exciting time to be probing chromosome dynamics and the diverse roles and functions of the proteins that influence chromosome compaction, segregation, organization, and transcription. The unique life cycle of *Streptomyces* provides an outstanding opportunity to explore chromosome dynamics and organization in diverse contexts, and investigations are revealing intriguing adaptations that allow these bacteria to thrive. An ambitious, long-term goal will be to achieve a systems-level understanding of how the activity of the diverse proteins summarized in Table 2 collectively functions to ensure chromosome integrity and functionality.

FUNDING

We would like to thank the Canadian Institutes of Health Research (MAE) and the Boris Family Foundation (MAE) for their support of this work. MJS and DJ acknowledge support from the National Science Centre, Poland: HARMONIA grant 2016/22/NZ1/00122 (MJS), SONATA grant 2018/31/D/NZ1/00287 (MJS) and OPUS grant 2018/31/B/NZ1/00614 (DJ).

Conflict of interest. The authors declare no conflict of interest.

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