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Reconciling DNA replication and transcription in a hyphal organism: Visualising transcription complexes in live *Streptomyces coelicolor*

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Summary

Reconciling transcription and DNA replication in the growing hyphae of the filamentous bacterium *Streptomyces* presents several physical constraints on growth due to their apically extending and branching, multigenomic cells and chromosome replication being independent of cell division. Using a GFP translational fusion to the β`-subunit of RNA polymerase (*rpoC-egfp*), in its native chromosomal location, we observed growing *Streptomyces* hyphae using time-lapse microscopy throughout the lifecycle and under different growth conditions. The RpoC-eGFP fusion co-localised with DNA around 1.8 μm behind the extending tip, whereas replisomes localise around 4-5 μm behind the tip, indicating that at the growing tip, transcription and chromosome replication are to some degree spatially separated. Dual-labeled RpoC-egfp/DnaN-mCherry strains also indicate that there is limited co-localisation of transcription and chromosome replication at the extending hyphal tip. This likely facilitates the use of the same DNA molecule for active transcription and chromosome replication in growing cells, independent of cell division. This represents a novel, but hitherto unknown mechanism for reconciling two fundamental processes that utilise the same macromolecular template that allows for rapid growth without compromising chromosome replication in filamentous bacteria and may have implications for evolution of filamentous growth in microorganisms, where uncoupling of DNA replication from cell division is required.

Introduction

The processes of transcription and chromosome replication occupy the same cellular template and understanding how conflicts between transcription and replication are reconciled is fundamental to understanding the complexities of bacterial growth and dynamics bacterial nucleoid[1,1,2]. In eukaryotes this problem is solved by segregating growth and replication in to separate stages within the cell cycle. In bacteria, this is not the case and spatial organisation of the nucloids is dependent on the growth habits and morphology of the specific bacterium[3]. Bacterial RNAP is highly sensitive to environmental cues and is subject to significant compaction and expansion forces due to the action of DNA-binding proteins, DNA supercoiling, macromolecular crowding, interaction with cytoskeletal proteins and transertion[4,5] impacting on other cell processes such as DNA replication.

Streptomyces are filamentous saprophytic bacteria that have a complex lifecycle, where a single unigenomic spore gives rise to a multi-compartment, multi-genomic vegetative hyphal mass that can forage for nutrients through tip extension. In response to nutrient limitation or stress, specialised multigenomic aerial hyphae are raised in to the air that form septa, resulting in the formation of a unigenomic compartment which completes development in to a mature spore[6,7]. This hyphal growth habit is remarkably similar to that of the filamentous fungi and represents an excellent example of how two groups of organisms have adapted to life in soil through convergent evolution. Several aspects of *Streptomyces* biology challenge our understanding of bacterial nucleoid structure/function and cell division, its links to chromosome replication and segregation and how this is reconciled with transcriptional activity. The large (8-10 Mbp) linear chromosome found in *Streptomyces*, appears to be largely uncondensed during vegetative growth[8] but is highly ordered in terms of its structure and transcriptional activity[9] and unlike the majority of bacteria it can be replicated independently of cell division[10]. *Streptomyces* are unusual amongst bacteria as many of the genes required for cell division are dispensable for vegetative growth such as *ftsZ*, *ftsQ*, and *mreB,* contrary to that observed in unicellular bacteria[10-12]. The temporal and spatial location and activity of key cellular proteins and nucleoids in *Streptomyces* is likely to have significant implications for our understanding of growth and development in hyphally growing bacteria. It is known that chromosome replication does not occur at the apex of hyphal tips in *Streptomyces*[8,13,14] yet it is asynchronous and non-uniform along extending hyphae[3,8]. What is less well understood is whether there is any hierarchical

organisation of transcription in growing *Streptomyces* hyphae. In unicellular bacteria transcriptional foci or patches occur in discrete locations in rapidly growing cells and are associated with the rRNA operons in bacterial chromosomes[15-18]. Recently we have begun to understand the evolutionary mechanisms that minimise these conflicts in unicellular bacteria such as chromosome organisation, avoidance of co-occupancy and recycling of stalled replisomes/RNA polymerase (RNAP) holoenzyme on DNA[1,2]. In *Streptomyces* however, the hyphal lifestyle represents a fundamental evolutionary problem, that is, to reconcile the issues of chromosome replication and transcription in tandem with the structural complications of the presence of linear chromosomes, branching and chromosome partitioning [3] and that chromosome replication is independent of cell division. To attempt to understand this problem we made a translational fusion of *rpoC* with *egfp* in its native chromosomal location and studied the dynamics of transcription throughout the lifecycle of *Streptomyces* using time-lapse microscopy in live cells.

Materials and Methods

Bacterial strains, plasmids, growth conditions and conjugal transfer from *E. coli* to *Streptomyces*

The *S. coelicolor* strains and cosmids used in this study are summarised in Table 1. All strains were grown on mannitol and soya flour (MS) agar[19], solid nutrient agar[20] or minimal medium with mannitol[21] . Conjugation from the *E. coli* strain ET12567 (*dam*$^-$ *dcm*$^-$ *hsdS*) containing the driver plasmid pUZ8002, was used to bypass the methyl-specific restriction system of *S. coelicolor* [21].

Construction of the RpoC-eGFP fusion strains

The *rpoC-egfp* fusion was created using ReDirect technology[22] in its native chromosomal location. The *egfp-aac(3)IV-oriT* cassette was amplified using oligonucleotides containing 39 nucleotide homologous extensions to chromosomal sequence of the 3' end of *rpoC* (SCO4655) and its adjacent flanking region (For - 5'-CCGCTGGAGGACTACGACTACGGTCCGTACAACCAGTACCTGCCGGGCCCGGGCTGCCGGGCCCGGAGGTGAGCAAGGGCGAGGAGCT-3' and Rev - 5'-CTCGGGGTGACCGCCCTTCGGTCGTATCAAGCTGCCCGCTTCCGGGGATCCGTCGACC-3') as used by Ruban-Osmialowska et al.,[8] in cosmid D40A, creating cosmid pLN301 (*rpoC-egfp*). The cosmid, pLN301 was moved in to the non-methylating *E. coli* strain ET12567/pUZ8002 to facilitate conjugation in to *S. coelicolor*, creating strain sLN301 (M145; *rpoC-egfp*) and was confirmed by sequencing and Southern hybridization (data not shown). Cosmid pLN301 was also moved in to the *relA* deletion strain M570 (*hyg* resistant) and mutant strains were selected on hygromycin and apramycin resistance, kanamycin sensitivity, creating sLN401. In addition pLN301 was conjugated in to DJ542, an unmarked *dnaN-mCherry* fusion. Strains were confirmed by sequencing and Southern hybridization (data not shown).

Microscopy

Using fluorescent microscopy and a previously established time-lapse fluorescent microscopy procedure[23] we monitored RpoC-eGFP as a reporter of RNAP spatial and temporal dynamics under a range of conditions (see Results). Antibiotic concentrations were as previously published (32 μg/ml for rifampicin [24]; and 13 μg/ml

for chloramphenicol [25]). Nucleic acid staining was achieved using SYTO42 (10 μM final concentration; Life Technologies Corp.) and membranes were stained using FM4-64 (2 μM final concentration; Life Technologies Corp.) according to the manufacturers instructions. Images were captured using a Nikon TE2000S inverted fluorescence microscopy. Exposure times were 20 ms for phase-contrast and 100 ms for fluorescence imaging, with the following filter settings - FITC filter (Ex 492/18; Em 520/20) for eGFP; DAPI filter (Ex 403/12; Em 455/10) for SYTO42; and TRITC filter (Ex 572/23; Em 600/20) for mCherry and FM4-64. Images were analysed using IPLab scientific imaging software version 3.7 (Scanalytics, Inc., Rockville, USA). Statistical analysis was performed using Microsoft Office Excel software.

Results and Discussion

RpoC-eGFP patches show dynamic localisation throughout the lifecycle of *Streptomyces coelicolor*.

To determine the location and dynamics of RNAP during the complex lifecycle of *S. coelicolor* we constructed a fusion of eGFP to the β` subunit of RNAP core enzyme (SCO4655 [15-18,26]). The *rpoC-egfp* fusion strain (sLN301) was found to sporulate normally and to grow at the same apical extension rate as the wild-type strain, enabling us to conclude that the fusion protein was functional (Fig. 1). We observed the location of RNAP throughout the lifecycle of *S. coelicolor* (Fig. 1) by monitoring RpoC-eGFP localization in combination with fluorescence stains for nucleic acids (SYTO42) and cell membranes (FM4-64).

RNAP was distributed throughout the apically extending germ tubes of sLN301 (*rpoC-egfp*) and co-localised with nucleic acids stained with SYTO42 (Fig. 1 A-D). Localisation of RNAP and nucleic acids was found to be in close proximity to the extending hyphal tip (< 1 μm). As the extending hyphae mature, the distance between RNAP and the apically extending tip increases. These branching vegetative hyphae exhibit nucleic acid (nucleoid) patches that co-localise with RNAP in discrete areas within the hyphae (Fig. 1. E-H; See below also). Moreover the distance from the tip to the first RNAP patch appears to be around 2 μm throughout the vegetative mycelium (1.8 μm ±0.3 μm; n=29), suggesting that transcription is spatially constrained at the extending tip as observed in other hyphae. We also observed areas of intense membrane staining which are reminiscent of the cross-membranes observed previously in *Streptomyces*[27].

Examining the distribution RNAP during the growth of aerial hyphae indicated that RNAP and nucleic acids were distributed throughout the extending aerial hyphae without showing the discrete pattern behind the extending tip observed in vegetative hyphae (Fig. 1, I-L). This may represent the requirement for complete distribution of transcriptional activity throughout the aerial hyphae for the maturation of spore chains. Examination of mature spore chains shows that RNAP co-localised with the condensed and segregated nucleoids within the septated spore chains (Fig. 1, M-P).

RNAP tracks behind the extending hyphal tip.

To characterize the dynamics of RNAP in extending hyphae time-lapse images of *S. coelicolor* sLN301 (*rpoC-egfp*) were generated as phase-contrast images merged with GFP images (FITC filter) every 30 minutes during growth on minimal medium plus mannitol as a carbon source. RpoC-eGFP was observed in discrete patches and tracked behind the extending hyphal tip (Fig. 2A) at a mean distance of either 2.0 μm (±0.4 μm; n=14) or 1.7 μm (±0.2 μm; n=15) when grown on minimal medium with mannitol or nutrient agar respectively. The dimensions of the patches being 2.5 μm (+/- 1.6 μm; n=116). No difference was observed in the average patch length between the two different media. The emerging branches on the vegetative hyphae also showed the same distribution pattern of RpoC-eGFP patches as the extending primary hyphae. There appears to be some variation in the intensity of the RNAP-eGFP patches within the hyphae, although no obvious pattern could be determined, it may be that this variation is due to the differences in expression levels of various regions in the genome, such as the rRNA operons which has been shown in a range of organisms including *Streptomyc*es[15-18,24,28].

RNAP patches and replisomes do not consistently co-localise

Examining vegetative hyphae by phase contrast, RNAP-eGFP (FITC filter) and fluorescent staining of nucleic acids (SYTO42) and membranes (FM4-64) it can be seen that RNAP patches clearly co-localize with DNA (Fig. 2B). However, nucleic acids stained by SYTO 42 extends to the hyphal tip, whereas RNAP-eGFP was never observed at the tip of extending hyphae. When compared to the patches for replisomes, measured by Wolanski et al.,[14] at 5.3 μm (± 2.0 μm) behind the hyphal tip, the RNAP-eGFP patches were found located at a mean of 1.8 μm behind the extending tip suggesting there is a spatial separation of transcription and DNA replication at the hyphal tip. These data, obtained from single tagged strains, suggest that one or more chromosomes are actively transcribing at the extending tip, yet active replication occurs behind this. To further examine this spatial separation hypothesis, a double fluorescent strain *dnaN-mCherry*/*rpoC-egfp* (sLN501) was constructed. In sLN501 (*dnaN-mCherry*/*rpoC-egfp*) RNAP patches were observed to lag behind the tip, as previously observed and DnaN-mCherry tagged replication factories were

located distal to these. Discrete RpoC-eGFP patches, un-associated with DnaN-mCherry were observed proximal to the extending tip (Fig. 2C), further supporting our hypothesis of a degree of spatial separation of transcription and DNA replication at the apical tip of extending *Streptomyces* hyphae. A detailed analysis on hyphal tips grown on minimal medium supplemented with mannitol, showed that the tip-proximal RpoC-eGFP and DnaN-mCherry did not co-localized in 42% of the tips examined (n = 85). These data suggest there is a hierarchy of chromosome occupancy at the tip of extending hyphae that is summarized in our model (Fig. 3). Whilst the molecular mechanism underpinning this spatial constraint is currently unknown, it is thought that avoiding co-occupancy of the DNA template occurs, at least to some extent, in eukaryotes[29]. The unusual combination of linear chromosomes and apical growth in *Streptomyces,* coupled with DNA replication being independent of cell division and chromosome segregation, suggests that this mechanism may have evolved to allow active transcription at the actively growing tips, independent of DNA replication and cell division. This is consistent with the replisome trafficking data of Wolanski et al.,[14] and intriguingly could involve the pleiotrophic regulator AdpA, which has recently been shown to control chromosome replication through competition with DnaA at *oriC*[30].

RNAP shows *relA*-dependant pausing during nitrogen starvation

To investigate how environmental cues may affect RNAP dynamics in *S. coelicolor* we examined the effect of the stringent response on RNAP localisation. The highly phosphorylated guanosine nucleotide ppGpp is known to mediate growth rate dependent gene expression in bacteria through direct interaction with RNAP during the stringent response[31,32]. In *Streptomyces*, ppGpp is synthesised by RelA, and has previously been shown to influence control over antibiotic production and morphological development in response to nutrient limitation[33-35], however, what is not known is how RelA influences the dynamics of RNAP within *Streptomyces* cells in response to nutrient downshift. To test this, we grew *S. coelicolor* sLN301 (WT *rpoC-egfp*) and sLN401 (*ΔrelA rpoC-egfp*) on cellophane discs placed upon on solid nutrient agar (Rich medium, amino acid/peptide based nitrogen source). Once cells were growing exponentially, cellophane squares were removed and applied to minimal medium containing sodium nitrate as the sole nitrogen source (30 mM;[36]) to induce nitrogen-starvation and the stringent response. Following nitrogen downshift, the dynamics of RNAP patches was followed (Fig. 4), in strain sLN301 (WT *rpoC-egfp*)

cell growth paused and RpoC-eGFP patches remained static, presumably during the stringent response and the synthesis of ppGpp by RelA. After 60 mins mycelial growth resumed, but from new branch points in the mycelium and following 120 mins, apical growth was within the normal distribution range of RpoC-eGFP patches. The resumption of growth via branching is intriguing and may involve the serine/threonine protein kinase, AfsK. It is known that branching is affected by environmental conditions[37] and that AfsK plays a role in the onset of secondary metabolism and sporulation, both nutrient dependent processes[38-40]. It has been shown that AfsK co-localizes and directly regulates DivIVA in *Streptomyces*[40,41]. Induction of AfsK results in branching and it is believed that phosphorylation of DivIVA results in disassembly of the apical polarisome and the assembly of new growth patches at branch points. Interestingly this could be a possible mechanism of altering growth habit in response to nutrient limitation, increasing the nutrient foraging ability of bacterial colonies. Repeating the experiment with sLN401 (*ΔrelA rpoC-egfp*) resulted in no cessation of growth and no increased branching following nitrogen-downshift. Intriguingly this suggests a role for the stringent response in reprogramming the growth habit (apical growth and branching) of *Streptomyces* in response to nitrogen-downshift, however neither AfsK or DivIVA were identified as direct targets in a microarray study of a *ΔrelA* mutant and a ppGpp inducible strain[42], suggesting there is an as yet unknown mechanism integrating these signals.

Disruption of transcription or translation results in altered RNAP dynamics in hyphae

To further understand the dynamics of RNAP in live *S. coelicolor* hyphae, we used antibiotic rifampicin to inhibit transcription and chloramphenicol to inhibit translation. *S. coelicolor* sLN301 (WT *rpoC-egfp*) was grown in the absence of each antibiotic on cellophane, once cells were growing exponentially, cellophane squares were removed and applied to the same medium containing ca 50 % of the minimum inhibitory concentrations (MIC) of each antibiotic (Fig. 5). Treatment of *S. coelicolor* sLN301 (WT *rpoC-egfp*) with rifampicin resulted in no cessation of the apical extension rate of hyphae, however RpoC-eGFP patches became dispersed, consistent with dis-association of RNAP from the nucleoid (Fig. 5); resulting in an overall increase in the size of fluorescent patches from 2.5 μm (± 1.5 μm; n=54) in untreated to 4.3 μm (± 3.0

μm; n=30). After two-hours rifampicin treatment, the distance from the hyphal tip to tip-proximal RpoC-eGFP decreased from 2.0 μm (± 0.4 μm; n=14) in untreated to 1.0 μm (±0.4; n=17). Rifampicin inhibits initiation and re-initiation of transcription through targeting β-subunit of RNAP core enzyme and this dispersal of RNAP patches following rifampicin treatment has also been observed in *Escherichia coli*[17]. Treatment of sLN301 (WT *rpoC-egfp*) with chloramphenicol resulted in a cessation of apical extension over a 120 min period and condensation of the RpoC-eGFP patches (Fig. 5), which is consistent with observations in other organisms[43]. The RpoC-eGFP patches also move away from the apical tip following treatment 2.0 μm (± 0.4 μm; n=14) in untreated to 4.5 μm (± 2.5 μm; n=15). Moreover, it has also been shown that active transcription is required for such compaction[17] suggesting that the compaction observed in *S. coelicolor* indicates that transcriptional activity is occurring in these patches and that active transcription is not occurring at the tip as shown above (Fig.1). The coupling of transcription and translation in bacteria has potentially profound effects on the structure of the nucleoid[17], the two antibiotics used in this study both inhibit translation, but in different ways; chloramphenicol directly inhibits translation, but does not prevent transcription, yet rifampicin inhibits transcription and due to the coupling of these processes in bacteria it also inhibits translation[17]. It has also been shown that transcriptional activity is adjusted in bacteria to meet the translational needs of cells under various growth conditions [44] suggesting that mechanisms to reconcile potentially conflicting key cellular processes such as transcription, translation and DNA replication can help reduce the extreme effects such process can have on growth and nucleoid structure.

Summary

The tip growth habit of *Streptomyces* challenges our understanding of how transcription and replisome occupancy of the same template in bacteria can occur. One way to resolve this is to spatially separate the two processes. Intriguingly, eukaryotic organisms temporally separate key cellular processes such as growth and replication. The data presented here suggest that the tip of the actively growing *Streptomyces* hyphae spatially separates DNA replication and transcription. In these rapidly extending areas of the mycelium, transcription and replication on the same template may lead to collisions, and separating these transcribing nucleoids from replicating nucleoids offers an attractive means to achieving this. Whilst the mechanism of this spatial separation is currently unknown, spatial or temporal separations of conflicting processes is an attractive mechanism to maximise apical growth with minimal conflict between transcription and DNA replication. This may be especially important for soil organisms such as *Streptomyces* or fungi that, through convergent evolution, exhibit similar apical growth habits in a resource-limited ecological niche.

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Author Contributions

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Data curation: LN and PAH

Formal analysis: LN and PAH

Funding acquisition: PAH

Methodology: LN and PAH

Project administration: PAH

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Conflicts of interest

The authors declare that there are no conflicts of interest

Ethical statement

No ethical approval was required**.**

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488 **Table 1.** Strains and plasmids used in this study

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Strain or plasmid	**Genotype/comments**	**Source or reference**
***S. coelicolor* strains**		
M145	Prototrophic, SCP1$^-$ SCP2$^-$	21
sLN301	Prototrophic, SCP1$^-$ SCP2$^-$; *rpoC-egfp*	This work.
M570	Δ*relA*	33
sLN401	Δ*relA; rpoC-egfp*	This work.
DJ542	M145 *dnaN-mCherry* - unmarked with antibiotic resistance	Jakimowicz, Unpublished
sLN501	M145, *rpoC-egfp* fusion in a DJ542 background – dual GFP & mCherry fluorescence	This work.
Cosmids		
D40A	SuperCos derived cosmid vector with a genomic fragment containing the *rpoC* gene.	45
pLN301	Cosmid D40A with an in-frame *eGFP* fusion to the 3` end of *rpoC* gene	This work.

Figure legends

Fig. 1. RpoC-eGFP patches show dynamic localisation throughout the lifecycle of *Streptomyces coelicolor*. Representative images of a germinating spore in phase contrast on minimal medium with mannitol **(A)**, germinating spore stained with SYTO 42 (DNA staining; **B**), RpoC-eGFP localisation in a germinating spore **(C)**, germinating spore stained with FM4-64 (membrane stain; **D**). Representative images of vegetative hyphae in phase contrast **(E)**, vegetative hyphae stained with SYTO 42 (DNA staining; **F**), RpoC-eGFP localisation in a vegetative hypha **(G)**, vegetative hyphae stained with FM4-64 (membrane stain; **H**), Representative images of aerial hyphae in phase contrast **(I)**, aerial hyphae stained with SYTO 42 (DNA staining; **J**), RpoC-eGFP localisation in an aerial hypha **(K)**, aerial hypha stained with FM4-64 (membrane stain; **L**). Representative images of a spore chain in phase contrast **(M)**, a spore chain stained with SYTO 42 (DNA staining; **N**), RpoC-eGFP localisation in a spore chain **(O)**, a spore chain stained with FM4-64 (membrane stain; **P**).

Fig. 2. RpoC-eGFP patches track behind the extending hyphal tip. (A) Time-lapse images of growing *S. coelicolor* hyphae (LN301; *rpoC-egfp*) showing the absence of RNAP-eGFP patches at the tip of extending vegetative hyphae on nutrient agar. (See also Supplementary video 1 - http://dx.doi.org/10.6084/m9.figshare.1181785) **B: RpoC-eGFP patches co-localise with DNA, but not at the hyphal tip.** Representative images of a vegetative hypha in phase contrast, stained with SYTO 42 (DNA staining), RNAP-eGFP, FM4-64 (membrane stain) and a multiprobe image (RNAP-eGFP in green and FM4-64 in red). **C: The majority of RpoC-eGFP patches do not co-localise with DnaN-mCherry at the hyphal tip, but do co-localise behind the tip.** Representative images of a vegetative hypha in phase contrast **(A),** DnaN-mCherry **(B)** RNAP-eGFP **(C)** and a multiprobe image **(D)** of RNAP-eGFP (green) and DnaN-mCherry (Red).

Fig. 3: Schematic representation of a hyphal tip (polarisome), indicating the locations of chromosomes (blue lines), transcription (green spots; this work) and replisome location(red spots) [14-18] suggesting there is a spatial separation of transcription and chromosome replication at the hyphal tip.

Fig. 4. RpoC-eGFP patches in Wild-Type *S. coeliocolor* exhibit pauses following nitrogen-downshift when compared to a Δ*relA* mutant. Time-lapse images of growing *S. coelicolor* hyphae (sLN301; *rpoC-egfp*) in nitrogen rich (nutrient agar) medium over 180 min. (See also Supplementary video 2 - http://dx.doi.org/10.6084/m9.figshare.1181781). Time-lapse images of growing *S. coelicolor* hyphae (sLN301; *rpoC-egfp*) following nitrogen downshift over 180 min. (See also Supplementary video 3 - http://dx.doi.org/10.6084/m9.figshare.1181780**).** Time-lapse images of growing M570 *S. coelicolor* hyphae (Δ*relA; rpoC-egfp*) following nitrogen downshift over 180 min. (See also Supplementary video 4 - http://dx.doi.org/10.6084/m9.figshare.1181782)

Fig. 5. RpoC-eGFP patches exhibit altered dynamics following inhibition of either transcription or translation. Time-lapse images of growing *S. coelicolor* hyphae (sLN301; *rpoC-egfp*) without any antibiotic treatment on nutrient agar. Time-lapse images of growing *S. coelicolor* hyphae (sLN301; *rpoC-egfp*) following treatment with chloramphenicol (Cm; 13 mg ml^{-1}). See also Supplementary video 5 - http://dx.doi.org/10.6084/m9.figshare.1181783. Time-lapse images of growing *S. coelicolor* hyphae (sLN301; *rpoC-egfp*) following treatment with rifampicin (rif; 32 mg ml^{-1}). Arrows are to indicate areas that change upon treatment. See also Supplementary video 6 - http://dx.doi.org/10.6084/m9.figshare.1181784 .

Supplementary data is all available on Figshare

Supp Video 1: **RpoC-eGFP patches tracking behind the extending hyphal tip.** Video of growing *S. coelicolor* hyphae (LN301; *rpoC-egfp*) showing the absence of RNAP-eGFP patches at the tip of extending vegetative hyphae. Images taken at 10 min intervals and converted to video using IPLab scientific imaging software version 3.7 (Scanalytics, Inc., Rockville, USA).
http://dx.doi.org/10.6084/m9.figshare.1181785

Supp Video 2: RpoC-eGFP patches in Wild-Type *S. coelicolor.* Video of growing *S. coelicolor* hyphae (sLN301; *rpoC-egfp*) in nitrogen rich (nutrient agar) medium over

180 min. Images taken at 10 min intervals and converted to video using IPLab scientific imaging software version 3.7 (Scanalytics, Inc., Rockville, USA). http://dx.doi.org/10.6084/m9.figshare.1181781

Supp Video 3: RpoC-eGFP patches in Wild-Type *S. coeliocolor* exhibit pauses following nitrogen-downshift. Video of growing WT *S. coelicolor* hyphae (sLN301; *rpoC-egfp*) following nitrogen downshift over 180 min. Images taken at 10 min intervals and converted to video using IPLab scientific imaging software version 3.7 (Scanalytics, Inc., Rockville, USA).
http://dx.doi.org/10.6084/m9.figshare.1181780

Supp Video 4: RpoC-eGFP patches in a Δ*relA* mutant of *S. coeliocolor* exhibit pauses following nitrogen-downshift. Video of growing *S. coelicolor* hyphae (Δ*relA; rpoC-egfp*) following nitrogen downshift over 180 min. Images taken at 10 min intervals and converted to video using IPLab scientific imaging software version 3.7 (Scanalytics, Inc., Rockville, USA).
http://dx.doi.org/10.6084/m9.figshare.1181782

Supp Video 5: **RpoC-eGFP patches exhibit altered dynamics following inhibition of translation.** Video of growing *S. coelicolor* hyphae (sLN301; *rpoC-egfp*) following treatment with chloramphenicol (Cm; 13 mg ml^{-1}). Images taken at 10 min intervals and converted to video using IPLab scientific imaging software version 3.7 (Scanalytics, Inc., Rockville, USA).
http://dx.doi.org/10.6084/m9.figshare.1181783

Supp Video 6: **RpoC-eGFP patches exhibit altered dynamics following inhibition of transcription.** Video of growing *S. coelicolor* hyphae (sLN301; *rpoC-egfp*) following treatment with rifampicin (rif; 32 mg ml^{-1}). Images taken at 10 min intervals and converted to video using IPLab scientific imaging software version 3.7 (Scanalytics, Inc., Rockville, USA). http://dx.doi.org/10.6084/m9.figshare.1181784

Fig. 1

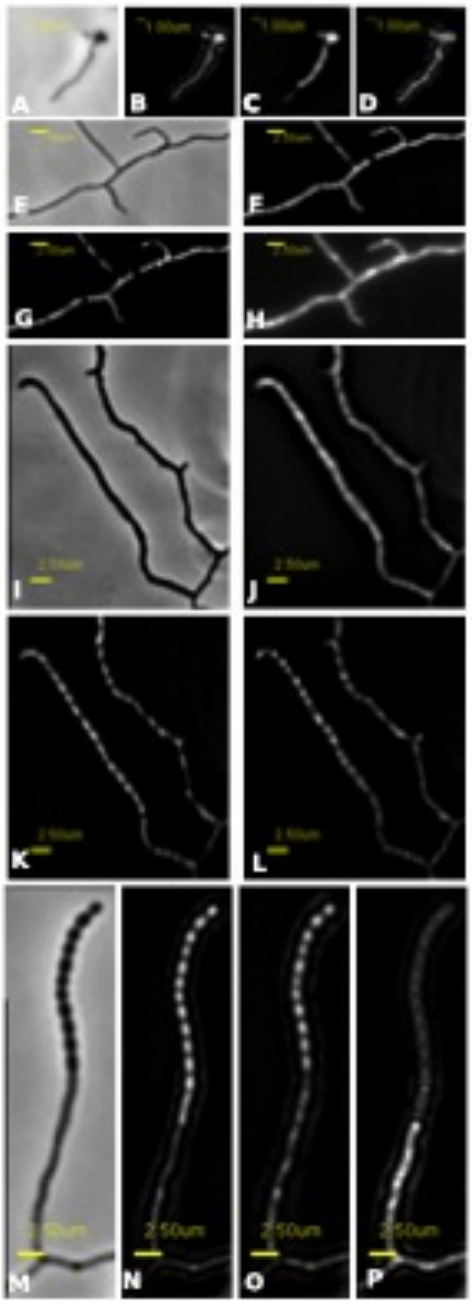

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Fig. 2

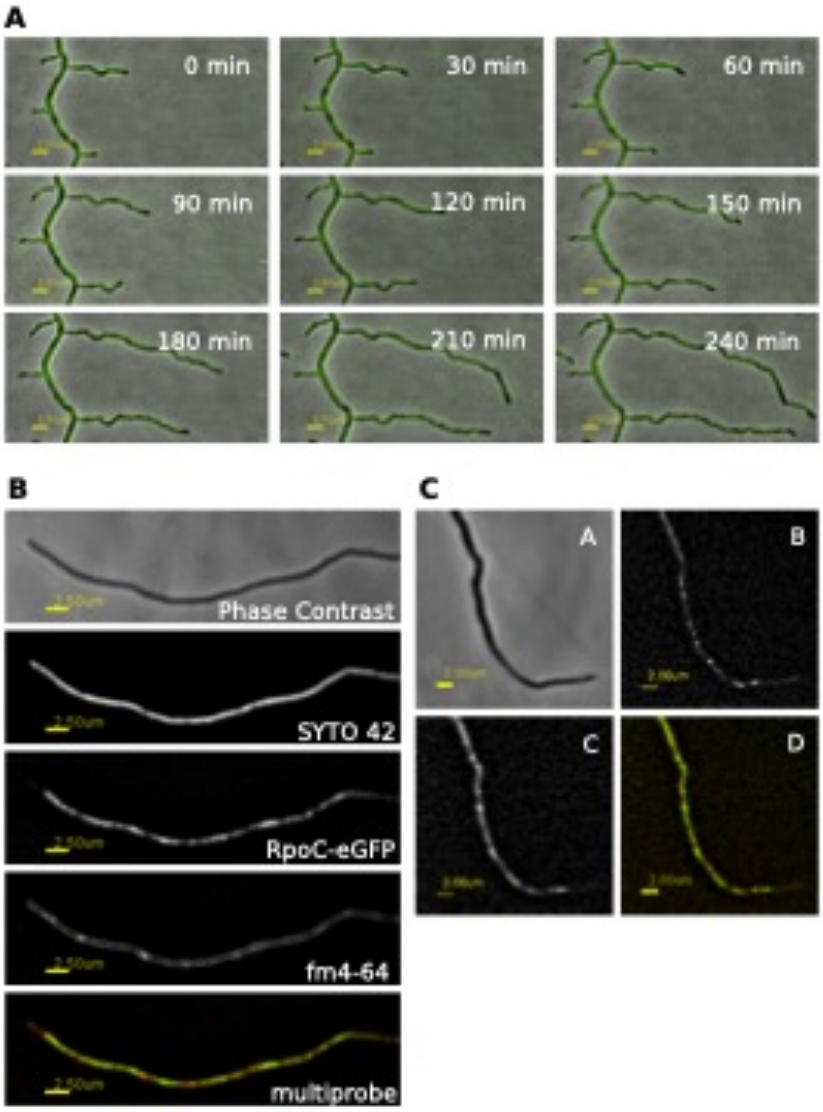
A
0 min
30 min
60 min
90 min
120 min
150 min
180 min
210 min
240 min
B
Phase Contrast
SYTO 42
RpoC-eGFP
fm4-64
multiprobe
C
A
B
C
D

Fig. 3

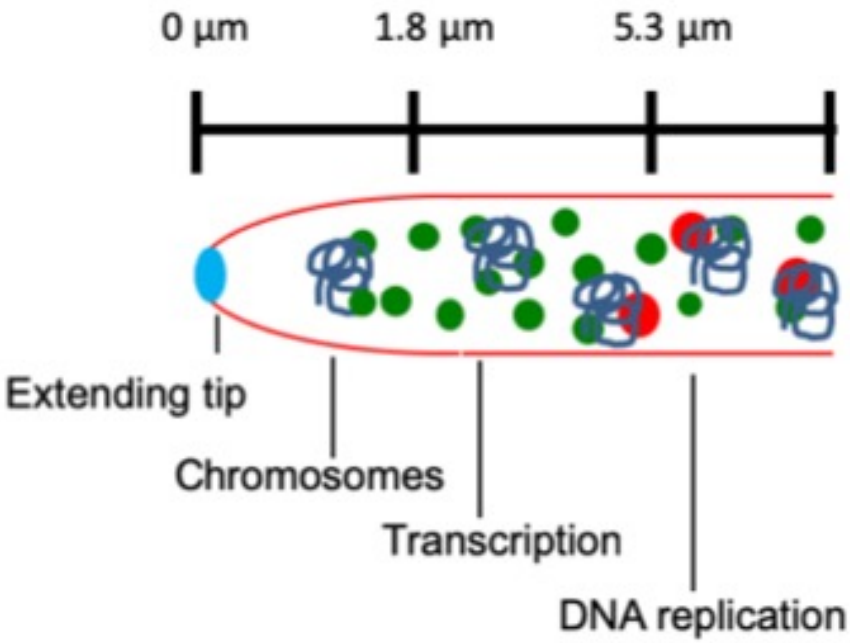
0 µm
1.8 µm
5.3 µm
Extending tip
Chromosomes
Transcription
DNA replication

Fig. 4

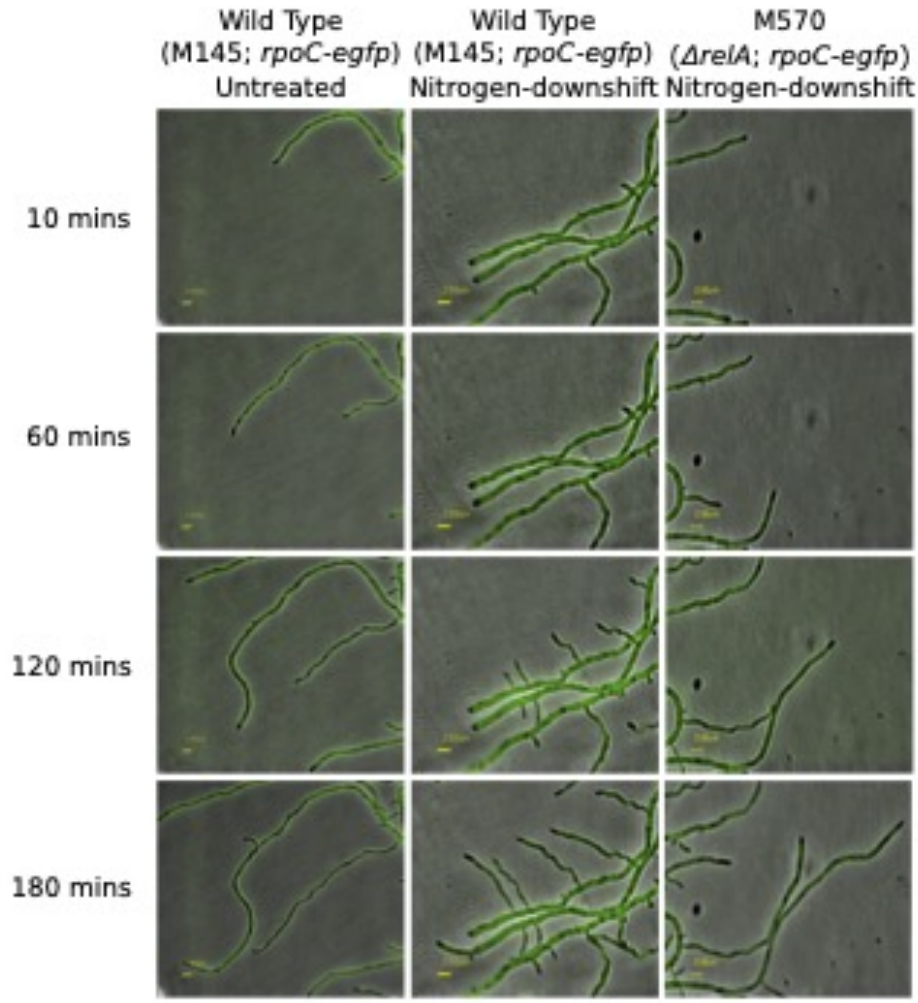
Wild Type
(M145; rpoC-egfp)
Untreated
Wild Type
(M145; rpoC-egfp)
Nitrogen-downshift
M570
(ΔrelA; rpoC-egfp)
Nitrogen-downshift
10 mins
60 mins
120 mins
180 mins

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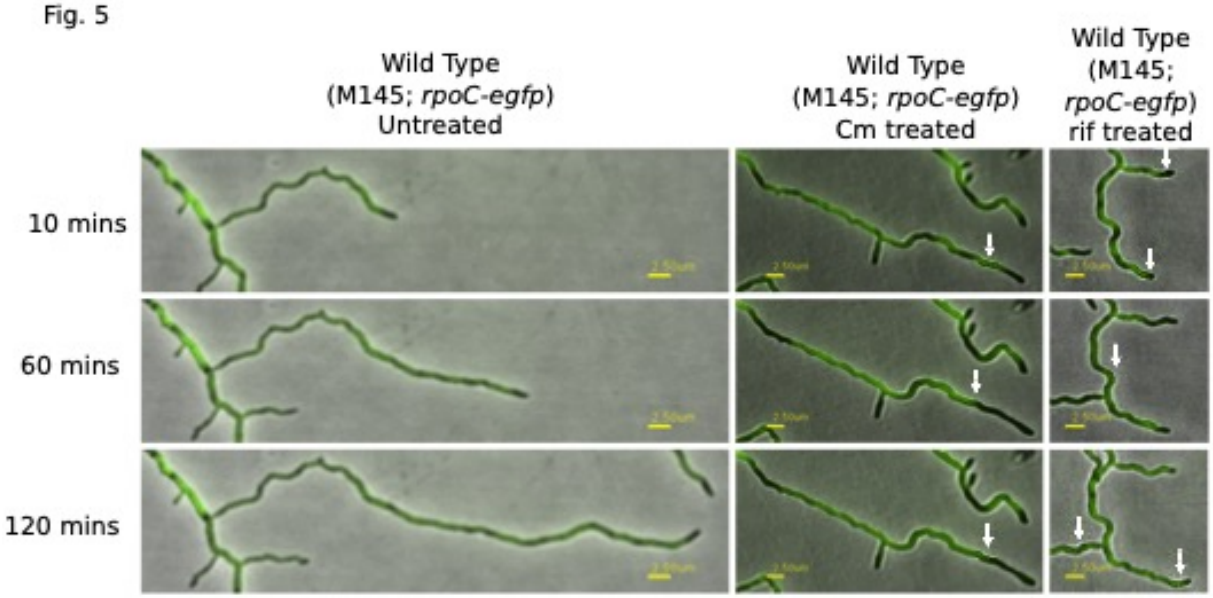
Fig. 5



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