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

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Key Words: *Streptomyces*, RNA polymerase dynamics, Transcription, Translation, DNA replication.

27 **Summary**

28

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

48

49

50 **Introduction**

51 The processes of transcription and chromosome replication occupy the same cellular
52 template and understanding how conflicts between transcription and replication are
53 reconciled is fundamental to understanding the complexities of bacterial growth and
54 dynamics bacterial nucleoid^{1,1,2}. In eukaryotes this problem is solved by segregating
55 growth and replication in to separate stages within the cell cycle. In bacteria, this is not
56 the case and spatial organisation of the nucleoids is dependent on the growth habits
57 and morphology of the specific bacterium³. Bacterial RNAP is highly sensitive to
58 environmental cues and is subject to significant compaction and expansion forces due
59 to the action of DNA-binding proteins, DNA supercoiling, macromolecular crowding,
60 interaction with cytoskeletal proteins and transcription^{4,5} impacting on other cell
61 processes such as DNA replication.

62 *Streptomyces* are filamentous saprophytic bacteria that have a complex lifecycle,
63 where a single unigenomic spore gives rise to a multi-compartment, multi-genomic
64 vegetative hyphal mass that can forage for nutrients through tip extension. In response
65 to nutrient limitation or stress, specialised multigenomic aerial hyphae are raised in to
66 the air that form septa, resulting in the formation of a unigenomic compartment which
67 completes development in to a mature spore^{6,7}. This hyphal growth habit is remarkably
68 similar to that of the filamentous fungi and represents an excellent example of how two
69 groups of organisms have adapted to life in soil through convergent evolution. Several
70 aspects of *Streptomyces* biology challenge our understanding of bacterial nucleoid
71 structure/function and cell division, its links to chromosome replication and segregation
72 and how this is reconciled with transcriptional activity. The large (8-10 Mbp) linear
73 chromosome found in *Streptomyces*, appears to be largely uncondensed during
74 vegetative growth⁸ but is highly ordered in terms of its structure and transcriptional
75 activity⁹ and unlike the majority of bacteria it can be replicated independently of cell
76 division¹⁰. *Streptomyces* are unusual amongst bacteria as many of the genes required
77 for cell division are dispensable for vegetative growth such as *ftsZ*, *ftsQ*, and *mreB*,
78 contrary to that observed in unicellular bacteria¹⁰⁻¹². The temporal and spatial location
79 and activity of key cellular proteins and nucleoids in *Streptomyces* is likely to have
80 significant implications for our understanding of growth and development in hyphally
81 growing bacteria. It is known that chromosome replication does not occur at the apex
82 of hyphal tips in *Streptomyces*^{8,13,14} yet it is asynchronous and non-uniform along
83 extending hyphae^{3,8}. What is less well understood is whether there is any hierarchical

84 organisation of transcription in growing *Streptomyces* hyphae. In unicellular bacteria
85 transcriptional foci or patches occur in discrete locations in rapidly growing cells and
86 are associated with the rRNA operons in bacterial chromosomes¹⁵⁻¹⁸. Recently we
87 have begun to understand the evolutionary mechanisms that minimise these conflicts
88 in unicellular bacteria such as chromosome organisation, avoidance of co-occupancy
89 and recycling of stalled replisomes/RNA polymerase (RNAP) holoenzyme on DNA^{1,2}.
90 In *Streptomyces* however, the hyphal lifestyle represents a fundamental evolutionary
91 problem, that is, to reconcile the issues of chromosome replication and transcription
92 in tandem with the structural complications of the presence of linear chromosomes,
93 branching and chromosome partitioning ³ and that chromosome replication is
94 independent of cell division. To attempt to understand this problem we made a
95 translational fusion of *rpoC* with *egfp* in its native chromosomal location and studied
96 the dynamics of transcription throughout the lifecycle of *Streptomyces* using time-
97 lapse microscopy in live cells.
98

99 **Materials and Methods**

100

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

103 The *S. coelicolor* strains and cosmids used in this study are summarised in Table 1.
104 All strains were grown on mannitol and soya flour (MS) agar¹⁹, solid nutrient agar²⁰ or
105 minimal medium with mannitol²¹. Conjugation from the *E. coli* strain ET12567 (*dam*⁻
106 *dcm*⁻ *hsdS*) containing the driver plasmid pUZ8002, was used to bypass the methyl-
107 specific restriction system of *S. coelicolor*²¹.

108

109 **Construction of the RpoC-eGFP fusion strains**

110 The *rpoC-egfp* fusion was created using ReDirect technology²² in its native
111 chromosomal location. The *egfp-aac(3)IV-oriT* cassette was amplified using
112 oligonucleotides containing 39 nucleotide homologous extensions to chromosomal
113 sequence of the 3' end of *rpoC* (SCO4655) and its adjacent flanking region (For - 5'-
114 CCGCTGGAGGACTACGACTACGGTCCGTACAACCAGTACCTGCCGGGCCCGG
115 GCTGCCGGGCCCGGAGGTGAGCAAGGGCGAGGAGCT-3' and Rev - 5'-
116 CTCGGGGTGACCGCCCTTCGGTCGTATCAAGCTGCCCGCTTCCGGGGATCCG
117 TCGACC-3') as used by Ruban-Osmialowska et al.,⁸ in cosmid D40A, creating cosmid
118 pLN301 (*rpoC-egfp*). The cosmid, pLN301 was moved in to the non-methylating *E.*
119 *coli* strain ET12567/pUZ8002 to facilitate conjugation in to *S. coelicolor*, creating strain
120 sLN301 (M145; *rpoC-egfp*) and was confirmed by sequencing and Southern
121 hybridization (data not shown). Cosmid pLN301 was also moved in to the *relA* deletion
122 strain M570 (*hyg* resistant) and mutant strains were selected on hygromycin and
123 apramycin resistance, kanamycin sensitivity, creating sLN401. In addition pLN301
124 was conjugated in to DJ542, an unmarked *dnaN-mCherry* fusion. Strains were
125 confirmed by sequencing and Southern hybridization (data not shown).

126

127 **Microscopy**

128

129 Using fluorescent microscopy and a previously established time-lapse fluorescent
130 microscopy procedure²³ we monitored RpoC-eGFP as a reporter of RNAP spatial and
131 temporal dynamics under a range of conditions (see Results). Antibiotic
132 concentrations were as previously published (32 µg/ml for rifampicin²⁴; and 13 µg/ml

133 for chloramphenicol²⁵). Nucleic acid staining was achieved using SYTO42 (10 µM final
134 concentration; Life Technologies Corp.) and membranes were stained using FM4-64
135 (2 µM final concentration; Life Technologies Corp.) according to the manufacturers
136 instructions. Images were captured using a Nikon TE2000S inverted fluorescence
137 microscopy. Exposure times were 20 ms for phase-contrast and 100 ms for
138 fluorescence imaging, with the following filter settings - FITC filter (Ex 492/18; Em
139 520/20) for eGFP; DAPI filter (Ex 403/12; Em 455/10) for SYTO42; and TRITC filter
140 (Ex 572/23; Em 600/20) for mCherry and FM4-64. Images were analysed using IPLab
141 scientific imaging software version 3.7 (Scanalytics, Inc., Rockville, USA). Statistical
142 analysis was performed using Microsoft Office Excel software.

143

144

145 **Results and Discussion**

146

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

149

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

158 RNAP was distributed throughout the apically extending germ tubes of sLN301 (*rpoC-*
159 *egfp*) and co-localised with nucleic acids stained with SYTO42 (Fig. 1 A-D).
160 Localisation of RNAP and nucleic acids was found to be in close proximity to the
161 extending hyphal tip ($< 1 \mu\text{m}$). As the extending hyphae mature, the distance between
162 RNAP and the apically extending tip increases. These branching vegetative hyphae
163 exhibit nucleic acid (nucleoid) patches that co-localise with RNAP in discrete areas
164 within the hyphae (Fig. 1. E-H; See below also). Moreover the distance from the tip to
165 the first RNAP patch appears to be around $2 \mu\text{m}$ throughout the vegetative mycelium
166 ($1.8 \mu\text{m} \pm 0.3 \mu\text{m}$; $n=29$), suggesting that transcription is spatially constrained at the
167 extending tip as observed in other hyphae. We also observed areas of intense
168 membrane staining which are reminiscent of the cross-membranes observed
169 previously in *Streptomyces*²⁷.

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

177

178 **RNAP tracks behind the extending hyphal tip.**

179

180 To characterize the dynamics of RNAP in extending hyphae time-lapse images of *S.*
181 *coelicolor* sLN301 (*rpoC-egfp*) were generated as phase-contrast images merged with
182 GFP images (FITC filter) every 30 minutes during growth on minimal medium plus
183 mannitol as a carbon source. RpoC-eGFP was observed in discrete patches and
184 tracked behind the extending hyphal tip (Fig. 2A) at a mean distance of either 2.0 μm
185 ($\pm 0.4 \mu\text{m}$; n=14) or 1.7 μm ($\pm 0.2 \mu\text{m}$; n=15) when grown on minimal medium with
186 mannitol or nutrient agar respectively. The dimensions of the patches being 2.5 μm
187 (+/- 1.6 μm ; n=116). No difference was observed in the average patch length between
188 the two different media. The emerging branches on the vegetative hyphae also
189 showed the same distribution pattern of RpoC-eGFP patches as the extending primary
190 hyphae. There appears to be some variation in the intensity of the RNAP-eGFP
191 patches within the hyphae, although no obvious pattern could be determined, it may
192 be that this variation is due to the differences in expression levels of various regions
193 in the genome, such as the rRNA operons which has been shown in a range of
194 organisms including *Streptomyces*^{15-18,24,28}.

195

196 **RNAP patches and replisomes do not consistently co-localise**

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

211 located distal to these. Discrete RpoC-eGFP patches, un-associated with DnaN-
212 mCherry were observed proximal to the extending tip (Fig. 2C), further supporting our
213 hypothesis of a degree of spatial separation of transcription and DNA replication at the
214 apical tip of extending *Streptomyces* hyphae. A detailed analysis on hyphal tips grown
215 on minimal medium supplemented with mannitol, showed that the tip-proximal RpoC-
216 eGFP and DnaN-mCherry did not co-localized in 42% of the tips examined (n = 85).
217 These data suggest there is a hierarchy of chromosome occupancy at the tip of
218 extending hyphae that is summarized in our model (Fig. 3). Whilst the molecular
219 mechanism underpinning this spatial constraint is currently unknown, it is thought that
220 avoiding co-occupancy of the DNA template occurs, at least to some extent, in
221 eukaryotes²⁹. The unusual combination of linear chromosomes and apical growth in
222 *Streptomyces*, coupled with DNA replication being independent of cell division and
223 chromosome segregation, suggests that this mechanism may have evolved to allow
224 active transcription at the actively growing tips, independent of DNA replication and
225 cell division. This is consistent with the replisome trafficking data of Wolanski et al.,¹⁴
226 and intriguingly could involve the pleiotrophic regulator AdpA, which has recently been
227 shown to control chromosome replication through competition with DnaA at *oriC*³⁰.

228

229 **RNAP shows *relA*-dependant pausing during nitrogen starvation**

230 To investigate how environmental cues may affect RNAP dynamics in *S. coelicolor* we
231 examined the effect of the stringent response on RNAP localisation. The highly
232 phosphorylated guanosine nucleotide ppGpp is known to mediate growth rate
233 dependent gene expression in bacteria through direct interaction with RNAP during
234 the stringent response^{31,32}. In *Streptomyces*, ppGpp is synthesised by RelA, and has
235 previously been shown to influence control over antibiotic production and
236 morphological development in response to nutrient limitation³³⁻³⁵, however, what is not
237 known is how RelA influences the dynamics of RNAP within *Streptomyces* cells in
238 response to nutrient downshift. To test this, we grew *S. coelicolor* sLN301 (WT *rpoC*-
239 *egfp*) and sLN401 (Δ *relA rpoC-egfp*) on cellophane discs placed upon on solid nutrient
240 agar (Rich medium, amino acid/peptide based nitrogen source). Once cells were
241 growing exponentially, cellophane squares were removed and applied to minimal
242 medium containing sodium nitrate as the sole nitrogen source (30 mM;³⁶) to induce
243 nitrogen-starvation and the stringent response. Following nitrogen downshift, the
244 dynamics of RNAP patches was followed (Fig. 4), in strain sLN301 (WT *rpoC-egfp*)

245 cell growth paused and RpoC-eGFP patches remained static, presumably during the
246 stringent response and the synthesis of ppGpp by RelA. After 60 mins mycelial growth
247 resumed, but from new branch points in the mycelium and following 120 mins, apical
248 growth was within the normal distribution range of RpoC-eGFP patches. The
249 resumption of growth via branching is intriguing and may involve the serine/threonine
250 protein kinase, AfsK. It is known that branching is affected by environmental
251 conditions³⁷ and that AfsK plays a role in the onset of secondary metabolism and
252 sporulation, both nutrient dependent processes³⁸⁻⁴⁰. It has been shown that AfsK co-
253 localizes and directly regulates DivIVA in *Streptomyces*^{40,41}. Induction of AfsK results
254 in branching and it is believed that phosphorylation of DivIVA results in disassembly
255 of the apical polarisome and the assembly of new growth patches at branch points.
256 Interestingly this could be a possible mechanism of altering growth habit in response
257 to nutrient limitation, increasing the nutrient foraging ability of bacterial colonies.
258 Repeating the experiment with sLN401 ($\Delta relA rpoC-egfp$) resulted in no cessation of
259 growth and no increased branching following nitrogen-downshift. Intriguingly this
260 suggests a role for the stringent response in reprogramming the growth habit (apical
261 growth and branching) of *Streptomyces* in response to nitrogen-downshift, however
262 neither AfsK or DivIVA were identified as direct targets in a microarray study of a $\Delta relA$
263 mutant and a ppGpp inducible strain⁴², suggesting there is an as yet unknown
264 mechanism integrating these signals.

265

266 **Disruption of transcription or translation results in altered RNAP dynamics in** 267 **hyphae**

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

278 μm ; n=30). After two-hours rifampicin treatment, the distance from the hyphal tip to tip-
279 proximal RpoC-eGFP decreased from $2.0 \mu\text{m}$ ($\pm 0.4 \mu\text{m}$; n=14) in untreated to $1.0 \mu\text{m}$
280 (± 0.4 ; n=17). Rifampicin inhibits initiation and re-initiation of transcription through
281 targeting β -subunit of RNAP core enzyme and this dispersal of RNAP patches
282 following rifampicin treatment has also been observed in *Escherichia coli*¹⁷. Treatment
283 of sLN301 (WT *rpoC-egfp*) with chloramphenicol resulted in a cessation of apical
284 extension over a 120 min period and condensation of the RpoC-eGFP patches (Fig.
285 5), which is consistent with observations in other organisms⁴³. The RpoC-eGFP
286 patches also move away from the apical tip following treatment $2.0 \mu\text{m}$ ($\pm 0.4 \mu\text{m}$;
287 n=14) in untreated to $4.5 \mu\text{m}$ ($\pm 2.5 \mu\text{m}$; n=15). Moreover, it has also been shown that
288 active transcription is required for such compaction¹⁷ suggesting that the compaction
289 observed in *S. coelicolor* indicates that transcriptional activity is occurring in these
290 patches and that active transcription is not occurring at the tip as shown above (Fig.1).
291 The coupling of transcription and translation in bacteria has potentially profound
292 effects on the structure of the nucleoid¹⁷, the two antibiotics used in this study both
293 inhibit translation, but in different ways; chloramphenicol directly inhibits translation,
294 but does not prevent transcription, yet rifampicin inhibits transcription and due to the
295 coupling of these processes in bacteria it also inhibits translation¹⁷. It has also been
296 shown that transcriptional activity is adjusted in bacteria to meet the translational
297 needs of cells under various growth conditions⁴⁴ suggesting that mechanisms to
298 reconcile potentially conflicting key cellular processes such as transcription,
299 translation and DNA replication can help reduce the extreme effects such process can
300 have on growth and nucleoid structure.

301

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305

306

307 **Summary**

308 The tip growth habit of *Streptomyces* challenges our understanding of how
309 transcription and replisome occupancy of the same template in bacteria can occur.
310 One way to resolve this is to spatially separate the two processes. Intriguingly,
311 eukaryotic organisms temporally separate key cellular processes such as growth and
312 replication. The data presented here suggest that the tip of the actively growing
313 *Streptomyces* hyphae spatially separates DNA replication and transcription. In these
314 rapidly extending areas of the mycelium, transcription and replication on the same
315 template may lead to collisions, and separating these transcribing nucleoids from
316 replicating nucleoids offers an attractive means to achieving this. Whilst the
317 mechanism of this spatial separation is currently unknown, spatial or temporal
318 separations of conflicting processes is an attractive mechanism to maximise apical
319 growth with minimal conflict between transcription and DNA replication. This may be
320 especially important for soil organisms such as *Streptomyces* or fungi that, through
321 convergent evolution, exhibit similar apical growth habits in a resource-limited
322 ecological niche.
323

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327

328 **Author Contributions**

329 Conceptualization: PAH

330 Data curation: LN and PAH

331 Formal analysis: LN and PAH

332 Funding acquisition: PAH

333 Methodology: LN and PAH

334 Project administration: PAH

335 Supervision: PAH

336 Visualization: LN

337 Writing – original draft: LN and PAH

338 Writing – review and editing: LN and PAH

339

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346

347 **Conflicts of interest**

348 The authors declare that there are no conflicts of interest

349

350 **Ethical statement**

351 No ethical approval was required.

352

353

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- 487

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

489

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

490 **Figure legends**

491

492 **Fig. 1. RpoC-eGFP patches show dynamic localisation throughout the lifecycle**
493 **of *Streptomyces coelicolor*.** Representative images of a germinating spore in phase
494 contrast on minimal medium with mannitol (**A**), germinating spore stained with SYTO
495 42 (DNA staining; **B**), RpoC-eGFP localisation in a germinating spore (**C**), germinating
496 spore stained with FM4-64 (membrane stain; **D**). Representative images of vegetative
497 hyphae in phase contrast (**E**), vegetative hyphae stained with SYTO 42 (DNA staining;
498 **F**), RpoC-eGFP localisation in a vegetative hypha (**G**), vegetative hyphae stained with
499 FM4-64 (membrane stain; **H**), Representative images of aerial hyphae in phase
500 contrast (**I**), aerial hyphae stained with SYTO 42 (DNA staining; **J**), RpoC-eGFP
501 localisation in an aerial hypha (**K**), aerial hypha stained with FM4-64 (membrane stain;
502 **L**). Representative images of a spore chain in phase contrast (**M**), a spore chain
503 stained with SYTO 42 (DNA staining; **N**), RpoC-eGFP localisation in a spore chain
504 (**O**), a spore chain stained with FM4-64 (membrane stain; **P**).

505

506 **Fig. 2. RpoC-eGFP patches track behind the extending hyphal tip. (A)** Time-lapse
507 images of growing *S. coelicolor* hyphae (LN301; *rpoC-egfp*) showing the absence of
508 RNAP-eGFP patches at the tip of extending vegetative hyphae on nutrient agar. (See
509 also Supplementary video 1 - <http://dx.doi.org/10.6084/m9.figshare.1181785>) **B:**
510 **RpoC-eGFP patches co-localise with DNA, but not at the hyphal tip.**
511 Representative images of a vegetative hypha in phase contrast, stained with SYTO
512 42 (DNA staining), RNAP-eGFP, FM4-64 (membrane stain) and a multiprobe image
513 (RNAP-eGFP in green and FM4-64 in red). **C: The majority of RpoC-eGFP patches**
514 **do not co-localise with DnaN-mCherry at the hyphal tip, but do co-localise**
515 **behind the tip.** Representative images of a vegetative hypha in phase contrast (**A**),
516 DnaN-mCherry (**B**) RNAP-eGFP (**C**) and a multiprobe image (**D**) of RNAP-eGFP
517 (green) and DnaN-mCherry (Red).

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519

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

524

525 **Fig. 4. RpoC-eGFP patches in Wild-Type *S. coelicolor* exhibit pauses following**
526 **nitrogen-downshift when compared to a $\Delta relA$ mutant.** Time-lapse images of
527 growing *S. coelicolor* hyphae (sLN301; *rpoC-egfp*) in nitrogen rich (nutrient agar)
528 medium over 180 min. (See also Supplementary video 2 -
529 <http://dx.doi.org/10.6084/m9.figshare.1181781>). Time-lapse images of growing *S.*
530 *coelicolor* hyphae (sLN301; *rpoC-egfp*) following nitrogen downshift over 180 min.
531 (See also Supplementary video 3 - <http://dx.doi.org/10.6084/m9.figshare.1181780>).
532 Time-lapse images of growing M570 *S. coelicolor* hyphae ($\Delta relA$; *rpoC-egfp*) following
533 nitrogen downshift over 180 min. (See also Supplementary video 4 -
534 <http://dx.doi.org/10.6084/m9.figshare.1181782>)

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536

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

546

547 **Supplementary data is all available on Figshare**

548

549 **Supp Video 1: RpoC-eGFP patches tracking behind the extending hyphal tip.**
550 Video of growing *S. coelicolor* hyphae (LN301; *rpoC-egfp*) showing the absence of
551 RNAP-eGFP patches at the tip of extending vegetative hyphae. Images taken at 10
552 min intervals and converted to video using IPLab scientific imaging software version
553 3.7 (Scanalytics, Inc., Rockville, USA).

554 <http://dx.doi.org/10.6084/m9.figshare.1181785>

555

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

558 180 min. Images taken at 10 min intervals and converted to video using IPLab scientific
559 imaging software version 3.7 (Scanalytics, Inc., Rockville, USA).
560 <http://dx.doi.org/10.6084/m9.figshare.1181781>

561

562 **Supp Video 3: RpoC-eGFP patches in Wild-Type *S. coelicolor* exhibit pauses**
563 **following nitrogen-downshift.** Video of growing WT *S. coelicolor* hyphae (sLN301;
564 *rpoC-egfp*) following nitrogen downshift over 180 min. Images taken at 10 min intervals
565 and converted to video using IPLab scientific imaging software version 3.7
566 (Scanalytics, Inc., Rockville, USA).

567 <http://dx.doi.org/10.6084/m9.figshare.1181780>

568

569 **Supp Video 4: RpoC-eGFP patches in a $\Delta relA$ mutant of *S. coelicolor* exhibit**
570 **pauses following nitrogen-downshift.** Video of growing *S. coelicolor* hyphae ($\Delta relA$;
571 *rpoC-egfp*) following nitrogen downshift over 180 min. Images taken at 10 min intervals
572 and converted to video using IPLab scientific imaging software version 3.7
573 (Scanalytics, Inc., Rockville, USA).

574 <http://dx.doi.org/10.6084/m9.figshare.1181782>

575

576 **Supp Video 5: RpoC-eGFP patches exhibit altered dynamics following inhibition**
577 **of translation.** Video of growing *S. coelicolor* hyphae (sLN301; *rpoC-egfp*) following
578 treatment with chloramphenicol (Cm; 13 mg ml⁻¹). Images taken at 10 min intervals
579 and converted to video using IPLab scientific imaging software version 3.7
580 (Scanalytics, Inc., Rockville, USA).

581 <http://dx.doi.org/10.6084/m9.figshare.1181783>

582

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

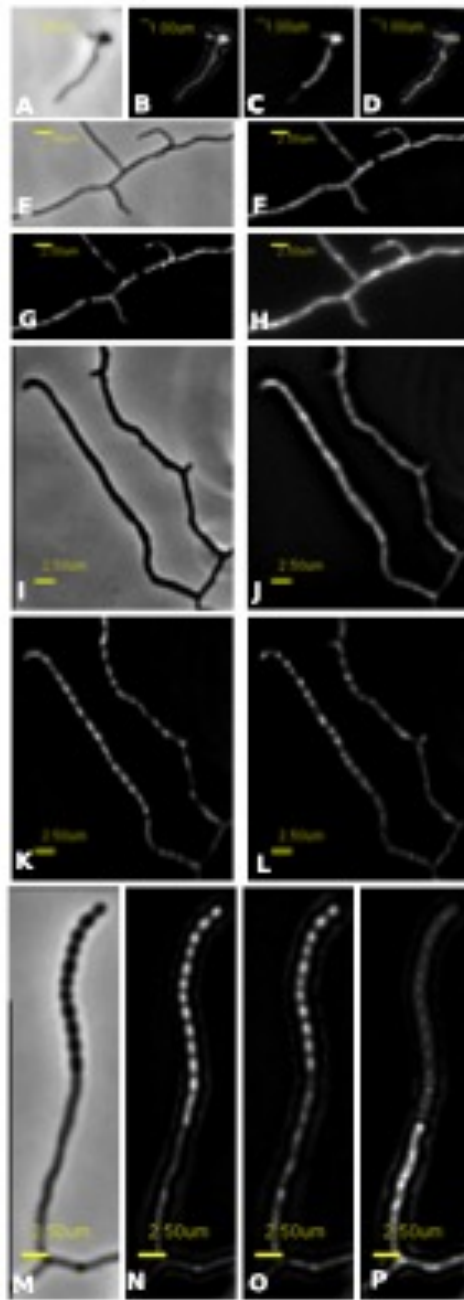
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Fig. 1



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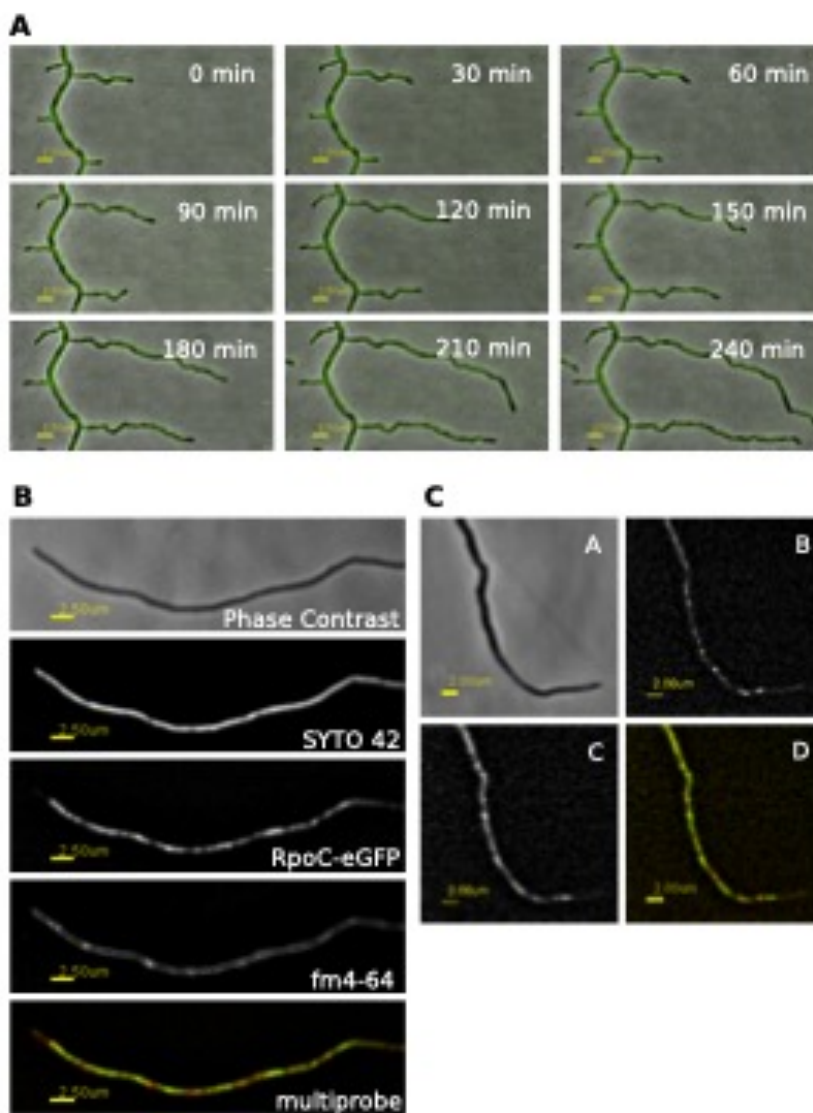
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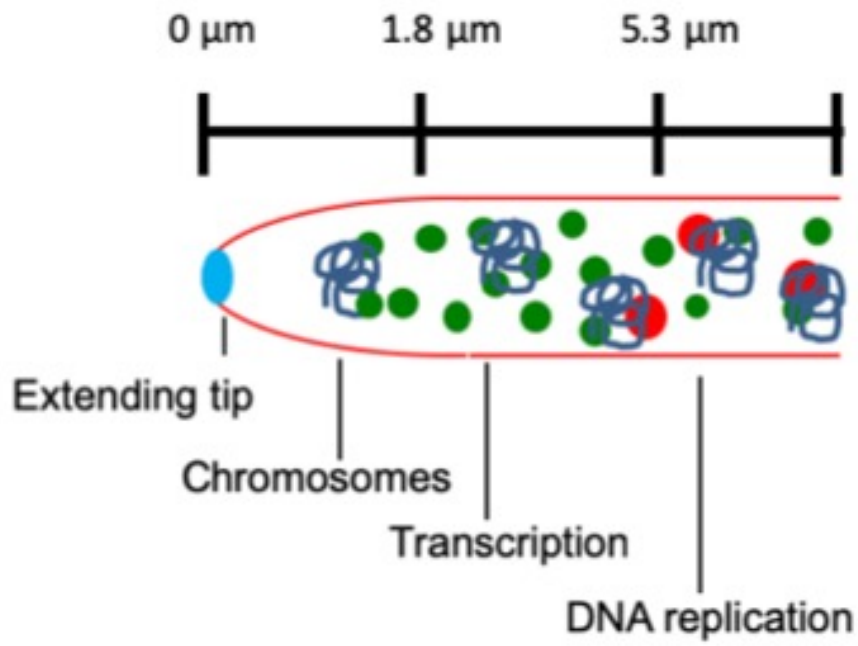
Fig. 2



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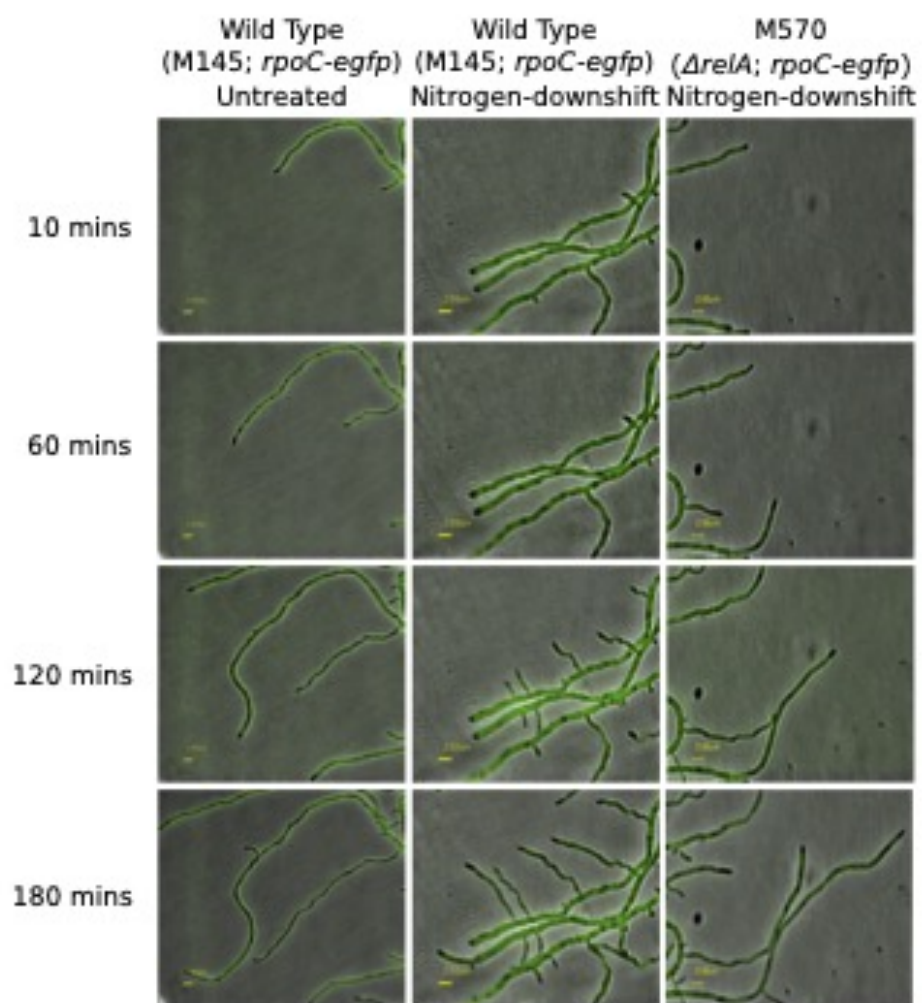
Fig. 3



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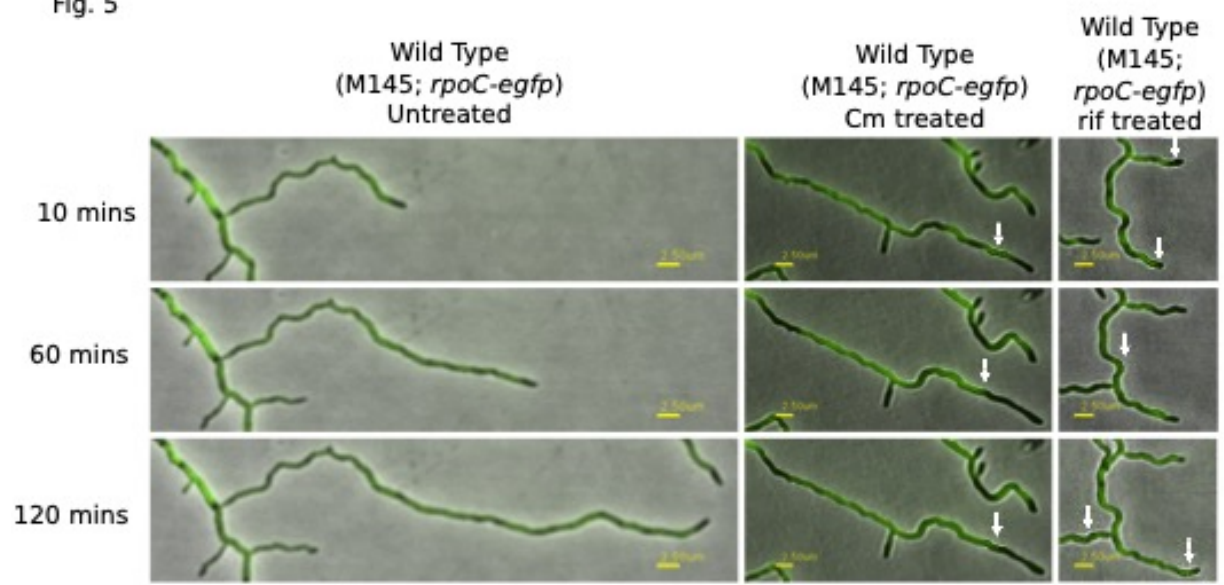
Fig. 4



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Fig. 5



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