

The absence of MaoP protein function disrupts control of DNA replication and transcriptional regulation during the stringent response



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INTRODUCTION

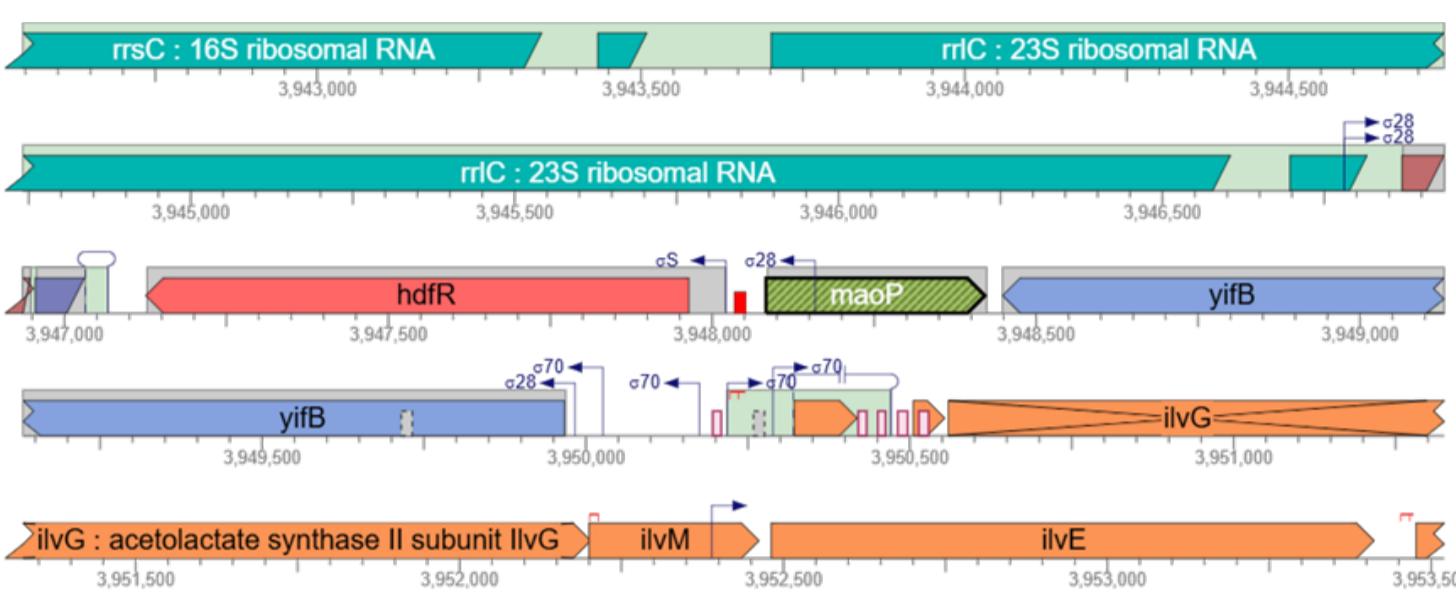


Fig. 1. Genomic context of the *maoP* gene, residing in vicinity of the *rrnC* operon in the *ori* region (source: EcoCyc)

The *Escherichia coli* MaoP protein, along with SeqA, MukBEF, and MatP, is part of a conserved group of proteins found in Enterobacteria that have coevolved with the Dam methylase. Previous studies have demonstrated that inactivation of either MaoP or a single *maoS* sequence located adjacent to the *maoP* gene leads to altered mobility of the origin (*ori*) macrodomain and disrupts its long-range interactions with other chromosomal regions¹. These findings led to the hypothesis that MaoP functions as a chromosome-organizing protein specific to the *ori* macrodomain.

In this study, we show that the loss of MaoP function results in significant changes in transcriptional activity at several promoters, particularly those located in the vicinity of the *maoS/maoP* locus. Furthermore, the transcriptional response of these genes to elevated levels of the alarmone ppGpp during the stringent response is impaired in the *maoP* deletion mutant. As a consequence, we observed deregulated initiation of DNA replication from *oriC* during the stringent response in cells lacking MaoP activity. Unlike wild-type cells, *maoP* mutants failed to inhibit the initiation of new replication rounds upon ppGpp induction.

Collectively, our findings suggest that MaoP contributes to the coordination of chromosome organization with transcriptional regulation and replication initiation, and plays a role in modulating the bacterial response to environmental stress.

METHODS

Chromosomal content analysis was performed on early exponential phase cultures grown with aeration at 37°C, as described². Then, 3-ml samples were collected, treated with 150 µg/ml rifampicin, and 10 µg/ml cephalaxin and incubated for 4 h at 37°C with mixing. Cells were harvested, washed with TBS and fixed with cold 70% ethanol overnight, followed by RNA digestion with RNase A for 4 h. Chromosomal DNA was stained with 2 mM Sytox Green (Invitrogen) and DNA content was measured with BD FACS Calibur at 488 nm Argon Ion laser (Fig. 2). MG1655 (WT) strain grown in AB medium containing different carbon sources was used as a standard (Fig. 3).

Flow cytometry after replication run-out

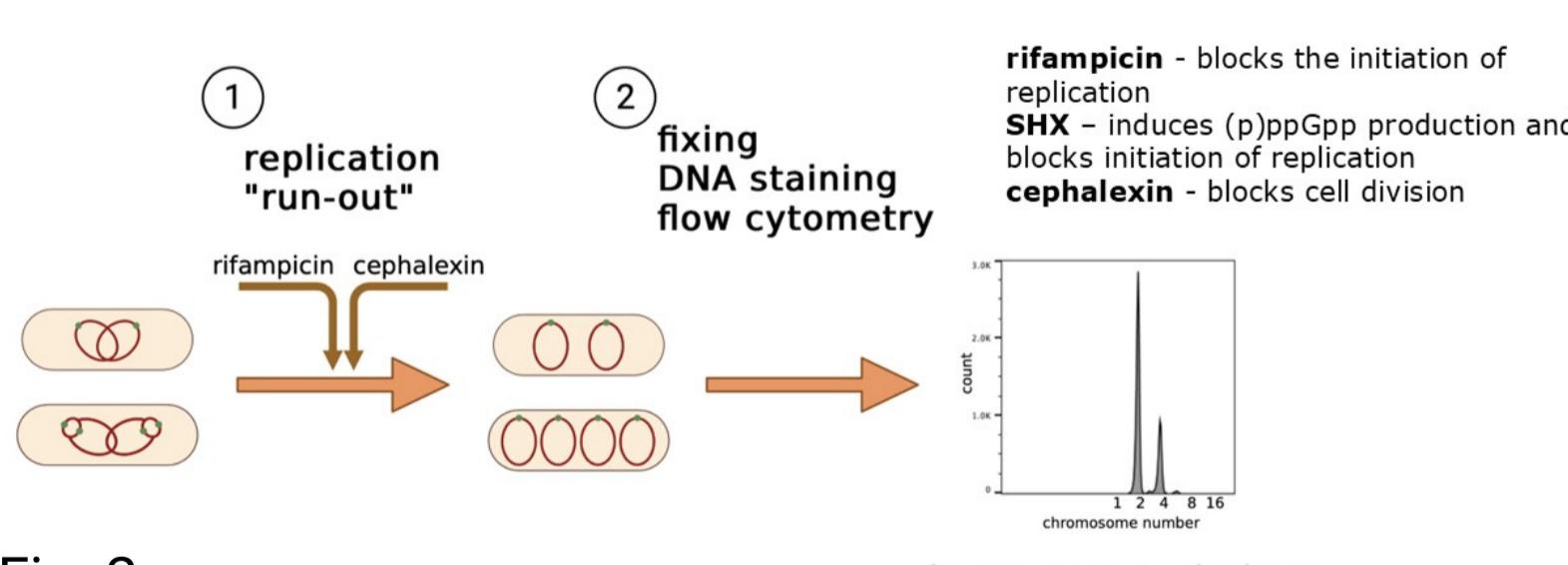


Fig. 2.

Gene expression analysis by qPCR (Fig. 3) RT-qPCR was performed using the LightCycler 480 system (Roche) in 96-well plates. Specific primers were incorporated into a SYBR Green I dye-based reaction mixture at a final concentration of 1 pmol/µl cDNA generated during reverse transcription was diluted 1:20 and added to the wells in duplicate for each sample. CT values were determined using the absolute quantification/second derivative algorithm. Relative gene expression was calculated as fold change according to the formula $x = 2^{-\Delta\Delta C_t}$, where $\Delta\Delta C_t$ represents the difference between the CT values of the target genes (*rRNA leader*, *iraP*, *argC*, and *ilvC*) measured before and after 10 or 60 min of SHX treatment in the wild-type and *ΔmaoP* strains.

REFERENCES

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3. Ferullo & Lovett (2008) doi: 10.1371/journal.pgen.1000300
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RESULTS

Fig. 3. Cell cycle progression is not inhibited in the *ΔmaoP* strain after the stringent response induction. A) Chromosome content analysis by flow cytometry after replication run-out in the wild-type strain (MG1655) growing in minimal media supporting different growth rates. B) *ΔmaoP* strain displays slower growth rate and DNA replication rate in minimal medium with glucose and casamino acids (caa) as a carbon source. C) overlay of chromosomal content in the wild-type (blue) and *ΔmaoP* strain (red) in M9 containing glucose and casamino acids. D) replication run-out after the stringent response induction with serine hydroxamate. In the wild-type strain, SHX treatment inhibits the initiation of DNA replication while permitting completion of the ongoing replication rounds. In the *ΔseqA* mutant, as shown before³, as well as in the *ΔmaoP* strain, replication initiation continues after SHX addition. This is shown by the lack of discrete peaks corresponding to integer number of chromosomes in bacterial cells.

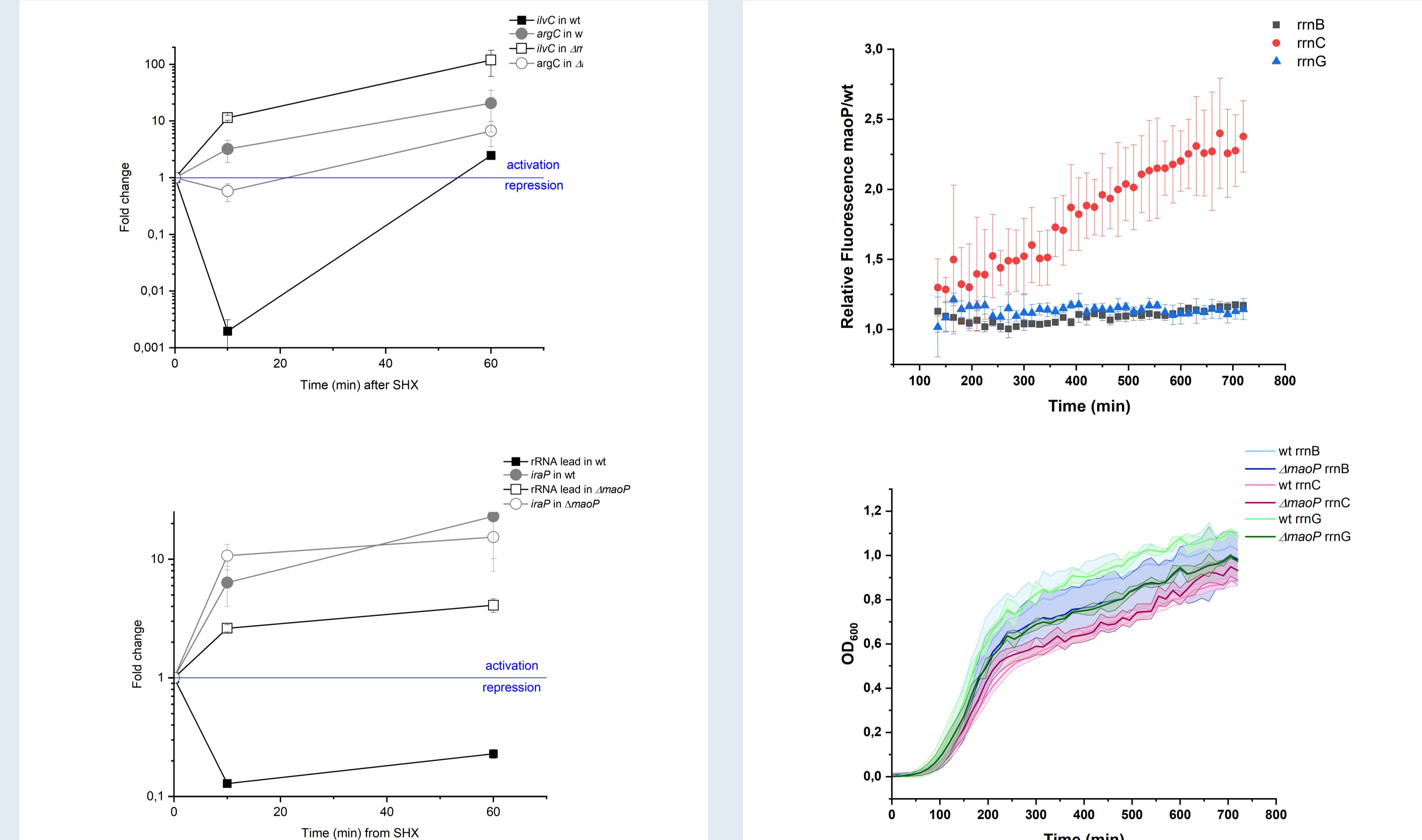
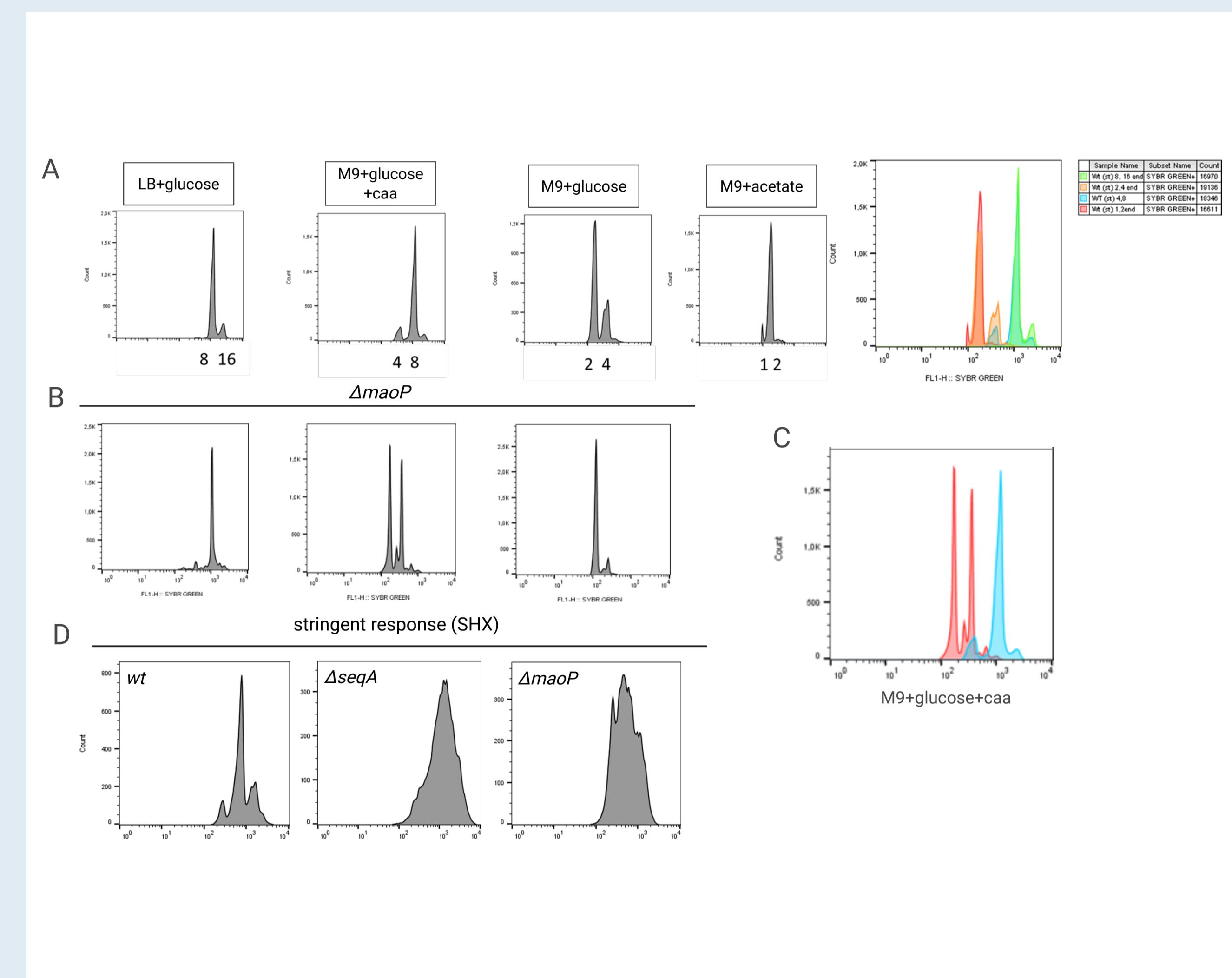


Fig. 4. (left) Deletion of the *maoP* gene alters transcription of rRNA (rRNA leader) and amino acid synthesis genes, both these adjacent to *maoP* (*ilvC*), and located further away on the chromosome (*argC*). Transcription of other amino acids pathways genes, like *leuA*, *trpC*, *ilvM* was also significantly changed in the *ΔmaoP* background (data not shown). rRNA promoters were previously demonstrated to be inhibited ppGpp⁴, whereas *iraP*, conversely, is activated by ppGpp⁵. Transcript levels were measured by RT-qPCR (Methods).

Fig. 5 (right) Transcription of *rrnC* operon is increased in the absence of *maoP* function. Transcription fusions of ribosomal operon promoters with mScarlet coding sequences were constructed. Overnight bacterial cultures were diluted 1:1000 and grown in 96-well plates at 37°C with shaking, OD₆₀₀ and fluorescence (560/600 nm) readings were taken in BioTek plate reader. Relative fluorescence was calculated in relation to OD.

CONCLUSION:

The MaoP protein function affects transcription of adjacent ribosomal operon and regulation of amino acids synthesis genes, as well as transcriptional control and DNA replication during the stringent response. We are currently investigating the impact of MaoP on chromosome structure and global transcription program during normal growth and under the stringent response.