MICROBIAL GENETICS

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Book of abstracts

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Resistance to colistin in *Pseudomonas aeruginosa* biofilms is modulated by the *arn* operon and pH

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Colistin is a cyclic antimicrobial peptide that interacts electrostatically with the lipid A moiety of LPS leading disruption of the outer membrane (OM) and, ultimately, cell death. In *Pseudomonas aeruginosa,* remodelling of the OM through aminoarabinosylation of lipid A leads to the emergence of colistin-resistant strains. Additionally, we observed that biofilms formed by colistin-sensitive strains were resistant to this drug. Considering that *P. aeruginosa* lives in a mild acidic environment in the airways of patients with cystic fibrosis, we observed greater biofilm resistance at pH 6 compared to pH 7, with a 2- to 4-fold increase in the colistin MBIC (Minimal Biofilm Inhibition Concentration). Biofilm resistance to colistin would suggest an upregulation of the *arn* operon. Accordingly, we observed that arnT, the last enzyme involved in aminoarabinosylation, was markedly upregulated in biofilms compared to planktonic cells in both reference and clinical *P. aeruginosa* Col^S strains. Furthermore, biofilm resistance to colistin was markedly lower in in *Δarn* mutant respect to the parental wt strains, with an average 8-fold reduction in MBIC. Similarly, inhibition of ArnT activity by FDO and FDO-H (doi:10.1093/jac/dkaa200; 10.1021/acs.joc.0c01459) significantly reduced MBIC values in all tested clinical isolates. Overall, our results demonstrate that upregulation of the *arn* operon contributes to intrinsic colistin resistance in *P. aeruginosa* biofilms, irrespective of the development of resistance in planktonic cells. Targeting the ArnT enzyme appears to be a promising strategy for restoring the efficacy of colistin in clinical settings where biofilm-associated infections are prevalent, such as chronic pulmonary colonization in cystic fibrosis.

Does transcription-replication interaction impact cellular metabolism?

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Abstract

Bacterial chromosome replication is one of the main drivers of gene expression throughout the cell cycle. In species like *Escherichia coli*, high growth rates induce a switch to mero-oligoploidy, where multiple active replication forks create a gene dosage gradient along the chromosome (multiplicity), from origin to terminus. Origin-proximal genes are present in higher copy numbers and may show increased expression, whereas terminus-proximal genes may be comparatively underrepresented. While the impact of DNA replication on gene expression is well-established, it remains unclear whether this effect also extends to other cellular processes, particularly cellular metabolism. Here, we integrate genomic position, multiplicity, and expression data into the *E. coli* genome-scale metabolic model to explore the role of the multiplicity-driven unbalanced expression of *ori*- vs. *ter*-proximal genes on the metabolic phenotype of the cell. We further formalize the theoretical concept that gene expression results from both regulatory control and gene copy number, and thereby try to quantify the relative contribution of multiplicity to overall expression and its metabolic impact. To experimentally support these predictions, we are currently implementing an integrated ^{13}C-based fluxomics/RNA-seq framework in *E. coli* grown under controlled glucose-limited conditions to induce distinct growth rates and, consequently, multiplicity profiles for a direct comparison/tuning of model predictions. Our work will reveal whether the effect of transcription-replication interactions, besides impacting gene expression, also propagate to the metabolic level or, rather, cells have evolved any mechanism to buffer it.

The *Pseudomonas aeruginosa* DedA protein PA4011 functions as a C55-PP phosphatase via a PAP2-like domain

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The recycling of the lipid carrier undecaprenyl phosphate (C55-P) across the cytoplasmic membrane is a key step of the peptidoglycan biosynthetic pathway. Recent studies have proposed that proteins of the DedA family mediate the flipping of C55-P back to the cytoplasmic side. However, during the recycling process, undecaprenyl pyrophosphate (C55-PP) must first be dephosphorylated.

Pseudomonas aeruginosa has six DedA proteins, one of which, PA4029, has been demonstrated to act as a C55-P flippase. Notably, besides the DedA domain, the PA4011 protein also contains a PAP2-like domain homologous to those present in the *Escherichia coli* phosphatases YbjG, PgpB, and LpxT, which, together with UppP, are involved in C55-PP dephosphorylation. Homologs of UppP and LpxT are also present in *P. aeruginosa*.

By generating double deletion mutants and a triple conditional mutant in PA4011, *uppP* and/or *lpxT*, we confirmed that PA4011 contributes to C55-P(P) recycling by acting as a C55-PP phosphatase. Indeed, deletion of *uppP* in the PA4011 mutant increased its sensitivity to the C55-P synthesis-targeting antibiotic fosmidomycin and caused growth arrest at 25°C, a condition that strongly reduces *P. aeruginosa lpxT* expression. Accordingly, growth was completely inhibited upon LpxT depletion in ΔPA4011 Δ*uppP* cells. Moreover, expression of a PA4011 variant mutated in a conserved catalytic residue and of PAP2-like domain alone demonstrated that PA4011 activity relies on its phosphatase domain. Notably, deletion of PA4011 and/or *uppP* also reduces the emergence of colistin resistance, likely due to increased LpxT-mediated transfer of phosphate from C55-PP to lipid A, which hampers its aminoarabinosylation. Finally, although inactive in C55-PP dephosphorylation, both the DedA domain alone and the catalytically-inactive mutant of PA4011 appear to have C55-P flipping activity, as their expression restored fosmidomycin resistance in the ΔPA4029 mutant.

Large-scale analysis of genetic diversity in Patescibacteria across environments

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Patescibacteria, also referred to as the Candidate Phyla Radiation (CPR), represent a vast monophyletic division within the bacterial domain, comprising diverse lineages with reduced genomes, limited metabolic capabilities, and symbiotic lifestyles. Due to these features, CPR bacteria remain largely uncultivated and are often underdetected or misclassified in 16S rRNA gene surveys. Thus, these bacteria are commonly detected and analysed using shotgun metagenomics. Despite several reports of CPR occurrence across diverse natural and human-associated sources, their global distribution and habitat preferences remain incompletely understood.

Here, we performed a large-scale study on publicly available metagenomic datasets to investigate the environmental distribution and ecological associations of CPR lineages. We developed a machine learning–based classification approach leveraging the RecA protein as a marker for the detection and classification of CPR bacteria. We applied this approach to the MGnify protein database, which contains protein sequences from tens of thousands of metagenomic samples with associated biome information, to describe CPR diversity across environmental sources. Our results indicate that CPR bacteria are widespread in freshwater environments, with lineage-specific enrichments in wastewater and human microbiomes, alongside an expansion of human-associated lineages, consistent with potential adaptation to the human host. Overall, we provide a comprehensive overview of the global distribution patterns in CPR bacteria by applying a robust marker-based bioinformatic pipeline on an extensive metagenomic database.

Investigating the role of the protein LysX2 of *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis (*Mtb*), the causative agent of tuberculosis, survives within the host through sophisticated mechanisms of environmental adaptation and resistance to immune-mediated killing, largely supported by the unique properties of its cell wall. Among the factors influencing cell wall composition, MprF-like proteins play a key role by modulating surface charge through the aminoacylation of phospholipids. *Mtb* encodes two members of this family, LysX and LysX2. While LysX has been extensively characterized, the function and mechanism of LysX2 remain poorly understood.

In this study, we investigated the role of LysX2 in its natural host, *Mtb*, by generating a knock-out strain using the ORBIT technique. Our results demonstrate that LysX2 is required for rapid adaptation to mildly acidic pH. Despite this deficiency, the KO strain compensates through the overexpression of *rv1169c*, encoding the PE11 protein involved in maintaining cell wall integrity. RNA-seq analysis further revealed the upregulation of oxidative stress response genes, including *sigH*, *katG*, and *rv2466*, in the KO strain.

Functional assays showed that the LysX2-deficient strain exhibits increased sensitivity to oxidative and nitrosative stress, as well as to vancomycin, indicating enhanced cell wall permeability and altered surface properties. High-performance thin-layer chromatography (HPTLC) lipid analysis revealed the accumulation of triglycerides under acidic conditions, consistent with a stress adaptation response. Finally, infection experiments using the murine RAW264.7 macrophage cell line demonstrated that the KO strain is significantly more susceptible to macrophage-mediated killing than the wild type.

Collectively, these findings identify LysX2 as a critical virulence factor in *Mtb*, contributing to resistance against key macrophage defense mechanisms. Further studies are needed to elucidate its molecular target and mechanism of action, potentially opening new avenues for therapeutic intervention.