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Review Article

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Intracellular Transport of Enzymes in Bacterial Cell

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Abstract

Background: Although little is known about the location and movement of enzymes within bacterial cells, this information is crucial for comprehending how metabolism is regulated spatially. The heavy RF synthase, a sizable protein complex with a capsid structure formed by RibH and an encapsulated RibE homotrimer, which regulates substrate-channeling, has been the focus of intensive Research on the four key enzymes (Rib enzymes) in the riboflavin (RF) production pathway in vitro using Bacillus subtilis, a Gram-positive model bacteria. Unfortunately, little is understood about these enzymes' activity and mobility in vivo.

Results: We looked at where the Rib enzymes were located and how they were moving around in the cytoplasm of B. subtilis. We present evidence for limited diffusion at the cell poles and otherwise Brownian motion by employing single particle tracking to characterise the diffusion of rib enzymes in living cells. Most RibH particles exhibited evident nucleoid blockage and a high level of restricted motion, which are largely eliminated following Rifampicin treatment, demonstrating that confinement is reliant on ongoing transcription. In contrast, RibE is primarily diffusive within the cell and only exhibits RibH nanocompartment encapsulation. In single cells, we localise several diffusive populations and discover that fast diffusion mostly crosses nucleoids in the cell centres whereas slower, constrained subdiffusion takes place at the congested cell poles.

Conclusions: Our findings demonstrate that active enzyme mobility varies locally within the bacterial cytoplasm, establishing metabolic compartmentalization primarily at the cell poles.

Keywords: Enzymes; RF synthase; Bacteria; Growth

Introduction

While it has long been believed that the bacterial cytoplasm does not permit for such spatial regulation due to the quick mixing of its components by passive diffusion, eukaryotic cells utilise cell compartments for spatial separation and regulation of their metabolism (Brownian motion). Since the discovery of bacterial cell biology, we have come to understand that bacteria are not just "bags of enzymes," but rather exhibit a high degree of internal structure in both time and space. However, it has not been clear whether the non-compartmentalized bacterial cell offers subcellular regions where metabolic enzymes might prefer to localise. Early research has shown that the bacterial cytoplasm can even have glass-like properties that depend on the size of the particle studied as well as on the metabolic state of the cell. It has been proposed that the vast central nucleoid of bacteria is a subcellular region that is abundant in DNA and proteins and enzymes with DNA-binding properties, and consequently depleted of cytoplasmic proteins. Nucleoids may be a physical barrier that creates concentration gradients between the cell poles and the nucleoid(s) in question. Additionally, it has been suggested that the fundamental physical basis driving the micro-compartmentation of components in the bacterial cytoplasm as a result of molecular crowding is aqueous phase separation. Spaces near the cell poles and at the locations of future cell division between separated nucleoids may contain localised metabolism, according to the hypothesis that nucleoid central occupancy may prevent space available for cytoplasmic proteins. Due to colocalized active translation, these regions are likely to have a very dynamic composition. As a result, they also have unique biophysical characteristics, such as high molecular crowding, which reduce enzyme diffusion and may encourage interactions between enzymes acting in subsequent reactions. According to modelling techniques, the huge central nucleoid that has been observed in various bacteria causes volume exclusion effects that lead large complexes like ribosomes (2 MDa) to collect at the bacterial cell poles. It has been suggested that the greater hydrodynamic interaction potential (resulting from bigger molecule surfaces) and entropic effects of larger assemblies will further improve their polar distribution. Large complexes can exclude smaller molecules (the depletant; this can be water, ions, metabolites, or proteins) from their occupied interfacial volumes when they come close together (caused by weakly attractive forces, e.g., Lennard-Jones Potential), which increases the system's entropy because many (excluded) smaller particles exhibit less order than the unspecific complex interaction. Smaller proteins were projected to gravitate towards less populated places in contrast to larger assemblies because those regions had a lesser proportion of their excluded volume. We set out to examine four enzymes/enzyme complexes with markedly different sizes that were predicted to be cytoplasmic in the Gram positive model bacterium Bacillus subtilis in order to answer the question of whether the metabolism of rod-shaped bacterial cells might be concentrated in particular subcellular regions. B. subtilis is able to synthesis riboflavin (Vitamin B2, RF) from a limited number of carbon and nitrogen sources, similar to many other bacteria, plants, and fungi. In an operon with several internal promoters, four Rib enzymes—2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5′-phosphate deaminase/5-amino-6-ribosylamino-2,4—are arranged (1H,3H) Guanosine triphosphate (GTP) and ribulose-5-phosphate (R5P) are converted to RF in a six-step process by the enzymes -pyrimidinedione 5′-phosphate reductase (RibDG), GTP-Cyclohydrolase II/3,4-dihydroxy-2-butanone-4-phosphate-

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synthase (RibAB), dimethylribityl-luma. A bifunctional flavokinase/FAD adenylyltransferase known as RibC converts RF to Flavin mononucleotide (FMN), one of its biologically active derivatives, or to Flavin adenosine dinucleotide (FAD), the other. The encoding gene ribC is not a component of the rib-operon structure, which consists of five genes in total, four of which are necessary for RF-biosynthesis. In contrast, the rib-fifth operon's gene encodes for a GCN-5-related N-acetyltransferase, for which only its crystal structure is currently known. The bifunctional enzymes RibAB and RibDG, which are thought to be present as dimers and tetramers, respectively, carry out the first four steps of biosynthesis. The first reaction is catalysed by ribAB, starting with the hydrolysis of GTP, opening of the guanine ring, removal of formate, and release of 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5-phosphate. The bifunctional [1-10] enzyme RibDG performs the following two reactions. It first deaminates DAROPP to produce 5-diamino-6-ribosylamino-2,4(1H,3H)-pyrimidinedione 5-phosphate (AROPP), and then it further reduces the ribityl-side chain to produce 5-amino-6-ribitylamino-2,4 (1H,3H)-pyrimidinedione 5-phosphate (ARIPP). The latter substance is additionally dephosphorylated by a general phosphatase with broad substrate specificity or a previously unidentified specialised phosphatase. The five-carbon precursor molecule R5P, which comes from the pentose phosphate pathway, is transformed into 3,4-dihydroxy-butanone-phosphate by RibAB in a second reaction branch. 5-amino-6-ribitylamino-2,4 (1H,3H)-pyrimidinedione (ARIP), the dephosphorylated product, and DHBP are both substrates for RibH, which catalyses their condensation to produce 6,7-dimethyl-8-ribityllumazine (DMRL). It's interesting to note that RibH creates capsids, which are structurally best defined as pentamer-based dodecamers. These 60-mer capsids contain homotrimeric RibE in their lumen, producing substrate channelling for DMRL created by RibH, which is subsequently taken up by RibE to form RF and ARIP as products. There has been a thorough analysis of the precise production method and developments in biotechnology. The "heavy RF synthase" or "3-60-complex" enzyme complex has historically been the focus of extensive structural and biochemical study. using published structures of RibH from B subtililis Rib H from B subtilis. Due to its advantageous encapsulation properties, it has been thought of as a potential pharmacological target and may potentially have uses in biotechnology, the production of vaccine carrier molecules, and other areas. We looked at the spatial organisation of Rib enzymes in light of the division of the RF biosynthesis pathway into an apparently compartmentalised and a compartmentalised component. We chose B. subtilis because it is frequently used to produce RF on an industrial basis. In order to provide evidence for transcription-dependent subcellular compartmentalization of the four Rib enzymes encoded by the rib-operon, we created functional fluorescent protein fusions to each of the four Rib enzymes. We also examined the interactions between the four Rib enzymes, with a focus on the two partner proteins of the heavy RF synthase.

Methods

Strains of bacteria and growth environments

The list of bacterial strains utilised in this investigation may be found in the Supplementary file. We employed the E. coli strain XL-1 Blue for the molecular cloning of plasmids and their proliferation (Stratagene). For plasmid preparations, all E. coli strains were routinely grown in [5-8] liquid LB medium at 37 °C with constant shaking (200 rpm) and the addition of the appropriate antibiotics to ensure selective pressure. We followed the manufacturer's instructions while using the Plasmid Mini Kit (Qiagen) to create plasmids. Using established techniques, competent E. coli XL-1 was prepared and subjected to a heat shock.

Creation of plasmids

We created pSG1164-linker-mVenus using the integrative single-crossover plasmid pSG1164 in order to produce C-terminal mV fusion proteins in place of homologous wild-type proteins.

Building of strain

We transformed PY79 wt with chromosomal donor DNA from the appropriate 168 deletion strain received from the BGSC in order to create deletion strains. The resulting transformants were carefully chosen for their PY79 phenotype, RF deficiency, and Kanamycin resistance before being employed for transformation.

Discussion

The movement of molecules within cells or at their interfaces is the basis of all life. The mobility of cytoplasmic proteins within bacterial cells has, by default, been assumed to be purely dependent on free diffusion for many decades, despite the fact that Brownian motion and guided movement have been investigated in considerable detail in eukaryotic cells. It's interesting to note that a recent study found that by fluidizing the cytoplasm, metabolic activity increases diffusion. Enzymes routinely change their shape in metabolically active cells when they attach to a substrate, release a product, or interact with another protein. Inert macromolecules like big viral protein capsids are hypothesised to be able to escape from their [9, 10] immediate local surroundings through the rearrangements of congested regions brought on by these intra- and intermolecular dynamics. It appears that ribosome crowding also causes restricted motion of other enzymes because this effect is also significantly diminished in the absence of active transcription, resulting in more uniform subcellular diffusion of RibH. Hence, we suggest that limited diffusion around nucleoids is a typical characteristic, particularly for big enzymes or enzyme complexes, creating subcellular regions of enzyme and metabolite crowding that may accelerate general metabolism in a rapidly expanding bacterial cell. Recent research has revealed that, in contrast to widely held beliefs, nucleoids in E. coli cells do not present diffusion barriers, supporting our findings.

Results

We created C-terminal mVenus (mV) fluorescent protein (FP) fusions, whose coding sequences were incorporated into the corresponding original gene loci, in order to analyse the dynamics of rib enzymes. The RibDG-mV fusion required a xylose promoter to drive ongoing transcription of downstream genes. For all other fusions, intrinsic promoters in the rib-operon made sure that transcription would continue. In this manner, fusion enzymes serve as the cell's sole source of the protein while the original promoter controls the expression of fusion protein mRNA. The growth of cells on minimal medium was supported by all fluorescent protein fusions described in this work, demonstrating that they could functionally take the place of the wild-type (wt) proteins. By using in-gel fluorescence detection, we confirmed that only full-length fusion proteins were present. Single particle tracking (SPT) slimfield microscopy was used to acquire trajectories generated from FPs, see accompanying Additional file 2: Video S1 as an illustration), followed by tracking using U-track and post-processing of point-spread functions (PSF), and then statistical analysis of trajectories using the SMTracker programme. While RibE-mV appeared to be evenly distributed throughout the cytoplasm and

was most likely to be found in the centre of cells (keeping in mind that Bacilli cells are spherocylindrical), we saw the strongest bias for RibH-mV to be located near the cell poles or in the middle of cells, which was most pronounced in mid-sized to large cells. Both the cell centre shortly before cell division, when cells contain two separated nucleoids, which cells will have as the future new pole, and the subcellular regions where the nucleoid is absent are considered to be cell poles. As a result, RibH-mV exhibits evident nucleoid occlusion (NO), just as was shown for ribosomes that were actively translating. We discovered that 28.1% of the RibDG-mV trajectories were freely diffusive without any discernible shift from a free to a confined state or vice versa using at least five consecutive steps of restricted motion. Contrarily, we discovered that 67.5% of trajectories underwent, at least in part, constrained movement with characteristically tiny JDs before these molecules began to travel in the direction of free diffusion, as determined by a larger JD distribution between successive steps. Hence we define this fraction as we consider this sub population showing "mixed diffusive behaviour." We consider this fraction as sub population showing mixed diffusive behaviour. Transitions between confinement and free diffusion in the instance of RibDG happened equally frequently, demonstrating an equilibrium process with no preference for one state.

Author Contributions

The diagnosis and treatment of this cat were handled exclusively by Jennifer Weng and Harry Cridge. This report was written by Jennifer Weng, and Harry Cridge gave it a critical appraisal. The final draught of the manuscript has received the approval of both Jennifer Weng and Harry Cridge.

Conflict of Interest

According to the authors, there are no conflicts of interest that might be thought to compromise the objectivity of the research presented.

Ethics Statement

The case described in this report was handled as part of the regular clinical caseload at the university teaching hospital; an IACUC or other ethical approval was not necessary. All facets of this patient's care had the owner's consent.

References

1. Pérez-Rodríguez M, del Pilar Cañizares-Macías (2021) Metabolic biomarker modeling for predicting clinical diagnoses through microfluidic paper-based analytical devices. Microchem J 165.
2. Singhal A ,Prabhu MS, Giri Nandagopal (2021) One-dollar microfluidic paper-based analytical devices: Do-It-Yourself approaches
3. Wang S, Blair IA, Mesaros C (2019) Analytical methods for mass spectrometry-based metabolomics studies. Advancements of Mass Spectrometry in Biomedical Research: 635-647.
4. Jang KS, Kim YH (2018) Rapid and robust MALDI-TOF MS techniques for microbial identification: a brief overview of their diverse applications. Journal of Microbiology 56:209-216.
5. Kim E, Kim J, Choi I, Lee J, Yeo WS, et al. (2020) Organic matrix-free imaging mass spectrometry. BMB reports 53:349.
6. Wang Y, Han Y, Hu W, Fu D, Wang G (2020) Analytical strategies for chemical characterization of bio-oil. Journal of separation science 43:360-371.
7. Ishii K, Zhou M, Uchiyama S (2018) Native mass spectrometry for understanding dynamic protein complex. Biochim Biophys Acta Gen Subj 1862:275-286.
8. Takeo E, Sasano R, Shimma S, Bamba T, Fukusaki E, et al. (2017) Solid-phase analytical derivatization for gas-chromatography–mass-spectrometry-based metabolomics. Journal of bioscience and bioengineering 124:700-706.
9. Micalizzi G, Vento F, Alibrando F, Donnarumma D, Dugo P, et al. (2021) Cannabis Sativa L.: A comprehensive review on the analytical methodologies for cannabinoids and terpenes characterization. Journal of Chromatography A 1637: 461864.
10. Han Y, Hu W, Fu D, Wang G (2020) Analytical strategies for chemical characterization of bio-oil. Journal of separation science 43:360-371.