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Quantifying Current Events Identifies A Novel Endurance Regulator

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Abstract

In non-growing microbes, proteome turnover is reduced and identification of newly synthesized, low abundance proteins is challenging. Babin and colleagues recently utilized bioorthogonal noncanonical amino acid tagging (BONCAT) to identify actively synthesized proteins in non-growing *Pseudomonas aeruginosa*, discovering a regulator whose influences range from biofilm formation to secondary metabolism.

Keywords

Pseudomonas aeruginosa; BONCAT; transcription; SutA; non-growing cells

Poor growth conditions are frequently encountered by bacteria, and cessation of cell division is a common coping mechanism. For example, when faced with nutrient depletion, some bacteria, such as *Bacillus subtilis*, form spores, which can subsist under stressful conditions for long periods of time and reawaken when the environment has become favorable again [1]. Other bacteria, such as *Mycobacterium tuberculosis*, may lie latent within a host for many years and reactivate when the host's immune system is compromised [2]. In addition, when assaulted with high concentrations of bactericidal antibiotics, bacteria that had ceased growing beforehand are more likely to tolerate the drug than those that were growing at the time of treatment [3]. Further knowledge of non-growing phenotypic states will improve our understanding of bacterial ecology and enhance our ability to treat latent infections. However, one challenge that stands in the way of gaining such knowledge is the difficulty of distinguishing proteins synthesized by the modest translational capacity of non-growing cells from the myriad of proteins lingering from previous growth states. In essence, the turnover rate of their proteomes lengthens considerably, which can hinder the identification of newly synthesized proteins that play a role in microbial endurance under stressful

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conditions. In a recent article published in *PNAS* [4], Babin and colleagues addressed this challenge by employing bioorthogonal noncanonical amino acid tagging (BONCAT) [5] to identify actively synthesized proteins in non-growing *Pseudomonas aeruginosa*, and they discovered a novel transcriptional regulator with a broad array of influence, which they have termed SutA (survival under transitions A).

P. aeruginosa is an opportunistic pathogen that is a common cause of pneumonia in cystic fibrosis patients and immunocompromised individuals, a prevalent resident of biofilms on prosthetic devices, and a frequently observed species in chronic wound infections [6]. During infection, *P. aeruginosa* could encounter anoxic environments within biofilms, where alternative, exogenous electron acceptors, such as nitrate, are absent or in low abundance [7]. Since *P. aeruginosa* is poorly suited to propagate in such environments, it enters a state where it is non-growing but some metabolic and other essential processes remain active [8]. In order to better understand the factors that enable *P. aeruginosa* to survive such conditions, Babin and colleagues endeavored to identify newly synthesized proteins, which could be at very low abundance, in non-growing *P. aeruginosa* using BONCAT, which labels all proteins being actively synthesized with non-canonical amino acids that have useful chemical handles [5].

The BONCAT method is performed by pulse-labeling cultures with an amino acid analog, and thus the analog is only incorporated into proteins translated after pulsation. Labeled proteins can be enriched from the rest of the proteome by ligating a probe to the amino acid analog, and then performing chromatography followed by mass spectrometry to identify the labeled proteins. For their study, Babin and colleagues utilized the methionine surrogate L-azidohomoalanine (Aha), which is able to be incorporated into proteins by the wild-type translational machinery. However, it is worth noting that the BONCAT method can be applied in a cell-specific manner by using another methionine analog, azidonorleucine (Anl), which requires a mutant tRNA synthase for incorporation, and therefore, restricts labeling to cells in which the mutant synthase is expressed [9]. In their study, Babin and colleagues enriched for labeled proteins by reacting protein lysate with the dialkoxydiphenylsilane (DADPS) biotin-alkyne probe and incubating with streptavidin resin. After cleavage of the DADPS linker, proteins were eluted and analyzed with liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Applying the above method to anaerobic *P. aeruginosa* cultures in minimal media with arginine as the sole carbon source, Babin and colleagues discovered a previously uncharacterized protein, SutA [4]. Upon further investigation, SutA was found to contribute to biofilm formation, pyocanin production, and the ability of cells to transition under fluctuating conditions. Deletion of SutA resulted in smaller biofilms and biofilms with altered morphology as compared to those of wild-type, whereas its overexpression resulted in larger biofilms. Removal of SutA also increased pyocanin production, which is linked to *P. aeruginosa* virulence, whereas increased SutA abundance resulted in decreased pyocanin production. Additionally, competition experiments between wild-type and a SutA deletion strain showed that wild-type cells had an advantage when cultures were alternated between aerobic growth in rich media and anaerobic growth in minimal media. Babin and colleagues went on to perform immunoprecipitation assays followed by LC-MS/MS, to find that SutA

coprecipitates with several components of the RNA polymerase core enzyme. Furthermore, chromatin IP (ChIP)-sequencing (seq) and RNA-seq experiments demonstrated that SutA works together with RNA polymerase to increase transcription of ribosomal protein and rRNA genes. Genome-wide analysis of the data indicated that SutA upregulates genes involved in energy production and maintenance, while it downregulates genes associated with defense mechanisms and motility.

Babin and colleagues' study not only provides novel insight into the ability of *P. aeruginosa* to endure anaerobic, fermentative conditions, but is also gives an elegant example of how the challenge of low proteome turnover in non-growing cells can be addressed with BONCAT. Given its versatility, which has been demonstrated by its application to a range of mammalian cells and several prokaryotic species [10], BONCAT is a powerful tool to apply to understand the physiology of bacteria when they are in non-growing states.

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