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Uridine to uracil catabolism controls commensal-to-pathogen transition

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How gut cells distinguish between beneficial symbionts and deleterious pathobionts is a central question in the gut-microbiota field. In this issue of Cell Host and Microbe, Kim et al demonstrate that the nucleoside catabolism pathway that controls bacterial uracil and ribose production is an essential trigger of the commensal to pathogen transition.

Theoretically, recognition of microbial-associated molecules by the host pattern recognition receptors is expected to trigger the production of immune effectors directed against the invading microorganisms. This is, however, not always true since the digestive tract of animals, including ours, which contains an abundant population of bacteria, is not, thankfully, constantly inflamed. It, therefore, seems that the gut of animals has developed a tissue-specific solution to this problem. While gut cells are able to preserve resident bacteria that are essential for many aspects of host physiology such as metabolism and behavior, they selectively eliminate pathogens that are deleterious for the host. The present study uses the genetically tractable model drosophila to address this conundrum and to identify the molecular events that make a bacterium either a gut fly symbiont or pathobiont.

With its powerful genetics tools and relatively simple commensal gut community, the drosophila is a very versatile model system to study gut-microbe interactions. In addition to show a high degree of morphologic and ultrastructural resemblances to the vertebrate intestine, it shares functional similarities such as a capacity for stem cell renewal upon injury and the production of bactericidal reactive oxygen species (ROS) in response to pathogen colonization. Previous works, mainly from the Lee's lab, has shown that if the production of ROS by enterocytes is activated upon colonization by opportunistic pathogens such as *Erwinia carotorova carotovora*¹⁵ (*Ecc*¹⁵) or *Pseudomonas entomophila*, it remains silent when gut cells are in contact with commensal strains such as *Acetobacter pasteurianus*. This is explained by the specific production and release of the nucleobase uracil by pathogenic bacteria, but not by symbionts, Uracil acts as a ligand for the intestinal DUOX, a nicotinamide adenine dinucleotide phosphatase oxidase, that controls ROS production in enterocytes. Bacterially produced uracil is, therefore, a pathogen-specific signature that is used by the host to distinguish between pathogens and symbionts. In the present report, Kim et al, reveal why only pathogenic and not commensal bacteria release uracil in the gut lumen and demonstrate that *in vivo* genetic perturbation of these signals is sufficient to transform a commensal into a pathogen and *vice versa*.

The study starts with a comparative metabolomic analysis of gut lumen of flies orally infected with *Ecc*¹⁵. The results show that the *Ecc*¹⁵ gut colonization is associated with an increase of the levels of uracil, paralleled with a decrease of the corresponding nucleoside, uridine. This "uridine-in uracil out flux", as it is called by the authors, is confirmed by mass spectrometry analysis of *Ecc*¹⁵ cell culture supernatant. Experiments using uridine radiolabeled either on the nucleobase or on the ribose moieties, further confirmed that *Ecc*¹⁵

bacteria are capable of utilizing uridine and catabolizing it into ribose, that stays in the cell, and uracil that is secreted. This raises the possibility that the ability of a given bacteria to metabolize or not uridine to uracil could be sufficient to categorize it as commensal or pathogen. Consistently, the screening of 10 *Acetobacteraceae* species, shows a perfect correlation between the ability of a given species to catabolize uridine into uracil and to trigger Duox-dependent ROS production in the fly gut. By performing comparative genomic studies, the authors reveal that the gene coding for the nucleoside hydrolase (NH), an enzyme that converts nucleoside into nucleobase and ribose, is only present in pathobiont genomes. This suggests that symbionts are unable to induce uracil-dependent ROS production in the fly gut because they lack at least one enzyme essential to metabolize uridine into uracil. Consistently, genetic inactivation of the NH gene in the pathobionts *Gluconobacter morbifer* and in *Ecc¹⁵* abolished their ability to produce uracil, to induce intestinal ROS production and to trigger enterocytes cell death. Elimination of a single gene from the nucleoside metabolic pathway is therefore sufficient to transform an aggressive pathobiont into a gut-friendly symbiont. Reciprocal experiments in which the NH protein is ectopically expressed in the symbiont *Acetobacter pasteurianus*, further demonstrate that the absence of NH-dependent nucleoside catabolism is a pre-requisite to avoid chronic DUOX activation in enterocytes and to allow the establishment of a peaceful bacteria gut interaction typical of symbiosis.

In the second part of the manuscript, the authors evaluate the role of this pathobiont specific nucleoside pathway on the bacterial side. Comparative RNA seq analysis of wild type *Ecc¹⁵* versus *Ecc¹⁵* mutants for the nucleoside catabolism pathway, reveals that the uridine catabolism is controlling the expression of some mediators of the quorum-sensing (QS) system. More specifically, nucleoside catabolism is required for the full production of acyl homoserine lactone (AHL), one of the primary quorum-sensing signals used by Gram-negative bacteria to regulate gene expression in a density-dependent manner. Further genetic dissection demonstrates that the ribose generated inside the cells by nucleoside catabolism is the trigger that upregulates QS regulators expression to maintain QS activation at high levels. Using both *in vitro* and *in vivo* approaches, the authors finally demonstrate that this nucleoside pathway-dependent QS module is also controlling the expression of a well-characterized *Ecc¹⁵* virulent factor called Evf (*Erwinia virulence factor*). Previous work has shown that *Ecc¹⁵* are capable of persisting in the *drosophila* gut by the sole action of this protein Evf. The present work demonstrates that while Evf transcription is activated in wild type *Ecc¹⁵* when they colonize the fly gut, this activation does not happen when using *Ecc¹⁵* mutant genetically unable to catabolize uridine. In conclusion, this study demonstrates how the presence of genes coding for the uridine metabolic pathway in a bacteria genome is sufficient to transform a symbiotic bacterium into a pathogenic one. It convincingly demonstrates that this metabolic flux is important not only to produce the uracil signal that will induce colitogenic DUOX-dependent ROS production in the enterocytes but also as a coordinating mechanism for virulence and survival of the pathogen in the fly gut environment (Figure 1).

The next challenge is to identify the source of uridine that fuels uracil production by gut pathobionts. Preliminary results using germ-free or holidic media suggest that enterocytes, rather than gut microbiota or food, are the main source of it. It will be of interest to know how this uridine is produced by gut cells and whether comparable uridine levels are also found in other fly fluids such as the hemolymph that could be colonized by pathogenic bacteria. The universality of the mechanisms revealed by this study is an open question. Some bacteria such

as *Lactobacillus brevis* strain EW was shown to be colitogenic in an uracil-dependent manner. While ribose is shown here to induce quorum sensing gene expression, it is a quorum sensing inhibitor that prevents biofilm formation in certain *Lactobacillus* species. It is also likely that additional pathogen signals beyond uracil may interact with the identified pathways, and that signals produced by symbionts could dampen immune responses to defend the gut epithelium against ROS-induced damages. Finally, while this work brings an essential piece into the puzzle of DUOX-dependent ROS production by pathogen-infected fly gut, one player remains elusive: the putative GPCR (s) that upon uracil sensing stimulates DUOX activation.

Figure 1: While symbionts are tolerated by the gut epithelium, pathogens that can induce damages are specifically eliminated. The gut lumen contains high levels of the nucleobase uridine. Pathogens are equipped with proteins that transport (NupC) uridine inside the cell and metabolize (NH) it into uracil and ribose. Intracellular ribose sustains quorum sensing and virulence factors expression, both required for inducing host cell damages. Detection of bacteria-born uracil by enterocytes induces the production of DUOX-dependent ROS that kill infecting pathogens. The symbionts that do not express members of the uridine pathway are unable to catabolize it. They do not produce QS and virulence factors. Since they do not release the DUOX ligand uracil, they do not trigger ROS production and are well tolerated by the gut epithelium.