Trends in Microbiology

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Opinion

Human–gut bacterial protein–protein interactions: understudied but impactful to human health

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The human gut microbiome is associated with a wide range of diseases; yet, the mechanisms these microbes use to influence human health are not fully understood. Protein–protein interactions (PPIs) are increasingly identified as a potential mechanism by which gut microbiota influence their human hosts. Similar to some PPIs observed in pathogens, many disease-relevant human–gut bacterial PPIs function by interacting with components of the immune system or the gut barrier. Here, we highlight recent advances in these two areas. It is our opinion that there is a vastly unexplored network of human–gut bacterial PPIs that contribute to the prevention or pathogenesis of various diseases and that future research is warranted to expand PPI discovery.

Highlights

The human gut microbiome is associated both positively and negatively with human disease.

The mechanisms for these associations are not completely understood; yet, a few disease-relevant interactions between gut bacterial proteins and human proteins have recently been identified.

Many of the human–gut bacterial protein–protein interactions (PPIs) identified to date impact human health and disease by targeting the immune system and the gut barrier.

With newer technologies such as artificial intelligence and high-throughput interaction screens, we expect the discovery rate of disease-relevant human–gut bacterial PPIs to increase substantially, as we see for many pathogens.

A paradigm shift for studying human-bacterial interactions

The gut microbiome is associated with a wide range of human diseases, from intestinal diseases such as colorectal cancer (CRC) [1] and inflammatory bowel disease (IBD) [2] to extraintestinal diseases such as diabetes mellitus [3] and Alzheimer's disease [4]. Despite the profound modulatory effects that the microbiome has on human health [5], our understanding of the biomolecular mechanisms that microbes use to influence host physiology is incomplete. Much of what is currently known about the gut microbiome's influence on host physiology relates to small molecules such as short-chain fatty acids and secondary bile acids [6–8], and there has been relatively less focus on the potential of modulatory proteins. Each year, a growing number of bacterial proteins are found to directly interact with human proteins and contribute to disease (Figure 1). However, the attention on bacterial proteins, and more specifically human–bacterial PPIs, is rooted in pathogenicity studies of several well-studied bacteria, such as *Mycobacterium tuberculosis* [9] and *Salmonella enterica* serovar Typhimurium [10]. By contrast, only a few PPIs have thus far been identified between gut bacteria and their human hosts.

Some host–microbe PPIs involve bacterial proteins known as microbe-associated molecular patterns (MAMPs) containing highly conserved molecular motifs recognized by pattern recognition receptors (PRRs) that are part of the host's innate immune system. The history of how the term 'MAMP' arose is indicative of the growing appreciation for human–bacterial interactions. Coined by a pioneering immunologist, Charles Janeway, in 1989, the concept of immune-detectable motifs in bacteria was originally termed 'pathogen-associated molecular pattern' (PAMP) [11]. However, over a decade later, the field shifted to the term 'MAMP' to encompass the many commensals that also harbor detectable protein motifs such as flagellin and non-protein motifs such as lipopolysaccharide (LPS) [12]. Mirroring the historical shift of PAMP to MAMP, human–bacterial PPIs, once primarily considered a pathogen-specific phenomenon, are now including human-associated microbiota that are implicated in various diseases, playing

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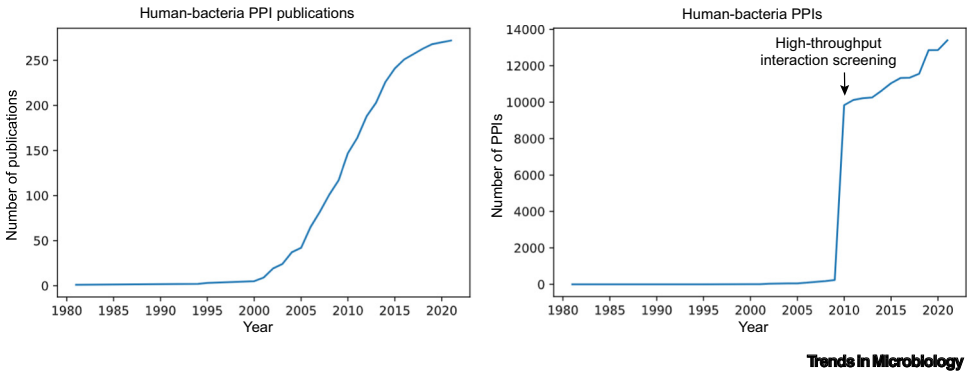

Figure 1. Human–bacteria protein–protein interaction (PPI) publications and discovered PPIs increased substantially over the past two decades. Information on PPIs between humans and bacteria was collected from three publicly available databases; a few additional PPIs were manually collected. The publications each PPI originated from were determined using National Center for Biotechnology Information Entrez, and the year was noted for each PPI and relevant publication. PPI data were collected in August 2022.

both protective and pathogenic roles. Their effects are not limited to PRR–MAMP signaling and include other surface receptor engagements, as well as secreted proteins with internal targets. Moreover, gut bacteria and their products may travel beyond the gut into more distal tissues, such as the pancreas and joints [13,14], increasing their potential for influencing various aspects of host physiology. Here, we discuss the growing recognition of human–gut bacterial PPIs, focusing on two pathways in which they have been more deeply studied: immune signaling and gut barrier regulation.

Gut bacterial proteins directly interact with immune system components

One way many microbes, both pathogenic and commensal, interact with their human hosts is through modulation of the immune system (Figure 2). Unsurprisingly, the most well-known example of an immune-modulating human–bacterial PPI involves bacterial flagellin, a MAMP, and human Toll-like receptor (TLR)5, a PRR. When flagellin is recognized by TLR5, a signaling cascade is triggered which activates NF-κB, a proinflammatory transcription factor that controls the expression of various inflammatory cytokine genes, ultimately leading to an inflammatory antibacterial response [15]. Although this finding dates back over two decades, recent work on flagellin variants encoded by different gut bacterial species shows that some flagellins have evolved to avoid TLR5 detection altogether, termed ‘evaders’ [16], or to strongly bind to TLR5 without eliciting an inflammatory response. The latter phenomenon, termed ‘silent recognition’, may help to explain how the immune system tolerates commensal flagellin while still remaining responsive to various pathogenic flagellins in the gut [17]. However, among the host–microbiome PPIs that have been identified to date, no other involves a protein as taxonomically widespread as flagellin.

Several species-specific effector proteins have been shown to bind to and manipulate members of the NF-κB pathway downstream of various PRRs. As expected, some pathogens reduce NF-κB activity, impairing the hosts’ inflammatory defenses. The *Shigella flexneri* protein OspJ, for example, prevents the ubiquitylation and degradation of the NF-κB inhibitory protein IκB, leading to prolonged NF-κB inhibition and reduced inflammation [18]. Conversely, *Listeria monocytogenes* induces inflammation; its *Listeria* adhesion protein activates NF-κB, and the subsequent inflammation increases the permeability of the gut barrier, allowing it to translocate into other host tissues [19]. Similar to these pathogens, some gut bacteria also modulate the NF-κB inflammatory response. Microbial anti-inflammatory molecule (MAM) is a secreted NF-κB protein inhibitor made by *Faecalibacterium prausnitzii*, a gut commensal inversely associated

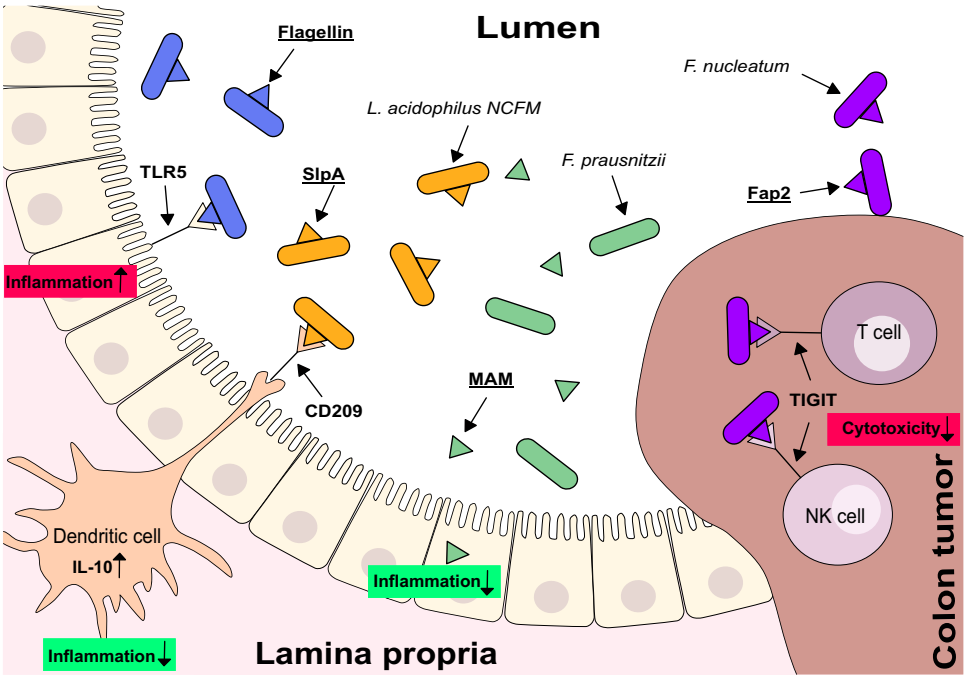

Figure 2. Gut bacterial proteins are immune-modulatory and target various host proteins. Flagellin from various gut bacteria (in blue) binds to TLR5 on gut epithelial cells, triggering the activation of proinflammatory NF-κB and the subsequent increase in inflammation. SlpA from *Lactobacillus acidophilus* NCFM (in yellow) targets CD209 on dendritic cells, inducing anti-inflammatory IL-10 in the gut. MAM from *Faecalibacterium prausnitzii* (in green) enters epithelial cells and reduces inflammation by blocking NF-κB activity, although the specific human protein interactor is unidentified. Fap2 from *Fusobacterium nucleatum* (in purple) specifically targets colon tumor tissue and binds to TIGIT on T cells and NK cells, inhibiting their antitumor cytotoxicity. Abbreviations: IL- interleukin; MAM, microbial anti-inflammatory molecule; NF-κB, nuclear factor-κB; NK, natural killer; SlpA, S layer protein A; TIGIT, inhibitory receptor T cell immunoglobulin and ITIM domain; TLR5, Toll-like receptor 5.

with multiple diseases, including two types of IBD (Crohn's disease and ulcerative colitis), type 2 diabetes mellitus, and CRC [20]. What is most striking is that this bacterial protein has a cytoplasmic target, and simply exposing Caco2 intestinal cells grown in culture to MAM is sufficient to reduce NF-κB activation, suggesting that secreted MAM enters epithelial cells and interrupts NF-κB signaling directly. Although the specific human protein interactor has not been confirmed, MAM colocalizes with IκκB, a regulatory kinase in the NF-κB pathway, suggesting protein interaction [21]. More research is needed to determine whether reducing inflammation via PPIs with components of the NF-κB pathway is common for gut bacteria.

Probiotics, a category of live microbes that provide health benefits to their hosts when consumed, have also been studied for their anti-inflammatory effects [22], a phenomenon that may be due in part to PPIs. For example, S layer protein A from *Lactobacillus acidophilus* NCFM, a probiotic often used in fermented foods and dietary supplements [23], helps promote gut immune cell homeostasis by binding directly to dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN) (also known as CD209), increasing the expression of anti-inflammatory cytokine interleukin 10 (IL-10) [24]. Similar to the NF-κB pathway, pathogens also have proteins that induce IL-10 production and promote the development of chronic infections. The protein PPE18 from the pathogen *M. tuberculosis*, for example, interacts with TLR2 on macrophages and activates p38 mitogen-activated protein kinase (MAPK), a kinase critical for IL-10 induction [25]. Aside from

proteins, many gut bacteria also produce small molecules such as short-chain fatty acids that induce IL-10 through G protein-coupled receptor 43, exemplifying how gut bacteria are already known to regulate IL-10 and protect against excessive intestinal inflammation [26]. Since defects in IL-10 production and signaling are associated with inflammatory diseases such as IBD and some autoimmune diseases, and because gut bacteria are already known to play important roles in maintaining gut homeostasis through other means, research is warranted to further explore whether additional gut bacterial proteins modulate IL-10 levels in the gut [27].

Bacterial proteins can also impact the immune system's response to tumors. *Fusobacterium nucleatum*, a common oral bacterium that is frequently detected in the gut microbiomes of patients with CRC, particularly those with adenocarcinomas, inhibits tumor cytotoxicity via its protein Fap2. Fap2 binds to and activates inhibitory receptor T cell immunoglobulin and ITIM domain (TIGIT) on natural killer (NK) cells and T cells, inhibiting their antitumor cytotoxicity and promoting tumor immune evasion [28]. Interestingly, since Fap2 is also a carbohydrate-binding protein, or lectin, that specifically binds to the glycan Gal-GalNAc, it is likely that the interaction between Fap2 and TIGIT is mediated through these bound glycans, providing an additional mechanism for host-bacterial protein interaction. A second *F. nucleatum* protein, FadA, promotes tumorigenesis in an orthogonal way. FadA mediates binding to E-cadherin on CRC cells, mediating *F. nucleatum* attachment and internalization, which consequently stimulates CRC cell proliferation through the activation of the Wnt/β-catenin pathway, a signaling pathway central in tumorigenesis [29,30]. An important note here is that tumor–microbe interactions are not restricted to the gut. Breast and lung tumors similarly have tumor-specific microbiota, suggesting that these microbes may also play some role in tumorigenesis and highlights the tumor environment as an interesting place to search for additional PPIs that mediate tumor–bacterial interactions [31].

Gut bacterial proteins regulate the gut barrier

The gut barrier protects the host against various external factors, including the microbiota, and a compromised barrier permits the translocation of microbial components that can trigger systemic inflammation in the host. An increase in gut permeability, controversially termed 'leaky gut', is observed in individuals with IBD, obesity, type 2 diabetes mellitus, and some autoimmune diseases, among others, and is thought to contribute to the pathogenesis of these diseases [32]. Thus far, a few disease-relevant human–gut bacterial PPIs have been identified that regulate the gut barrier (Figure 3). One such protein, Amuc_1100 from *Akkermansia muciniphila*, a gut commensal deficient in individuals with obesity and diabetes mellitus, has been noted for its beneficial effects on metabolic disease markers [33]. Amuc_1100, via its interaction with TLR2, can improve gut barrier integrity in obese and diabetic mice while simultaneously reducing high-fat diet-induced hypercholesteremia and fat mass gain [33]. Although TLR2 is an innate immune receptor, it also functions to regulate the expression of tight junction genes such as claudin 3 and occludin that play central roles in maintaining gut barrier integrity [34]. The previously discussed protein MAM from *F. prausnitzii* also modifies the gut barrier by interacting with proteins in the tight junction pathway, such as zonula occludens 1 (ZO-1), increasing gut barrier integrity in a diabetes mellitus mouse model [35]. Since proteins such as Amuc_1100 and MAM can ameliorate 'leaky gut' in a diabetes mellitus mouse model and both *A. muciniphila* and *F. prausnitzii* abundance are reduced in individuals with diabetes mellitus versus healthy individuals [35], it is possible that reduced levels of these proteins help prevent increased intestinal permeability and associated diseases.

Unlike MAM and Amuc_1100, various microbiota-derived proteases instead increase the permeability of the gut barrier, most of which are associated with IBD. The secreted metalloprotease

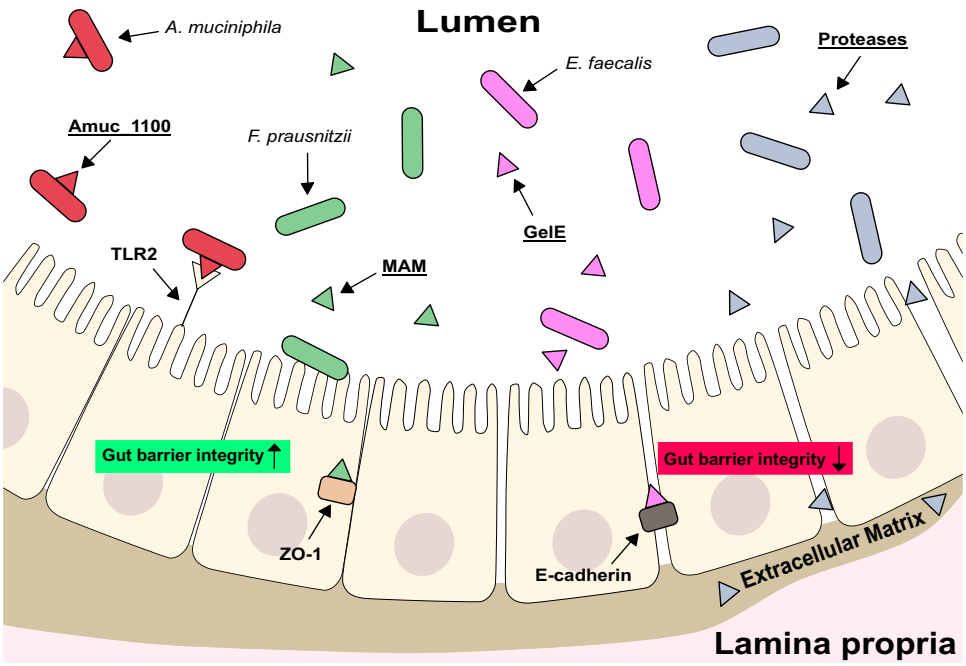

Figure 3. Gut bacterial proteins impact gut barrier integrity through direct interactions with host proteins. Amuc_1100 from *Akkermansia muciniphila* (in red) binds to TLR2 on epithelial cells, inducing higher expression of tight junction genes such as claudin 3 and occludin that increase barrier integrity. *Faecalibacterium prausnitzii*'s MAM (in green) enters epithelial cells and targets tight junction genes such as ZO-1, also increasing barrier integrity. The metalloprotease GelE from *Enterococcus faecalis* (in pink) binds to and degrades the adherens junction protein E-cadherin, decreasing strength of the intercellular barrier. Proteases from various gut bacteria (in gray) target and degrade components of the ECM, reducing overall gut barrier integrity. Abbreviations: ECM, extracellular matrix; MAM, microbial anti-inflammatory molecule; TLR2, Toll-like receptor 2; ZO-1, zonula occludens 1.

gelE from *Enterococcus faecalis* decreases gut barrier integrity by degrading the adherens junction protein E-cadherin on epithelial cells [36], similar to the stomach pathogen *Helicobacter pylori*'s protease HtrA [37]. For *H. pylori*, degrading E-cadherin facilitates its access to the intercellular space of epithelial cells and, consequently, the basal side of the gut barrier. Interestingly, as an opportunistic pathogen, *E. faecalis* has also been observed to translocate across the gut barrier, possibly via proteases such as gelE [38]. The extracellular matrix (ECM), an extracellular network of proteins that provide structural support and maintain the barrier between cells and the external environment, is another gut barrier PPI target. Various gut bacterial proteases, such as those from *Bacteroides fragilis*, degrade components of the ECM and may contribute to ECM remodeling, an integral part of IBD progression [39]. Last, ulcerative colitis-associated proteases from the gut microbe *Bacteroides vulgatus* induce barrier dysfunction and worsen colitis in mice, though their specific host targets have not been identified [40].

Concluding remarks and future perspectives

Gut bacterial proteins interact with several immune and gut barrier components, and their involvement in human disease is just now being appreciated. Since gut bacteria are in constant close contact with their human hosts, primarily with the epithelium and underlying immune cells, it is logical that some of their proteins interact with these surrounding tissues. Human–gut bacterial PPIs potentially occur by random chance and accidental affinity, existing without any selective pressures on either gut bacteria or their human hosts. However, there may be fitness benefits

Outstanding questions

How common is it for gut bacterial proteins to influence host physiology, as opposed to small molecules such as short-chain fatty acids?

What other host pathways are modulated by human–gut bacterial PPIs besides immune and gut barrier pathways?

How did these PPIs evolve between gut bacteria and humans? Do any of these PPIs confer fitness advantages for the bacteria? For the host?

How do gut bacterial proteins access the cells they interact with? Can they travel through the mucus layer and other protective layers of the gut to reach the epithelium? Or can they access the epithelium only if the gut barrier is already damaged?

How do secreted bacterial proteins such as MAM enter cells to interact with their target proteins? Is this a mechanism that extends to other gut bacterial proteins?

Can the disease amelioration noted in mouse studies by human–gut bacterial PPIs such as MAM and Amuc_1100 translate to humans? Can these proteins be used as therapeutics to reduce inflammation and help repair the integrity of the gut barrier?

that contribute to the evolution of host–gut bacterial PPIs. For example, the protein Bxa, a phage-encoded bacterial ADP-ribosyltransferase from *Bacteroides stercoris*, binds to human non-muscle myosin II proteins and induces both actin cytoskeleton changes and inosine secretion, which thereby increases the ability of *B. stercoris* to colonize the gut [41]. An alternative hypothesis was recently proposed: that host–microbiome interactions developed due to a dependency on bacteria for normal human physiological functions, termed 'evolutionary addiction' [42]. This hypothesis may help explain the presence of human–gut bacterial PPIs, specifically those involved in metabolic interdependence and intestinal and immune tissue maturation.

Some of these human–gut bacterial PPIs likely involve multifunctional proteins known as moonlighting proteins. Moonlighting often occurs within a bacterial cell, but, notably, one-fourth of known moonlighting proteins are virulence factors, involving interactions with human proteins [43]. Elongation factor thermo unstable (EF-Tu), for example, is a bacterial moonlighting protein that not only transports aminoacyl-tRNAs to the ribosome to facilitate translation in the cytoplasm but also can be surface localized or secreted, where it interacts with various human proteins such as fibronectin and factor H, promoting intestinal adhesion and immune evasion [44]. However, not all bacterial species' EF-Tu proteins are cell surface localized or secreted. Even though many bacterial proteins may have natural affinity for certain human proteins, a fitness benefit may be realized only if the proteins are used in a specific environmental context.

So far, discovering new PPIs has remained low throughput, largely directed by disease association studies that identify bacteria enriched or depleted in disease. Disease-relevant proteins are subsequently found by performing proteomics on fractions of the bacterial supernatants or membranes that produce an effect (decreased inflammation, increased barrier integrity, etc.). In general, human–gut bacterial interactions have been difficult to study, not only due to the vast number of coexisting species found in the microbiome but also due to the difficulty in culturing and genetically engineering many of these bacteria.

Recent technological advances are key in expanding our understanding of host–gut bacterial PPIs. High-throughput experimental approaches have emerged in recent years, although they have so far been aimed only at identifying human–pathogen PPI networks. A human–severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) PPI network, formed by 739 PPIs, was generated by systematically screening all pairs of SARS-CoV-2 proteins and human proteins using a high-throughput yeast two-hybrid assay [45]; however, this experimental method is feasible only for one organism with a limited number of genes. Some improvements have been made to increase the throughput of yeast two-hybrids and have been applied to human protein interactions with *Bacillus anthracis*, *Francisella tularensis*, and *Yersinia pestis* [46]. A human–*Acinetobacter baumannii* PPI network, conversely, was generated using mass spectrometry of crosslinked proteins in *A. baumannii*-infected lung epithelial cells [47]. Although mass spectrometry-based methods are similarly promising for developing a human–gut bacteria PPI network, this method needs modification to eliminate the need for human cell infection, which is inapplicable to many gut microbes.

Computational approaches hold promise for predicting PPIs between human proteins and gut bacterial proteins that can later be experimentally verified. A recent computational study, for example, analyzes metagenomic data from different disease cohorts and, using sequence homology to known PPIs, predicts over 1000 gut bacterial protein clusters to be involved in human–gut bacterial PPIs associated with CRC, IBD, obesity, and type 2 diabetes mellitus [48]. Artificial intelligence (AI) was also recently adapted to predict protein structure and interaction and is a major step in PPI discovery [49]. AlphaFold, a revolutionary AI tool used to predict a

protein's 3D structure from their amino acid sequence, maintains a high level of accuracy and can be trained to predict PPIs [50,51]. By leveraging the potential of these technologies to explore human–gut bacterial PPIs at a larger scale, we anticipate the rate of human–gut bacterial PPI discovery to increase exponentially, enabling us to address some of the outstanding questions that remain (see Outstanding questions).

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Declaration of interests

The authors have no interests to declare.

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