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MINI REVIEW

Immune activity at the gut epithelium in the larval sea urchin

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Abstract

The embryo of the purple sea urchin has been a fruitful model for the study of developmental gene regulatory networks. For similar reasons, the feeding sea urchin larva provides a gene regulatory model to investigate immune interactions at the gut epithelium. Here we describe what is known of the gut structure and immune cells of the sea urchin larva, and the cellular and gene expression response of the larva to gut-associated immune challenge. As a focused example of how the sea urchin larva can be compared with vertebrate systems, we discuss the expression and function of the IL-17 signalling system in the course of the larval immune response.

Keywords Mucosal immunity · IL-17 · Inflammation · *Vibrio* · Barrier immunity

The larval sea urchin gut

As the barrier separating animal hosts from their associated microbiota, the gut epithelium is a site of intense immune activity. Although the immune system is typically considered in a protective context (*i.e.*, preventing microbial pathogenesis), the gut also actively promotes the growth of beneficial microbiota, which subsequently has broad implications on host health, including physiology, neurology, and behavior (McFall-Ngai et al. 2013). To mediate these relationships, the luminal microbiota interact not only with gut epithelial cells, but also with an assembly of mesodermally derived hematopoietic cells with immune functions (McDermott and Huffnagle 2014). Parsing the specific contributions of each of these cell types and microbial species using in vivo vertebrate models is challenging not only from a technical perspective but also due to the biological complexity of vertebrates.

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As an alternative strategy to understand fundamental aspects of the close relationship between animal and microbial life, sea urchin larvae provide a unique model system (Buckley and Rast 2017). Most sea urchin species have biphasic life cycles that include a long-lived (several months), planktonic, pluteus larval stage that precedes metamorphosis into the adult form. This feeding larval stage is morphologically simple and transparent, allowing for detailed microscopy (Fig. 1). Additionally, as these animals are a long-used model system in developmental biology (Ernst 2011), development of sea urchins is well-established, and many experimental techniques are available. Finally, as deuterostomes, sea urchins share a genetic inheritance with vertebrates, where immunology is best described (Hibino et al. 2006).

Sea urchin larvae are characterized by a tripartite gut formed by a monolayer of epithelial cells. Broadly, the gut is divided into a foregut, midgut, and hindgut, although gene expression patterns reveal much more complexity (Fig. 1a; Annunziata et al. 2014). The foregut, also called esophagus, opens to a highly innervated and sensory ciliary oral area. The coordinated action of the ciliated band surrounding the mouth creates micro-currents to facilitate larva feeding (Strathmann 1975). The single-layer ciliated epithelium in the foregut is encompassed by strands of pharyngeal smooth muscle cells that mediate swallowing once the action of pharyngeal cilia has formed a food bolus. The foregut is separated from the midgut by the cardiac sphincter, a constricting ring of myoepithelial cells (Burke 1981). The larval midgut (also termed the stomach) is the major site of digestion. The anterior region of the midgut is characterized by an organized ring of

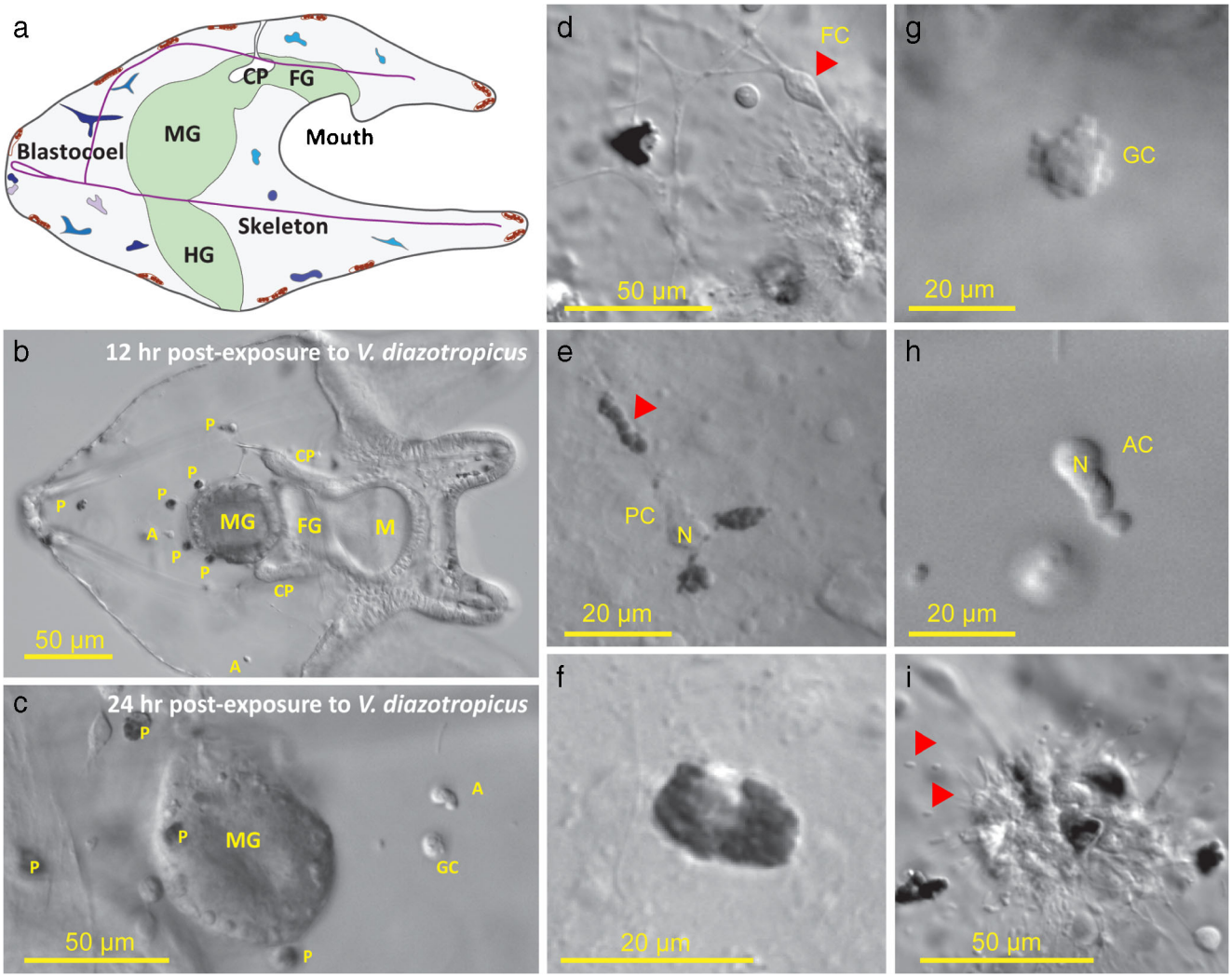

Fig. 1 Morphologically simple sea urchin larvae undergo stereotypic changes in cell behavior in response to immune challenge. **a** In pluteus stage, feeding sea urchin larvae have a tripartite gut (green), mesodermally derived immune cells, and a calcite skeleton (purple) that supports the distinct larval shape. A schematic of larval anatomy is shown from the lateral view. Under immunoquiescent conditions, granular pigment cells (red) are typically apposed to the aboral ectoderm, whereas several types of morphologically distinct blastocoelar cells (shown in shades of blue) are distributed throughout the larva. **b** In response to seawater-based exposure to the pathogenic *Vibrio diazotrophicus*, pigment cells (P) migrate from the ectoderm to the gut and the midgut epithelium constricts. The image shown is from the aboral (dorsal) view, 12 h after bacterial exposure. **c** After 24 h of exposure to *V. diazotrophicus*, the larval midgut is extremely constricted. **d** Phagocytic filopodial cells (FC) in blastocoel at site of *Vibrio lentus* injection. Arrowhead indicates nucleus. **e** Pigment cell (PC) with three pigment laden pseudopods near ectoderm in immunoquiescent larva. N indicates nucleus. Arrowhead indicates pigment granule. **f** Rounded pigment cell (PC) in blastocoel of larva challenged with *Vibrio*. **g** Globular cell (GC) in larval blastocoel. **h** Amoeboid cell in larval blastocoel. N indicates position of nucleus. **i** Agglomeration of filopodial cells, pigment cells, and globular cells on at site of *Vibrio lentus* injection on hour after exposure. Select bacterial cells are indicated with arrowheads. Images in **b** to **i** are extracted from time lapse series and cells are identified both by morphology at different focal planes and by behavior over time. MG, midgut; HG, hindgut; CP, coelomic pouch; M, mouth; P, pigment cells; A, amoeboid cells; GC, globular cells

secretory cells that exhibit molecular similarities to vertebrate cells of the exocrine pancreas (Perillo et al. 2016). The rest of the midgut is primarily composed of secretory columnar epithelial cells that exhibit irregular microvilli on the luminal face. Specialized, spindle-shaped cells are also present throughout the midgut that appear to phagocytose algae in some echinoderm larvae (Burke 1981). The pyloric sphincter separates the midgut from the larval hindgut (intestine). The hindgut consists of a relatively simple squamous epithelium.

Within this small limited size and straightforward morphology, the larval gut lumen is a tightly regulated environment. Using ion pumps and transporters, sea urchin larvae maintain alkaline conditions within the midgut (pH = 9.1; Stumpp et al. 2015). Larvae also support complex assemblages of microbiota that shift during development and in response to diet (Carrier and Reitzel 2018). Finally, larvae control the movement of food through the digestive tract using nitric oxide derived from endodermally derived enteric neurons to relax

the pyloric sphincter between the midgut and hindgut (Yaguchi and Yaguchi 2019). Thus, within a relatively simple morphology, the larval sea urchin gut exhibits a remarkable cellular and functional complexity that, in many ways, mirrors that of the adult forms, albeit on a much smaller scale. This provides an attractive model system for study of intricate regulatory mechanisms. As described in the following sections, the larval gut epithelium plays a central role in coordinating the system-wide immune response.

The sea urchin larval gut interacts with immune cells during infection

Echinoderm species have been used to investigate several aspects of the animal immune response (Smith et al. 2018). In particular, the long-lived adult sea urchins provide a rich source of circulating immune cells (coelomocytes) that are used to study unique transcriptional responses to microbial challenge (e.g., Ghosh et al. 2010). Additionally, studies in the sea star *Asterina pectinifera* have identified phagocytic, mesodermally derived cells that express distinct sets of genes with immune functions (Furukawa et al. 2009, 2012; Liu et al. 2011). The best characterized model system for understanding system-wide immune responses, however, is in the larval stage of the purple sea urchin (*Strongylocentrotus purpuratus*; Buckley and Rast 2017).

The cellular immune system of *S. purpuratus* larva is composed of several types of morphologically distinct mesenchymal cells (Gibson and Burke 1985; Tamboline and Burke 1992; Ho et al. 2016). These cells emerge from a ring of secondary mesoderm (also known the non-skeletal mesoderm [NSM]) during gastrulation and are specified through a regulatory program that shares key similarities with vertebrate hematopoiesis (Solek et al. 2013; Schrankel et al. 2016). Interactions among immune cell types and with the gut epithelial cells imply that, as in vertebrates, immunity is coordinated through a complex program of cell-cell interactions (Ho et al. 2016; Buckley et al. 2017).

The mesodermally derived immune cells are distributed throughout the larval body plan: some cell types are located throughout the blastocoel space of the main body cavity whereas others localize to the larval arms or lie in close apposition to the epithelial layers of the ectoderm or gut (Buckley and Rast 2017). The most phagocytic cells of these cell types are a subset of *filopodial cells*, which are characterized by long filopodia that form a mesh-like network throughout the blastocoel. A subset of these filopodial cells are able to recognize and engulf invading bacteria or other foreign particles present in the blastocoel (Furukawa et al. 2009; Ho et al. 2016). This cell type is most likely what Elie Metchnikoff observed in his seminal descriptions of phagocytic behaviors (Metchnikoff 1891). A related cell type, *ovoid cells*, is also highly phagocytic, but has a round morphology and lack long cell processes. Ovoid cells likely emerge quickly from a different cell type, as they are absent in immune quiescent animals but appear within tens of minutes after blastocoelar injection of foreign particles. A rarer, but highly motile type of immune cells within sea urchin larvae is known as *amoeboid cells*. Their functions remain unknown, but, in response to immune challenge, these comma-shaped cells frequently interact with other immune cell types as well as gut epithelial cells (Ho et al. 2016). Morphologically, amoeboid cells are nearly identical to the motile form of adult coelomocyte cell type known as colorless spherule cells (Smith et al. 2018). Larval immunity is also mediated by *globular cells* that appear to be secretory in nature, and are also present in small numbers in the blastocoel, but tend also to concentrate at the tips of the arms and the larval apex. These cells probe epithelia with filopodia and are marked by the expression of *MacpfA*, which encodes a perforin/MPEG-like factor (Solek et al. 2013). Orthologs of these evolutionarily conserved proteins exert antibacterial functions by forming pores in bacterial cell walls (McCormack et al. 2013). Finally, the highly granular *pigment cells* have a central role in the larval immune response. These red cells contain echinochrome A, a potentially antibacterial naphthoquinone that may be secreted to modulate iron levels (Service and Wardlaw 1984; Lebedev et al. 2008; Coates et al. 2018).

Injecting foreign particles into the larval blastocoel elicits behavioral responses in nearly all types of immune cells. Larvae also undergo a stereotypic inflammatory response following microbial perturbation in the gut lumen (Ho et al. 2016). In response to the presence of model pathogenic bacteria *Vibrio diazotrophicus* in the seawater (and as a consequence also in the larval gut), immune cells rapidly migrate to the basal (blastocoelar) face of the mid- and hindgut epithelium. The midgut becomes severely restricted and feeding largely ceases. Most strikingly, pigment cells, which are typically distributed along the outer ectoderm, traverse the blastocoel to the gut (Fig. 1b, c). Once they reach the gut, pigment cells closely interact with the epithelial cells, sometimes inserting granule-filled pseudopodia into the gut lumen. This surveillance-like behavior is carried out also by the amoeboid cells.

The larval inflammatory response is strictly dependent on bacterial species and concentration; some bacteria do not elicit any obvious cellular response. This suggests that sea urchin larvae rely on sophisticated recognition systems to detect potentially pathogenic strains or danger signals (Buckley and Rast 2015). At the onset of this response, bacteria are not observed within the larval blastocoel but instead remain restricted to the gut lumen (Ho et al. 2016). Under standard laboratory conditions (i.e., in the absence of specific immune challenge), the mesodermally derived immune cell types are typically distributed throughout the animal. Pigment cells localize to the aboral ectoderm, whereas the blastocoelar cells migrate through and disperse across the blastocoel. The gut epithelium thus acts as a physical barrier between the larval

immune cells and microbes in the lumen. Given this barrier and the rapid, system-wide activation of the cellular immune in response to pathogenic microbes, one hypothesis is that the initial microbial recognition occurs in gut epithelial cells, which subsequently recruit peripheral immune cells through the expression of cytokines or signaling molecules. The larval gut thus plays a central role in discriminating among bacterial strains and mounting an appropriate immune response.

Gene activity in immune response

An active role for the gut epithelial cells in the infection process is indicated by changes in gene expression from early after initial *Vibrio* exposure. In a sampling of genes with greater than three-fold change in transcript prevalence during *V. diazotrophicus* exposure, more than half of localized transcriptional changes occur in gut epithelial cells. These include cytokines (see IL-17 below), cytoplasmic signal mediators (NFKBIZ and TNFAIP3), transcription factors (CEBPα and CEBPγ), and effectors (e.g., a Soul domain protein; Buckley et al. 2017). Many of the highly regulated genes in the gut are specific to echinoderms or widespread in invertebrates but absent in vertebrates and common ecdysozoan model organisms. The larval sea urchin therefore provides a model to explore systems that are shared among deuterostomes including vertebrates as well as important response gene systems that are widely shared among invertebrates but have been ignored because they have been lost vertebrates and common invertebrate model systems.

The immune cells themselves also upregulate a suite of genes with various kinetics during immune response to gut-associated *Vibrio* exposure. These include receptors (e.g., SRCR domain proteins in migrating pigment cells) and effectors (e.g., the Transformer [185/333] genes in an activated subset of filopodial cells and complement factors in pigment cells). Many of these cells upregulate genes with different kinetics that may indicate response to different signals and cues. For example, pigment cells begin to upregulate a scavenger receptor (SRCR143) shortly

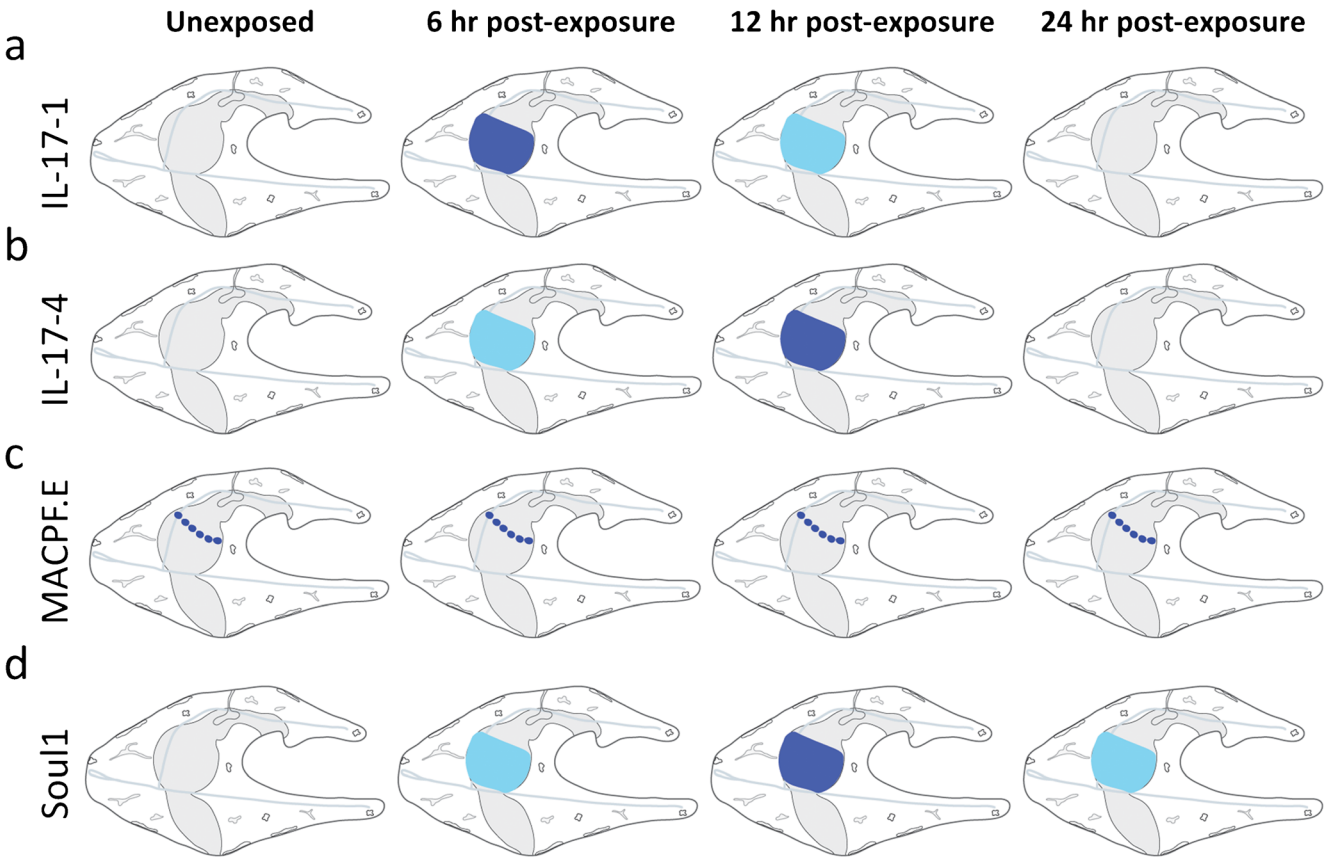

Fig. 2 Immune challenge with *Vibrio diazotrophicus* induces dynamic changes in gene expression in the gut. Diagrams illustrate the expression of four genes in unchallenged larvae at after 6, 12, and 24 h of exposure. The blue color indicates the gene expression territory; darker blue indicates higher expression levels. **a** *IL-17-1* genes are expressed from a closely related multigene subfamily in the posterior midgut and sometimes hindgut early in the immune response. Expression of IL-17-1 decreases to low levels by 12 h and is usually undetectable by in situ hybridization at 24 h. **b** *IL-17-9* is a single copy gene that is also transiently expressed in the midgut but with delayed kinetics. **c** *MACPF.E* is a perforin/MPEG-like effector expressed from a ring of separated gut epithelial cells in the anterior midgut and does not change in levels upon immune challenge. **d** *Soul1* is a SOUL domain protein expressed in the gut downstream of IL-17 signaling. Members of this domain family are heme-binding proteins that may play a role in iron regulation

after *Vibrio* exposure, but filopodial cells upregulate the *Transformer* genes only late in the infection process probably in direct response to *Vibrio* invasion of the blastocoel through the gut epithelium (Ho et al. 2016). The relative morphological simplicity of the larva allows these programs to be teased apart.

Gut expression of IL-17 family members activates the immune response

One of the fundamental molecular similarities between the immune response in the gut epithelia of mammals and sea urchins is the role of interleukin-17 (IL-17). Like most cytokines, IL-17 is best characterized in mammals (McGeachy et al. 2019). Specifically, IL-17A expression defines a subset of mammalian T cells that exhibit pro-inflammatory effects (Th17 cells; Korn et al. 2009). However, increasing evidence suggests that in addition to this lymphocyte-derived expression, two additional IL-17 proteins (IL-17C and IL-17E) are also produced in epithelial tissues (Ramirez-Carrozzi et al. 2011; Song et al. 2011; Pfeifer et al. 2013). In gut and respiratory tissues, IL-17 cytokines are rapidly upregulated in response to immune disturbance, and signaling through their cognate receptors activates a suite of genes that function in the innate immune response (Chang et al. 2011). Similarly, transcriptional profiling of sea urchin larvae responding to bacterial infection reveals that the most strongly upregulated genes early in infection were a set of IL-17 cytokines (Buckley et al. 2017).

The *S. purpuratus* genome sequence encodes 35 IL-17 genes that form 10 distinct subfamilies. Due to the evolutionary divergence between echinoderms and chordates (~ 550 million years; Erwin et al. 2011) as well as the rapid diversification of cytokine molecules (Alcami 2003; Secombes and Cunningham 2004), it is not possible to infer direct relationships between the IL-17 paralogs in sea urchins and mammals. In bacterially infected larvae, two of these subfamilies (IL17-1 and IL17-4) are expressed exclusively in gut epithelial cells (Fig. 2a, b). Following exposure to specific strains of bacteria, these genes are rapidly upregulated (within 4 h).

Notably, this early activation of IL-17 precedes a secondary wave of immune gene expression (Ho et al. 2016) (Fig. 2d). Interfering with IL-17 signaling in vivo results in impaired expression of downstream immune effector genes. This suggests that a function for IL-17 in the mucosal immune response and the associated regulatory circuitry are ancient aspects of immune response that may have been co-opted in vertebrate adaptive systems (Buckley et al. 2017).

Conclusions

In nearly all bilaterians, the gut is a site of intense, multidirectional interaction between the mesodermally derived cells of the immune system and the microbiota of the lumen, both beneficial and pathogenic. The gut epithelium is a primary mediator of these ancient interaction networks. The sea urchin larva has been demonstrated as an efficient model in which to study these complex and ancient regulatory programs in the context of an organism that has phylogenetic affinities to vertebrates while also retaining largely unexplored invertebrate response systems.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

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