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Quorum Sensing in Gram-Negative Bacteria

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Abstract: It has become increasingly and widely recognised that bacteria do not live as isolated entities but instead exist as communities that exploit elaborate systems of intercellular communication to facilitate their adaptation to changing environmental conditions. A well-characterised example of such intercellular communication is quorum sensing. Quorum sensing depends on the production of diffusible signal molecules termed autoinducers or pheromones, which enable a bacterium to monitor its own cell population density. A variety of physiological processes in a range of bacterial species is regulated by quorum sensing. Examples include bioluminescence, antibiotic biosynthesis, swarming, biofilm differentiation, conjugation and the production of virulence determinants in animal, fish and plant pathogens. The best studied common signalling molecules found in Gram-negative bacteria are N-acyl derivatives of homoserine lactone (acyl HSLs). In this paper, the current state of research concerning acyl HSL-mediated quorum sensing in Gram-negative bacteria is reviewed.

Key Words: Quorum sensing, acyl homoserine lactones, bacterial signalling, gene expression

Gram-Negatif Bakterilerde Çevreyi Algılama

Özet: Bakterilerin izole varlıklar olarak yaşamadıkları, değişen ortam koşullarına uyumlarını kolaylaştırmak için karmaşık hücreler arası haberleşme sistemleri kullanan toplukluklar halinde bulundukları giderek artan bir yaygınlıkla kabul edilmektedir. Bu tip hücreler arası haberleşmenin iyi karakterize edilmiş bir örneği, çevreyi algılamadır. Çevreyi algılama, bir bakteriye kendi hücre populasyon yoğunluğunu izlemesine olanak veren autoinducer veya feromon olarak adlandırılan sinyal moleküllerinin üretimine bağımlıdır. Değişik bakteri türlerinde, çeşitli fizyolojik işlemler, çevreyi algılama ile regüle edilir. Bu işlemlere örnek olarak, biyolüminesens, antibiyotik biyosentezi, biyofilm oluşumu, konjugasyon ve hayvan, bitki ve balık patojenleri tarafından oluşturulan virülens etkenlerinin üretimi verilebilir. Gram-negatif bakterilerde en yaygın olarak bulunan sinyal molekülleri N-acyl homoserine lakton türevleridir (acyl HSLs). Bu makalede, Gram-negatif bakterilerdeki acyl-HSL aracılı çevreyi algılama üzerindeki araştırmaların bugünkü durumunun bir derlemesi yapılmıştır.

Anahtar Sözcükler: Çevreyi algılama, acyl homoserine laktonlar, bakteriyel sinyal, gen ekspresyonu

Introduction

Prokaryotes have evolved complicated signal transduction mechanisms to perceive sensory information and so facilitate their adaptation to changing environmental conditions including changes in temperature, pH, osmolarity and nutrient availability. Such mechanisms frequently involve a two-component sensory transduction system consisting of a sensor protein that detects the environmental stimulus and a second component that acts as a regulator controlling the expression of particular genes, thus facilitating an adaptive response.

Signal transduction and gene regulation through the phosphorylation of two regulatory components is now recognised as one of the major global regulatory networks in bacteria (1). However, not all types of sensor-regulator circuits relay information via phosphoryl transfer. Alternative signalling systems mediated by small diffusible molecules termed autoinducers or pheromones have long been recognised to be involved in the control of gene expression.

Numerous signalling molecule-mediated sensing and response pathways have now been identified and many fall within the scope of a form of regulation which is known as quorum sensing.

Quorum sensing is commonly used to describe the phenomenon whereby the accumulation of a low-molecular-mass signalling molecule enables individual cells to sense when the minimal population unit or 'quorum' of bacteria has been achieved for a concerted action to be initiated (2). This system relies on two major components, a small diffusible signalling molecule which

accumulates in a population density-dependent manner and a transcriptional activator protein which, in concert with the signalling molecule, activates the expression of relevant genes. This review will focus on the wide range of quorum sensing systems that employ *N*-acyl-homoserine lactones (acyl HLs) as the signalling molecule. Long thought to be unique to *Vibrio fisheri* and certain closely related marine bioluminescent bacteria, it has now become evident that acyl HLs are produced by a wide variety of terrestrial and marine bacteria including some Gram-positive and Gram-negative bacteria and they have been shown to control a diverse range of cell density-dependent factors (Table). Figure 1 illustrates the structures of some *N*-acyl homoserine lactone molecules produced by Gram-negative bacteria. Recent studies have shown that some bacteria produce multiple AHLs, each controlling different phenotypes. AHLs signalling was first described in *V. fisheri* and has become a model for studies of quorum sensing. This review will, therefore, begin with quorum sensing in *V. fisheri*.

Quorum sensing in *V. fisheri*

One of the most intensively investigated quorum sensing systems is the regulation of bioluminescence in *V. fisheri*. *V. fisheri* is a marine bioluminescent, facultatively aerobic, Gram-negative bacterium which lives both as a specific symbiont in the light organs of certain marine fish and squid and as a free-living organism in seawater. When at low cell density in seawater, cultures of this bacterium appear dark. However, when at high cell density within the light organs, the population emits light and becomes bioluminescent (3). The animals, marine fish and squid, use the bacterial light in a variety of luminous displays associated with avoiding predators, locating food and finding mates. The bacteria are provided with nutrients for growth in a habitat free of other micro-organisms. In *V. fisheri*, bioluminescence is dependent on the accumulation of an autoinducer. The autoinducer in *V. fisheri* was identified as *N*-(3-oxohexanoyl) homoserine lactone (OHHL).

Table. Some examples of bacterial N-acyl homoserine lactones and their associated phenotypes.

Organisms	Regulated Phenotype(s)	Signal molecule(s)
Aeromonas hydrophila	Biofilms, exoproteases	BHL, HHL
Aeromonas salmonicida	Exoprotease	BHL, HHL
Agrobacterium tumefaciens	Plasmid conjugation	OOHL
Chromobacterium violeceum	Exoenzymes, cyanide, violacein	HHL
Erwinia carotovora	(5R)-Carbapen-2-em-3-carboxylic acid antibiotic	OHHL
Erwinia carotovora (Eca)	Virulence factors: protease, cellulases, pectinases	OHHL
Erwinia stewartii (Est)	Exopolysaccaride synthesis	OHHL
Escherichia coli	Cell division	Unknown
Pseudomonas aeruginosa	Virulence factors including rhamnolipid, cyanide elastase, hemolysine.	BHL+OdDHL
Pseudomonas aureofaciens	Production of phenazine	HHL
Rhizobium leguminosarum	Expression of rhizosphere genes	HtDeHL
Serratia spp. *ATCC 39006*	Carbapenem antibiotic, pigment (Prodigiosin)	BHL+HHL
Serratia liquefaciens	Swarming motility	BHL
Vibrio fisheri	Bioluminescence	OHHL
Vibrio harveyi	Bioluminescence	HBHL
Yersinia enterocolitica	Unknown	HHL+OHHL
Xanthomonas campestris	Extracellular enzymes and polysaccaride virulence determinants	OOHL

OHHL *N*-3-(oxohexanoyl)-L-homoserine lactone
BHL *N*-butanoyl-L-homoserine lactone
HHL *N*-hexanoyl-L- homoserine lactone
OOHL *N*-3-(oxooctanoyl)-L-homoserine lactone
HBHL *N*-3-(hydroxybutanoyl)-L-homoserine lactone
OdDHL *N*-3-(oxododecanoyl)-L-homoserine lactone
HtDeHL *N*-3R-(hydroxy-7-cis-tetradecanoyl)-L- homoserine lactone

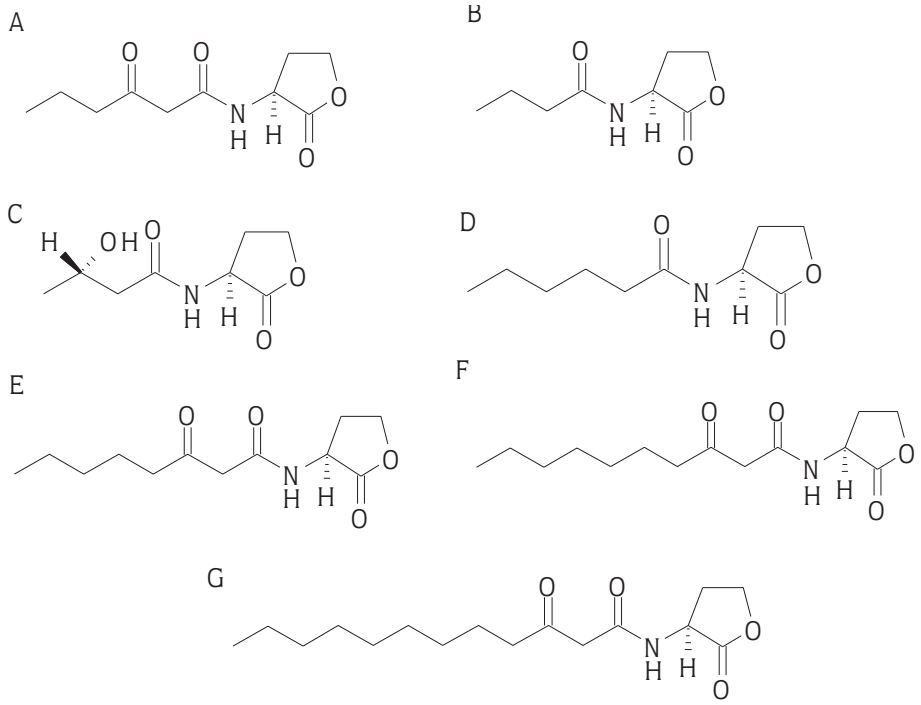

Figure 1. Some of the more common microbial acyl HSLs: (A) *N*-(3-oxohexanoyl)-L-homoserine lactone (OHHL): (B) *N*-butanoyl-L-homoserine lactone (BHL): (C) *N*-(3-hydroxybutanoyl)-L-homoserine lactone (HBHL): (D) *N*-hexanoyl-L-homoserine lactone (HHL): (E) *N*-(3-oxooctanoyl)-L-homoserine lactone(OOHL): (F) *N*-(3-oxodeconoyl)-L-homoserine lactone (ODHL): (G) *N*-(3-oxododecanoyl)-L-homoserine lactone (OdDHL).

The bioluminescence gene, *lux*, cluster of *V. Fisheri* consists of eight genes (*luxA-E, luxG, luxI* and *luxR*) (Figure 2) (4,5). The rightward operon contains the genes required for autoinducer synthesis (*luxI*) and light production (*luxCDABEG*). The product of the *luxI* gene is the autoinducer synthase protein which is necessary for the synthesis of *N*-(3-oxohexanoyl)-L-homoserine lactone (OHHL) (6). The leftward operon consists of a transcriptional activator, *luxR* (7). At low cell densities *luxI* is transcribed at a basal level and OHHL slowly accumulates in the medium until it reaches a sufficiently high concentration. It is then thought to interact with the autoinducer domain of LuxR forming a complex. The LuxR-OHHL complex then binds to the *lux* promoter region upstream of *luxI*, known as the *lux* box, and strongly stimulates transcription of the *luxICDABEG* operon (Figure 2) (8,9). This causes an induction of luminescence and generates a positive feedback loop, leading to further expression of *luxI* and more OHHL.

LuxR-LuxI type quorum sensing in other bacteria

Quorum sensing in *Pseudomonas aeruginosa*

In recent years, the micro-organism on which most quorum sensing related studies have been initiated is *P. aeruginosa*. *P. aeruginosa* is an important human pathogen which is responsible for opportunistic infections in cancer, AIDS and cystic fibrosis (CF) patients (10-12). A wide variety of extracellular enzymes contribute to the virulence of *P. aeruginosa*. These include elastase, protease, hemolysins, exotoxin A, rhamnolipid biosurfactants and phospholipase. These exofactors are collectively capable of causing extensive tissue damage in humans and other mammals (13,14).

Regulation of the genes encoding these exoproducts is controlled through quorum sensing systems. Unlike in *V. fisheri*, in *P. aeruginosa*, two quorum-sensing systems have been identified, i.e., LasR/I and RhlR/I (VsmR/I) (15,16). Each system is comprised of (i) a specific *N*-acyl homoserine lactone signal (autoinducer) and (ii) an

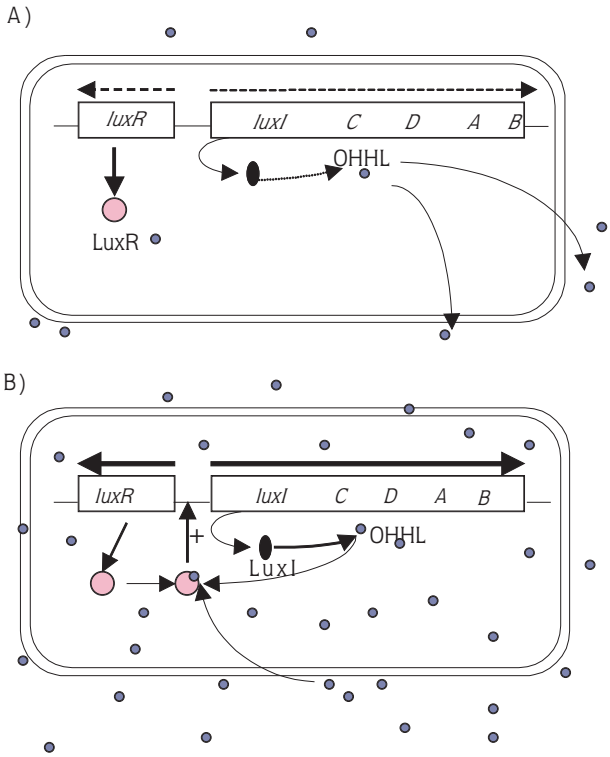

Fig. 2. The regulation of bioluminescence in *Vibrio fisheri*.

A) At low population density, *luxR* and *luxI* are transcribed at a low level and there is insufficient accumulation of OHHL.

B) When the intracellular concentration of OHHL reaches a certain threshold, an OHHL-LuxR complex activates transcription of the *luxICDABE* operon resulting increased levels of OHHL and bioluminescence.

autoinducer-dependent transcriptional activator (R protein). In the *las* system, LuxI homologue LasI is involved in the synthesis of the autoinducer *N*-(3-oxododecanoyl)-L-homoserine lactone (OdDHL) (Table) (17,18). LuxR homologue LasR and OdDHL is required for the activation of virulence genes including encoding elastase (*lasB*), protease (*lasA*), alkaline protease (*apr*) and toxin A (*toxA*) (19).

The discovery of a second quorum sensing-based system revealed that quorum sensing in *P. aeruginosa* is more complex than originally thought (20-22). The *rhl* quorum sensing system consists of the transcriptional activator proteins RhlR and RhlI which are responsible for the synthesis of two autoinducers, predominantly *N*-butonoyl-L-homoserine lactone (BHL) and a small amount of *N*-hexanoyl-L-homoserine lactone (HHL) (Table) (23). The *rhl* system regulates the production of rhamnolipids, elastase, pyocyanin, cyanide and lipase (16,24). It has been demonstrated that the *las* and *rhl* regulatory systems are connected via a hierarchical cascade (25,26).

P. aeruginosa also employs quorum sensing to control the formation of differentiated biofilms (27). Biofilm differentiation is thought to protect the organisms from host defences and provide increased resistance to antibiotics. Davies et al., (28) have shown that the production of OdDHL via LasI is necessary for the formation of normal biofilm.

Quorum sensing in other pseudomonads

Many other species of pseudomonads possess quorum sensing systems. One of these species is *Pseudomonas aureofaciens. P. aureofaciens* produces phenazine antibiotics which are responsible for both suppression of fungal take-all disease of wheat and enhanced survival of this organism within the wheat rhizosphere in competition with other organisms (29). The production of this antibiotic is growth-phase dependent. The phenazine antibiotic genes are transcriptionally regulated, from at least two divergently transcribed operons, by PhzR (a LuxR homologue). The analysis of the regulation of phenazine production also identified a LuxI homologue, PhzI. Together these two proteins (PhzI/PhzR) comprise an *N*-hexanoyl-L-homoserine lactone (HHL) response system. It has been shown that PhzR activates phenazine production in conjunction with HHL produced by PhzI via the transcriptional activation of the phenazine biosynthetic gene *phzB* (30,31).

Quorum sensing in *Erwinia carotovora*

E. carotovora is an opportunistic phytopathogen that causes soft-rot in several plant species (32). It is of economic importance due to the diseases it causes in numerous commercial crop plants such as the potato, carrot, turnip, celery, cucumber, onion and pineapple.

The pathogenicity of *E. carotovora* depends on its ability to produce large quantities of exoenzymes, including pectate lyases (Pels), pectin lyases, cellulases (Cels) and proteases (Prts), that enable them to macerate the parenchymatous tissue of plants (33,34). The production of enzymes by only a few cells would not have an effect on the plant tissue, and more likely, it would elicit a defence reaction from the plant host. Therefore, it is crucial that the timing of exoenzyme production by *E. carotovora* is tightly regulated in order to evade and overcome these defences.

The quorum sensing component of exoenzyme regulation can provide information regarding population density and contribute to the coordinate control of the genes in the exoenzyme regulon(s). Regulation through quorum sensing relies on the LuxRI homologues ExpR and ExpI. ExpI is responsible for the synthesis of the pheromone OHHL and inactivation of *expI* leads to a down regulation of exoenzyme biosynthesis in *E. carotovora* and the subsequent loss of virulence (35,36). In contrast, the interruption of *expR* has no significant effect upon virulence, exoenzyme synthesis or OHHL production. Interestingly, the overproduction of *expR* results in decreased exoenzyme production and this is relieved by additional exogenous OHHL (37). These findings have led to the proposal that ExpR might act as a repressor of exoenzyme synthesis by sequestering the level of OHHL.

In addition to the agricultural importance of *Erwinia* spp., some strains of *Ecc* have been found to produce a simple b-lactam antibiotic, 1-carbapen-2-em-3-carboxylic acid (carbapenem) (38). Production of this antibiotic is thought to increase the organism's fitness for survival in the rhizosphere by reducing the number of antibiotic-sensitive competitor bacteria (39).

The commercial potential of this natural b-lactam and the genetic tractability of *Ecc* initiated molecular genetic studies of carbapenem production in this organism. One such molecular genetic study of an *Ecc* carbapenem mutant has led to the identification of a novel cluster of eight genes (*carA-H*) responsible for the production of carbapenem (40). Molecular genetic analysis suggests that the first five genes in the cluster, *carABCDE,* are required for antibiotic biosynthesis. The next genes in the cluster, *carF* and *carG,* encode a carbapenem resistance mechanism (41). The function of CarH is unknown.

The *car* cluster is located immediately downstream from the *carR* gene. The product of this gene has been demonstrated to be a member of the LuxR-type family of transcriptional activators (37). Like most of the LuxR family regulators, CarR relies on a pheromone signalling molecule for its function . The major pheromone in *Ecc* has been identified as *N*-(3-oxohexanoyl)-L-homoserine lactone (OHHL), which is the same molecule as that responsible for the autoinduction of bioluminescence in *V. fisheri*. This molecule is produced by the unlinked *carI* gene in a cell-density-dependent manner and results in the cell-density- dependent expression of the car genes via *carR*.

Quorum sensing in other *Erwinia* spp.

It has been found that strains of *E. carotovora* subsp. *atroseptica*, *E. herbicola* and *E. chrysanthemi* produce various different acyl HSLs. The *expI* gene product is responsible for the synthesis of OHHL, HHL and DHL. Like *Ecc*, a *luxR* homologue is located adjacent to *expI* (42,43).

E. stewartii is the etiological agent of Stewart's wilt of corn. The pathogenesis of *E. stewartii* is correlated with the ability to produce large amount of extracellular polysaccaride (EPS). The production of EPS is controlled by quorum sensing (44). Two genes, *esaR* and *esaI*, encode regulatory proteins. EsaI has been shown to be responsible for OHHL production and EsaR is the cognate gene regulator. It has been recently reported that strains containing an *esaR* mutation produce high levels of EPS in the absence of OHHL. This indicated that in *E. stewartii* EsaR functions as a repressor, in contrast to most other LuxR homologues (45).

Quorum sensing in *Agrobacterium tumefaciens*

A. tumefaciens is a Gram-negative soil bacterium that causes crown gall tumours in plants via the transfer of oncogenic DNA to the nucleus of its host. The major virulence determinant of *A. tumefaciens* is a large plasmid known as the tumour inducing, Ti, plasmid. Upon infection, a region of the Ti plasmid, the T-DNA, is transferred from *A. tumefaciens* to the plant cell where it is integrated into the nuclear genome (2,46,47).

The Ti plasmid, *tra*, genes are positively regulated by the quorum sensing proteins TraR and TraI, which are homologues of LuxI/LuxR, in conjunction with a diffusible compound *N*-3-(oxooctanoyl)-L-homoserine lactone (AAI) (48,49). The second regulatory determinant is TraM (product of the *traM* gene), which inhibits the activation of *tra* genes by TraR and AAI (2). This is thought to be a mechanism whereby TraM sequesters TraR, preventing TraR-mediated AAI induction at low cell density, until the appropriate environmental conditions arise (50).

In addition to the oncogenes, the T-DNA carries genes for biosynthesis of opines which can be utilised by *A. tumefaciens* as a sole carbon source enabling a competitive advantage over other soil bacteria. The opines function as signals to induce conjugation and different opines induce different genes. Only when the respective opines are present does expression of the *tra* genes take place. For example, the transcriptional

activator OccR activates the occ operon in the presence of octopine and this leads to *traR* expression (51,52).

Quorum sensing in *Rhizobium leguminosarum*

Species of *Rhizobium* sustain symbiotic relationships with leguminous plants via the formation of nitrogen-fixing nodules on roots (53,54). In *Rhizobium* spp. most of the genes required for legume nodulation (*nod*) and symbiotic nitrogen fixation (*nif*) are often encoded on large, so-called symbiotic (Sym) plasmids. The Sym plasmid pRL1JI contains a transcriptional activator, RhiR, that is homologous to LuxR in *V. fisheri*. This LuxR homologue activates the *rhiABC* operon. The protein products of these genes are strongly expressed in the rhizosphere around legume roots, but not within legume nodules (55). The activation of *rhiABC* genes is dependent on a homoserine lactone made by RhiI. RhiI, the LuxI homologue, has been identified very recently and shown to be regulated by RhiR in a cell-dependent fashion (56,57). Flavonoids which induce *nod* gene expression repress *rhiI* expression. This suggests that the plant can influence the level of homoserine lactone production.

Acyl HSL-based quorum sensing in other Gram-negative bacteria

Many other bacterial species utilise quorum sensing for the regulation of specific phenotypes. Two fish pathogens, *Aeromonas hydrophia* and *Aeromonas salmonicida*, possess LuxRI homologues termed AhyRI and AsaRI respectively (58). Another fish pathogen, *Vibrio anguillarum*, express the LuxRI homologue VanRI (59). *Burkholderia cepacia* is commonly associated with lung infections of CF patients and express LuxRI homologues CepR and CepI, which are involved in the production of siderophore and protease (60). In *Chromobacterium violaceum*, purple pigment, chitinolytic activity, antibiotic and virulence factor production are all regulated by HHL (61,62). Many species belonging to the genus *Yersinia* including *Y. enterocolitica*, *Y. pestis*, and *Y. pseudotuberculosis* express quorum sensing systems (63-65). The opportunistic human pathogen *Serratia marcescens* makes the red pigment, prodigiosin, and carbapenem antibiotic and the production of these is regulated via a quorum sensing system (66,67). *Xenorhabdus nematophilus* is a major insect pathogen that utilises an acyl HSL-based quorum sensing system. HBHL has been shown to play a key role in the development of virulence by *X. nematophilus* (68).

Concluding remarks

The discovery that bacteria are able to communicate with each other changed our general perception of many single, simple organisms in our world. Understanding how bacterial cells communicate with each other has a number of important practical implications for the control of pathogen organisms, and for the screening and exploitation of bacteria that produce antibiotics and other high value products. Since many important plant and animal pathogens use quorum sensing to regulate virulence, strategies intended to interfere with their signalling systems will likely have many potential applications. The disruption of signalling systems offers an opportunity to prevent the bacteria from responding to the signal and thereby prevent the expression of virulence factors. Biotechnological research is now focused on the development of AHL antagonists. In medicine, such molecules have a potential use as antimicrobial drugs. Similarly, in agriculture, AHL antagonists could protect crops from damage caused by pathogens such as *E. carotovora*. In biotechnology, quorum sensing could be used to control fermentation processes either by triggering early production of a desired metabolite or by making the onset of synthesis of a toxic product dependent on the addition of an exogenous AHL.

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