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QS-DEPENDENT VIRULENCE REGULATION IN PSEUDOMONAS AERUGINOSA

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P.aeruginosa is an opportunistic pathogen with high level of behavior versatility and, thereafter, adaptability to a wide range of various external conditions. Preferably it infects immunocompromised patients with cancer or AIDS, or those having breaches in normal barriers caused by burns, indwelling medical devices, or prolonged use of broad-spectrum antibiotics. It causes severe infection in the respiratory tract and lung tissue of cystic fibrosis patients, and is an important nosocomial pathogen - circulating hospital strains of P.aeruginosa is a common problem for intensive care units, surgical and burn-wound clinics because of their remarkable survivability. Serious (often unsolvable) problems in etiotropic treatment has been created by capacity to resist antibiotics, either intrinsically (because of constitutive expression of β-lactamases and efflux pumps, combined with low permeability of the outer-membrane) or following acquisition of resistance genes (e.g., genes for β-lactamases, or enzymes inactivating aminoglycosides or modifying their target), over-expression of efflux pumps, decreased expression of porins, or mutations in quinolone targets. Worryingly, these mechanisms are often present simultaneously, thereby conferring multiresistant phenotypes.

P.aeruginosa has an impressive composition of cell-associated and extracellular virulence factors, and majority of the last one expressed in cell-density dependent manner with maximum protease activity occurring during the late logarithmic and early stationary phases of growth [1]. As the sensibility to rather limited array of effective antibiotics decrease - rising population density facilitate its aggressive properties, while products of host cell lysis in turn is an excellent plastic and energetic source and predetermine the population growth continuation.

Like in many other bacteria, cell-density dependent phenotypic variability has been ensured by the "quorum sensing" (QS) – the system consists of the autoinducer synthase and transcriptional regulator, which is activated in response to rising environmental autoinducer concentration [2]. P.aeruginosa has complicated QS with multilayer regulatory network that stipulate high degree adequacy of population behaviour to multiple different environmental conditions. At the top of this system are disposed genes lasR and lasI, with significant homology to the *luxR* and *luxI* genes of Vibrio fischeri – the first described bacterial QS system [3]. Likewise *luxR-luxI* in V.fischeri - *lasR-lasI* utilized acylated homoserine lactone (AHL) as an autoinducer. Transcriptional activator LasR positively regulated by cognate AHL - N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL), which is synthesized by the LasI (AHL-synthase) and accumulated in environment as population density arise.

Besides a number of virulence genes (including lasB, encoding elastase; lasA, encoding LasA protease; aprA, encoding alkaline metalloproteinase; toxA, encoding exotoxin A and some others [1,3-5]) LasR regulates expression of lasI, creating an autoinduction feedback loop [6]. An additional gene that controlled production of AHL, *rsaL*, is under the regulatory control of LasR-3-oxo-C12-HSL, the product of which negatively regulates QS by inhibiting lasI expression [**7**]. The DNA binding site of the RsaL protein on the *rsaL-lasI* bidirectional promoter partially overlaps the binding site of the LasR protein, which is consistent with the hypothesis that RsaL and LasR could be in binding competition on this promoter [8]. A mutation in the *rsaL* gene leads to producing of dramatically higher amounts of AHL with respect to the wild type, highlighting the key role of this negative regulator in controlling QS [**7**].

Second AHL signalling system described in P. aeruginosa – *rhlR- rhlI* - consists of the transcriptional activator RhlR and the autoinducer synthase RhlI, which directs the synthesis of N-butyryl-L-homoserine lactone (C4-HSL) [9]. The RhlR-C4-HSL complex regulates expression of rhlAB, lasB, aprA, the stationary-phase sigma factor RpoS, and production of the secondary metabolites pyocyanin and cyanide [10, 11]. Compared with the wild type, the rhlR and rhlI mutants both showed defects in the production of elastase, LasA protease, rhamnolipid, and pyocyanin. [11]. Observed functional interference is not stipulated by defined structural similarities between LasR and RhlR or the similarities between the two AHLs. The recognizing receptors (R-proteins) are not significantly activated by their noncognate AHLs: LasR is not activated by C4-HSL and 3-oxo-C12-HSL is capable of only low-level RhlR activation [12]. Similarly, genes that are primarily activated by one system are only minimally activated by the other, indicating that specific recognition sequences must be present in the operator regions of these target genes that predetermine which quorum-sensing system is required for induction. Thus, relationships between the two systems are arranged in a hierarchical fashion with the las system being the dominant regulator [13].

In addition to 3-oxo-C12-HSL and C4-HSL, which are the major AHLs produced by P.aeruginosa grown in vitro, minor AHL products can also be detected [14]. Biological function of these noncognate AHLs remains unclear, but it is supposed [15] that minor AHLs may act as competitive inhibitors of autoinduction. Autoinducer synthase use S-adenosylmethionine and the appropriate fatty acid conjugated to acyl carrier protein as substrates and, when one of the enzymatic steps of the fatty acid biosynthetic pathway becomes rate limiting, 3-oxo-C12-HSL is no longer produced at detectable levels; instead, the shorter-chain-length HSLs 3-oxo-C10-HSL, 3-oxo-C8-HSL and 3-oxo-C6-HSL are preferentially generated. Thus, during decreasing availability of the 3-oxo-acyl-ACP substrate precursors LasI (which synthesize majority, if not all, of the 3-oxo-HSLs found in P.aeruginosa culture supernatants) switched over to another signal type production. Consequently, the inhibitory effect of noncognate AHLs may be used as means of precise control of target genes expression.

Pseudomonas aeruginosa also possesses a non-AHL extracellular signal: 2-heptyl-3-hydroxy-4-quinolone (PQS), or Pseudomonas Quinolone Signal, which is directly implicated in the QS system, 4-hydroxy-2-alkylquinolines (HAQs), - a family of compounds released by P. aeruginosa starting at the end of exponential growth and 4-hydroxy-2-heptylquinoline (HHQs), - a precursor of PQS biosynthesis, which is also secreted from the cell and may act as a signalling molecule [16,17]. It was demonstrated that the highly hydrophobic PQS signal is exported through membrane vesicles [18], in contrast to the AHL signals, which either diffuse directly across the cell membrane (butyryl-HSL) or transported by an efflux pump (3-oxododecanoyl-HSL) [19].

PQS is required for the expression of rhlR and rhlI and for the production of rhl -dependent virulence factors at the onset of stationary phase [20]. Simultaneously, PQS production is dependent on the las system [21, thereby the PQS signal functions as a link between the las and rhl QS systems and represent additional layer of regulation [22].

Finally, it is necessary to mention, that a major set of transcriptional responses regulators are the two-component systems [23] and QS regulatory components are coupled and subjected to regulation by other global regulatory networks. No systematic approach has been undertaken to elucidate how the quorum-sensing systems of *P. aeruginosa* are integrated into the global regulatory network of the cell, but separated information has been accumulated. It was shown, that a mutant defective in the response regulator *gacA* exhibit reduced and delayed formation of C4-HSL and also reduced expression of *lasR*[25]; a CRP homologue termed Vfr was shown to be required for basal-level *lasR* expression [26]; a regulator termed RsaL has been described that is thought to repress transcription of *lasI*[27]; a deletion of *qscR* in *P. aeruginosa* resulted in premature expression of the quorum sensing-related genes *lasI*, *rhlI*, *hcnA*, and *phzA* [28]; a deletion in the posttranscriptional regulator RsmA led to advanced expression of *lasI*, *rhlI*, and *hcnA*[29]. Similarly it was reported influence on QS-regulation MvaT [30], VqsR [31], as well as the sigma factors RpoS [32], RpoN [33] and MvfR[34]. Particularly Whiteley et al. [32] have shown that the stationary-phase sigma factor RpoS negatively regulates C4-HSL production and that, in an *rpoS* mutant, expression of *rhlI*, *hcnA*, and *phzA* is advanced.

We are not intending to highlight all of them, but trying to explain the relationships complexity by example. Transcriptional regulator MvfR controls the transcription of two co-regulated operons, *pqsABCDE* and *phnAB*, the products of which, with the exception of *pqsE*, mediate the synthesis of a large array of HAQs, including HHQ, the direct precursor of PQS [17]. It has been shown, that *pqsE* mutant is not defective in the production of HAQs or PQS, but still is deficient in production of pyocyanin, lectin and HCN, and expression of the corresponding synthetic operons *phz1*, *lecA* and *hcnABC* [35]. Dissection of how *mvfR* is interwoven into the *P. aeruginosa* QS circuitry reveals that the MvfR system, through the essential contribution of PqsE, positively regulates a subset of genes dependant on both LasR and RhlR [34]. Animal studies show that MvfR contributes to *P. aeruginosa* virulence by controlling the transcription of genes not under RhlR regulation, and that reduced virulence of a *mvfR* mutant is caused not only by deficiency in HAQs/PQS production but also by the loss of *pqsE* expression [34]. It also was demonstrated, that PQS enhances the *in vitro* DNA-binding affinity of MvfR to the *pqsA-E* promoter, to suggest it might function as the *in vivo* MvfR ligand. Gaoping Xiao et. al. (2006) [36] find that HHQ binds to the MvfR ligand-binding-domain and potentiates MvfR binding to the *pqsA-E* promoter leading to transcriptional activation of *pqsA-E* genes. They also show that HHQ is highly produced *in vivo*, where it is not fully converted into PQS, and demonstrate that it is required for MvfR-dependent gene expression and pathogenicity. PQS is fully dispensable, as *pqsH* mutant cells, which produce HHQ but completely lack PQS, display normal MvfR-dependent gene expression and virulence [36].

In conclusion we allow ourselves to draw an analogy with another field, in which the methodology of molecular biology allows to hope for resolution of the problems that are unsolvable at present. The regulatory processes that govern cell proliferation and differentiation became central in basic research of oncogenesis and it seems that the processes that govern bacterial reproduction and functional phenotype must be placed in microbiology in a similar way.

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QS-DEPENDENT VIRULENCE REGULATION IN PSEUDOMONAS AERUGINOSA
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The discovery of the cell-to-cell communication in the bacterial populations led to the understanding that prokaryotes are capable of coordinated activity. That facilitates development of a very attractive field of research, within which exists ability to reveal fundamentally new patterns that control course of events during bacterial infection, and, appropriately, preconditions for development of new therapeutical approaches also exists. For the recent years different kinds of quorum sensing systems, that support the perception of population quantitative conditions and ensure its application for synchronisation of the certain gene expression, were described in different species of microorganisms. One of the most studied objects is P.aeruginosa. Its adaptive potential provides the ability to exist in different environmental conditions, to infect a wide range of the organisms (from Protozoa to Homo sapiens) and to modulate population behaviour aggressiveness in compliance with its suitability degree.
Keywords: bacterial populations, cell-to-cell communication, quorum sensing, P.aeruginosa

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QS-ЗАЛЕЖНА РЕГУЛЯЦІЯ ВІРУЛЕНТНОСТІ У PSEUDOMONAS AERUGINOSA
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Відкриття механізмів міжклітинної комунікації в бактерійних популяціях призвело до визнання факту здатності мікроорганізмів до координованої діяльності та сприяло розвитку надзвичайно привабливого напрямку досліджень, в межах якого можливо виявлення принципово нових закономірностей розвитку бактеріальних інфекцій та, відповідно, формуються передумови для виникнення принципово нових рішень в галузі боротьби з інфекційною патологією. За останні роки у різних видів мікроорганізмів було описано різні типи QS (quorum sensing) систем, що забезпечують сприйняття популяцією інформації про власні кількісні параметри та її використання для синхронізації експресії певних комплексів генів. Одним з найбільш досліджуваних об'єктів є P.aeruginosa. Її адаптаційний потенціал зумовлює здатність до існування в різноманітних умовах зовнішнього середовища, вірулентність по відношенню до широкого спектру організмів (від Protozoa до Homo sapiens) та варіабельність ступеню агресивності колективної поведінки популяції. Експресія факторів патогенності відбувається узгоджено, диференційовано та у відповідності зі ступенем ситуативної доцільності.
Ключові слова: P.aeruginosa, бактерійна популяція, сигнальна трансдукція

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QS-ЗАВИСИМАЯ РЕГУЛЯЦИЯ ВИРУЛЕНТНОСТИ У PSEUDOMONAS AERUGINOSA
Крестецкая С.Л., Нестеренко А.М.
Открытие механизмов межклеточной коммуникации в бактериальных популяциях позволило осознать тот факт, что микроорганизмы способны к координированной деятельности и способствовало возникновению чрезвычайно перспективного направления исследований, в рамках которого возможно выявление принципиально новых закономерностей развития бактериальной инфекции, и, соответственно, формируются предпосылки возникновения принципиально новых решений в области борьбы с инфекционной патологией. За последние годы у разных видов микроорганизмов были описаны разные типы QS (quorum sensing) систем, обеспечивающих восприятие популяцией информации о собственных количественных параметрах и её использование для синхронизации экспрессии определённых комплексов генов. Одним из наиболее исследуемых объектов является P.aeruginosa. Её адаптационный потенциал обеспечивает способность к существованию в разнообразных условиях внешней среды, вирулентность в отношении широкого спектра организмов (от Protozoa до Homo sapiens) и вариабельность степени агрессивности коллективного поведения популяции: экспрессия факторов патогенности происходит согласованно, дифференцированно и в соответствии со степенью ситуативной целесообразности.
Ключевые слова: P.aeruginosa, бактериальная популяция, сигнальная трансдукция