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Regulatory Mechanisms Involved in the Pathoadaptation of Extraintestinal Pathogenic *Escherichia coli*

Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie doktorsexamen framläggs till offentligt försvar i Major Groove, NUS byggnad 6L, onsdagen den 20 februari, kl. 09:00.
Avhandlingen kommer att försvaras på engelska.

Fakultetsopponent: Professor Eliora Ron (President of IUMS, School of Molecular Cell Biology & Biotechnology, Faculty of Life Sciences, Tel Aviv University, Israel)

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Title

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Abstract

Establishment of commensal bacteria within a new niche of their host usually promotes the transition from commensalism to pathogenicity. Extraintestinal Pathogenic *Escherichia coli* (ExPEC) represent different pathovars with biphasic lifestyle – they can reside in the gut as commensals or they can escape and cause diseases elsewhere in the human body. Depending on the disease they are linked to, ExPEC can be divided into Uropathogenic *E. coli* (UPEC) and Newborn Meningitis-causing *E. coli* (NMEC). Pathoadaptive mutations linked to c-di-GMP signaling were investigated in the NMEC strain IHE3034 which lacks the main global stress regulator RpoS. Deletion of *ycgG2*, shown by us to encode an YcgG allozyme with c-di-GMP phosphodiesterase (PDE) activity, and the restored RpoS led to a decrease in the S-fimbriae, otherwise robustly produced in artificial urine, hinting that the urinary tract could serve as a habitat for NMEC. We showed that NMEC were capable of aerobic citrate utilization in the presence of a co-substrate - a property that normally does not exist in *E. coli*. We also found that citrate utilization is a property of ExPEC, since we reconstituted it in *E. coli* UTI89 (a cystitis isolate) *via* inactivation of its RpoS, and since a set of pyelonephritis *E. coli* isolates use citrate aerobically in the presence of glucose. The main reason for this metabolic capability is the absence of RpoS which leads to the production of the citrate transporter CitT. Furthermore, we highlighted the deletion of the *fec* operon (required for the ferric citrate uptake) in a large group of different ExPEC strains and we showed that NMEC can use CitT for *in vitro* ferric citrate uptake dependent on YcgG2 as an alternative system. Another pathoadaptive mutation which influences the fitness of ExPEC is the *clyA* (cytolysin A) gene inactivation, resulting from different deletions in different ExPEC genomes. When we restored the *clyA*$^{+}$ locus, the UPEC strain 536 displayed increased susceptibility to antimicrobial peptides and urea. We also showed that the ClyA expression in 536 was increased by the presence of the DNA-binding regulator SfaX and another stand-alone PDE similar to YcgG2, called SfaY. We also studied the role of *sfaY* - a gene coding for another stand-alone c-di-GMP PDE. The expression of *sfaY* is under the regulation of the main promoter of the horizontally acquired *sfa* gene cluster. The latter is responsible for the regulation and assembly of the virulence-associated S-fimbriae, *via* which ExPEC bacteria bind to sialylated receptors. We found that NMEC are competent for filamentation because of a c-di-GMP-dependent program under the control of a phase-variation event which selectively turns 'ON' the *sfa* promoter in a subpopulation of bacteria. When SfaY is present, c-di-GMP levels are reduced, thus inducing the SOS stress response *via* the canonical LexA-RecA pathway. The signaling resulted in an internal differentiation of the bacterial population into a subpopulation exhibiting a filamentous morphotype (bacteria with induced SOS stress response) and a subpopulation of small motile and non-motile bacteria. Hence, this molecular program could serve as a clue to explain the formation of the intracellular bacterial communities observed during urinary tract infection by UPEC.

Keywords

ExPEC, PAIs, c-di-GMP, citrate, metabolism, filamentation, toxins

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