Turn Up the Heat: Environmental cues and virulence gene regulation in uropathogenic *Escherichia coli*

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RimJ and *pap* **regulation:**

Lyn LeClerc '04 Jessica Slack '06 Jill Angelosanto '05 Amy Malhowski '05 Sooyeon Choi '06 Alia Black MHC '01 Michelle Ploutz '03 Stacie Eliades '02

Microarrays:

Sarah Young '03 Amy Malhowski '05 Nabila Noor '06

Support:

National Institutes of Health Blakeslee Genetics Fund Smith College Howard Hughes Medical Institute GCAT (microarrays) Bob Zimmerman (S5 protein)

What good bacteria do

- aid in digestion
- produce vitamins B, K
- compete with pathogenic bacteria=

microbial antagonism

Lansing M. Prescott, John P. Harley, Donald A. Klein, Microbiology, 4e. Copyright @ 1999 The McGraw-Hill Companies, Inc. All rights reserved.





Environmental regulation: A common theme in bacterial pathogenesis

- Temperature
- pH
- Osmolarity
- Oxygen
- Carbon dioxide
- Iron
- Growth phase
- Stress

Escherichia, Shigella, Yersinia Salmonella, Vibrio Escherichia, Salmonella, Pseudomonas Corynebacterium Bacillus, Streptococcus Escherichia, Corynebacterium Salmonella, Shigella Escherichia, Neisseria

Adapted from Escherichia coli and Salmonella Cellular and Molecular Biology

Sensing Host Environments



External environment

Figure adapted from Microbiology by Prescott, Harley, and Klein

Pyelonephritis-associated pili



Photo courtesy of Dr. David Low

Bacterial Colonization



Photo from Microbiology by Prescott, Harley, and Klein



Urinary tract infections (UTI)

UTIs affect the bladder (cystitis) and the kidneys (pyelonephritis)

40% of women will experience a UTI in their lifetime

Significant problem in hospitals due to catheters

UTIs are predominantly due to Escherichia coli (70-90% of infections)

7 million cases of acute cystitis and 250,000 cases of pyelonephritis annually in USA

Over 90% of the isolates from pyelonephritis contain pap DNA sequences

Antibiotic resistant organisms have been isolated

Photo courtesy of Dr. David Low

Operon structure of P pili





Tip-associated proteins

papBA-lacZYA transcriptional fusion



LacZ

encodes β -galactosidase

cleaves substrate in medium to give color reaction serves as reporter of transcription from promoter

papBA-lacZYA transcriptional fusion



Phase ON= blue colony phenotype (Lac+) ->Transcription from *papBA* promoter

Phase OFF= white colony phenotype (Lac-) ->No transcription from the *papBA* promoter

Regulation of *pap* occurs at the transcriptional level



 $23^{\circ}C M9 glycerol \Rightarrow$

$\Leftarrow 37^{\circ}C M9 glycerol$



Common environmental cues control *pap* transcription





Microarray questions

⇒What other genes are controlled by temperature, similar to *pap*?

⇒How do these genes help the bacterium adapt to its environment?

Microarray design





MC4100 *pap-lac* ZYA fusion + or - *rimJ*, 37°C and 23°C

2.5 ug/slide/condition or genotype Genisphere kit

U. Wisconsin glass slides, MG1655 Provided through GCAT ~4200 PCR products, 2X

Axon GenePix GProcessor

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)



http://biochemistry.yonsei.ac.kr/biochem_molecular/gene_cloning_09.php



http://www.accessexcellence.org/AB/GG/microarray.html





http://www.biology.ucsc.edu/mcd/research.html

Overview of temperatureregulated genes

- 45 genes upregulated \ge 3X at 37°C
- Control:*pap-lac* fusion increased 12.5 fold
- Majority (34) focused to transport and metabolism
- 11 genes of unknown/putative function

- 74 genes upregulated \ge 4X at 23°C
- 44 number of genes with putative/unknown function
- Many devoted to environmental adaptation

Genes upregulated at 37°C

- o Iron acquisition genes: cirA, fecR, fes
- o Amino acid synthesis: cys, his
- o Transporters: galT, lamB, livK, malM, mglABC
- o Transposon related sequences: IS5 transposase

Iron acquisition required for UPEC virulence

- Knock out of one iron acquisition pathway in UPEC strain CFT073 decreased the ability of mutant to compete with wild type for virulence in mouse model
- Genome of uropathogenic *E. coli* is enriched with iron acquisition genes



RT-PCR confirms thermoregulation of *cirA/fes* transcription

