Evolutionary and molecular origins of a phenotypic switch in *Pseudomonas fluorescens* SBW25

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Jenna Gallie

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Abstract

Survival in the face of unpredictable environments is a challenge faced by all organisms. One solution is the evolution of mechanisms that cause stochastic switching between phenotypic states. Despite the wide range of switching strategies found in nature, their evolutionary origins and adaptive significance remain poorly understood. Recently in the Rainey laboratory, a long-term evolution experiment performed with populations of the bacterium *Pseudomonas fluorescens* SBW25 saw the *de novo* evolution of a phenotypic switching strategy. This provided an unprecedented opportunity to gain insight into the evolution and maintenance of switching strategies.

The derived 'switcher' genotype was detected through colony level phenotypic dimorphism. Further microscopic examination revealed the cellular basis of phenotypic switching as the bistable (ON/OFF) expression of a capsule. Transposon mutagenesis demonstrated that the structural basis of the capsule was a colanic acid-like polymer encoded by the Pflu3656-*wzb* locus. Subsequently, whole genome re-sequencing enabled elucidation of the series of mutational events underlying the evolution of capsule bistability: nine mutations were identified in the switcher. Present in both forms of the switcher, the final mutation – a point mutation in a central metabolic pathway – was shown to be the sole mechanistic cause of capsule switching; it 'set the stage' for a series of molecular events directly responsible for bistability.

Two models were proposed to explain capsule switching at the molecular level: the genetic amplification-reduction model, and the epigenetic feedback model. Collective results of biochemical and genetic assays proved consistent with the epigenetic model, whereby a decrease in flux through the pyrimidine biosynthetic pathway activates an already-present feedback loop. Subsequent analysis of a second switcher (evolved independently of and in parallel with the first) revealed a radically different genetic route leading to phenotypically and mechanistically similar capsule switching.

In addition to providing the first empirical insight into the evolutionary bases of switching strategies, the work presented in this thesis demonstrates the power of natural selection – operating on even the simplest of organisms – to forge adaptive solutions to evolutionary challenges; in a single evolutionary step, selection took advantage of inherent intracellular stochasticity to generate an extraordinarily flexible phenotype.

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Table of Abbreviations

Abbreviation	Meaning
ACP	Acetylated cellulosic polymer
AP-PCR	Arbitrary primed-polymerase chain reaction
BLAST	Basic local alignment search tool
bp	base pairs
c-di-GMP	Cyclic-dimeric-guanosine monophosphate
CP	Carbamoyl phosphate
CPSase	Carbamoyl phosphate synthetase
DGC	Di-guanylate cyclase
dNTP	dinucleotide triphosphate
DUF	Domain of unknown function
g	gram/gravity
GDP/GTP	Guanosine di-/tri- phosphate
GDP-Fuc	GDP-L-fucose
Gm	Gentamicin
HK	Histidine kinase
KB/kb	King's medium B/kilobase
Km	Kanamycin
LB	Lysogeny broth
M-W-W test	Mann-Whitney-Wilcoxon test
NF	Nitrofurantonin
OD	Optical density
PDE	Phosphodiesterase
REE	Reverse evolution experiment
RR(r)	Response regulator (receiver)
SM	Smooth morph
SSI	Site-specific inversion
SSM	Slipped-strand mispairing
Tc	Tetracycline
TCSTP	Two-component signal transduction pathway
UDP-Gal	UDP-D-galactose
UDP-Gluc	UDP-D-glucose
UDP-GlucA	UDP-D-glucuroinc acid
UMP/UDP/UTP	Uracil mono-/di-/tri- phosphate
WS	Wrinkly spreader
X-gal	5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside