Exploring the origins of multicellularity using experimental populations of *Pseudomonas fluorescens* SBW25: Deciphering the genetic basis of an environmentally-responsive developmental switch

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Abstract

The evolution of multicellularity was a significant evolutionary event that occurred on numerous independent occasions in the history of life. It is useful to consider this in the Darwinian population framework: a population may participate in evolution by natural selection given that it satisfies the criteria of – variation, reproduction, and heredity. The transition from unicellular to multicellular life represented the emergence of Darwinian properties at a new hierarchical level, and the shift of Darwinian individuality from the level of the individual cell to the cooperating cell collective. This required a mechanism of reproduction of the collective; best conceived with nascent multicellular life cycles, likely manifest through clonal development and single-cell bottlenecks to mediate conflict between levels of selection. For the origin of multicellularity, transitioning between phases of the life cycle was also dependent on the evolution of developmental processes that integrate the activity of the individual cells and the collective. An experiment previously conducted in the Rainey laboratory explored the origins of multicellularity using *Pseudomonas fluorescens* SBW25, selecting for the evolution of a developmental program to transition between the soma-like SM and germ-like WS phases of the life cycle. Derived from this experiment was the TSS-f6 genotype, that demonstrates an environmentally-responsive capacity to change phenotype – resembling a primitive multicellular organism able to transition through the life cycle under developmental regulation. Whole-genome sequencing revealed the mutational history of TSS-f6, with a substitution in the *wspA* gene necessary for the phenotype; the WspA chemoreceptor hypothesised to sense environmental oxygen. Suppressor analysis of the TSS-f6 phenotype revealed the underlying activation pathways: for the WS phenotype – the *wsp* & *wss* operons, and *mut* genes; and the SM phenotype – *pflu5960*, *amrZ*, and *wspE*. From this genetic dissection a simple model was proposed for the TSS-f6 developmental switch, though the role of *wspE* and the DNA mismatch repair system remain unexplained. The TSS-f6 genotype provided the opportunity to gain mechanistic insight into the emergence of a nascent life cycle under the control of a developmental program, and thus the origins of multicellularity and development in itself.

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Table of Abbreviations

Abbreviation	Meaning
ALI	Air liquid interface
Amp	Ampicillin
AP-PCR	Arbitrary primed-polymerase chain reaction
BLAST	Basic local alignment search tool
bp	base pair
c-di-GMP	Cyclic-dimeric-guanosine monophosphate
DGC	Di-guanylate cyclase
DNA	Deoxyribonucleic acid
dNTP	dinucleotide triphosphate
g	gram or gravity
G	guanine
GPM	Genotype-phenotype map
hr	hour
kb	kilobase pair
KB	King's broth
Km	Kanamycin
LB	Lysogeny broth
LSWS	Large spreading wrinkly spreader
min	minute
Nf	Nitrofurantoin
PCR	Polymerase chain reaction
PDE	Phosphodiesterase
Rif	Rifampicin
rpm	revolutions per minute
sec	second
SM	Smooth morphology
Tet	Tetracycline
TSS	Temperature sensitive switcher
WS	Wrinkly spreader
WT	Wild type