Isolation and Characterization of Host Mutations that Suppress the Bacteriophage Lambda (λ) Rex Phenotype

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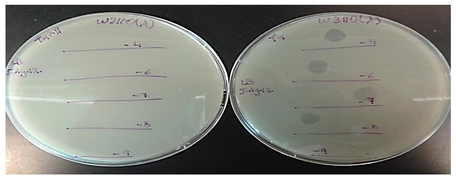
Introduction

- the λ T4*rII* exclusion (Rex) phenotype: is the ability of the *rex* genes (*rexA, rexB)* of bacteriophage Lambda (λ) to prevent mutant bacteriophage T4*rII* plaque formation on *E. coli* hosts lysogenized by λ.



- More than six decades have now passed since the discovery of Rex by Seymour Benzer in 1955, yet the mechanism behind this elusive exclusion system remains a mystery.
- The Rex system is encoded by two genes of λ (*rexA*, and *rexB*); the expression of which is primarily regulated by the repressor gene *cI* from the P_M promoter.

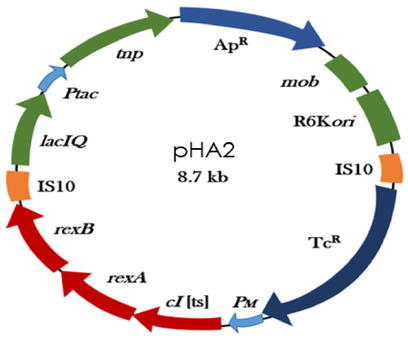
Factors affect the onset and/or abrogation of Rex:

1- T4*rII* infection: results in rapid membrane depolarization and a harsh cellular environment that in many ways resembles stationary phase metabolism and morphology.
2- Disruption of the RexA:RexB balance: can lead to same manifestations without infection.
3- Ionic environment: Monovalent cations like H^+, Na^+ in the cellular environment are essential for the exclusionary phenotype. Divalent cations such as Ca^{2+} Mg^{2+}, or polyamines in culture can attenuate the exclusion activity.

- Despite some cell killing, infected lysogens can recover from Rex activation. Rex may thus be a mutualistic protection mechanism that protects both itself and the host cell from external infection.
- It has long been believed that additional host proteins like the Outer Membrane Proteins (Omp) are involved in such a pleiotropic phenotype.
- We aimed to generate, isolate and characterize *E. coli* mutants that **attenuate Rex** to uncover important clues about the functionality of Rex and the mechanism of its activation.

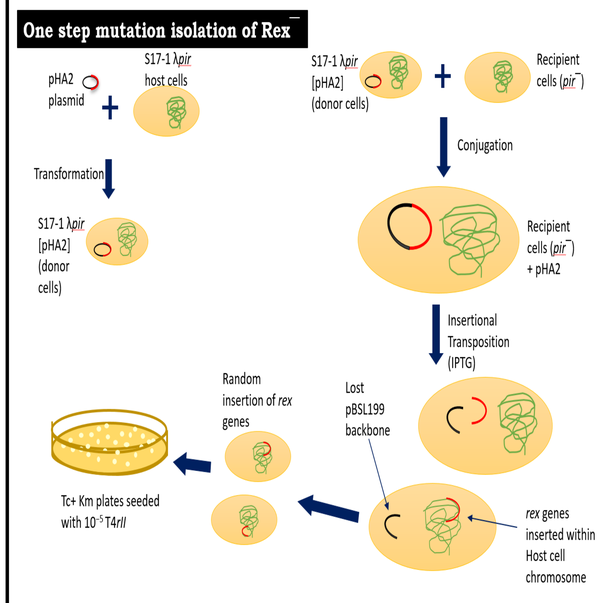
Methods

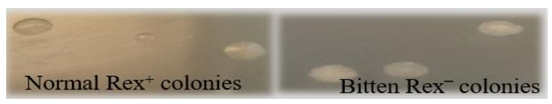
We have designed a system for the rapid one-step isolation of host mutations that abrogate Rex in order to identify the host genes involved and to elucidate the mechanism of this enigmatic exclusion system.



Construction of the suicide *rex*⁺ vector (pHA2): R6K *ori*: origin of replication which needs π replication protein (*pir* gene)to initiate plasmid replication. Tn*10*: transposable element includes two IS10 insertion sequences flanking tetracycline resistance marker (Tc^R) and the transposase gene. *cI-rexA-rexB* : gene of interest conferring natural *rexA-rexB* expression scenario*(rex⁺)*

Mutagenize and isolate *rex*⁺ *E. coli* mutants that impact Rex activity:





Results

- We have isolated 13 host mutations of *E. coli* K-12 that attenuated the Rex phenotype as well as identifying for the first time several outer membrane protein genes including: *ompA*, *ompF*, *ompW*, and *ompX* that are involved in imparting Rex activity.
- Confirming isolated *rex*⁺ *E.coli* mutant(s) for sensitivity to T4*rII* plating:

E.coli JW0427-1 # of Mutant	Mutations (*ΔclpP*) (*rex*⁺)	Relative Efficiency of Plating (T4*rII*)	Rex Activity
JW0427-1	*rex*⁻	1.0	< 1 x 10^{-7}
JW0427-1 (λ)	*λ⁺(rex⁺)*	< 1 x 10^{-7}	1.0
JW0427-1	*rex*⁺	< 1.0 x 10^{-7}	1.0
# JW-HA 1	*rex*⁺	2.3 x 10^{-5}	< 100 fold
# JW-HA 2	*rex*⁺	7.0 x 10^{-6}	< ~100 fold
# JW-HA 3	*rex*⁺	1.5 x 10^{-5}	< 100 fold
# JW-HA 4	*rex*⁺	1.0 x 10^{-3}	< 10K fold
# JW-HA 5	*rex*⁺	1.7 x 10^{-5}	< 100 fold
# JW-HA 6	*rex*⁺	1.4 x 10^{-4}	< 1K fold
# JW-HA 9	*rex*⁺	5.0 x 10^{-6}	< 10 fold
# JW-HA 11	*rex*⁺	1.6 x 10^{-5}	< 100 fold
# JW-HA 12	*rex*⁺	1.3 x 10^{-5}	< 100 fold
# JW-HA 19	*rex*⁺	2.0 x 10^{-4}	< 1K fold
# JW-HA 20	*rex*⁺	8.0 x 10^{-6}	< ~100 fold
# JW-HA 21	*rex*⁺	1.6 x 10^{-5}	< 100 fold
# JW-HA 25	*rex*⁺	1.8 x 10^{-5}	< 100 fold

1-T4/T4*rII* plating efficiency on Δ*omp* (*rex*⁺) infective centers:

Test of viable candidate genes postulated to influence Rex activity:

E. coli Strain	*Δomp* location	Plasmid	T4(wt) Activity	Efficiency of Plating T4*rII*	Rex
BW25113 wt	_	pHA1*	0.33	< 3.0 x 10^{-7}	1.0
JW0554-1	*ΔompT*	pHA1	0.16	< 2.0 x 10^{-7}	1.0
JW0799-1	*ΔompX*	pHA1	0.2	3.0 x 10^{-4}	< 10^3
JW0912-1	*ΔompF*	pHA1	0.15	3.0 x 10^{-5}	< 10^2
JW0940-6	*ΔompA*	pHA1	3.0x 10^{-4}	< 4.0 x 10^{-7}	1.0
JW1248-2	*ΔompW*	pHA1	0.22	1.0 x 10^{-4}	< 10^3
JW1312-1	*ΔompG*	pHA1	0.12	< 2.0 x 10^{-7}	1.0
JW1371-5	*ΔompN*	pHA1	0.3	< 4.0 x 10^{-7}	1.0
JW2203-1	*ΔompC*	pHA1	<1 x10^{-7}	< 1.0 x 10^{-7}	_ **
JW3368-1	*ΔompR*	pHA1	3.0x10^{-5}	<4.0 x 10^{-7}	1.0
JW3846-1	*ΔompL*	pHA1	0.13	1.7 x 10^{-5}	< 10^2

* PHA1 is pUC19 (*rex*+) plasmid
**Δ*ompC* mutant was not able to form plaques as OmpC is the absorption site for T4 phage, so neither phage can infect this strain.

2- T4*rII* plating efficiency on Δ*omp* λ*cI-857* lysogens following the infective center assay:

E. coli Strain	*Δomp* (del) Location	Relative Efficiency of Plating T4*rII*	Rex Activity
BW25113(wt)	_	< 3.0 x 10^{-7}	1.0
JW0554-1	*ΔompT*	< 2.0 x 10^{-7}	1.0
JW0799-1	*ΔompX*	4.5 x 10^{-4}	< 10^3
JW0912-1	*ΔompF*	3.3 x 10^{-5}	< 10^2
JW0940-6	*ΔompA*	5.0 x 10^{-4}	< 10^3
JW1248-2	*ΔompW*	1.0 x 10^{-3}	< 10^4
JW1312-1	*ΔompG*	< 2.0 x 10^{-7}	1.0
JW1371-5	*ΔompN*	< 4.0 x 10^{-7}	1.0
JW2203-1	*ΔompC*	< 1.0 x 10^{-7}	1.0
JW3368-1	*ΔompR*	< 4.0 x 10^{-7}	1.0
JW3846-1	*ΔompL*	3.0 x 10^{-6}	< 10

3- Cell viability of T4*rII*-infected Δ*omp* (*rex*⁺) [pHA1] hosts:

E. coli Strain	*Δomp* Mutation Location	Plasmid	Relative Efficiency of Plating (T4*rII*)	Reduction in Cell Viability
BW25113 WT	-	*(rex⁺)*	8 x10^{-4}	1.0
BW25113 WT	-	No plasmid *(rex⁻)*	< 2 x10^{-7}	< 10^3
JW0554-1	*ΔompT*	*(rex⁺)*	4 x10^{-4}	1.0
JW0799-1	*ΔompX*	*(rex⁺)*	1.2 x10^{-6}	< 10^2
JW0912-1	*ΔompF*	*(rex⁺)*	1 x10^{-6}	< 10^2
JW0940-6	*ΔompA*	*(rex⁺)*	5 x10^{-6}	< 10^2
JW1248-2	*ΔompW*	*(rex⁺)*	7 x10^{-6}	< 10^2
JW1312-1	*ΔompG*	*(rex⁺)*	9 x10^{-3}	~1.0
JW1371-5	*ΔompN*	*(rex⁺)*	5 x10^{-4}	1.0
JW2203-1	*ΔompC*	*(rex⁺)*	≈1	~ 0
JW3368-1	*ΔompR*	*(rex⁺)*	7 x10^{-3}	~1.0
JW3846-1	*ΔompL*	*(rex⁺)*	9 x10^{-4}	~1.0

Involvement of *E. coli omp* mutants in Rex activity:

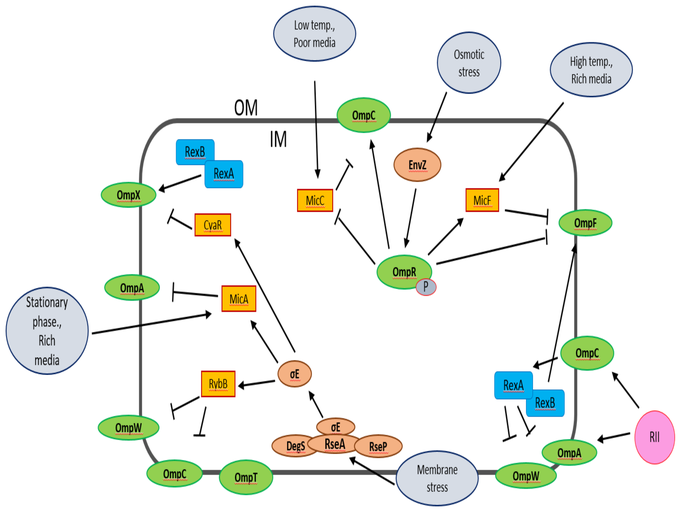


Figure keys: Green ovals are Outer membrane proteins; Pink circles are RII protein of T4 phage; Blue boxes are Rex proteins of λ; yellow boxes are small RNAs that regulate the transcription of *omp* genes and the expression of Omp proteins; blunted arrows indicate inhibition/downregulation/ blocking expression; regular arrows indicate stimulation/upregulation/ initiation expression; OM: outer membrane; IM: inner membrane.

Conclusion and significance of this work

- This project aims to understand the mechanism behind the λ Rex phenotype through identifying and understanding the functions of non-essential host proteins that are involved in Rex activity
- We have shown for the first time that OmpA, OmpW, OmpX and OmpF proteins are involved in Rex activity, supporting the model, whereby Rex activation shunts cells into a stationary phase-like state to prevent T4*rII* growth
- Understanding the mechanism and the functions of *omp* genes that are involved in the suppression of Rex activity is critical to understanding the function of the *rex* genes and the exclusion mechanism
- Identifying the isolated gene that were knocked-out and attenuated Rex⁻ phenotype, we definitely can get noteworthy clues about *rex* genes functions and their mechanism of exclusion
- Using the Tandem affinity purification technique to tag Rex and RII proteins and study their interactions and then to identify and localize the Rex proteins in *E. coli* (λ lysogens)
- Understanding Rex can provide important clues and strategies for use toward eukaryotic exclusion systems and control mechanisms

Acknowledgments:

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