Prof. Gil Segal Research

The research in the lab is focused on the study of bacterial pathogenesis systems. We wish to understand the molecular mechanisms by which these systems function, the ways in which they are regulated and the evolutionary events that facilitate their establishment as pathogenesis systems. We have chosen to study two bacterial pathogens: ***Legionella pneumophila*** – the causative agent of a sever pneumonia in humans known as Legionnaires' disease and the potential bio-terrorism agent ***Coxiella burnetii*** – the causative agent of Q-fever. Both these pathogens utilize a conserved type-IV secretion system, the Icm/Dot system, to inject bacterial proteins (termed effectors) into the host cells. These effectors modulate host cellular functions during infection. The components of the type-IV secretion system itself and the arsenal of effectors which are translocated into host cells during infection are the primary pathogenesis determinants of these bacteria.

The projects in the lab focus on different aspects of microbial pathogenesis:

- Host cell processes modulated by bacterial pathogenesis determinants (effectors)

- The regulatory network that controls the expression of pathogenesis related genes

- Functional genomics of *Legionella* and *Coxiella* pathogenesis components

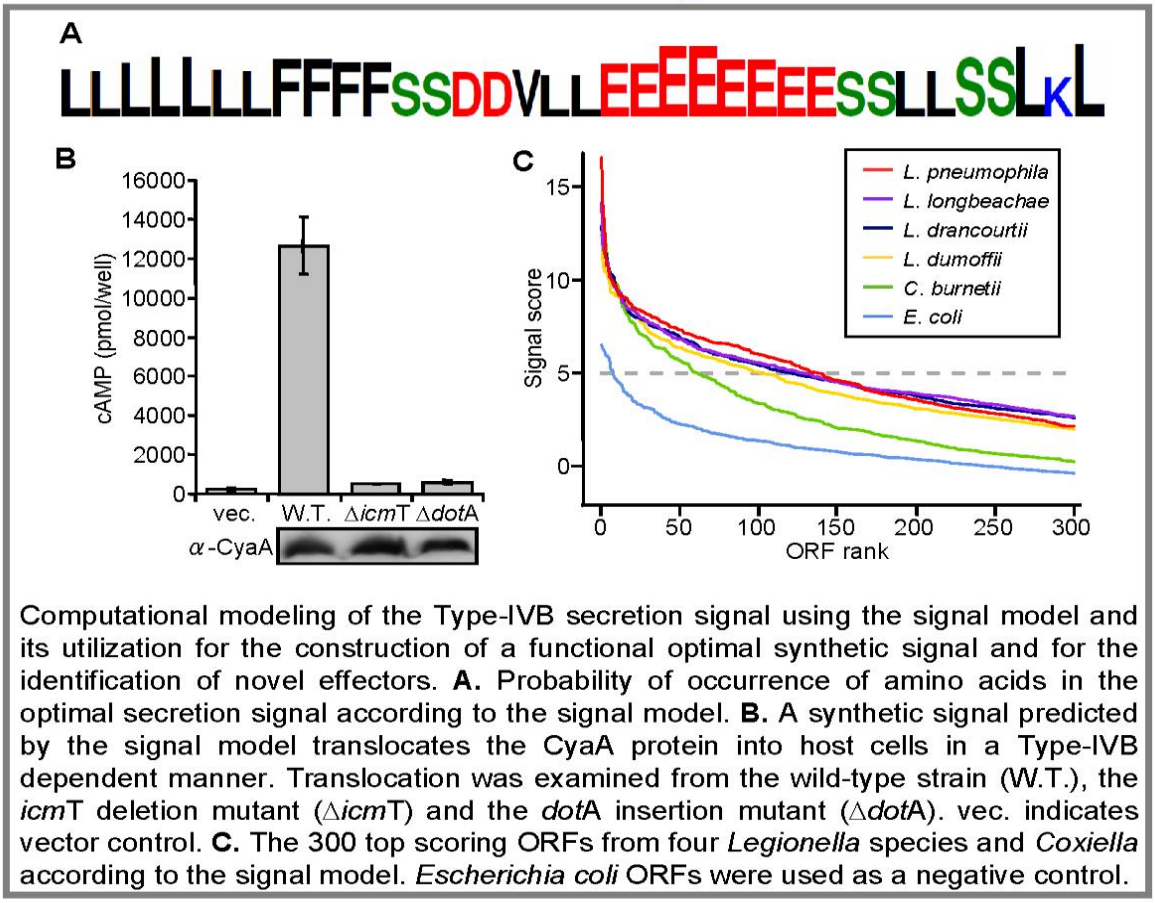
- Host-pathogen lateral gene transfer and its contribution to pathogenesis and co-evolution

Some examples of our research are shown below

1. Identification of *Legionella* and *Coxiella* effector proteins

The main pathogenesis system of *Legionella* and *Coxiella* is the Icm/Dot type-IV secretion system which translocates effector proteins into host cells. Identification of the effector proteins which are translocated into host cells by this secretion system is an essential step in the study of these pathogens. Our lab utilized several experimental and bioinformatics approaches in order to identify effector proteins which include: 1) identification of effectors according to their regularly elements; 2) identification of effectors due their genomic location; 3) identification of effectors using a machine learning approach; and 4) identification of effectors using their translocation signal.

Identification and characterization of the Icm/Dot effectors translocation signal



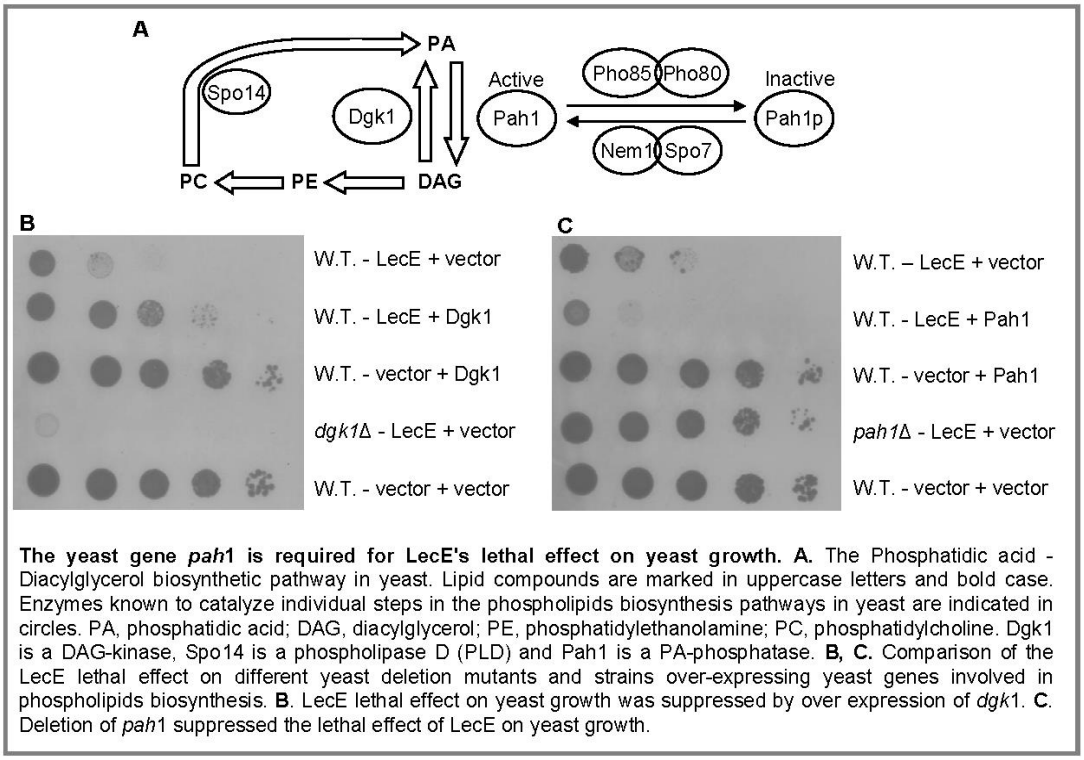
Computational modeling of the Type-IVB secretion signal using the signal model and its utilization for the construction of a functional optimal synthetic signal and for the identification of novel effectors. **A.** Probability of occurrence of amino acids in the optimal secretion signal according to the signal model. **B.** A synthetic signal predicted by the signal model translocates the CyaA protein into host cells in a Type-IVB dependent manner. Translocation was examined from the wild-type strain (W.T.), the *icm*T deletion mutant (△*icm*T) and the *dot*A insertion mutant (△*dot*A). vec. indicates vector control. **C.** The 300 top scoring ORFs from four *Legionella* species and *Coxiella* according to the signal model. *Escherichia coli* ORFs were used as a negative control.

Proc. Natl. Acad. Sci. USA. (2013) 110(8): E707-E715

2. Functional characterization of effector proteins using yeast genetics

It was shown before, using effectors from different pathogenic bacteria, that ectopic expression of effectors sometimes result with lethal effect on yeast growth. The lethal effect on yeast growth suggests that a conserved and essential eukaryotic process, which is modulated by the effectors in the host cell, was also modulated in the yeast cell, resulting in an inhibition of yeast cell growth. In addition, effectors sometimes target a cellular pathway that is conserved but is not normally rate-limiting for yeast growth and in order to identify such effectors different stress conditions as well as yeast mutants are used. Identification of effectors that cause strong lethal effect on yeast growth in any of these conditions can lead to the identification of their target proteins by using the yeast over-expression and deletion libraries. Using these approaches we uncovered the involvement of the effector LecE in phospholipids metabolism and discovered several effectors with are involved in the modulation of ER - Golgi vesicular trafficking.

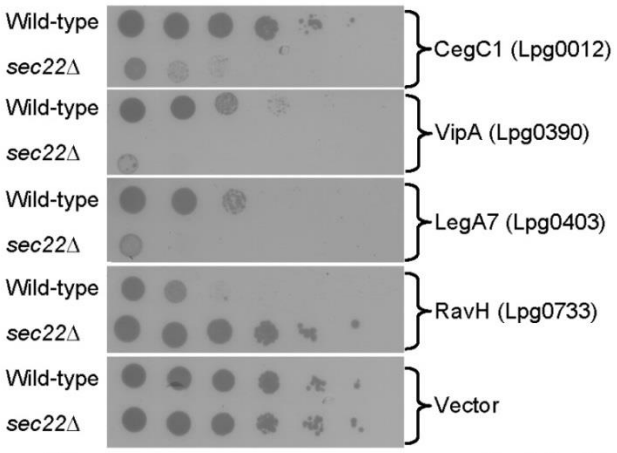
A suppressor screen identified the involvement of LecE in phospholipids metabolism



The yeast gene *pah*1 is required for LecE's lethal effect on yeast growth. A. The Phosphatidic acid - Diacylglycerol biosynthetic pathway in yeast. Lipid compounds are marked in uppercase letters and bold case. Enzymes known to catalyze individual steps in the phospholipids biosynthesis pathways in yeast are indicated in circles. PA, phosphatidic acid; DAG, diacylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine. Dgk1 is a DAG-kinase, Spo14 is a phospholipase D (PLD) and Pah1 is a PA-phosphatase. **B, C.** Comparison of the LecE lethal effect on different yeast deletion mutants and strains over-expressing yeast genes involved in phospholipids biosynthesis. **B**. LecE lethal effect on yeast growth was suppressed by over expression of *dgk*1. **C**. Deletion of *pah*1 suppressed the lethal effect of LecE on yeast growth.

PLoS Pathog. (2012) 8(11):e1002988

A synthetic lethality screen in yeast identified several *Legionella* effectors involved in ER - Golgi vesicular trafficking



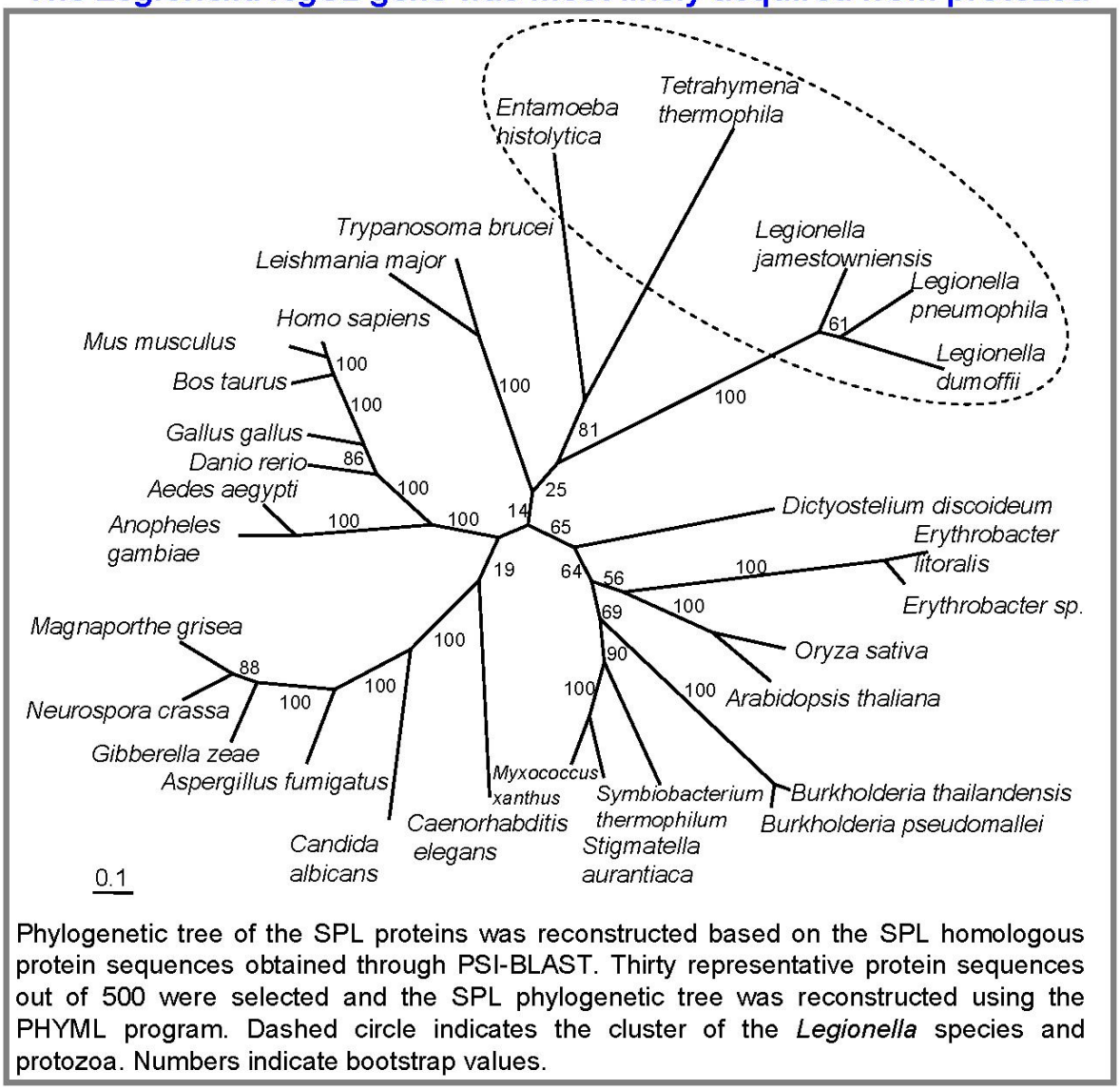
Several *L. pneumophila* effectors cause a synthetic growth defect in the yeast *sec22* deletion mutant. Comparison of the degree of lethal effect caused by effectors expressed in the wild-type yeast and the *sec22*Δ mutant (*sec22* encodes for a SNEAR protein that has a critical role in ER – Golgi vesicular trafficking). Yeast containing the effectors indicated on the right were platted in ten-fold serial dilutions under inducing conditions (Galactose). The effectors were over expressed in the wild-type *S. cerevisiae* BY4741 (upper dilutions in each pair) and the *sec22*Δ mutant (lower dilutions in each pair).

J. Bacteriol. (2014) 196:681-692

3. Study of effectors horizontally transferred from amoebae to Legionella

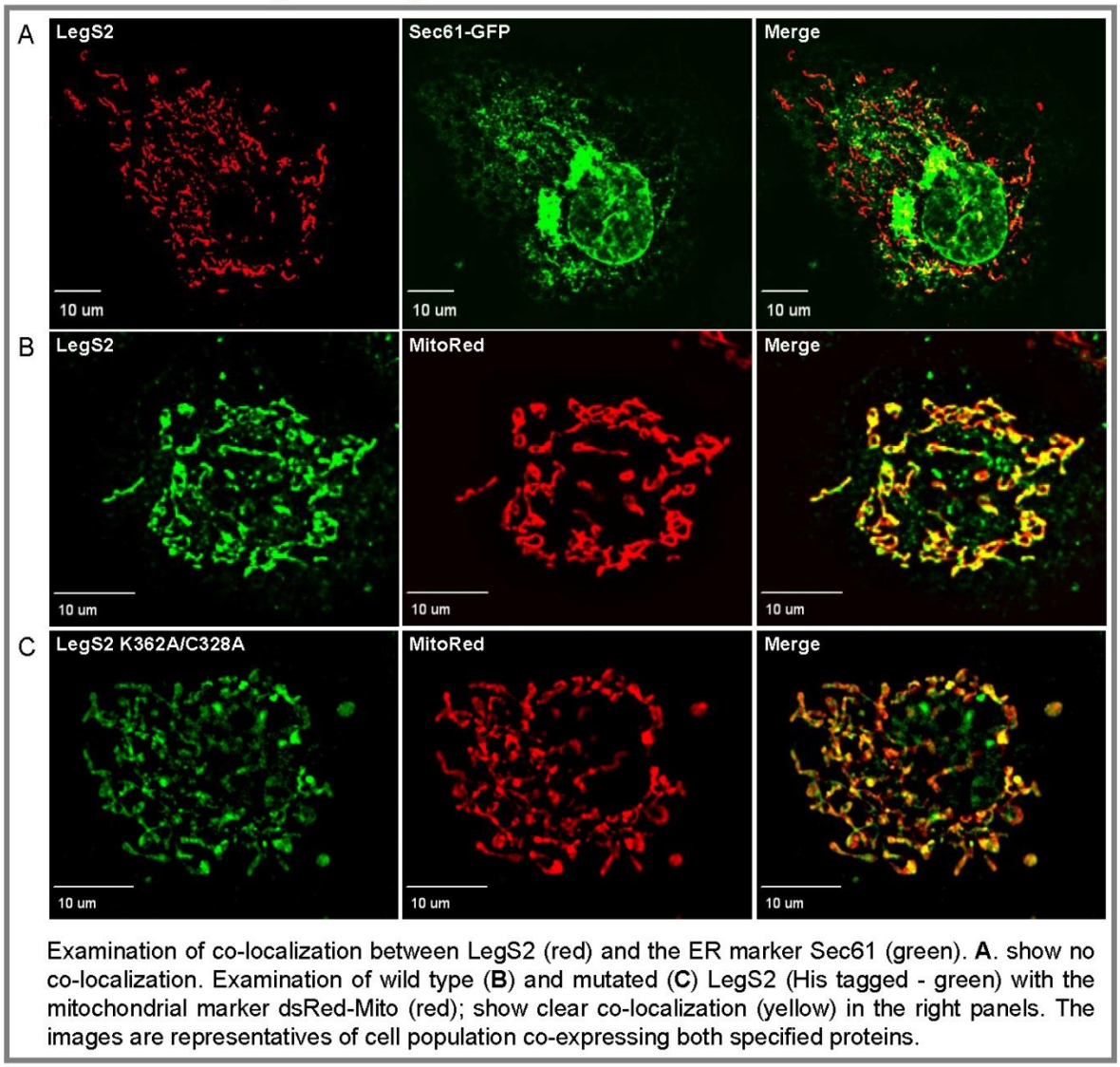
The effector LegS2 of *Legionella pneumophila* is a homologue of the highly conserved eukaryotic enzyme sphingosine-1-phosphate lyase (SPL). Phylogenetic analysis revealed that it was most likely acquired from a protozoan organism early during Legionella evolution. The LegS2 protein was found to translocate into host cells using a C-terminal translocation domain absent in its eukaryotic homologues. LegS2 was found to complement the sphingosine-sensitive phenotype of the yeast *Saccharomyces cerevisiae* SPL-null mutant and this complementation depended on evolutionary conserved residues in the LegS2 catalytic domain. Interestingly, unlike the eukaryotic SPL that localizes to the endoplasmic reticulum, LegS2 was found to be targeted mainly to host cell mitochondria.

The *Legionella legS2* gene was most likely acquired from protozoa



Phylogenetic tree of the SPL proteins was reconstructed based on the SPL homologous protein sequences obtained through PSI-BLAST. Thirty representative protein sequences out of 500 were selected and the SPL phylogenetic tree was reconstructed using the PHYML program. Dashed circle indicates the cluster of the *Legionella* species and protozoa. Numbers indicate bootstrap values.

LegS2 is targeted to the host cell mitochondria



Examination of co-localization between LegS2 (red) and the ER marker Sec61 (green). **A**. show no co-localization. Examination of wild type (**B**) and mutated (**C**) LegS2 (His tagged - green) with the mitochondrial marker dsRed-Mito (red); show clear co-localization (yellow) in the right panels. The images are representatives of cell population co-expressing both specified proteins.

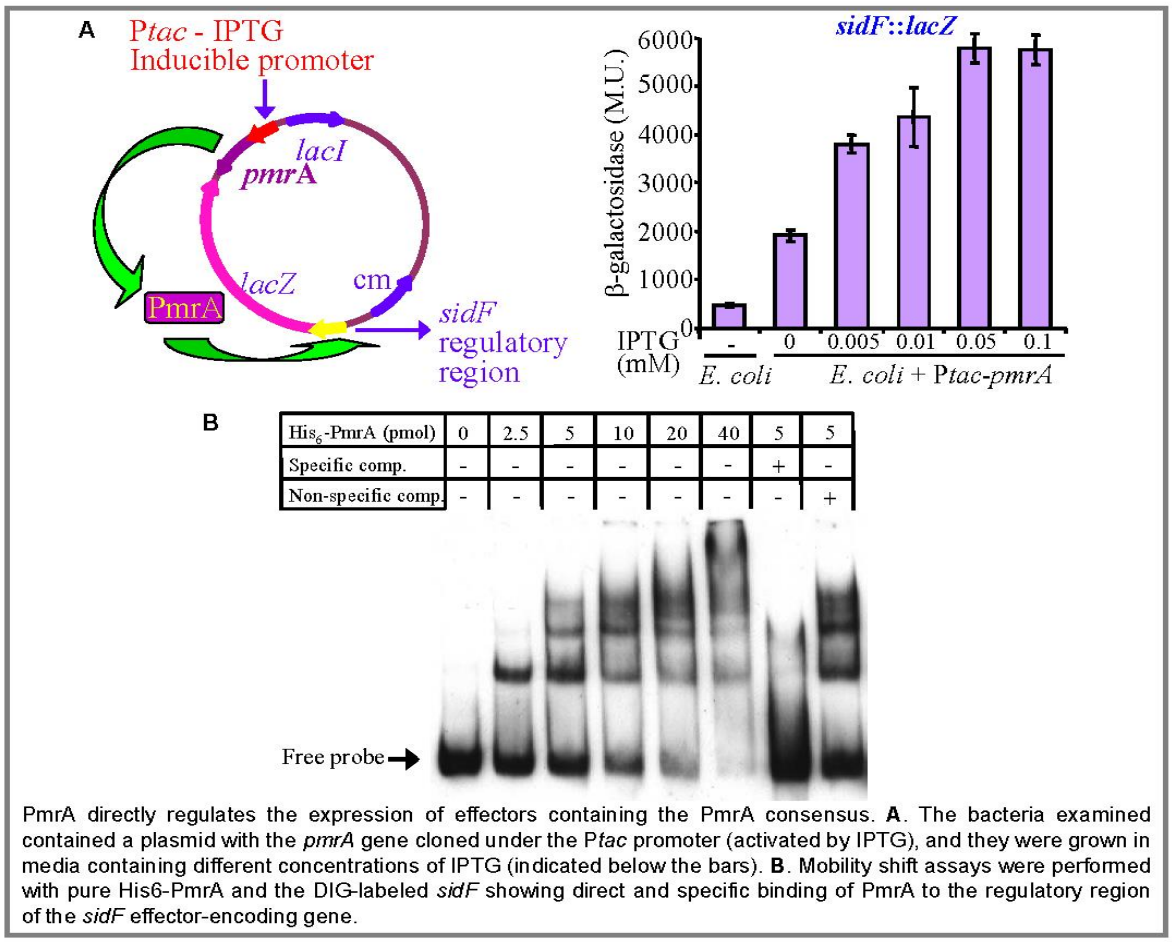
Cell. Microbiol. (2009) 11:1219-1235

4. The regulatory network that controls the expression of the *Legionella* pathogenesis system

To establish its replication niche *Legionella* translocate about 300 effector proteins into the host cells during infection. This enormous number of effectors should be coordinated at the level of gene expression, in order to be expressed and translocated at the correct time and appropriate amounts. One of the predominant ways in bacteria to regulate virulence gene expression is by the use of two-component systems (TCSs). To date, our lab has identified three TCSs directly involved in the regulation of Icm/Dot effector-encoding genes: the PmrAB, CpxRA and LetAS TCSs. According to our current knowledge, these three TCSs control the expression of about 80 effector-encoding

genes. The regulation by different TCSs divides the effectors into groups of co-regulated effector-encoding genes that are probably co-expressed at a similar time during infection and might perform related functions.

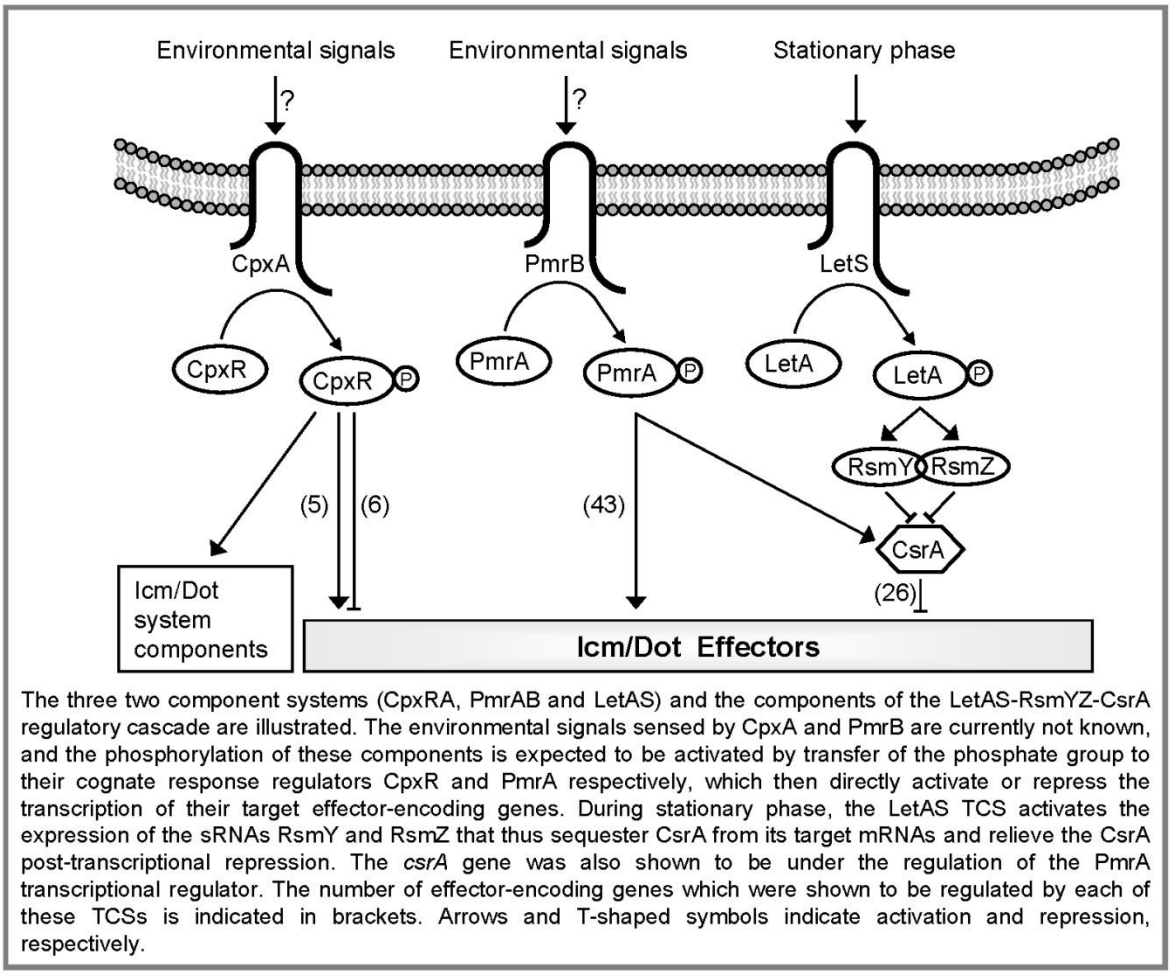
Characterization of effectors regulated by PmrA



PmrA directly regulates the expression of effectors containing the PmrA consensus. **A**. The bacteria examined contained a plasmid with the *pmrA* gene cloned under the P*tac* promoter (activated by IPTG), and they were grown in media containing different concentrations of IPTG (indicated below the bars). **B**. Mobility shift assays were performed with pure His6-PmrA and the DIG-labeled *sidF* showing direct and specific binding of PmrA to the regulatory region of the *sidF* effector-encoding gene.

Mol. Microbiol. (2007) 63:1508-1523

Model of the regulatory systems that control the expression of the *Legionella* Icm/Dot effector-encoding genes



The three two component systems (CpxRA, PmrAB and LetAS) and the components of the LetAS-RsmYZ-CsrA regulatory cascade are illustrated. The environmental signals sensed by CpxA and PmrB are currently not known, and the phosphorylation of these components is expected to be activated by transfer of the phosphate group to their cognate response regulators CpxR and PmrA respectively, which then directly activate or repress the transcription of their target effector-encoding genes. During stationary phase, the LetAS TCS activates the expression of the sRNAs RsmY and RsmZ that thus sequester CsrA from its target mRNAs and relieve the CsrA post-transcriptional repression. The *csrA* gene was also shown to be under the regulation of the PmrA transcriptional regulator. The number of effector-encoding genes which were shown to be regulated by each of these TCSs is indicated in brackets. Arrows and T-shaped symbols indicate activation and repression, respectively.

The PmrA-PmrB story - Mol. Microbiol. (2007) 63:1508-1523
The CpxR-CpxA story - J. Bacteriol. (2008) 190:1985-1996
The LetAS-RsmYZ-CsrA story - J. Bacteriol. (2014) 196:681-692
The FIS story - J. Bacteriol.(2014) 196:4172-4183
The regulation of effectors (Review) - Curr. Top. Microbiol. Immunol. (2013) 376: 35-52

Lab pictures

The Segal lab - 2013

The Segal lab - 2011

The Segal lab - 2007