Regulation of \textit{cpeCDESTR} by Two Sensory Pathways Controlling Complementary Chromatic Adaptation in \textit{Fremyella diplosiphon}.

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The freshwater cyanobacterium \textit{Fremyella diplosiphon} is able to reversibly change between red-light absorbing phycocyanin and green-light absorbing phycoerythrin in response to the ratio of red to green light to maximize photosynthetic efficiency in a process called complementary chromatic adaptation (CCA). At least two separate photosensory systems control CCA; the Rca system, which functions in red light to control red-light gene induction and inhibit green-light induced operons, and the Cgi system, an as yet unknown system controlling green-light induced genes.

Regulation of the \textit{cpeCDESTR} operon is critical during CCA for a number of reasons. Encoded within the operon is CpeR, which is both necessary and sufficient for expression of some green-light induced genes. This suggests a serial mode of gene regulation, where \textit{cpeC} and CpeR are expressed in green light, inducing expression of other green-light genes. Also a promoter element we have named the L-box is present in front of \textit{cpeC} and in the promoters of the red-light induced \textit{pcyA} and \textit{cpc2} genes. Data demonstrates that the L-box within the \textit{cpeC} promoter acts to repress its transcription in red light conditions, and that this repression is mediated by RcaC, a transcription factor within the Rca system which simultaneously induces expression of red light genes. In addition, we have obtained evidence that the Cgi system operates post-transcriptionally, acting upon the 5' leader region of \textit{cpeC}, down-regulating \textit{cpeC} further during red-light growth. These findings support regulation of \textit{cpeC} as a key step in the control of CCA, where two separate sensory systems feed in to control regulation of \textit{cpeC} and those genes controlled by CpeR.
Bid regulates reovirus-induced apoptosis and neurovirulence

Angela Berger and Pranav Danthi

Reovirus infection leads to apoptosis both in cultured cells and in vivo. NF-κB-driven transcription of proapoptotic cellular genes is required to mediate the effector phase of the apoptotic response. Although both extrinsic and intrinsic death-signaling pathways are implicated in reovirus-induced apoptosis, mechanisms by which either of these pathways are activated and their relationship to NF-κB signaling following reovirus infection are unknown. Proapoptotic Bcl-2 family member Bid is activated by proteolytic cleavage following reovirus infection. To understand the regulation of cell-death networks activated by reovirus, we examined proapoptotic signaling following infection of Bid-deficient cells. Although reovirus produced equivalent yields in wild-type and Bid-deficient cells, it failed to induce apoptosis in cells that lack Bid. Furthermore, NF-κB activation was required for Bid cleavage and subsequent proapoptotic signaling. These data suggest a critical role for Bid in reovirus-induced apoptosis and place Bid downstream of NF-κB in the apoptosis program initiated by reovirus. Our ongoing work is aimed at defining how NF-κB activation results in the cleavage-induced activation of Bid. Toward this end, we are comparing gene expression profiles of known cell-death regulators in the presence and absence of functional NF-κB using RT-PCR and reporter gene expression assays. In addition to revealing how effector apoptosis pathways are stimulated following reovirus infection, these studies will provide insight into mechanisms by which NF-κB serves a prodeath function.

1. Initial studies carried out by Pranav Danthi while at Vanderbilt University
Mutations in Sindbis Virus subgenome 5’UTR affect host shut off of translation and viral genome translation.
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Sindbis virus is the type species of the alphavirus genus and is maintained in a vector-host relationship between mosquitoes and warm-blooded vertebrates. Virus replication requires infection of a host cell and Sindbis infection of vertebrate cells results in severe cytopathology, while infection of arthropod cells results in a persistent noncytopathic infection. To investigate the mechanism of translation of Sindbis Virus subgenomic RNA, we made sequential 10 base pair deletions of the subgenomic RNA 5’ untranslated region (UTR). The titer of virus Toto1101-5’SG-UTR-Δ11-20 was 2 logs lower than wild type and showed a mixed plaque phenotype. To determine what affect this mutation had on translation of the subgenomic RNA, cells were infected with WT or 5’SG-UTR-Δ11-20 and radiolabeled for one hour at 4, 8, and 12 hours post infection. Synthesis of capsid protein from the subgenomic RNA was reduced for all time points as compared to WT. While WT infection shut off host translation of actin by 8 hours post infection, shut off of host cell translation was impaired after infection by 5’SG-UTR-Δ11-20 and actin translation was observed at 12 hours post infection. Viral genomic RNA translation also continued as radiolabeled Sindbis nonstructural protein nsP2 was observed at 8 and 12 hours post infection. The hyperphosphorylation of nsP3 in WT infection was not observed after infection by 5’SG-UTR-Δ11-20 at any time point. These data suggest that mutations in the 5’ UTR of Sindbis virus subgenomic RNA can alter the shut off of host cell translation. This also suggests that translation of the viral genome and post translational modification of viral proteins are affected by translation of the subgenomic RNA.
Defining the role of YopK in the Regulation of *Yersinia pestis* Type III Secretion

By Rebecca Dewoody, predoctoral student in MBG

*Yersinia pestis*, the causative agent of plague, is a deadly human pathogen that has resulted in an estimated 200 million deaths over time. As one of its major virulence factors, *Y. pestis* uses a type III secretion system (TTSS) to translocate effector proteins from the bacterial cell into the host cytosol. Termed Yops for *Yersinia* outer proteins, they work inside the host cell to prevent phagocytosis, circumvent host immunity and induce apoptosis. My project focuses on a unique *Yersinia* outer protein, YopK, which is known to regulate the TTSS and thus the amount of effector proteins injected into host cells. A \textit{yopK} deletion mutant has a very interesting virulence phenotype: during \textit{in vitro} experiments a \textit{yopK}$^{-}$ strain shows increase injection of effector yops while during \textit{in vivo} infection the \textit{yopK}$^{-}$ strain is essentially avirulent. Recent work has revealed that YopK is injected into host cells in a TTS dependent manner. My preliminary work suggests that YopK is in fact working within the host cell to regulate secretion, but the mechanism of regulation as well as host interacting factors are at present unknown. Current work to determine YopK localization after transient transfection will determine whether YopK is interacting with the TTSS pore complex in the host cell membrane. Transfection of a YopK expression vector into host cells has also shown increased host necrosis suggesting that YopK is cytotoxic in addition to its role in TTS regulation.
Regulation of *Escherichia coli* DNA Polymerase IV Activity by Polyphosphate Kinase
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Patricia L. Foster (Indiana University Department of Biology, Bloomington, Indiana)

*Escherichia coli* DNA Polymerase IV (Pol IV) is an error-prone Y-family polymerase that can replicate damaged DNA. As Pol IV is present at relatively high copy number even when cells are growing normally, its activity must be tightly regulated. One such regulator is polyphosphate kinase (Ppk). In *ppk* mutants, the activity of Pol IV is greatly reduced even though the amount of Pol IV protein is unaffected. Screening of a plasmid-based genomic library revealed a chromosomal intergenic region that, when overexpressed, suppresses our *ppk* mutant phenotypes. Mutational and molecular biological analysis suggest that this region contains a small RNA, but overexpression of the small RNA only suppresses some of our *ppk* mutant phenotypes. Therefore, other elements in this region must also be important for regulating Pol IV activity. This region is being further characterized in order to elucidate the mechanism by which Ppk regulates Pol IV activity.
Initial characterization of a functional ortholog of copper sensing repressor CsoR in *Streptococcus pneumoniae* D39

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The copper sensing transcriptional repressor CsoR has been characterized in *Bacillus subtilis* and *Mycobacterium tuberculosis*. It plays a critical role in copper homeostasis by regulating the transcription of a copper efflux system encoded by the *cso* operon. A subfamily of functional orthologs of CsoR that we designate CsoR(Ox) is found in a subset of gram-positive pathogens. This subfamily lacks a key Cu(I)-coordinating histidine conserved among Cu-CsoRs, but retains the two conserved cysteine residues that we hypothesize might be involved in as yet unknown redox response. To test this, we have performed a DNA microarray experiment on a *Streptococcus pneumoniae* D39 ∆CsoR(Ox) (SPD_0073) strain. Results indicate increased expression of an operon encoding the enzyme complex that catalyzes the oxidation of pyruvate to acetyl-CoA (SPD_1028-1024). This is consistent with CsoR(Ox) acting as a repressor since deletion of CsoR(Ox) would lead to constitutive expression of the target operon. The promoter region of this operon harbors a potential operator site that is similar to the operator site upstream of the Cu(I) chaperone *copZ* recognized by the bona fide Cu-CsoR in *Bacillus subtilis*. Initial biochemical assays indicate that the recombinant *Spn* CsoR(Ox) binds this DNA sequence in an electrophoretic mobility shift assay, but binding is abolished upon pretreatment with 10 mM hydrogen peroxide. The nature of the CsoR(Ox) oxidation products are being determined by mass spectrometry, as is the specificity of the DNA binding, and the redox potential of the cysteine pair in CsoR(Ox). *Spn* strains encoding a dominant-negative mutant of CsoR(Ox) should lead to a better understanding of the CsoR(Ox) regulon in *Spn*.

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Effects of whole genome duplication in *Paramecium*: a preliminary analysis of the *P. biaurelia* genome sequence

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Whole genome duplication (polyploidization) can have significant and lasting effects on a species and its descendent lineages. New gene duplicates provide substrate for the evolution of novel genes and gene functions, and differential loss of duplicate genes in subpopulations may lead to reproductive isolation and rapid speciation. The published genome of the ciliate *Paramecium tetraurelia* shows evidence for at least three whole genome duplications in the past. In addition, paralogs from the most recent duplication event seem to be highly maintained. Here we provide preliminary data from the genome sequence of a closely related species *P. biaurelia*, which diverged from *P. tetraurelia* after the most recent polyploidization. Further analyses of the *P. biaurelia* genome and comparison with *P. tetraurelia* will shed light on the long-term effects of whole genome duplication, including identification of possible parallel and differential evolution of the two daughter lineages.
Impact of acetyl phosphate, ATP and hydrogen peroxide on pneumococcal metabolism

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In a previous study we showed how the pyruvate oxidase, SpxB, is regulated and its role in virulence and protection against endogenously produced H$_2$O$_2$ in *Streptococcus pneumoniae*. Currently we have constructed a combination of mutants in *spxB* and other important enzymes in central metabolism like phosphotransacetylase (Pta) and acetate kinase (AckA). With these mutants we can look at the effects of absence or accumulation of Ac-P, ATP and H$_2$O$_2$ in pneumococcal metabolism.

In this study we show that AckA is conditionally essential and that suppressor mutations are acquired only if the levels of endogenously produced H$_2$O$_2$ are over 40% (relative to WT). The suppressor mutations map to *cps2E, spxB* and *spxR* causing the loss of capsule and the levels of H$_2$O$_2$ to go below 40% suggesting the need for ATP in protection against endogenous H$_2$O$_2$. In contrast a mutation in *pta* is readily obtainable and the only effect observed is a decrease in colony size which goes away anaerobically suggesting a higher sensitivity to H$_2$O$_2$.

We also show that a triple *spxB acka pta* mutant lacks Ac-P but it is viable and has no significant growth defects. The absence of acetyl phosphate seems to
cause changes in two important TCS involved in virulence, CiaRH and VicRK as observed by microarray analysis. With further studies we hope to show a correlation between ATP pools and sensitivity to H$_2$O$_2$ and study how Ac-P regulates these TCS.
Conservation of disease resistance pathways in Arabidopsis and Soybean
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The major goal of the Innes lab is to better understand the molecular mechanisms that lead to disease resistance in plants. My project focuses on the recognition of the bacterial virulence factor, AvrB, and the resulting disease resistance response mediated by the soybean disease resistance protein, Rpg1-b. Previous work has shown that recognition of AvrB by RPM1 in Arabidopsis requires another protein, RIN4. My goal is to determine if soybean and Arabidopsis use the same molecular mechanisms to recognize and respond to AvrB by attempting to complement mutant Arabidopsis, which lacks the ability to recognize AvrB, with soybean genes. Currently, transgenic lines of Arabidopsis are being generated that have been transformed with either Rpg1-b, a GmRin4, or both. Preliminary data suggests that GmRin4C, but not GmRin4A or GmRin4B, is able to complement for the loss of AtRIN4 in RPM1-mediated defense against AvrB. However, whether Rpg1-b requires a GmRin4 in order to complement for the loss of RPM1 in Arabidopsis is still unclear. Once homozygous lines of transgenic Arabidopsis have been generated, hypersensitive response and bacterial growth assays will be performed to determine if recognition of AvrB can be regained.
SINDBIS NSP4 MUTATIONS AFFECT PROMOTER RECOGNITION AND 
HOST MACROMOLECULAR SYNTHESIS SHUTOFF  
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Sindbis virus RNA synthesis requires four virally encoded nonstructural proteins (nsPs). The nsPs perform a number of functions in addition to RNA synthesis, including modulating host cell macromolecular synthesis. The catalytic domain of the RNA synthetic complex is contained in nsP4, but complete virus replication requires the other nsPs, nonstructural polyprotein processing intermediates, and host factors. The details of RNA synthetic complex formation remain unclear, however it is evident that nsP4 is present in all active forms of the complex. Modeling software predicts that the novel amino terminal domain of nsP4 is disordered. Prior work indicates that this region of nsP4 may be essential for interaction with other components of the RNA synthetic complex, and complete deletion of the amino-terminal 97 amino acids of nsP4 does abolish de novo copying but not terminal adenylyl-transferase (TATase) activity. To further determine this domain’s role in complex formation and RNA synthesis, we undertook a reverse genetic screen of the domain, targeting conserved charged or turn inducing residues. The mutant viruses displayed a variety of plaque morphologies, from minute to wild type. The smaller plaque mutants also displayed delayed growth kinetics. As expected, plaque and growth phenotypes correlated with reduced viral RNA synthetic ability in mammalian cell culture. Additionally, these RNA synthesis defects could be divided into those that reduce total RNA synthesis, and those that reduced the ratio of subgenomic RNA:genomic RNA. The later phenotype was observed mainly in mutations made in residues 70 to 100, implying this region may be involved in promoter recognition or appropriate formation of the subgenomic RNA transcriptase complex. Perhaps most interestingly, several mutants also showed delayed or inhibited host transcription and translation shutdown, a phenotype not previously associated with mutations in nsP4. We are currently investigating the role nsP4 plays, either directly or indirectly, in promoter recognition and host macromolecular shutoff.
The retinal determination gene eyes absent is regulated by the EGFR pathway throughout development.

Summary
Members of the Eyes absent (Eya) protein family play important roles in tissue specification and patterning by serving as both transcriptional activators and protein tyrosine phosphatases. These activities are often carried out in the context of complexes containing members of the Six and/or Dach families of DNA binding proteins. eyes absent, the founding member of the Eya family is expressed dynamically within several embryonic, larval and adult tissues of the fruit fly, Drosophila melanogaster. Loss-of-function mutations are known to result in disruptions of the embryonic head and central nervous system as well as the adult brain and visual system including the compound eyes. In an effort to understand how eya is regulated during development we have carried out a genetic screen that is designed to identify genes that lie genetically upstream of eya and govern its expression. We have identified a large number of putative regulators including members of several signaling pathways. Of particular interest is the identification of both yan/anterior open and pointed, two members of the EGF Receptor signaling cascade. The EGFR pathway is known to regulate the activity of Eya through phosphorylation via MAPK. Our findings suggest that this pathway is also used to influence eya transcriptional levels. Together these mechanisms provide a route for greater precision in regulating a factor that is critical for the formation of a wide range of diverse tissues.
The specific mechanisms underlying the origin of morphological innovation are fundamental to evolutionary biology and yet have proven elusive for 150 years. Because new forms must follow from modifications of developmental programs, if we seek to understand how evolution works to generate novel features, it is a necessary first step to characterize the general and specific modifications that led to their appearance in one lineage but not another. The light-producing organs of fireflies lack even remote homology to ancestral structures and thus represent unambiguously novel traits of relatively recent invention whose developmental underpinnings should provide clues to their origin. It has been suggested that expression of existing gene batteries in novel contexts may be an important means by which new forms arise, and it is thought that pre-existing domains of underlying transcription factor expression might bias potential phenotypic outcomes. Several attributes of the firefly photic organ (often paired, ventrolaterally-positioned, sometimes serially repeated, and exhibiting proximodistal organization) suggest the potential co-option of abdominal appendage-patterning gene networks in their evolution. We have found, through preliminary expression studies, that genes associated with appendage formation in related organisms (i.e., Distal-less, dachshund) are present in the presumptive adult lantern in firefly pupae, and we will soon be characterizing the function of these genes and associated abdominal hox genes via larval and embryonic RNA interference. In addition, we are working toward the first characterization of the photic-organ transcriptome through random EST sequencing of several thousand clones from a normalized, photic organ enriched cDNA library.
Regulation of cellular morphogenesis is a key component of development. Cell shape is important not only to cell function but also to an organism’s overall morphology, particularly in plants. The purpose of this project is to study the organization of cortical microtubule arrays in Arabidopsis thaliana and to assess their influence on cellular morphogenesis. Cortical microtubules are long polymers that form a highly dynamic network just beneath the plant plasma membrane. Microtubules are hypothesized to direct the deposition of cellulose microfibrils in the cell wall, which, in turn, defines plant cell morphology. My research focuses on quantifying array dynamics over time under different developmental conditions and relating the microtubule array organization to cell expansion. This project aims to establish the relative influence of microtubule arrays on morphogenesis during plant growth.
MukB is required for chromosomal condensation in γ-proteobacteria. This protein shares structural homology with the ubiquitous Structural Maintenance of Chromosome (SMC) proteins whose members are required for chromosomal housekeeping functions. These proteins share common domain connectivity with globular N- and C- terminal domains that pack against one another to form an ABC-like ATPase domain. These termini are connected by a long antiparallel coiled coil domain which folds at a centralized dimerization domain. 

Presented here is a structural characterization of the coiled coil domain in which we used site specific cysteine crosslinking between interacting positions on opposing strands of the coiled coil. This analysis has identified several points of inter-strand interaction through the length of this domain. With this information we have developed a refined model where at least five segments of continuous coiled coil are joined by a reoccurring structural motif that may confer flexibility to MukB. Ongoing studies are designed to probe the structure and function of these motifs.
A Tale of Two Horns: Insights into Expression and Function of Patterning Networks in the Evolution and Development of Beetle Horns

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Abstract: The development of novel morphologies and the circumstances under which they arise and diversify are major research foci in evolutionary developmental biology. Horned beetles in the genus *Onthophagus* provide excellent opportunities to address the development of novel morphologies because they exhibit a great diversity of horn structures that lack direct homology to any other insect appendages. Horns develop as epidermal outgrowths from the prothorax and/or head but lack joints, muscle, or nerve tissue. Furthermore, size and location vary dramatically across species and between sexes. My research explores developmental patterning mechanisms responsible for the remarkable diversity in horn expression. Specifically, I am examining how members of two developmental regulatory networks (A: the *Hox* complex and B: the Appendage Patterning network) regulate the development of beetle horns and horn diversity in two different body regions: the prothorax and the head. A: Within the *Hox* complex, I focus on a possible role of *Sex Combs Reduced* (*Scr*) in horn development. *Scr* regulates segment identity in posterior head and prothorax across insect orders. Thus far, comparative gene expression studies and larval RNA interference (RNAi) suggest that while *Scr* maintains a conserved function in traditional body regions, it also plays a significant role in specifying both pupal and adult pronotal horns in a sex- and species-specific manner. B: Using similar approaches, I am also investigating the function of other candidate genes that play important roles in appendage development in other insects, including *decapentaplegic* (*dpp*) (principal member of the TGF-β pathway) and *pangolin* (*pan*) (a transcription factor in the *wingless* (*wg*) pathway). These pathways work cooperatively to not only determine where appendages will develop, but also initiate the patterning of the proximal-distal axis in growing appendages. Preliminary results suggest that both *dpp* and *pan* RNAi not only have direct effects on the development of traditional appendages, but also impact pronotal horn growth during late larval development. Combined, these results suggest extensive co-option of both *Hox* and Appendage Patterning gene networks during the development and evolution of *Onthophagus* beetle horns.
Characterization of a chimeric Alphavirus with a heterologous RNA synthetic complex
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The Alphavirus genus of *Togaviridae* encompasses a diverse group of arthropod-borne RNA viruses that have varied inflammatory manifestations of disease. During a single-cell replication cycle, Alphavirus RNA synthesis complexes must recognize multiple sequence-specific templates to efficiently generate antigenomes, genomes, and subgenomic RNAs. These complexes are composed of nonstructural polyproteins in various states of proteolytic processing, which in coordination with cellular factors specify the template for RNA synthesis. The consistent component in all manifestations of the RNA synthetic complex is cleaved nsP4, which has been identified as the viral RdRp. Replication complex interactions during an infectious cycle remain poorly understood. We have utilized a reverse genetic screen to genetically identify interactions within the replication complex by generating chimeric Alphaviruses with heterologous viral replication complexes. AURA nsP4 sequence was cloned into a Sindbis (SIN) TOTO1101 background, retaining the nsP3-4 cleavage junction (TOTO-AURAnsP4). We report that transfection of TOTO-AURAnsP4 RNA into mammalian BHK-21 cells resulted in observable cytopathology and the generation of plaque-forming virus. The growth of TOTO-AURAnsP4 virus was delayed relative to wild-type SIN, resulting in a small-plaque phenotype. Subsequent passage of virus resulted in reversions to near wild-type plaque morphology. Sequence analysis of revertant viruses showed no changes in the AURA nsP4 sequence, but rather second-site changes in the subgenomic promoter. These changes suggest that the introduction of a heterologous replication complex has reduced promoter-recognition leading to suppressor changes in the promoter sequence. We report on the characterization of chimera TOTO-AURAnsP4 replication relative to wild-type SIN and AURA virus, and the isolation and characterization of revertants and their implication in replication complex interactions.
The VicRK two-component system of \textit{S. pneumoniae} is a member of the essential YycFG family found in low G+C gram positive bacteria. Previously we showed that phosphorylated VicR response regulator (RR) is essential because of its strong positive regulation of the \textit{pcsB} gene, which mediates normal cell division. Little is known about the signals sensed by the VicK histidine kinase (HK). We characterized \textit{ΔvicK} mutants along with point mutations and domain deletions of VicK. \textit{ΔvicK} mutants are viable during aerobic growth, presumably due to cross talk. Contrary to a previous report, we found that \textit{ΔvicK} mutants were inhibited for anaerobic growth in BHI rich medium; therefore, the VicK HK is conditionally essential in pneumococcus. Anaerobic growth of \textit{ΔvicK} mutants was restored by ectopic expression of \textit{vicK} or constitutive expression of \textit{pcsB}. We show that the PAS domain, which sense oxygen in other bacteria, is not required for VicK function anaerobically. We found that the levels of VicK during anaerobic growth are three fold less than that of aerobic culture. This suggests that the levels of the VicRKX operon might also be lower anaerobically, which prevents cross talk that occurs during aerobic culture to phosphorylate VicR. The conditional essentiality of VicK during anaerobic culture is bypassed by two classes of suppressor mutations; high and low growth yield. The genome of a high yield suppressor was sequenced by the Illumina method, and we detected three missense point mutations in the suppressor strain. Most interestingly, we detected a mutation in the PnpR RR.
Characterization of the requirements for resistance to the DNA damaging agent 4-nitroquinoline-1-oxide in *Escherichia coli*

Ashley B. Williams and Patricia L. Foster

*Escherichia coli* has two Y-family DNA polymerases, DNA polymerase IV (Pol IV, DinB), and DNA polymerase V (Pol V, UmuDC). While the *in vivo* functions of Pol V are thought to be well understood, the roles of Pol IV in DNA metabolism are not as clear. Studies of Pol IV function have largely focused on its translesion synthesis (TLS) activity, a damage tolerance mechanism that allows continued cell division when DNA replication forks encounter lesions that block the replicative polymerase. We have characterized the relationships between Pol IV and various DNA repair pathways that confer resistance to the DNA damaging agent 4-nitroquinoline-1-oxide (4-NQO). Reaction of 4-NQO with DNA results base adducts that block the replicative polymerase, but that can be substrates for TLS by Pol IV. The data presented here support the model that Pol IV acts mostly in a pathway requiring nucleotide excision repair, most likely TLS, and that Pol IV-dependent resistance to 4-NQO does not require recombination.
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Title:
Effects of genetic background on pilA-swarming phenotype in M. xanthus

Abstract:
Responses in biological systems are context dependent. Context can be due to species composition, abiotic environment, social interactions, etc. At the genetic level, the effects of individual genes can be dependent on the genetic background in which they are expressed. Here, we test for variable effects of genetic background on the phenotypic expression of a gene of known effect, pilA, in a lab evolved strain of Myxococcus xanthus. Strain E8 evolved from a S-motility deficient ancestor (ΔpilA) after selection for increased surface swarming. E8 evolved a novel swarming mechanism that both increased swarming rate and dramatically altered swarm morphology. At each evolutionary time point, we performed transformations to re-introduce the pilA gene into the genome. Through this approach, we are able to monitor effects of the changing genetic background on both rate and morphology of pilA swarming. Preliminary results show that re-introduction of pilA rescues the ancestral swarming to wildtype levels, but at late time points strongly represses the novel swarming. Ongoing work will finish the transformations to achieve finer resolution of the effect of pilA effect on a changing genetic background. Two potentially interesting results could emerge from this study. A sharp decline in pilA swarming could be associated with specific mutations in E8. Alternatively, there could be a linear decline in swarming with additional mutations. In addition to demonstrating the importance of genetic background on gene effects, this work has potential implications for the evolution of pathways and the role of horizontal gene transfer in adaptive evolution.