Resolving the Molecular Mechanisms by Which DNA Mutations Alter the Function of a Genetic Switch

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ABSTRACT

Animal genomes possesses anywhere from tens of thousands to more than a million mutations that are genetic baggage from mutations that occurred in the past. Each mutation can either improve, reduce, or have no effect on fitness. Moreover, the effects of such mutations can depend on the presence or absence of other mutations, so called epistatic interactions. A goal of evolutionary-developmental biology research is to identify the mutations responsible for the evolution of form and function, and to understand the molecular mechanisms of their effects. This goal remains out of reach, as the effects of mutations and epistatic interactions are difficult to predict without knowing the function of the DNA sequence they reside in. This difficulty is heightened for mutations occurring in cis-regulatory element sequences that act as switches to control gene transcription. We are using a fruit fly model to test hypotheses about the molecular mechanisms by which mutations alter a genetic switch's activity, and whether these function-altering mutations are subjected to the tyranny of epistatic interactions. Specifically, we are investigating the Drosophila melanogaster dimorphic element that is a transcription-regulating switch for the bric-à-brac genes. Three mutations in the dimorphic element were identified that individually alter the level of bric-à-brac transcription. The presence or absence of epistatic interactions will be determined by measuring the activity of dimorphic elements from related species that have been engineered to possess the Drosophila melanogaster mutations. I will also test the hypothesis that these mutations impart their effects by creating or destroying binding sites for transcription factors. The results will provide a needed example where an understanding of molecular mechanisms bridges the gap between a cis-regulatory element's DNA sequence and its functional evolution.

Hypothetical Outcomes for Mutations

DNA Sequence	Name	fitness
GACTTGCAGCTTG CTGAACGTCGAAC	"wild type"	normal
GGCTTGCAGCTTG CCGAACGTCGAAC	mutation 1	increased
GACTTACAGCTTG CTGAATGTCGAAC	mutation 2	no change
GACTTGCAGCTCG CTGAACGTCGAGC	mutation 3	no change
GGCTTACAGCTTG CCGAATGTCGAAC	mutations 1 & 2	increased (no epistasis)
GGCTTGCAGCTCG CCGAACGTCGAGC	mutations 1 & 3	decreased (epistasis)
GGCTTACAGCTCG CCGAATGTCGAGC	mutations 2 & 3	no change

A major goal for genetics is to make a connection between the DNA sequence of individuals and their physical, chemical, and behavioral phenotypes.

Challenges to making the genotype-phenotype connection are the possibility of epistasis, and revealing what the molecular consequence are for mutations.

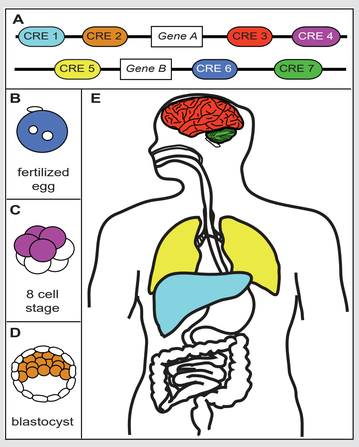
My thesis project is interested in identifying cases of epistasis in a sequence regulating gene expression and revealing the molecular reasons as to why it is occurring.

The Control of Gene Expression by CREs

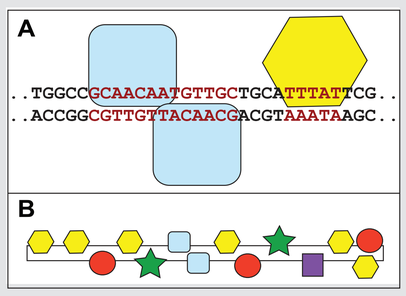
(A) Animal genomes are replete with sequences that contain information about the amino acid sequence for proteins (*Gene A* and *Gene B*) and sequences referred to as *cis*-regulatory elements or CREs that control the expression of genes.

(A) Genes often have more than one CRE and each CRE usually controls the expression of a gene (B-D) during a certain point in development and/or (E) in a particular body region.

The occurrence of epistasis in CREs has received little attention to date and if it occurs, it remains to be understood as to what molecular mechanism might be causing such an outcome.



The DNA Sequence Logic of Gene Expression Regulation



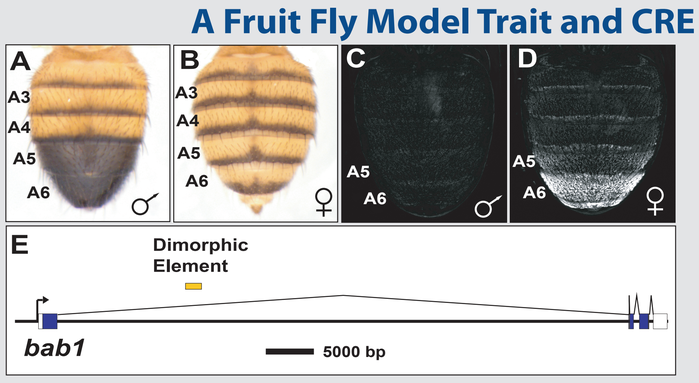
To understand the molecular consequences of CRE mutations, it is important to understand the general principles by which CREs function.

(A) Specific DNA sequences [in red] function as binding sites for transcription factors [blue and yellow shapes]. (B) The specific activity of a CRE results from the combination, or logic, of binding sites for numerous transcription factors.

Reporter Transgene Approach to Study the Gene Regulation Activities of CREs

The activity of CREs can be observed by linking them to a gene such as Green Fluorescent Protein (GFP) and visualizing where, when, and how much GFP is produced.

A Fruit Fly Model Trait and CRE Activity



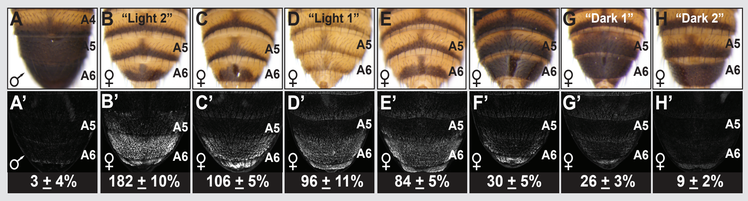
(A & B) Abdomen pigmentation is dimorphic in the species *Drosophila melanogaster*.

(C & D) GFP expression controlled by the CRE known as the Dimorphic Element, which patterns dimorphic Bab1 expression & ultimately pigmentation.

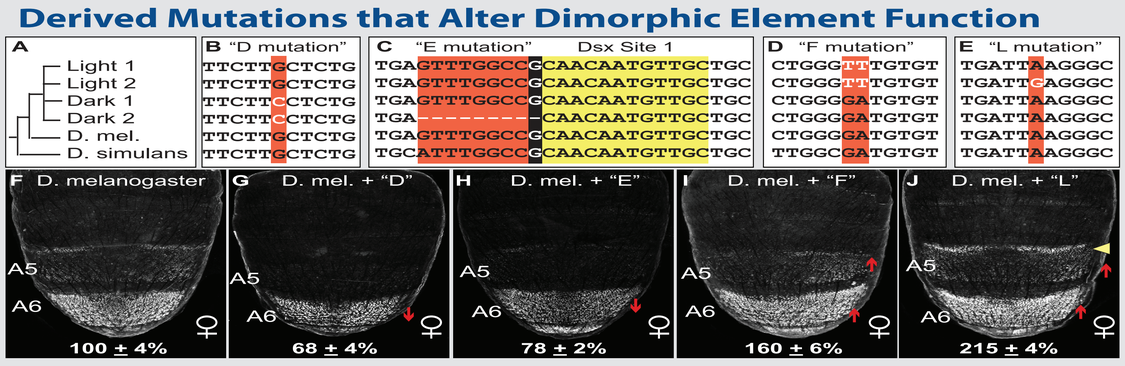
(E) The Dimorphic Element resides in the large first intron of the *bab1* gene.

Evolution in Pigmentation and Dimorphic Element Activity

Genetic differences in the Dimorphic Element have shaped female pigmentation diversity & CRE activity variation within (shown) & between species (not shown).



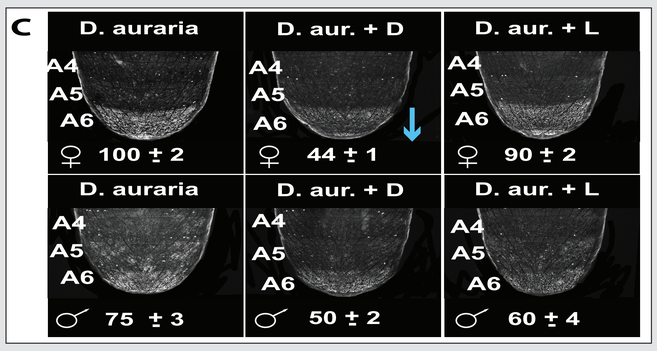
Derived Mutations that Alter Dimorphic Element Function

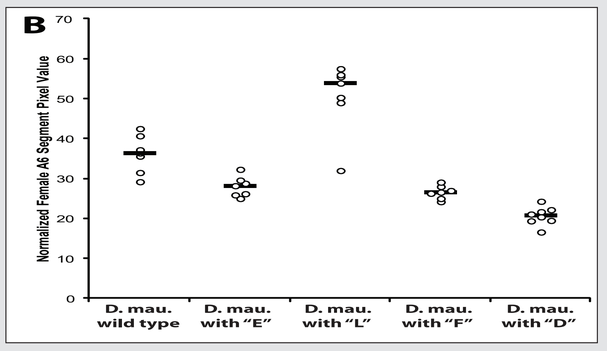


Derived Mutations Exhibit Conserved and Epistatic Effects

(A) Phylogeny of species whose dimorphic element are being evaluated as reporter transgenes in transgenic *D. melanogaster*.

Tests of the Derived mutations within the (B) *D. mauritiana* and

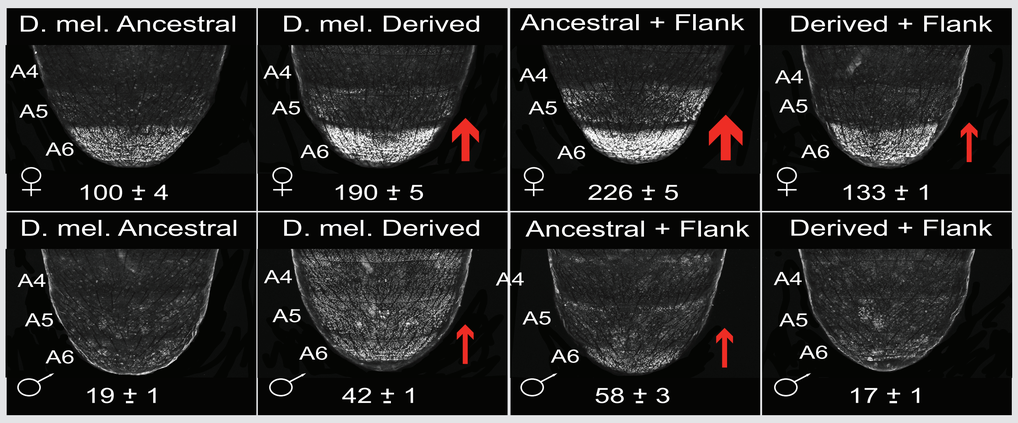




Do the Derived Mutations Alter Transcription Factor Binding Sites?

"L" Mutation Effects		Potential Outcomes & Interpretations	
Ancestral TGATTAAGGGC ACTAATTCCCG	normal CRE activity	Ancestral + flanks mutation GTAGGACTGTA CATCCTGACAT	if activity increases, then L mutation destroyed a repressor binding site
Derived "L" TGATTGAGGGC ACTAACTCCCG	increased CRE activity	Derived + flanks mutation GTAGGGCTGTA CATCCCGACAT	if activity increase lost, then L mutation destroyed an activator binding site

By introducing mutations to the nucleotides flanking the ancestral or derived mutant CRE sequence in a reporter transgene assay, we can infer whether the mutation created or destroyed a binding site.

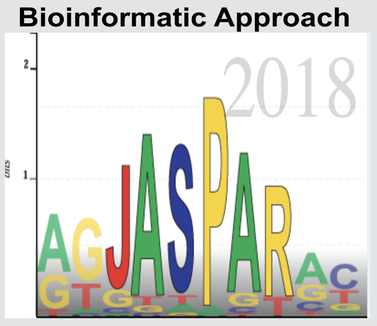


The outcomes for the "L" mutation are generally consistent with a model where the derived mutation weakened or destroyed a binding site for a repressive transcription factor.

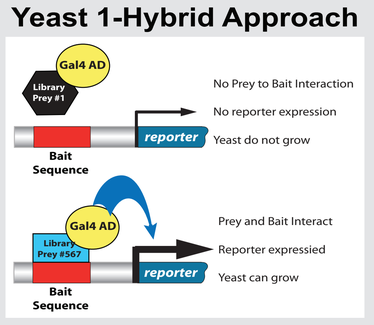
Which Transcription Factor's Binding Site Did the Derived Mutation Alter

The *D. melanogaster* genome has ~750 transcription factors. For cases of suspected transcription factor binding sites we will use a combination bioinformatic binding site predictions, yeast one-hybrid assays, and RNA-interference to implicate a specific transcription factor.

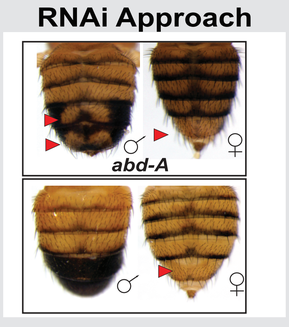
Bioinformatic Approach

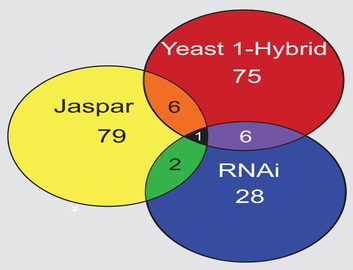


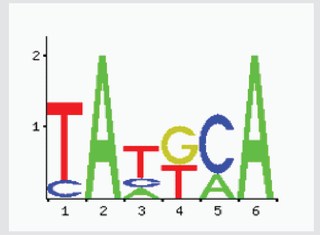
Yeast 1-Hybrid Approach



RNAi Approach







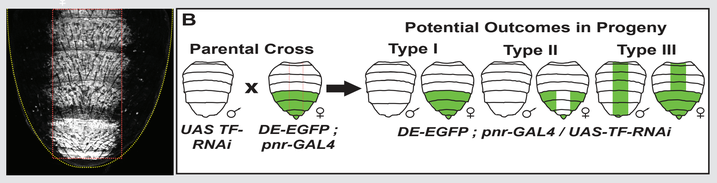
vvl

TTAGTTGATTAAGGGCGTGGC

TTAGTTGATTGAGGGCGTGGC

Vvl is the only factor identified by all 3 approaches.

Future Directions



We will validate factors, such as Vvl, by testing whether reduced expression by RNAi results in altered Dimorphic Element CRE activity.

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