Transgenic Animal Studies on the Evolution of Genetic Regulatory Circuitries
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Summary
The ability to transfer genes from one species to another provides a powerful method to study genetic regulatory differences between species in a homogeneous genetic background. A survey of several transgenic animal experiments indicates that the vast majority of regulatory differences observed between species are due to differences in the cis-acting elements associated with the genes under study. A corollary is that in almost all cases the host species provides the necessary regulatory proteins for expression of the transgenes in specific tissues in which the endogenous homolog is not expressed. Although the details of the cis-acting differences are unknown for most cases, it appears that these differences may consist of the acquisition or loss of unique elements or subtle variation of conserved elements. It is unknown whether much of this variation is directly related to adaptive evolution. The identification of the promoter/enhancer elements responsible for these differences is an important first step in examining the functional significance of this variation.

Introduction
Darwin's theory of evolution arose primarily from his studies of subtle variation within species and among closely related species\(^1,2\). The power of Darwin's approach derives from the examination of a few characters in a relatively homogeneous background, a form of reductionism practiced by most biologist today. During the past decade the development of methods to insert cloned genes back into the germline of several eukaryotic species has provided a new means to analyze genetic regulatory differences between species in a completely homogeneous trans-regulatory environment, far surpassing the degree of experimental control available to Darwin and his contemporaries. Britten and Davidson\(^3\) and others\(^4,6\) have argued that the evolution of gene regulation is the major mechanism of organismal evolution. Consequently, the ability to investigate genetic regulatory differences among species using transgenic organisms is a important technical development in the study of evolution. Previous studies on the mechanistic basis of genetic regulatory differences were largely restricted to the examination of F\(_1\) hybrids between two species\(^7,8\). Transgenic organisms bearing genes from different species provide several advantages over the study of species hybrids: (a) Theoretically, any eukaryotic gene from any species can be examined in a heterologous species no matter how distantly related the two species are; (b) The trans-acting regulatory environment is the product of a single species (i.e. the host), simplifying the interpretation of the resulting gene expression pattern; (c) In vitro mutagenesis can be used to precisely delineate the promoter/enhancer elements responsible for cis-acting differences and test the functional significance of such differences. One disadvantage of the transgenic system has been that transduced genes randomly insert at several places in the genome. Several independent transgenic lines are necessary in order to demonstrate that the observed pattern of expression of the transduced gene is not the result of the unique chromosomal environment of its insertion site\(^9\). Fortunately, methods to insert genes at specific sites in the genomes of Drosophila and mouse are being refined\(^10,11\) which will afford a completely homogeneous cis-regulatory environment for future studies.

Transgenic experiments are typically performed for reasons other than the study of regulatory evolution. Consequently, extensive developmental analyses of the expression of genes transduced into a heterologous species are rarely undertaken. Nonetheless, several cases of regulatory differences have been examined. I will review these examples and will also describe the efforts of my laboratory to combine transgenic experiments along with other molecular and developmental methods to study the evolution of gene regulation in Drosophila.

Human/Mouse \(\alpha_1\)-antitrypsin
The mRNA for the mouse \(\alpha_1\)-antitrypsin elastase inhibitor is abundantly expressed in the liver and yolk sac during fetal development and subsequently in adult liver and kidney\(^13\). Humans share this pattern but also express moderate levels of \(\alpha_1\)-antitrypsin mRNA in the gut, pancreas, and lung of the fetus and in the adult gut. When the human \(\alpha_1\)-antitrypsin was placed in the mouse genome, it was expressed in a broad pattern in fetal and adult tissues characteristic of the human endogenous gene as opposed to mouse endogenous \(\alpha_1\)-antitrypsin gene\(^12,16\). In addition the human \(\alpha_1\)-antitrypsin transgene mRNA was expressed in several other fetal and adult tissues not previously known to express \(\alpha_1\)-antitrypsin in either mice or humans. However, it is important to note that the expression pattern of the mouse and human transgene \(\alpha_1\)-antitrypsin has been examined more thoroughly than the endogenous human \(\alpha_1\)-antitrypsin gene. Consequently, the significance of the expression of the human transgene in these additional tissues (e.g. adult stomach and lung) is unknown. Nonetheless, it is clear that the broader tissue expression of \(\alpha_1\)-antitrypsin in humans compared to mouse is largely due to cis-acting differences between these two species. Koopman and coworkers argue that in addition to a set of common
trans-acting factors which both the human and mouse α₁-antitrypsin genes respond to, either the human gene must respond to a unique set of positive factors or the mouse gene must respond to a unique set of negative factors in order to elaborate the broader pattern observed by the human gene\(^{(16)}\). However, as I will argue in more detail below for another example, cis-acting differences may consist of subtle variation in conserved elements which change the threshold level of response to a conserved set of regulatory proteins. Thus it is not axiomatic that unique regulatory proteins are required to explain the difference in α₁-antitrypsin expression between mouse and human. Most of the additional tissues which express the human α₁-antitrypsin gene are epithelial, suggesting a common cellular lineage.

Does the broader expression pattern of the human α₁-antitrypsin gene represent an adaptation? Several anomalies have been associated with genetic mutations of the α₁-antitrypsin (Pi) gene, leading to the suggestion that these additional sites of α₁-antitrypsin expression are physiologically important\(^{(16)}\). Moreover, a carefully regulated balance of α₁-antitrypsin and elastase is apparently essential\(^{(17)}\) arguing that the pattern of α₁-antitrypsin expression is under strong selection. However, it is presently impossible to know the significance of expression of α₁-antitrypsin in other tissues since they are supplied with circulating α₁-antitrypsin as well. The broader expression of α₁-antitrypsin in humans could, in fact, be maladaptive and mice may be more highly evolved in this respect. A phylogenetic study of the expression of α₁-antitrypsin in mammals could be informative in this regard to determine whether one of the two patterns clearly represents the ancestral type.

**Cow/Mouse Oxytocin Regulation in the Testis**

Another example of cis-acting differences examined in transgenic mice involves the expression of oxytocin\(^{(18)}\). The bovine oxytocin gene is expressed in the seminiferous tubules of the testis but the mouse oxytocin gene is not. However, when placed into the mouse genome, the bovine oxytocin gene is expressed in the mouse seminiferous tubules. Once again, mice contain the necessary trans-acting regulatory environment for the expression of a heterologous gene in a tissue which does not support the expression of their homologous gene.

**Conservation of Vertebrate γ-Crystallin and Globin Gene Expression**

It is important to note that several examples of highly conserved tissue-specific regulation have been demonstrated in vertebrate gene transfer experiments. Many of these examples involve gene transfer between different mammalian species, but a few involve distantly related vertebrates, demonstrating remarkable regulatory conservation over hundreds of millions of years. For example, the mouse and rat γ-crystallin promoters can direct lens-specific expression of a reporter gene in transgenic *Xenopus laevis* tadpoles\(^{(19)}\). An equally impressive demonstration of regulatory conservation was reported by Dillon and coworkers for tissue and stage specific expression of *Xenopus laevis* globin genes in transgenic mice\(^{(20)}\).

**Moth/Drosophila Chorion Genes: Different Proteins but Conserved Regulation**

*Drosophila* has provided the major transgenic system for insects. The relative efficiency of transformation and shorter generation time allows for more detailed analysis of the evolution of gene regulation. I will first describe a case of remarkable conservation of gene regulation and then discuss several examples of regulatory divergence.

*Drosophila* and moth egg-shell chorion genes are apparently not homologous although they serve the same basic function. In addition the organization and structure of the *Drosophila* and moth chorion genes are quite different\(^{(21)}\). Nonetheless, when a moth chorion gene was inserted into the genome of *D. melanogaster*, it was expressed only in females with correct tissue and temporal specificity. A 272 bp promoter fragment from the moth chorion gene fused to a reporter gene was subsequently shown to contain the same specificity\(^{(22)}\). Mutagenesis experiments revealed that a repeated hexanucleotide sequence element was essential for the observed developmental specificity in these experiments. Both *Drosophila* and moth chorion genes contain dispersed copies of this hexanucleotide sequence in their promoter region despite the fact that they are not homologous genes. Apparently, these elements act as binding sites for a positive transcription factor present in both moths and fruit flies. Since these elements are very short and several copies should occur at random throughout any eukaryotic genome, it is unnecessary to suggest that these hexanucleotide elements are homologous between moth and *Drosophila*. Rather, I suggest that their presence and function in the moth and *Drosophila* chorion genes represent a case of molecular convergence.

**Drosophila ADH: Multiple Uses of Fat Body Enhancers**

The expression patterns of the alcohol dehydrogenase gene (Adh) from several different *Drosophila* species have been examined in *D. melanogaster* transgenic flies\(^{(23-26)}\). In general, ADH is expressed at high levels in the larval fat body but displays variable expression in the gut. Malpighian tubules of larvae and/or adults among various *Drosophila* species. The species *D. mulieri*, *D. grimshawi*, *D. affinisjuncta*, and *D. hawatensis* each display at least one tissue-specific pattern difference as compared to *D. melanogaster*. When introduced into the genome of *D. melanogaster*, each of the *Adh* genes from these species exhibit patterns that are virtually identical to their original
donor species. Thus the observed ADH pattern differences appear to be dictated almost entirely by cis-acting differences among the homologous Adh genes. An interesting corollary is that D. melanogaster contains the necessary set of regulatory proteins capable of directing each of the distinct Adh promoters of the donor species. This might imply that several regulatory proteins are capable of trans-activating Adh transcription depending upon the presence of their cognate promoter/enhancer elements. Fisher and Maniatis have shown that Adh gene of D. mulleri contains a set of fat body specific enhancers which can activate a neutral (non-Adh) promoter in the fat body([25]). In addition, these enhancers can stimulate high levels of expression in other tissues when Adh specific promoter elements are present. They argue that since the fat body is the only conserved site of expression among the species in the genus Drosophila, that the species-specific patterns of Adh expression evolved under the constraints of these enhancers.

**Fig. 1.** Aligned DNA sequences of the Gld promoter for D. melanogaster, D. pseudoobscura, and D. virilis. Asterisks denote identity among all three species. The conserved TTAGAAT elements are underlined. The melanogaster TTAGAAT element is present on the (-) strand whereas this element is on the (+) strand in D. pseudoobscura and D. virilis. A direct repeat of AATTTAGACC in melanogaster is underlined (-157 to -138) and denoted TTAGACCA. A palindromic sequence (GFul) is present in all three species at variable positions upstream of a TATA box element and the transcription start site.
Drosophila GLD: A Panoply of Expression Patterns

My laboratory has studied the genetic regulation and evolution of the glucose dehydrogenase gene (Gld) in Drosophila(27-37). In addition to the basic discrimination of cis- and trans-differences between species, we have identified some of the specific cis-elements responsible for regulatory differences between species and have
made a tentative identification of major trans-acting determinants as well. The larval expression pattern of GLD is evolutionary conserved among representative Drosophila species. A conserved palindromic sequence was shown to be responsible for high levels of expression in the anterior spiracular gland cells, a site of abundant GLD expression in the third larval instar. During metamorphosis, GLD protein and mRNA is expressed in the developing rectal papillae of D. melanogaster but not in D. pseudoobscura or D. virilis. We have shown that a sequence element denoted TTAGACCA is capable of directing the expression of a naive promoter-reporter gene to the rectal papillae in transgenic flies. A direct repeat of the TTAGACCA element occurs in the D. melanogaster Gld promoter (Fig. 1) but does not occur in the Gld promoters of D. virilis or D. pseudoobscura. This is perhaps the first identification of a specific promoter element responsible for an organ-specific pattern difference between species.

At the adult stage, GLD displays a surprising array of pattern variation among Drosophila species in the somatically derived reproductive organs (Fig. 2). In total, five male and six female pattern types were found among 50 Drosophila species. The male types include all possible combinations of the presence or absence of GLD expression in the anterior ejaculatory duct and ejaculatory bulb as well as one type displaying expression in the posterior ejaculatory duct. Unlike males which do not display a single conserved feature of their GLD expression, all females exhibit expression in the spermathecae and vaginal plate. The six female types are based upon variation in expression in the oviduct, seminal receptacle, and parovaria. Interestingly, GLD is also expressed in all of these organs during their preadult development (metamorphosis) in all species. For example, GLD is expressed in the ejaculatory bulb and duct during the metamorphic development of the male reproductive tract of D. melanogaster and D. virilis but then at the adult stage is only expressed in the ejaculatory duct of D. melanogaster and only in the ejaculatory bulb of D. virilis. It is important to note that although these organs are morphologically and functionally distinct, they are developmentally closely related to each. They are all derived from the genital imaginal disc and fate mapping experiments indicate close cellular lineages among these organs.

The adult GLD expression pattern of D. pseudoobscura is also quite distinct from D. melanogaster. Specifically, males express an extremely low level of GLD in the ejaculatory bulb (but no GLD is found in the ejaculatory duct). Females express GLD in their seminal receptacle and parovaria. When placed in the genome of D. melanogaster, the D. pseudoobscura Gld gene exhibits a pattern of expression in adult females that is identical to the pattern of the D. pseudoobscura host species in two of the five transformant strains (Table 1). The other three transformants strains lack only the seminal receptacle expression indicating some sensitivity to chromosomal background influences. Since the D. melanogaster Gld gene is not expressed in either the seminal receptacle or parovaria when present at its normal locus or at random sites in the genome, it is clear that cis-element differences occur between the D. pseudoobscura and D. melanogaster Gld genes. For the male transformants, the D. pseudoobscura Gld gene is expressed in the male ejaculatory duct and ejaculatory bulb in five independent transformant lines (Table 1). In three of these lines, the ejaculatory duct expression varies considerably from fly to fly. Thus the D. pseudoobscura gene has a strong potential for ejaculatory duct expression despite not being expressed in this organ at the adult stage of D. pseudoobscura. Like D. melanogaster Gld, the D. pseudoobscura Est-5/6 gene is expressed at high levels in the ejaculatory duct but the homologous gene is not expressed in this organ in D. pseudoobscura. However, unlike what was observed for Gld, when the D. pseudoobscura Est-5/6 transgene was inserted into D. melanogaster, it was not expressed in the ejaculatory duct (but was expressed in the eyes and hemolymph). Although one might assume that Gld and Est-5/6 share common regulatory elements since both are expressed in the ejaculatory duct of D. melanogaster, the promoter sequence elements responsible for Gld's expression in the ejaculatory duct are, in fact, not present in the promoter of Est-5/6.

To date, attempts to achieve the reciprocal transgenic experiment for the Gld gene (i.e. integration the D. melanogaster Gld gene into the genome of D. pseudoobscura) have been unsuccessful. The reciprocal transgenic experiment would be particularly useful for proving trans-differences in ejaculatory duct expression of GLD. However, we have transplanted the genital imaginal disc from D. melanogaster into third instar larvae of D. pseudoobscura. In several cases, the transplanted disc differentiated in parallel with the host reproductive organs, yielding an ejaculatory duct and ejaculatory bulb with normal morphology. The
Table 1. GLD expression patterns in D. melanogaster resulting from the presence of the D. pseudoobscura Gld gene or from partial sexual transformation

<table>
<thead>
<tr>
<th>Species/Strain</th>
<th>Phenotypic sex</th>
<th>Adult GLD pattern</th>
<th>Number of transgenic lines with pattern</th>
</tr>
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<tbody>
<tr>
<td>D. melanogaster</td>
<td>Male</td>
<td>ED</td>
<td></td>
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<tr>
<td></td>
<td>Female</td>
<td>SP, SA, &amp; SRA</td>
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<tr>
<td>D. pseudoobscura</td>
<td>Male</td>
<td>EB</td>
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<tr>
<td></td>
<td>Female</td>
<td>SP, SA, PO, SRA, &amp; SR</td>
<td></td>
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<tr>
<td>DpGld transgene</td>
<td>Male</td>
<td>ED&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>SP, SA, PO, &amp; SRA</td>
<td>3</td>
</tr>
<tr>
<td>tra feminized&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Male</td>
<td>ED &amp; EB</td>
<td>2</td>
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<td></td>
<td>Female</td>
<td>SP, SA, SRA, &amp; OD</td>
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<td>tra masculinized</td>
<td>Male</td>
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<td>Male&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ED &amp; EB</td>
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<td></td>
<td>Female</td>
<td>SP, SA, SRA, &amp; OD</td>
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<sup>a</sup>GLD activities from the DpGld transgene from the D. pseudoobscura Gld gene integrated into the D. melanogaster genome.

<sup>b</sup>These two lines also showed a variable degree of GLD expression in the ejaculatory duct (ED).

<sup>c</sup>tra feminized and tra masculinized indicate low apparent expression of tra in phenotypic males and suboptimal expression of tra in phenotypic females, respectively (D. melanogaster Gld).

<sup>d</sup>Temperature shift experiments were conducted on tra-2<sup>e</sup> temperature sensitive mutant flies immediately after sexual phenotype determination was set during late larval development. GLD patterns of such flies were determined at the adult stage (D. melanogaster Gld).

<sup>e</sup>ED=ejaculatory duct, EB=ejaculatory bulb, SP=spermathecae, SA=Spermovarial atrium, SRA=seminal receptacle atrium, SR=seminal receptacle, OD=oviduct.

donor ejaculatory duct but not the host ejaculatory duct exhibited high GLD activity. This result excluded the possibility that a hormonal difference was responsible for the differences in expression. Taken together, the transgenic and imaginal disc transplantation experiments suggest that the expression of GLD in the ejaculatory duct of D. melanogaster versus the absence of its expression in this organ in D. pseudoobscura at the adult stage is due to a cell-autonomous (i.e. non-hormonal) trans-acting differences between these two species at this stage of development.

For D. melanogaster we have been able to show that proper expression of the sex-determination pathway preceding metamorphic development is required for the specific adult female and male pattern types<sup>(35)</sup>. Genetic manipulations which result in a low level expression of opposite-sex determining factors during this period alters adult GLD determination. Specifically, GLD is expressed in both the ejaculatory duct and bulb in partially feminized adult males and in the spermathecae, vaginal plate, oviduct, seminal receptacle, and parovaria in partially masculinized adult females (Table 1). These findings are germane to several features of Gld regulation discussed above. The pattern displayed by feminized males and masculinized females is virtually identical (a) to the normal preadult pattern of this and other species, (b) to the normal adult pattern of some Drosophila species, and (c) to the pattern exhibited by the D. pseudoobscura gene in the D. melanogaster transgenic adults, with the exception of the female oviduct. These remarkable correlations suggest that although D. melanogaster and D. pseudoobscura exhibit dramatic pattern differences at the adult stage, rather subtle molecular differences may underlie them.

I propose that the interspecific variation in GLD expression in the adult reproductive tract is a consequence of the degree of spatial and temporal restriction imposed by the sex-determination genes and by subtle changes in the promoter elements of the Gld gene. One assumption of this model is that expression during preadult development and at the adult stage involves the same basic cis- and trans-acting factors in all species. Moreover, since these somatically derived reproductive organs share a close developmental history, an additional assumption can be made that the same cis- and trans-acting factors are responsible for expression in all of these organs. Under these two assumptions, regulatory differences among species would have to be achieved through changes in the concentration of regulatory proteins and/or changes in the binding affinity of these proteins either through mutational or posttranslational modification of the regulatory proteins or through nucleotide substitutions in the promoter/enhancer elements. Therefore, the differences in the expression of D. pseudoobscura Gld gene and D. melanogaster Gld in a D. melanogaster regulatory background would be a consequence of subtle alterations of the promoter/enhancer elements (as opposed to unique elements). The D. pseudoobscura gene may be able to respond to a lower threshold level of regulatory proteins in the adult male ejaculatory bulb and adult female seminal receptacle and parovaria. Consistent with this notion is the finding
that the *D. pseudoobscura* Gld gene is expressed several-fold higher in the *D. melanogaster* transformants than it is in its native state in *D. pseudoobscura*. I postulate that the alteration of sex-determination gene expression results in modulating the concentration of regulatory proteins to a level equivalent to that present in preadults, thereby generating a broader pattern of *D. melanogaster* Gld expression similar to that seen for *D. pseudoobscura* Gld. Preliminary results from mutagenesis experiments have identified two sequence elements which collectively can promote expression of a reporter gene to most of the internal somatic reproductive organs. Both elements are present in *D. melanogaster*, *D. pseudoobscura*, and *D. virilis* but minor sequence differences occur in these elements among the three species.

Two major related questions remain unresolved: Did the adult GLD patterns arise through the evolutionary accretion or restriction relative to the pre-adult pattern? Does the adult GLD pattern of variation among *Drosophila* species represent adaptive evolution? Preliminary genetic experiments with *D. melanogaster* indicate that although GLD is not essential for reproduction, it is required for normal female fertility when females are mated to males which have depleted sperm loads. GLD expression in one or both of the sperm storage organs is a conserved feature of all fifty *Drosophila* species and representative species from four other closely related genera whereas adult males of several species do not express GLD in any of their reproductive organs. Therefore, the simplest evolutionary interpretation is that GLD expression first arose in the female spermathecae as an adaptation for sperm storage. Later, the accretion of GLD expression in the other reproductive organs may have arisen as an augmentation to GLD’s function in sperm storage. In this regard it is interesting to note that *D. melanogaster* males transfer a large amount of secreted GLD from the ejaculatory duct to females during copulation (22).

Experiments are underway to determine whether this male contribution of GLD to females functionally augments sperm storage. Consideration of the developmental relationship of the somatic reproductive organs and their apparent universal expression of GLD during pre-adult development suggests an alternative hypothesis. Perhaps first, GLD was broadly expressed in the somatic reproductive organs at the adult stage (similar to the extant preadult pattern) and then became restricted to species-specific subset of these organs as a consequence of direct or indirect selection on GLD function. In *D. melanogaster* it is known that the developmental restriction of GLD expression in these organs requires the proper expression of the sex-determination genes. It is therefore possible that part of the variation in adult GLD patterns are due to differences in the balance between male and female sex-determination factors. Indeed, the degree of sexual dominance may determine the degree of sexual maturity of the adult reproductive organs. Incomplete sexual dominance could lead to a form of molecular neoteny where GLD is expressed in adult structures that previously expressed GLD during their preadult development. Indeed, Raff and Kaufman have noted that a common form of neoteny involves the temporal dissociation of development of somatic traits and the maturation of the gonads(40). (In the present example, the somatic traits are the somatic reproductive organs.)

**Do Differences in Tissue-specific Expression Represent Adaptations?**

Dickinson has proposed that much of the variation in gene regulation observed between closely related species may be an indirect consequence of changes in the regulatory networks(41). Since such networks are thought to be composed of a limited number of regulatory proteins which act through a combinatorial mechanism to regulate gene expression, changes in the expression of even one regulatory protein could influence a large number of downstream genes. Thus, an expression pattern change in one particular gene may not represent an adaptation but rather reflect natural selection on some other component of the combinatorial regulatory circuit. On the other hand, changes in cis-acting promoter/enhancer sequences of a terminal gene in regulatory pathway may only effect the expression of that gene. Although it would seem that examples of cis-acting differences ought to represent simple adaptations, Dickinson and others have argued that they are more likely the consequence of neutral mutations whose fate is dictated by random drift. Biochemists and molecular biologists are somewhat uncomfortable with this hypothesis because it implies that natural selection allows the wasting of energy by expressing genes at times and places where they are not needed. Nonetheless as molecular methods have led to detailed developmental analysis of a myriad of genes, it is becoming more evident that eukaryotic genes are almost always expressed in a broader pattern than expected based upon known genetic and biochemical functions of their gene products.

It is now possible in some cases to address the functional significance of regulatory differences between species via in vitro mutagenesis. For example, we have shown via in vitro mutagenesis that the TTA-GACCA element of the *D. melanogaster* Gld promoter is responsible for the species-specific expression in the preadult rectal papillae. The *D. pseudoobscura* Gld gene, deficient for both this element and rectal papillae GLD expression, can nonetheless rescue *D. melanogaster* Gld null mutants from lethality indicating that the rectal papillae expression is non-essential. It will take considerably more work to determine whether GLD expression in the rectal papillae of *D. melanogaster* represents a minor adaptation. In general, whether variation in gene expression patterns among species is the end-product of natural selection or the raw material for future adaptation will be a difficult issue to resolve.

**Subtle Variations on a Theme**

An important key to understanding gene expression
patterns is knowledge of cellular ontogeny. Different organs may have widely different functions and morphology, but if they arose developmentally from a common primordial tissue they may nonetheless share some elements of their regulatory circuitry as a consequence of their ontogeny. Evolutionary modifications of this circuitry as a result of random mutations may result in dramatic changes in organ-specific morphology, but if they arose developmentally from a promoter/enhancer elements or new regulatory proteins. On the other hand, regulatory changes could also be achieved by more subtle alteration of the DNA sequence of pre-existing promoter/enhancer elements or alterations of the regulatory proteins which affect their binding affinity or transactivation activity. Moreover, as amply demonstrated by the study of lambda phage, relatively small changes in the concentration of regulatory proteins can have dramatic effects on gene expression.

References


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