The technical difficulties of ideally recombining various portions of the chick epithelium with the dental papilla resulted in random associations, which contributed to the variable success in the grafts.

The ability of chick epithelium to participate in odontogenesis and to secrete enamel matrix proteins suggests that during evolution avian toothlessness was not a consequence of a change in the genetic coding in the oral epithelium for specific protein synthesis that persists in Reptilia and Mammalia. Rather, an upset of a developmental sequence or an alteration in the behavior of cranial neural-crest cells must have blocked the initiation of tooth development and subsequent synthesis of enamel matrix proteins. Correction of abnormal heart development by normal endoderm in cardiac mutant salamanders (*8*) gives another example in which an appropriate tissue interaction permits organogenesis to proceed and restores gene function. These data support a suggestion made by Jacob (*9*) in his discussion of the mechanisms of evolution. The notion that the genetic information for the synthesis of a specific product can remain quiescent in the genome and that perturbations of tissue interaction can alter gene expression should not surprise developmental biologists, who appreciate the interactions that result in embryogenesis, form, and evolution. These data provide evidence that phenotypic change in evolution need not involve loss of genetic information.

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Tissue Specificity of Enzyme Expression Regulated by Diffusible Factors: Evidence in *Drosophila* Hybrids

Abstract. *Pairs of hybridizable species of Hawaiian picture-winged* Drosophila *differ qualitatively in the distributions of specific enzymes in their tissues. An examination of the patterns of enzyme expression in the hybrids showed that, in three instances, absence of an enzyme from a specific tissue was dominant to presence. Since other developmental features indicated that both parental genomes were functioning, these results suggest that, in these cases, the pattern differences in the parental species were due to diffusible factors that affected expression of the relevant structural genes rather than to differences in the genes themselves or in* cis*-acting regulatory sites.*

The appearance of defined proteins in specific tissues at specific stages is a central feature of eukaryotic development. Useful information on the mechanisms that generate these patterns of gene expression can be obtained by identifying genetic variants that alter them. Such variants can be used to identify components of regulatory systems and to probe their function. "Regulatory" includes any process contributing to the observed tissue and the stage-specific appearance of the functional gene product. Most of the known genetic elements that regulate the development of specific proteins in eukaryotes are closely linked to the structural gene they affect and are either *cis*-acting or show additive inheritance consistent with that mode of action (*1*). A few unlinked or easily separable regulatory loci have been recognized. Some, surprisingly, also show additive inheritance (*1, 2*). It was recently shown that *cis*-acting regulatory sites can be recognized by examining the pattern of expression of distinguishable structural alleles that code for a given enzyme in hybrids produced between two species in which normal patterns of expression of that enzyme are markedly different (*3*). Here I report three examples in which absence of an enzyme from a specific tissue was dominant to presence, suggesting control by diffusible (*trans*-acting) favors. Additional examples of *cis*-acting control are also noted.

I used three species of Hawaiian picture-winged *Drosophila* (*4*) in this study. The stocks and hybrids of *D. grimshawi* and *D. orthofascia* were those reported in (*3*). A single stock of *D. formella* (M87G1) was used in crosses to the S30G10 stock of *D. grimshawi*. Matings were done with 8 to 12 females and 4 to 5 males in a 2.8 by 8 cm vial. All cultures were maintained by standard methods (*5*). The tissue distributions of octanol dehydrogenase (ODH) and aldehyde oxidase (AO) were determined by electrophoresis with extracts of dissected tissues in agarose gels (*3, 6*). Quantitative measurements were carried out with a Quick Scan integrating densitometer. Gels were washed (~ 2 hours) several times in a mixture of water, ethanol, and acetic acid (5:5:1) and were air-dried at room temperature on a glass plate. Calibration with a dilution series of each enzyme subjected to electrophoresis and stained in the standard way confirmed that staining intensity is linear with enzyme concentration under these conditions and over the relevant range (linear $r > .99$ for both enzymes).

There are some striking interspecific differences in the distributions of an AO (AO-1) and an ODH (ODH-2). In actively feeding third-instar larvae of *grimshawi*, AO-1 is barely detectable in the carcass (muscle and hypodermis) but is present at moderately high levels in the fat body. In contrast, *formella* larvae have very high levels of this enzyme in the carcass and almost none in the fat body. A further difference is seen in salivary glands of late third-instar larvae, with strong AO-1 activity present in this tissue in *formella* and only a trace in *grimshawi*.

Two forms of ODH are found at unusually high levels in several tissues of *orthofascia*. I will be concerned with the ODH-2 band that is readily detected in the Malpighian tubules and fat body of both second- and third-instar larvae of this species. *Drosophila grimshawi* larvae have no ODH in the Malpighian tubules and only ODH-1 in the fat body. These pattern differences are shown in Figs. 1 and 2 along with the hybrid patterns for comparison. Several stocks of *grimshawi* and *orthofascia* have been examined (*3*), and the characteristic pattern features are consistently present. Only one stock of *formella* was available, but the AO-1 expression characteristics in this stock are also present in a stock of the related species, *D. silvarentis*. With the exception of ODH-2 in the fat body (where nonspecific staining obscures the results), all of these pattern differences were qualitatively confirmed with intact tissues fixed in glutaraldehyde and

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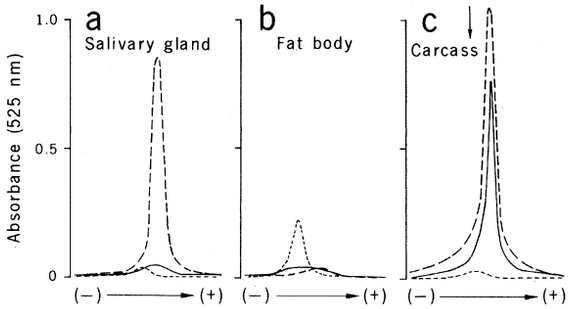

Fig. 1. Aldehyde oxidase in tissues of *D. grimshawi*, *D. formella*, and F_1 hybrids. Densitometer scans of AO-1 activity from the same tissue and stage of each parent and the F_1 hybrids are superimposed in each panel. The tissues are (a) late third-instar salivary gland, (b) feeding third-instar fat body, and (c) carcass. Tracings for *grimshawi* are in short dashes; for *formella*, long dashes; and for the hybrids, solid lines. The three tracings in (a) are from one gel and the six in (b) and (c) are from another. They were chosen from among six replicates from different individuals on the basis of having areas under the peaks in as close agreement as possible with the means of the six replicates. The arrow in (c) marks the position of a *grimshawi* AO-1 marker run on the same gel.

stained for enzyme activity (*7*). Thus the species-specific patterns are not due to differences in solubility of the enzyme or its stability in extracts.

In each of the above cases, when an enzyme is missing or barely detectable in a tissue of one species and is present in the same tissue in another species, the same enzyme is readily detectable in other tissues of both species. For example, AO-1 is strongly present in the adult head and ovary of both *grimshawi* and *formella*, and ODH-2 is seen in the larval and adult midgut and the adult head of both *grimshawi* and *orthofascia*. This indicates that the differences lie in pattern of expression rather than presence or absence of a structural allele capable of producing an active enzyme. In this interpretation, it is important that the enzyme seen in various tissues of a particular species is coded by the same structural gene and that the structural genes of the species being compared are homologous (*3*). The evidence strongly favors this interpretation in the case of AO-1 (*8*). The evidence is less complete for ODH (*9*). I favor the conclusion that ODH-2 is coded by a separate structural gene, but it remains possible that both forms of ODH are products of the same gene with some secondary modification occurring in a tissue-specific pattern.

Figure 1 shows the expression of AO-1 in the tissues of interest in *grimshawi* × *formella* hybrids compared to the parental patterns. In both the salivary glands and fat body, low activity of the enzyme is dominant to high activity. These two features are characteristic of opposite parental stocks, indicating that there is no general inactivation of one genome or the other. This is further confirmed by the fact that AO-1 is seen in the carcass, as in *formella*, whereas alcohol dehydrogenase (not shown) is present in the carcass and midgut, as in *grimshawi* but not *formella*. The AO-1 in the carcass is an electrophoretic form found in the *formella* stock used in the cross and not in the *grimshawi* stock, demonstrating that presence of AO-1 in that tissue in *formella* is due to a *cis*-acting gene. Thus, of the three aspects of AO-1 expression in this pair of species, two are determined by *trans*-acting factors (each parental pattern dominating in one case) and one is under *cis*-acting control.

Figure 2 shows comparisons between the pattern of ODH-2 expression in *grimshawi* × *orthofascia* hybrids and in parental stocks. In the fat body, absence is dominant to presence, and it is clear that both parental genomes are functioning (*3*). In Malpighian tubules, presence is dominant but the small amount suggests additive inheritance consistent with *cis*-active regulation. However, the products of the ODH alleles in the parental stocks are not electrophoretically distinguishable, so definite evidence on this point is lacking.

Apparently, some aspects of the program of expression of the structural gene studied here are controlled by factors capable of acting on both alleles in heterozygotes. Alternative explanations that account for the observed dominance of low activity in terms of phenomena not really related to how activity is normally regulated in vivo must be considered. An inhibitor in the specific tissue that lacks an enzyme in one species could, if produced in the hybrid, explain the dominance patterns. However, mixing experiments in vitro provided no evidence for such factors. Furthermore, the agreement between results obtained by electrophoresis of extracts and histochemical staining in intact tissues strongly suggests that the pattern differences are present in vivo—not generated in the extracts. Any mechanism that inactivates an enzyme in vivo in a particular pattern is, in the broad sense of the term, regulatory. Distribution of ODH-2 could reflect tissue-specific differences in the processing of the product of a single ODH structural gene. Again, if the two forms of ODH are functionally different, such processing should be considered regulatory.

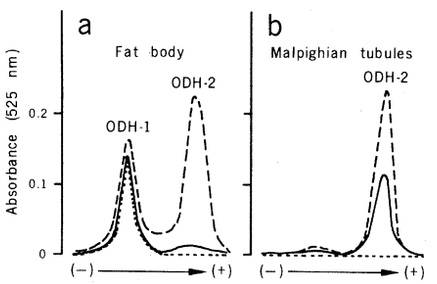

Fig. 2. Octanol dehydrogenase in tissues of *D. grimshawi*, *D. orthofascia*, and F_1 hybrids. (a) Densitometer scans of ODH in the fat body; (b) the same in Malpighian tubules (both from second-instar larvae). Tracings for *grimshawi* are in short dashes; for *orthofascia*, in long dashes; and for the hybrids in solid lines. All tracings are from a single gel and were chosen, from among four replicates, on the same basis as in Fig. 1.

Observations in F_1 hybrids allow one to make the important distinction between factors that influence the expression of a gene to which they are linked in the *cis* position and factors that influence the expression of genes to which they are not so linked. In the latter case, no inference can be drawn as to the location of the genes whose products exert that influence. Whether such genes are linked or unlinked to the structural genes they influence has little bearing on the plausible mechanisms of action. In either case, one must postulate a product capable of acting at a distance to silence gene expression at some level (*10*).

Nevertheless, the results of backcrosses are of interest. Information concerning the number of genes involved in producing a pattern of expression may be important in analyzing the mechanisms, and their location may be of evolutionary, if not functional, significance. The poor viability and fertility of *Drosophila* hybrids and the lack of established genetic markers in picture-winged species present problems in such an analysis. Preliminary results were obtained from a backcross of *grimshawi* × *formella* hybrid females to *formella* males. With respect to the most dramatic of the pattern differences, expression of AO-1 in the salivary gland, individuals showing full expression of both parental phenotypes were obtained. Total activity of AO-1 in salivary glands showed a clearly bimodal distribution, but with 17 individuals in the low range characteristic of *grimshawi* and only 5 in the high range characteristic of *formella*. Whether this approximately 3:1 segregation implies control by two loci, both of which

must be homozygous for the "*formella*" allele to allow high expression, or whether other factors, such as meiotic problems in the hybrid mothers or differential survival, are important cannot be decided at present. Unfortunately, none of the successful backcrosses thus far have been sufficiently marked with AO-1 electrophoretic variants to allow segregation of the regulatory pattern relative to structural alleles to be tested. (The electrophoretic difference noted in Fig. 1c is not fixed.)

Analysis of the mechanism of action of diffusible factors that influence tissue-specific enzyme expression will require biochemical methods. Of interest initially will be experiments to determine whether the posttranslational steps potentially included in the broad sense of gene regulation are important in these cases or whether the patterns reflect different rates of enzyme synthesis. The dramatic example of *trans*-acting regulation reported here should be amenable to biochemical analysis. Expression of AO-1 in salivary glands is a particularly attractive model for analysis of such regulation, since the presence of excellent giant chromosomes would permit joint use of cytological and biochemical methods.

The expression of AO-1 in *grimshawi* × *formella* hybrids provides evidence for a system involving both *cis*- and *trans*-acting factors in controlling various aspects of the program of a single enzyme. The results with ODH-2 in *grimshawi* × *orthofascia* hybrids are suggestive of the same thing. Similar conclusions have been reached with systems involving intraspecific variants in mice and *D. melanogaster* (*1*, *2*). Results that are understandable in terms of *trans*-acting regulators have also been obtained in studies of enzyme expression in interspecific hybrids in another group of *Drosophila* and in teleosts (*11*). Studies of enzyme expression in species hybrids may be useful in seeking models of developmental gene regulation and should yield important information about the evolution of new regulatory patterns.

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8. Cytological evidence (*4*) suggests that all members of the picture-winged group carry the same basic set of genes. All of the 26 species I examined have three nonallelic structural genes coding for proteins with aldehyde oxidase activity. The enzyme AO-1 is consistently distinguishable from the others by its electrophoretic mobility (it is always the slowest), substrate range (the other two appear to correspond to xanthine dehydrogenase and pyridoxal oxidase of *D. melanogaster*), and regulatory patterns present in all species (it always predominates in the adult head and is the only one in the ovary). Electrophoretic variants have been found in various species including *grimshawi* and *formella*. Mobility of AO-1 is shifted independently of the other two isozymes, whereas the AO-1 band in all tissues in which it is found is always affected coordinately.
9. All species have an ODH-1 that is homologous by criteria virtually identical to those applied to AO-1 here and to alcohol dehydrogenase (*3*). However, only 17 of 26 species examined have a distinguishable, faster-migrating band designated ODH-2. These two bands continue to be distinct when run in gels containing nicotinamide adenine dinucleotide, unlike the multiple forms of alcohol dehydrogenase also found in these flies (*3*). Electrophoretic variants of ODH-1 have been found in several species, and in no case is the mobility of ODH-2 coordinately affected. In one species (*D. affinidisjuncta*), one of two allelic forms of ODH-1 comigrates with ODH-2, obscuring the latter. This may be the situation in the species in which ODH-2 has not been found (and in all of which only a single form of ODH-1 has been seen).
10. A possible exception is suggested by cases in *D. melanogaster* in which the ability of homologs to synapse in tissues containing giant chromosomes influences the activity of specific genes [G. Korge, *Chromosoma* **62**, 155 (1977)]. Since homologs in the *grimshawi* × *formella* hybrids do not synapse well (H. L. Carson, unpublished data), this effect may be relevant. However, the best-documented case works in the direction opposite to that observed in the present experiments (that is, a normally inactive gene is activated when tightly paired with a normally active homolog), and it is difficult to imagine a negative influence of one unsynapsed chromosome on its homolog that does not involve a diffusible product.
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12. I thank H. L. Carson, in whose lab much of this work was done, and M. Rechsteiner and J. Hall, who made useful suggestions on alternative interpretations of the results. Supported by NIH grant 2 RO1 HD 10723 and NSF grant DEB 78-10679.

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Chronic Arthritis in Goats Caused by a Retrovirus

Abstract. *A virus was isolated from an adult goat with chronic arthritis and shown to belong to the retrovirus group by electron microscopy and biochemical methods. Inoculation of the virus into cesarean-derived specific-pathogen-free goats' kids produced arthritic lesions similar to those in the spontaneous disease. Virus was reisolated from the experimentally induced lesions.*

The concept that viruses can initiate chronic degenerative disease has been supported by studies of a variety of spontaneous retroviral diseases in animals (*1*-*4*). A report of RNA-dependent DNA polymerase in a particulate fraction of brains from patients with amyotrophic lateral sclerosis (*5*) raises further interest in this class of viruses as a cause of progressive degenerative disease in man. The present report describes a chronic disease in goats (caprine arthritis-encephalitis syndrome) characterized by progressive arthritis as well as demyelinating encephalomyelitis. The syndrome is caused by a retrovirus that we have designated caprine arthritis-encephalitis virus (CAEV).

We previously described the pathology of leukoencephalomyelitis in a herd of goats and its transmission with 220-nm filtrates of tissue suspensions (*6*, *7*). Clinical disease of the central nervous system in this herd occurred predominantly

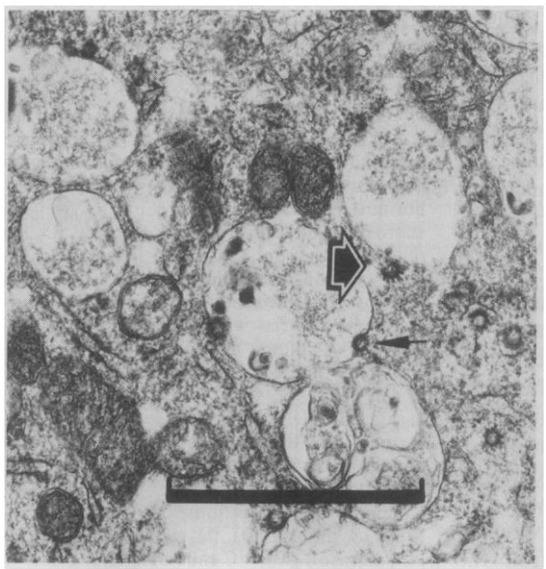

Fig. 1. Electron microscopy of CAEV-infected synovial membrane cells. Fetal caprine synovial membrane cells in plastic flasks at the eighth passage were infected with the 75-63 isolate of CAEV. At 8 days after infection the cells were removed by brief treatment with trypsin, fixed in glutaraldehyde, and centrifuged into a dilute (0.2 percent) agar suspension. The block was postfixed in osmium tetroxide, stained with 1 percent uranyl acetate, and sectioned on a Porter-Blum ultramicrotome. Sections were examined in a Phillips EM-200 electron microscope. The photograph shows particles in the cell cytoplasm and budding into intracellular spaces (small arrow). Membrane projections are visible on some of the particles (large arrow). Scale bar, 1 μm.

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