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9. Purified SOD-1 was used as assay standard. The SOD-1 was isolated from lysates of RBC's as described by McCord and Fridovich (*15*), except that chromatography (Sephadex G75) was included as the final step. The purified enzyme was proved homogeneous by polyacrylamide gel electrophoresis, had 2.0 moles of copper and 2.0 moles of zinc per mole of protein, and reacted fully with antibody after labeling with Bolton-Hunter reagent. The amount of enzyme was determined with an extinction coefficient of $E_{266}^{1\ mg/ml} = 0.558$ as in M. Stansell and H. Deutsch [*J. Biol. Chem.* **41**, 2509 (1966)].
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25 October 1979

Tooth Induction in Chick Epithelium: Expression of Quiescent Genes for Enamel Synthesis

Abstract. *Intraocular grafts of chick epithelium combined with mouse molar mesenchyme produced a variety of dental structures including perfectly formed crowns with differentiated ameloblasts depositing enamel matrix. The results suggest that the loss of teeth in Aves did not result from a loss of genetic coding for enamel synthesis in the oral epithelium but from an alteration in the tissue interactions requisite for odontogenesis.*

Embryonic induction has been shown experimentally to operate normally between tissues of different phylogenetic origins. Both neural induction (*1*) and ectodermal-mesenchymal interactions (*2*) have been examined in xenoplastic tissue combinations. Thus we may conclude that inductive signals are general and can be interpreted by interacting tissues despite genetic disparities. Further, once the inductive influence has been exerted, the responding tissue differentiates as expected from its genetic repertoire. Thus undifferentiated corneal epithelium of the chick responds to mouse dermis from embryonic back skin by producing feathers (*3*).

In contrast, in chorioallantoic membrane grafts of chick epithelium recombined with mouse molar dental papilla (*4*), the chick epithelium responded in an unanticipated manner (*5*). After 1 week's growth the chick epithelium showed an enamel organ-like arrangement and the dental papilla had begun to secrete dentin. Enamel matrix protein was not deposited, however, and epithelial cells adjacent to the dentin matrix were undifferentiated. One week was too short a time to permit development of a fully formed tooth. Nonetheless, the epithelium had assumed a mammalian character, as evidenced by its invasion into the mesenchyme. In addition, dentin secretion by differentiated mouse odontoblasts indicated that the epithelium was acting as an enamel organ; the initiation of dentin synthesis requires permissive tissue interaction with an enamel organ (*6*). Thus it was hypothesized that if a longer culture period were provided, complete odontogenesis might be achieved. The results reported here show that chick epithelium can secrete enamel matrix proteins and suggest that the genetic information for this synthesis was not lost during evolution.

We separated and then recombined the epithelium of the first and second pharyngeal arches of 5-day-old chick embryos with the mesenchyme from first mandibular molars of 16- to 18-day-old CD-1 mouse embryos. The isolations, separations, recombination, and grafting procedures have been described in detail elsewhere (*5*). The tissues were dissected in Hanks balanced salt solution containing 10 percent fetal calf serum and were separated into epithelial and mesenchymal components after digestion with 1 percent trypsin (Bacto-Difco) in the salt solution (1:250) for 2 hours at 4°C. Recombinations were incubated overnight on agar-solidified medium in a humidified atmosphere (37°C) to ensure that the recombined tissues would adhere to each other during grafting into the anterior chambers of the eyes of adult nude athymic mice. After 1, 2, or 4 weeks the grafts were harvested, decalcified, histologically processed, and stained with hematoxylin and Biebrich Scarlet Red. Fifty-five chimeric recombinants were examined.

A range of tissue responses was seen. The chick epithelium made some unusual invasions into the mouse mesenchyme, suggesting an attempt at enamel organ histogenesis (Fig. 1A). [These epithelial patterns resembled our earlier finding (*4*), and variations appeared in young and old grafts.] We found ten aberrant structures consisting of dentin and odontoblasts in molar-like configurations. The odontoblasts of these structures appeared normal, and dentinal tubules were visible (Fig. 1B). Dentinal tubules and well-defined odontoblastic layers clearly distinguished these structures from the ubiquitous spongy bone that was distributed randomly in these grafts. Spongy bone is the only hard tissue found in control grafts of dental mesenchyme alone. In one case, the aberrant dentinal structure was clearly developing in close association with keratinizing epithelium (Fig. 1C). Enamel matrix was not seen next to the dentin of these structures (the normal relationship).

Complete teeth developed in four grafts. This occurred when the chick epithelium also developed well, consisting of keratinizing epithelium and the mucus-secreting, ciliated epithelium (Fig. 1, D and E) characteristic of avian esophageal glands (*7*). In one case (Fig. 1, F and G), the tooth could be seen with transmitted light in the graft at the time of harvesting, permitting serial longitudinal sectioning. The tooth epithelium was contiguous with the epithelium of the keratinizing cyst and of the glands. The entire tooth structure was well formed, with root development in proper relation to the crown, but the latter did not have the typical first-molar morphology, since it lacked the cusp pattern usually present in intraocular grafts of first-molar rudiments. In other cases there were similar alterations of the enamel matrix deposition and crown morphology.

Several criteria were used to confirm the avian source of the epithelium tissue. First, control grafts of isolated mouse dental papilla were examined for possible contaminating remnants of mouse epithelium. These control grafts never

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displayed epithelial fragments and produced only spongy bone. In the experimental grafts, the epithelium was identified on the basis of the staining properties with hematoxylin as well as from nuclear characteristics. In addition, keratinizing chick epithelium is virtually devoid of keratohyalin granules compared to keratinizing mouse epithelium, which diagnostically has a well-defined granular layer. Further, the development of aberrant dentin configurations has never been observed in recombinations of mouse tissues. Even when the mouse epithelium is reduced to a small fragment, it will repopulate a graft after 1 month and form recognizable mouse molars. The aberrant structures found in association with keratinizing chick epithelium suggest that the ability of chick epithelium to participate in tooth formation is modified. Apparently, in these cases, chick epithelium supports the early stages of odontogenesis but does not continue its own differentiation by secreting enamel matrix protein in response to the dentin formed by the mouse papilla. On the other hand, complete expression of odontogenesis is possible when the chick epithelium grows optimally. In the best specimens, the presence of ciliated glandular epithelium [identical to chick esophageal glands epithelium (*7*)] in association with large keratinizing epithelial cysts devoid of keratohyalin granules provides strong evidence that the epithelium is indeed avian in origin. Since both the ectodermal and endodermal epithelia of the first and second pharyngeal arches were used in these grafts, the presence of ciliated glandular epithelium could be expected.

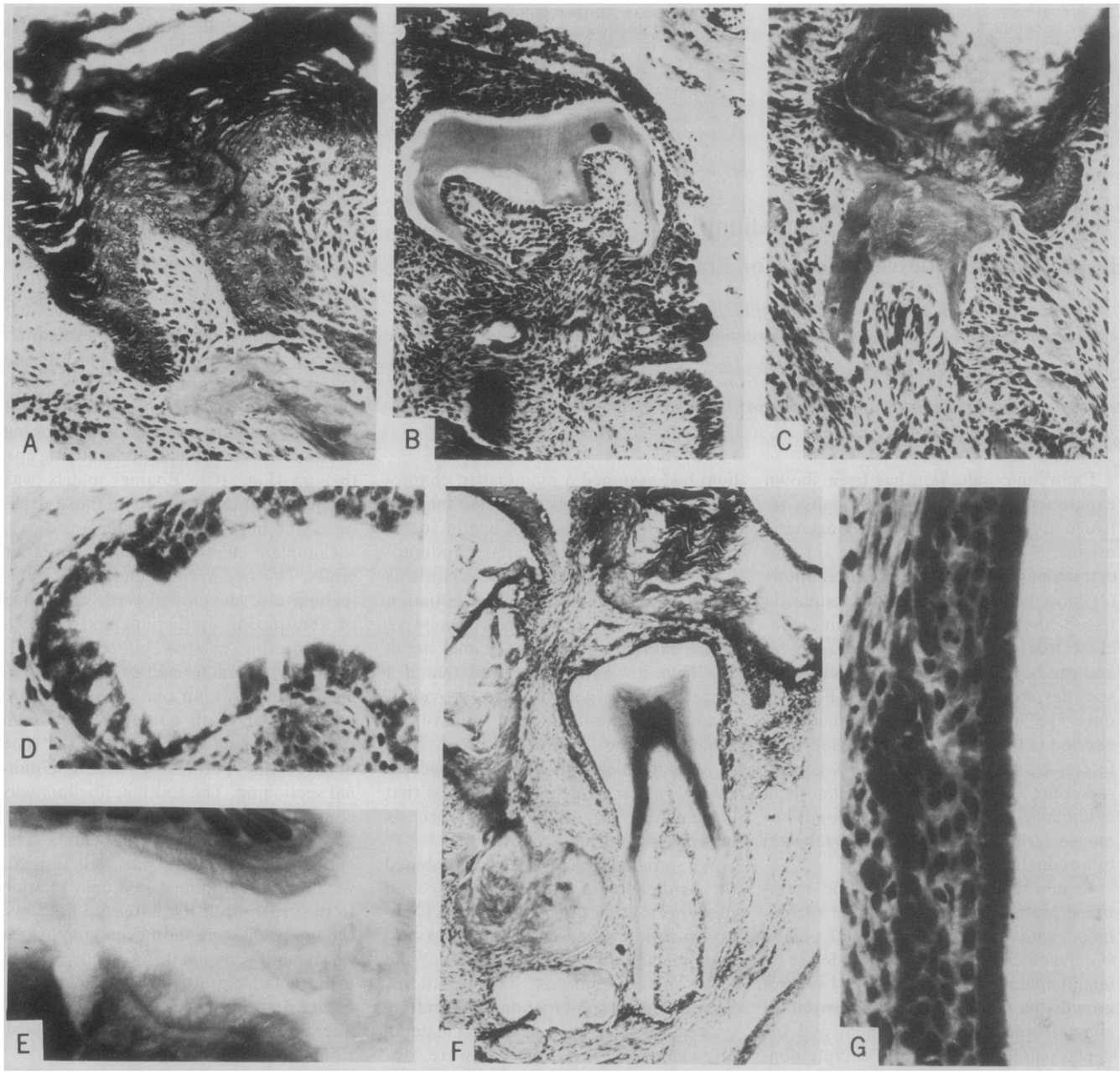

Fig. 1. (A) Chick epithelium assuming a primitive enamel organ-like configuration and incorporating mouse mesenchyme (×240). (B) Dentin in a molar shape. Odontoblasts and dentinal tubules are present. Enamel matrix is not visible (×150). (C) Molariform deposition of dentin in close association with keratinizing chick epithelium (×240). (D) Portion of a mucus-secreting, ciliated gland developing in the chick epithelium (×385). (E) Detail of the ciliated epithelium in the chick glandular epithelium (×1540). (F) Well-developed tooth arising from keratinizing and glandular epithelium. This combination consisted of molar mesenchyme isolated from a 16-day embryonic mouse and the pharyngeal arch epithelium from a 5-day chick embryo (×45). (G) Detail of the enamel organ epithelium from the tooth illustrated in (F). Reduced ameloblasts line the enamel space left after decalcification. Remnants of the enamel matrix protein were present in the enamel space (×615).

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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10. Supported in part by a grant from the University of Connecticut Research Foundation. We thank M. Bruckner for his technical assistance and E. Rallis for typing the manuscript.

30 November 1979

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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