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Contributions of heterospecific tissue recombinations to odontogenesis

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ABSTRACT Determining the functions of cell surface and substrate adhesion molecules during heterospecific tissue recombinations constitutes a significant problem in biology. The developing tooth organ provides a fine model to pursue this problem, especially in the context of instructive epithelial-mesenchymal interactions. The interpretation of many experimental heterospecific tissue combinations is difficult because of the complexity of the embryonic systems. According to some authors, the expressed phenotype corresponds to the genotype of the epithelium; on the contrary, other studies have demonstrated the leading role of the mesenchyme. The importance of cranial neural crest in tooth morphogenesis has been established. Lizard teeth maintain a continuous morphogenetic field throughout life (polyphyodont). These tissues can be considered as biological models and are ideally suited to study neural crest cell differentiation. Oral cavity of chick embryos show a rudiment resembling the dental lamina of amphibian, reptilian and mammalian embryos, though further odontogenic interactions between the epithelial and mesenchymal tissues are not operating. Some authors have suggested that genes involved in tooth formation, which have remained silent in birds for more than 200 million years, can be activated by appropriate signals. Chick epithelium combined with mesenchyme from mouse molar tooth produced dental structures with differentiated ameloblasts depositing enamel matrix. Quail neural crest combined with lizard dental bud showed quail cells with odontoblastic processes. Combinants (quail ectoderm-lizard papilla, and quail ectoderm-rabbit embryo papilla) showed differentiated chimeric-tooth-like structures. However, controversy persists regarding the ability of avian epithelium to express the ameloblast phenotype and to secrete enamel protein. Peptides extracted from dental papilla of tooth germs from adult lizards and dog embryos could have some significance in odontogenesis.

KEY WORDS: *teeth, heterospecific, cytodifferentiation, odontoblast, ameloblast*

Introduction

Tissue recombinations constitute interesting models to study the odontogenesis. By means of homospecific tissue recombinations of rodent embryos, Mina and Kollar (1987), and Lumsden (1988), showed that the odontogenic potential initially resides in the oral epithelium and that this information is transferred to the mesenchyme. At the cap and bell stages the dental papilla expresses the odontogenic potential (Kollar and Baird, 1969,1970; Ruch and Karcher-Djuricic, 1975; Thesleff, 1977; Ruch, 1990,1994). Isolated dental papilla will not give rise to odontoblasts without homochronic or heterochronic enamel organ epithelial tissue (Ruch *et al.*, 1976; Thesleff *et al.*, 1978; Thesleff and Hurmerinta, 1981).

Heterospecific tissue interactions have been less extensively studied. Karcher-Djuricic and Ruch (1973), and Ruch and Karcher-Djuricic (1975), showed that mouse bud stage molar tooth mesenchyme combined with embryonic chick mandibular epithelium did not allow neither odontoblast nor ameloblast differentiation. Hata and Slavkin (1978) suggested that dental mesenchyme differentiated into cartilage under the influence of avian limb epithelium. Kollar and Fisher (1980) reported that chick epithelium combined with mesenchyme from mouse molars produced dental structures including differentiated ameloblasts depositing enamel matrix. These authors suggested that during evolution avian toothlessness was not a consequence of a change in the genetic coding in the oral epithelium. Rather, an upset of a developmental sequence or an alteration in the behavior of cranial neural crest cells may have caused the genetic information for the synthesis of a specific product to remain quiescent in the genome. Cummings *et al.* (1981) designed experiments to test these findings. In contrast, their results indicated that quail epithelium combined with mouse mandibular molar dental papilla mesenchyme could differentiate into functional odontoblasts and produce dentine, but they did not observe differentiation of ameloblasts and the formation of enamel by quail epithelial cells. On the other hand, Arechaga *et al.* (1983) have carried out recombinations between mouse molar mesenchyme and quail epithelium from mandibular processes and limb buds. Neither odontoblasts nor chondrocytes differentiated.

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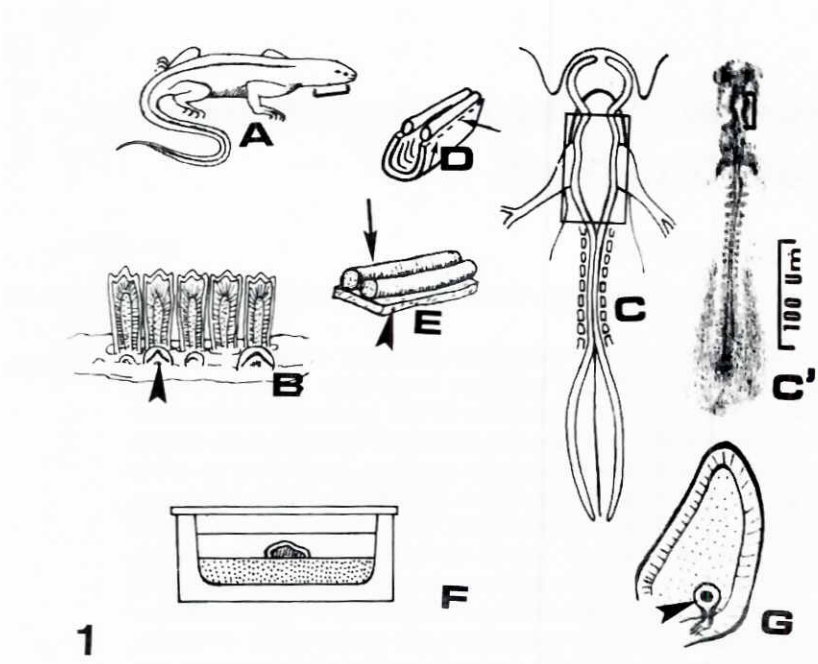
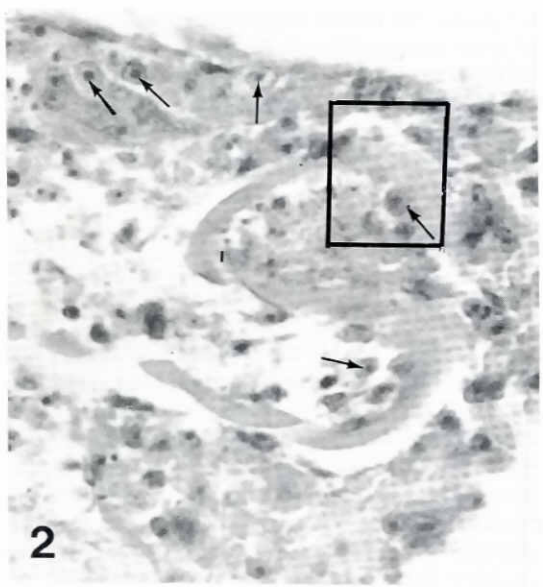




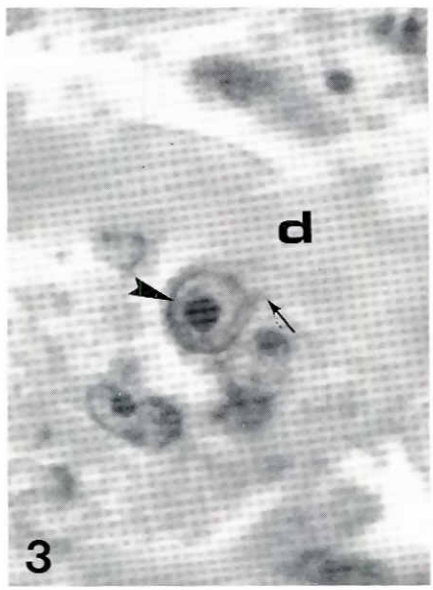


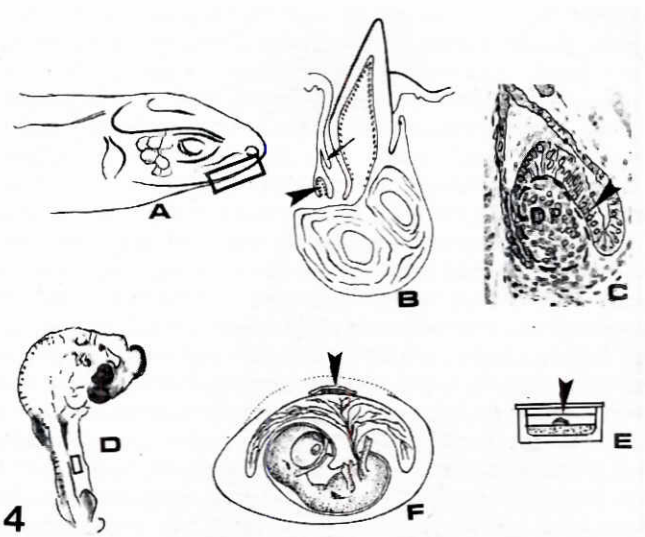
Fig. 1. ***Liolaemus gravenhorsti.*** **(A)** *Adult lizard. The area encompassed by the bracket shows the side of surgical removal of dental tissues.* **(B)** *Isolated fragment of dental lamina. Arrowhead points to teeth rudiments (polyphyodont condition). Arrow shows tritubercular teeth.* **(C)** *Dorsal view of 33 h quail embryo. The area marked by the rectangle shows the level of the surgical removal of cranial neural tube of Coturnix coturnix japonica. C. c. japonica stage 9.* **(D)** *Neural crest cells were relatively isolated from neural tube, and the surrounding ectodermal tissues were cut (arrows).* **(E)** *Heterospecific combinations between adult lizard tooth bud (preameloblasts and preodontoblast cells) and cranial neural crest of quail embryos (arrow) cultured in semisolid medium.* **(F)** *Explants were cultured for 84 h, and the growth medium was changed every day.* **(G)** *Drawing of lizard tooth rudiment after 84 h in vitro quail ectomesenchymatic cells could be seen developing in contact with dentin (arrowhead).*

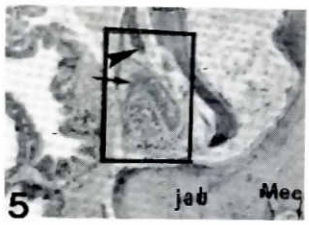
Fig. 2. View of a chimeric quail-lizard culture after 84 h *in vitro*. *Arrows point to quail cells, which have proliferated actively. Some quail cells are in contact with dentin. The area enclosed by the rectangle is magnified in Fig. 3. Feulgen and Rossenbeck staining. x190.*

Fig. 3. Note the presence of the positively Feulgen-stained nucleolus of a quail cell developing in close contact with dentin (arrowhead). *Arrow points to a cytoplasmic extension which is like an odontoblastic process. Dentin, d. Feulgen and Rossenbeck staining. x800.*

Cartilage appeared if the quail epithelium was contaminated with homologous limb mesenchyme and odontoblasts differentiated if the mouse dental papilla was contaminated with dental epithelium cells. These investigators suggested that one possible explanation of the discrepancies between their results and earlier studies was tissue contamination, and with regard to Kollar's work, the different culture techniques may explain the differences observed. In fact, Kollar and Fisher (1980) cultured the recombinants as transplants in the anterior chamber of the eye, where the tissues were vascularized, while Arechaga *et al.* (1983) culture the mouse dental papillae and quail mandibular epithelium on semi-solid coagulum, and in serumless and chemically defined medium. Arechaga *et al.* (1983) indicated that the absence of tissue contamination should always be documented using either biologic markers or cytophotometric DNA quantifications.

The importance of cranial neural crest in tooth morphogenesis demonstrated that mouse cephalic neural crest is the source of odontoblasts, dental papilla cells, peridental and some bone cells. It has been shown that the differentiation of odontoblasts can be triggered by various epithelial tissues, and that no other mesenchymal cells were capable of differentiating into odontoblasts, even if they were cultured with the enamel epithelium (Kollar and Baird, 1970; Ruch and Karcher-Djuricic, 1978). Mina and Kollar (1987) showed that mouse mandibular ectoderm (day 9-11) combined with 2nd arch mesenchyme (derived from rhombomere 4) also allowed molariform tooth morphogenesis. According to Ruch





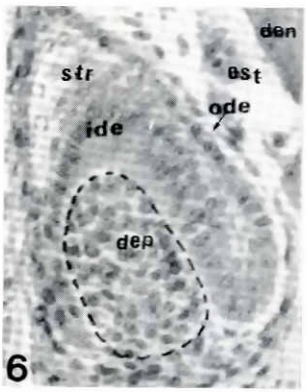
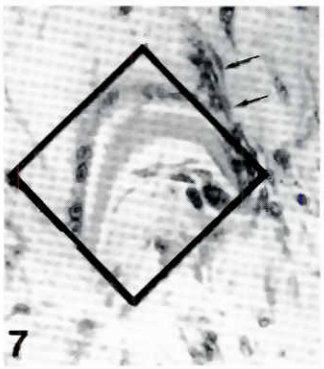
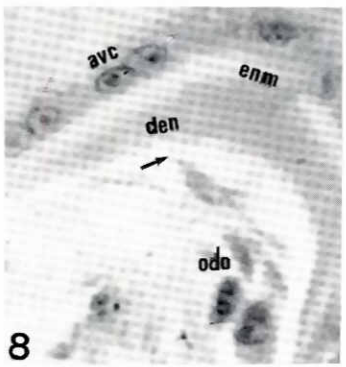
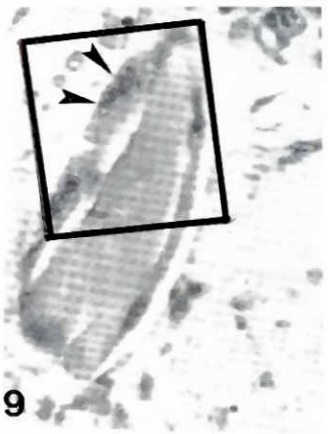


Fig. 4. *Liolaemus gravenhorsti.* **(A)** *Adult lizard. Rectangle shows the site of removal of the tooth rudiments.* **(B)** *Polyphyodont tooth and its relationship with replacement teeth. Arrowhead depict the site of removal of dental papilla that were combined with quail ectoderm. Arrow points to dental lamina.* **(C)** *Higher magnification of a tooth at bell stage showed by the arrowhead in* **B**. *Arrow points to inner dental epithelium. Dental papilla, dp.* **(D)** *View of a quail embryo at 72 h. Rectangle shows the level from which quail flank ectoderm was removed.* **(E)** *Heterospecific reaggregate between dental papillae of adult lizard and flank skin ectoderm of quail embryo in semi-solid medium plus liquid medium, culture for 48 h.* **(F)** *Chick chorioallantoic membrane (CAM) after 7 days of incubation. Arrowhead points to reaggregate, which was placed on the CAM at this age and cultured as a choriollantoic graft for 6 days.*







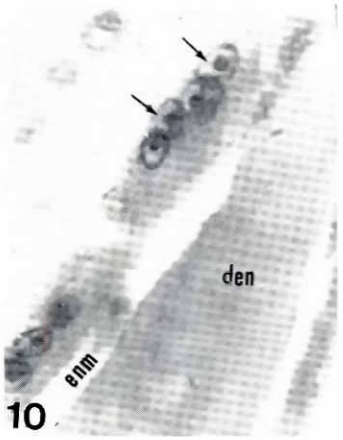


Fig. 5. Longitudinal sections of adult teeth and their relationship with the dental lamina *(arrowhead). Enamel organ of replacement teeth (arrow). Meckel's cartilage (Mec), jawbone (jab). Masson staining. x80.*

Fig. 6. Area marked in Fig. 5. *Broken lines depict the sites of removal of dental papillae that were combined with quail ectoderm and cultured as explants. Stellate reticulum (str), outer dental epithelium (ide), dental papilla (dep), odontoclast (ost), dentin (den). Masson staining. x300.*

Fig. 7. Chimeric dental crown with well-differentiated dentin surrounded by inner enamel epithelium made up of quail cells. *Arrows point to outer epithelium of enamel organ. Feulgen and Rossenbeck staining. x80.*

Fig. 8. Square in Fig. 7. *Dental papilla of lizard tissue has some cells that appear to be unpolarized odontoblasts (odo). Arrow points to a cytoplasmic extension that is similar to an odontoblastic process. A well-developed layer of dentin (den) is present. Quail cells are distinguished in these chimeric tissue associations because of their characteristic nuclei. These avian cell (avc) are cuboidal and apparently not polarized, yet they appear to have secreted an enamel matrix (enm) similar to that in the lizard control. Feulgen and Rossenbeck staining. x700.*

Fig. 9. Section of chimeric tooth and surrounding tissue after 8 days in explant. *Arrowheads point to quail cells in inner dental epithelial layer. Feulgen and Rossenbeck staining. x80.*

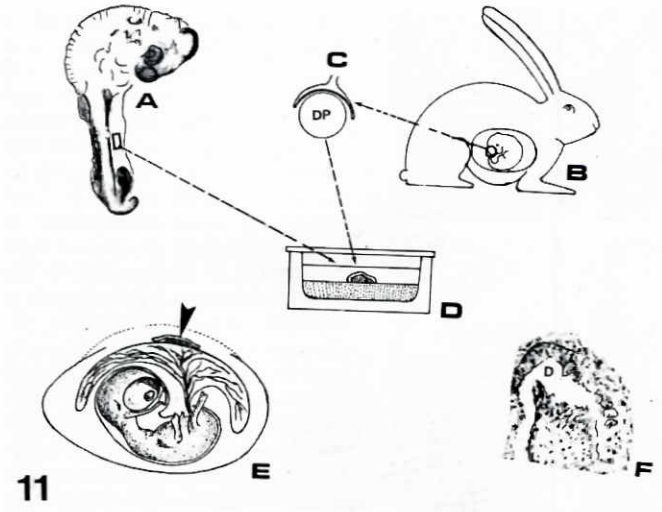
Fig. 10. Square in Fig. 9. *Differentiation of quail cells (arrows) and apparent formation of enamel extracellular matrix (enm) is observed. The cavity between dentin (den) and epithelium is probably partly due to preparation artifact resulting from extraction of the enamel protein. Feulgen and Rossenbeck staining. x700.*

(1994 and references therein), these data are clarified by evolutionary data: neural crest was probably involved in dermal odontode formation as well as in the pharyngeal dentition. The initial neural crest had widespread odontogenic potencies.

As we know, modern birds have a horny beak instead of the typical reptilian teeth of their ancestors of the Jurassic and Cretaceous periods, for example *Archeopterix* and *Archeornithes*. It is tempting to consider that during the development of the oral cavity in the chick the oral epithelium invaginates into underlying mesenchyme to form a rudiment resembling an early stage of the dental lamina of amphibian, reptilian and mammalian embryos (Lemus *et al.*, 1983), though further odontogenic interactions between the epithelial and mesenchymal tissues are not present.

Personal data

In order to investigate the possible bird neural crest odontogenic potential we carried out a series of experiments using neural crest of quail embryos and dental buds of adult lizards (Fig. 1). Experimental studies on lizard teeth have not been conducted in spite of the fact that most species are polyphyodont (i.e. successive generations of tooth germs progressively repeat the morphogenetic



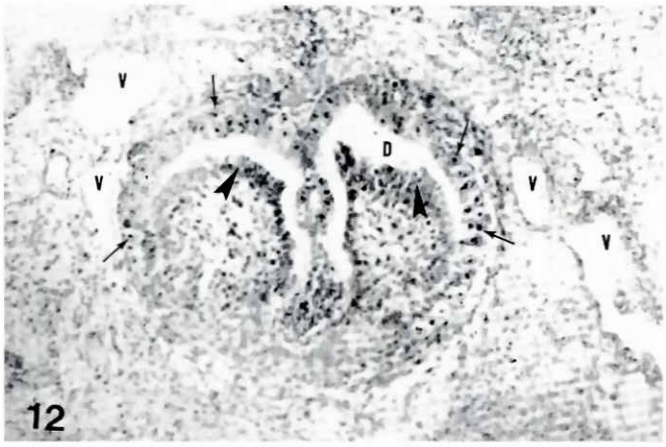


Fig. 11. (A) *Diagram of 72 h quail embryo. Rectangle shows the site of quail flank ectoderm which was removed.* **(B)** *Gravid female adult rabbit (Oryctolagus cuniculus). Circle represents the site of the microsurgical removal of the tooth buds.* **(C)** *Isolated tooth at the bell stage. The dental papilla (DP) were rinsed in Tyrode's (TS).* **(D)** *Reaggregates between dental papillae from rabbit embryo and flank-skin-ectoderm from quail embryo cultured for 48 h on semi-solid medium.* **(E)** *CAM previously incubated for 7 days.* **(F)** *The reaggregates were cultured as a chorioallantoic graft for 6 days.*

Fig. 12. Chimeric dental bud with well-differentiated dentin surrounded by inner epithelium made up of quail cells. *Dental papilla of rabbit shows polarized odontoblasts (arrowheads). Dentin (d). CAM shows numerous blood vessels (v) that surround the graft. Feulgen and Rossenbeck staining. x400.*

events involved in odontogenesis) (Lemus and Duvauchelle, 1966; Lemus, 1967; Lemus *et al.*, 1977, 1981). These tissues can be considered as biological models and are ideally suited to study neural crest cell differentiation. In fact, in adult lizards *Liolaemus gravenhorsti* and *Liolaemus tenuis* the dental tissues maintain a continuous morphogenetic field throughout life. This property is intrinsic, residing in the isolated tooth tissues and being relatively independent of external factors. For example, dental buds from adult lizards isolated and cultured *in vitro* were able to differentiate into teeth (Lemus *et al.*, 1980). Using the biological marker system (Le Douarin, 1973), quail neural crest was combined with lizard dental tissues (Lemus *et al.*, 1983). In these experiments we showed that some quail ectomesenchymal cells migrated into the dental pulp of already determined lizard tooth germs. In two of our best cases, quail cells had a cytoplasmic extension like that of typical odontoblastic processes (Figs. 2,3). In other experiments, Lemus *et al.* (1986) examined the inductive potential of dental papilla from adult lizard. The quail ectoderm-lizard papilla recombinants showed differentiated, chimeric, tooth-like structures (Figs. 4,5,6,7,8,9,10). In other experiments, Fuenzalida *et al.* (1990) showed that dental papilla from rabbit embryos (Lagomorpha) combined with quail ectoderm resulted in relatively well-developed teeth (Figs. 11,12). Dental papillae from lizard tooth at bell stage were also recombined with quail ectoderm and cultured in explant for 8 days. These control heterospecific recombinants differentiated into chimeric tooth structures, showing that the dental papillae were capable of producing developmental instructions. Lemus *et al.*, (1987), examined the differences in the electrophoretic distribution of soluble proteins with respect to the isoelectric point (IP) and the molecular weight (Mr) extracted from dental papilla isolated from tooth germs at bell stage of adult lizards (polyphyodont species) and dog embryos (diphyodont species). A comparison was also made with proteins from dental pulps of adult dogs. Under SDS-PAGE and 2-D PAGE protein patterns from adult lizard and dog embryos disclosed two protein spots with an approximate Mr of 49000 and IP of pH ranges between 6 and 7.5 (Figs. 13,14).

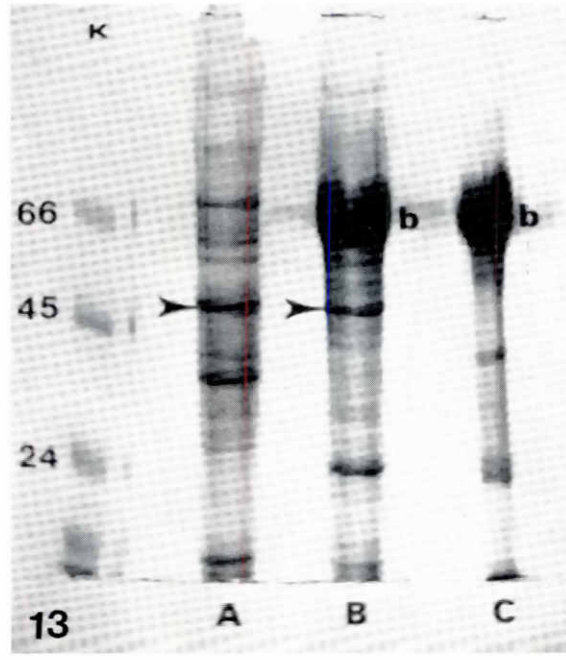


Fig. 13. SDS-PAGE of soluble proteins from dental papilla and pulls of polyphyodont lizard *Liolaemus tenuis tenuis* and diphyodont dog *Canis familiaris*. *Arrowheads point to bands with approximate Mr 49000. In embryo and adult dog protein patterns, a band of Mr 64000-85000 is present (b). Six replicates of the three samples were run in parallel at each opportunity. The gels were calibrated using molecular weight standards: BSA (Mr 66000), egg albumin (Mr 45000), pepsin (Mr 34700), trypsinogen (Mr 24000), beta-lactoglobulin (Mr 18400), and lysozyme (Mr 14300). The height of the gels was uniform. Relative mobility of each band was determined with reference to the dye front, and the Mr of the samples were computed from the linear regression from the mobilities of the molecular weight standards.*

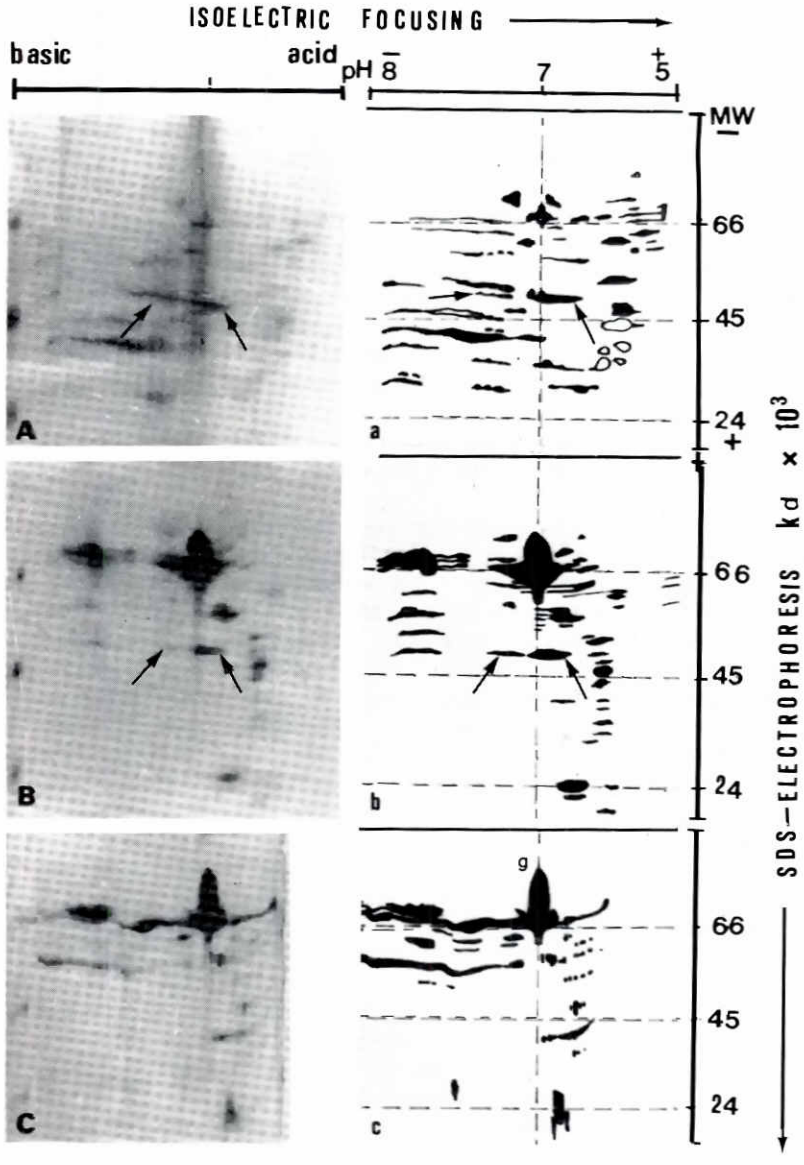


Fig. 14. Distribution of soluble peptide spots in both dental papilla from diphyodont (dog) and polyphyodont (lizard) species by 2-D PAGE. *Each spot corresponds to a different polypeptide chain. Peptides were first separated according to their isoelectric points by isoelectric focusing (IEF) from left to right. These proteins were then further fractionated according to their molecular weights by electrophoresis from top to bottom in the presence of SDS. Note that different proteins are present in very different amounts.* **(A,B,C)** *Photographs of spots.* **(a,b,c)** *Diagrams of the same patterns. Polyphodont species,* **A,a.** *Diphyodont species, dog embryos,* **B,b** *and adult dogs,* **C,c**. *Samples were dissolved in lysis buffer: 2% Nonidet P40, 2% Pharmalytes carrier ampholytes for IEF (Sigma Chemical Co.) at pH ranges 5-8 and 3-10, 5% mercaptoethanol, 9.5 M urea (O'Farrell, 1975). The pH gradient formed during the first dimension was measured with Biorad micro pH electrode immediately after electrophoresis. Arrows point to unidentified spots which seem to be unique to patterns obtained from lizard and dog embryos. Note that these spots have an approximate Mr of 49000 daltons and similar isoelectric point at pH 6-7.5.*

Discussion

The odontogenesis may be considered as a sequence of biochemical and physical interactions between dental epithelium and mesenchyme. A variety of factors such as fibronectin, type III collagen, tenascin, syndecan, growth factors peptides and receptors, homeobox genes and protooncogene products, retinoic acid receptors and carbohydrate moieties seem to play important roles in the developing tooth (Ruch *et al.*, 1982; Ruch, 1987, 1995; Thesleff *et al.*, 1990, 1991; Mark et al., 1991, 1992; Sasano *et al.*, 1992; Zeichner-David *et al.*, 1992; Hu et *al.*, 1993; Slavkin, 1993; Bloch-Zupan *et al.*, 1994a,b; Lemus et al., 1994). Although important progress has been made directed towards the elucidation of numerous developmental events related to odontogenesis, the molecular nature of the instructions remain unknown.

According to some authors, the expressed phenotype corresponds to the genotype of the epithelium (Sengel and Dhouailly, 1977); on the contrary, the mesenchyme might also change the prospective fate of the epithelium (Propper and Gomot, 1973; Kollar and Fisher, 1980). Other studies have demonstrated that the epithelium is required in the control of morphogenesis and differentiation of mesenchymal cells such as odontoblasts, chondroblasts, osteoblasts, and muscle cells (Arechaga et *al.*, 1983).

The results of heterospecific quail neural crest-lizard dental bud recombinants, suggest that during *in vitro* culture of polyphyodont lizard dental buds, some non-species-specific morphogenetic morphogens are produced. Such inductive signals are thus evidently nonspecific with respect to the age-related competence of the neural crest to respond. These

morphogens could provide regulatory developmental information directing cranial neural crest cells of the quail to express some dental characteristics which would be repressed during normal development (Lemus *et al.*, 1983). The most intriguing finding in our study is that even avian neural crest cells could acquire odontogenic potencies. We should continue our studies on these recombinations, and try to get more evidence on this point. Perhaps we could improve the culture system to achieve better survival and to extend the culture period, or apply the transplantation method of Lumsden, where the tissue pieces are transplanted into the anterior chamber of the eye. Recently, Trowsdale (1993) in a review of Genomic and Function of the MHC (major histocompatibility complex), has postulated that some duplicated MHC genes appear to be much older and yet have not suffered obvious mutations. That some genes are not necessarily dismantled when selection pressure is lifted is most spectacularly shown by the genes for tooth formation, which have remained silent in bird for more than 200 million years, but can be activated by appropriate signals. These data would support the suggestion made by Kollar and Fisher (1980), and Lemus *et al.* (1983).

Lemus *et al.* (1986) reported that combinations between quail ectoderm and lizard dental papilla showed relatively well developed teeth. Fuenzalida *et al.* (1990) established that the dental papillae dissected from 15 day rabbit embryos have already acquired odontogenic potential, as can be demonstrated by the enamel organ differentiation. Nevertheless, it is uncertain whether or not enamel was secreted; these points requires confirmation by immunocytochemistry. As we know, Mina and Kollar (1987), and Lumsden (1987) have shown that the information for tooth development in mice resides in the early odontogenic epithelium. Our results are in accordance with their findings. However controversy persists regarding the ability of avian epithelium to express the ameloblast phenotype and to secrete enamel proteins. Another approach, implying the characterization of dental papilla proteins, is an intriguing question too. The search for differences and similitudes between polyphyodonts and diphyodonts is very important. Probably a lot of similitudes exist as far as the epigenetic control of histo-morphogenesis and cytodifferentiation are concerned. Specific differences must surely exist as far as odontogenic potential of the dental lamina is concerned.

To date, few electrophoretic studies have been performed due, in part, to the limited amounts of material that can be obtained from each embryonic tooth germ (Slavkin, 1974; Kestler *et al.*, 1980). Lemus *et al.* (1983, 1987) examined the possible role of the dental papilla from polyphyodont lizard tooth germs in heterospecific recombinants. Advanced and relatively well constructed teeth were observed, with clear indications of hard tissue deposition in association with quail epithelium. Under SDS-PAGE and 2-D PAGE, Lemus *et al.* (1987) disclosed two peptide spots with an approximate Mr 49000 and approximate isoelectric point, pH 6-7.5 in dental papillae from adult lizard as well as dog embryo dental protein (Figs. 13,14). Apparently, these spots are unique to both patterns, and they were not observed in the adult dog protein pattern. These peptides may have some common significance in odontogenesis, since the existence of proteins from two divergent species of identical size and identical isoelectric point seems to be a relatively rare finding; nevertheless, the absence of contaminating dental tissues requires confirmation.

Conclusions

The morphogenesis as well as cell differentiation in the tooth germ are under the control of reciprocal interactions between epithelial and mesenchymal tissues, and their nature is still being actively studied in several laboratories. The interpretation of many experimental heterospecific tissue combinations is difficult because of the complexity of the embryonic system. It is difficult to distinguish instructional signals and instructional receptors. Moreover, the signals (epigenetic in nature) may represent modest motifs within a molecule (e.g. amino acid sequence, glycosilation, phosphorylation, disulfide bonding patterns, etc.) or groups of molecules.

New methodologies (*in situ* hybridization, combined with immunohistochemistry of homeobox genes, proto-oncogene, retinoic acid receptors, transcripts and proteins, etc.), may give further insights into the molecular nature and regulation of the reciprocal interactions that take place in interspecific tissue recombinations. With the advances in molecular biology, biological functions of individual matrix and cell surface molecules, as well as the control of their expression, can now be examined. It could be useful in our recombination experiments to analyze differentiation of the quail neural crest cells, by means of monoclonal antibodies to find differentiation markers for odontogenic mesenchyme.

With the aid of the new technologies it will perhaps be possible to answer some questions. For example: 1) when, where, and how does mesenchyme induce a responding epithelium to express unique patterns of morphogenesis and/or cytodifferentiation?; 2) does odontogenic potential exist in neural crest of modern birds?; 3) how is this potential repressed? and, 4) how is odontogenic information in the bird oral epithelium translated into the mesenchymal cells?

Acknowledgments

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