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Concepts of Epithelial-Mesenchymal Interactions During Development: Tooth and Lung Organogenesis

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One of the major problems in developmental biology concerns how differential gene activity is regionally controlled. One approach to this problem is the use of mesenchyme specification of epithelial-specific gene expression, such as, during tooth morphogenesis or lung morphogenesis. In the example of tooth morphogenesis, dental papilla ectomesenchyme induces de novo gene expression as assayed by detection of amelogenin transcripts, or immunodetection of amelogenin polypeptides within ameloblast cells. This process does not require serum supplementation or exogenous factors during epithelial-mesenchymal interactions in vitro. In contrast, lung morphogenesis requires hormones to mediate mesenchyme-derived influences upon type II epithelial cell differentiation and the production of pulmonary surfactant (eg, neutral and phospholipids, surfactant proteins). Glucocorticoids are required to stimulate the release of fetal pneumonocyte factor (FPF) from fibroblasts which, in turn, enhance the production of pulmonary surfactant. Thyroxin appears to regulate the relative responsiveness of progenitor type II cells to steroid-stimulated release of FPF. This review will highlight key concepts associated with these developing organ systems and emphasize the problem of regional controls which regulate epithelial cell-specific gene activity.

Key words: gene expression, amelogenins, cDNA, type II cells, pulmonary surfactant, ameloblasts, epithelial differentiation, regional mesenchymal specificity

One of the most interesting current problems in developmental biology concerns the mechanisms by which differential gene activity is regionally specified. What are the mechanisms by which regional mesenchymal specificity provides instructions for adjacent epithelial differentiation? Why are casein gene expressed in mammary gland acinar epithelial cells and not in other cell types? Why are amelogenin genes expressed in dental epithelial cells?

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This general type of gene regulation has been described as the "cell-type specificity of gene control"[1]. Several aspects of cell-type specific gene control seem extremely important: (i) identification of the DNA sequences which code for the functional mRNA transcripts; (ii) determination of the expression of epithelial cell-specific gene products (eg, insulin, casein, ovalbumin, and amelogenins); (iii) identification of the non-DNA or epigenetic molecules putatively responsible for cell-specific gene activity; and (iv) determination of the mechanism for epigenetic regulation of epithelial cell-specific differentiation. This review is designed to highlight the problem of instructive epithelial-mesenchymal interactions during tooth and lung morphogenesis.

EPITHELIAL-MESENCHYMAL INTERACTIONS

Following the primary induction processes associated with neurulation during early embryogenesis, secondary inductive or epithelial-mesenchymal interactions are described as associated with many different examples of organogenesis (eg, limb morphogenesis, heart, thymus, thyroid, salivary, mammary, lung and tooth organogenesis) [see reviews 2–5].

Epithelial-mesenchymal interactions are defined as tissue interactions which result in profound changes in one or both of the tissue interactants. These changes do not occur in the absence of epithelial-mesenchymal interactions. Several major problems remain in developmental biology regarding these intriguing processes: (i) how is the instructive information regionally partitioned during embryogenesis? (ii) in those examples of mesenchyme-derived specification for epithelial differentiation, what is the physico-chemical nature of the signal? (iii) what is the mode of transmission (eg, direct cell-cell contacts, cell-extracellular matrix interactions, long-range hormone-like mediators, etc.)? (iv) what is the process by which the responding epithelial cells receive the mesenchyme-derived information? and (v) how do the responding epithelial cells become determined for cell-specific gene activity? (Fig. 1.)

IDENTIFICATION OF DNA SEQUENCES WHICH CODE FOR FUNCTIONAL AMELOGENIN TRANSCRIPTS

The identification of DNA sequences which represent an authentic probe with which to identify mRNA transcripts is essential towards understanding cell-specific gene control. Recently, our laboratory reported the identification and construction of mouse amelogenin cDNA clones [6].

Available information indicates that there are two classes of mouse enamel extracellular matrix proteins: (i) enamelins of 62 kilodaltons (Kd) which are acidic glycoproteins; and (ii) amelogenins of 22–28 Kd which are proline-rich, hydrophobic polypeptides [7]. During the process of epithelial differentiation into ameloblasts (ie, amelogenesis), amelogenins represent approximately 90% of the total enamel extracellular matrix proteins [see review 8]. Amelogenins are soluble using either acetic acid or 4 M guanidine hydrochloride (GuHCl) extraction followed by 4 M GuHCL plus EDTA [9]. On the basis of relative solubility properties, electrophoretic mobilities under denaturing conditions on SDS gels, and amino acid composition analyses,

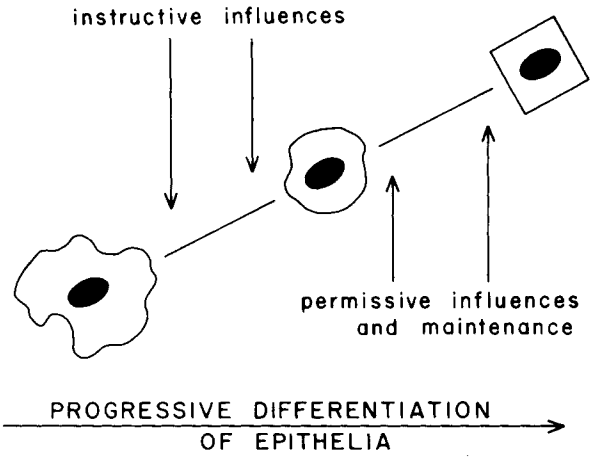

Fig. 1. Epithelial-mesenchymal interactions are requisite for epithelial differentiation in a large number of different organ systems (eg, mammary and salivary glands, thyroid, lung, and tooth organogenesis). Two types or processes are identified: (i) instructive interactions in which mesenchyme induces epithelia to differentiate into a specific phenotype (eg, ameloblasts which produce enamel extracellular matrix constituents) and (ii) permissive influences which maintain or stabilize a committed epithelial phenotype (eg, mediators of type II cells production of pulmonary surfactant).

enamelins and amelogenins appear to represent two different classes of enamel extracellular matrix proteins [7–9].

Partial N-terminal amino acid sequence for several mammalian amelogenins have shown that the first 33 amino acid residues were identical [10–12]. Therefore, porcine, bovine, murine, and primate amelogenins appear to be identical for the first N-terminal 33 amino acids and then show some degree of substitution in the remaining sequence data [see reviews 8,13].

Polyclonal antibodies produced in rabbits against porcine, bovine, or murine enamel proteins have been found to be monospecific for enamel proteins [7–9]. Antibodies produced against purified amelogenins were found to be cross-reactive with purified enamelins, suggesting that both enamelins and amelogenins share a dominant epitope [8]. In addition, phylogenetic studies have reported that rabbit anti-mouse ameloginin as well as rabbit anti-bovine amelogenin antibodies were antigenically cross-reactive with all vertebrate enamel matrix proteins (eg, Pacific hagfish, shark, fish, frog, alligator, rodents, lagamorphs, bovine, porcine, and primates) [13–17].

In order to obtain a cDNA probe for one of the amelogenins, mRNAs for mouse enamel proteins were isolated and partially characterized as assessed by cell-free translation and subsequent immunoprecipitation of the labeled translation products [6, 18]. Subsequent synthesis and cloning of epithelial cell-specific amelogenin cDNA, and identification of amelogenin clones by differential hybridization and hybrid-selected translation assays have been reported [6]. The results of the cell-free translation studies indicated that mouse ameloblasts synthesized four enamel proteins of 62, 28, 26, and 22 Kd, and each of these gene products appeared to share a dominant epitope as determined by their common immunoprecipitation. We have produced a cDNA clone which specifically hybrid-selected the translation of one of the three

amelogenins. These findings suggest the presence of four functional enamel-specific mRNAs [6]. Southern blot analysis of mouse and human genomic DNAs indicated that amelogenin-homologous sequences were detected in both mouse and human genomes [6].

DETERMINATION OF THE EXPRESSION OF EPITHELIAL CELL-SPECIFIC GENE ACTIVITY

A number of experimental embryological studies have demonstrated that cap stage mouse molar ectomesenchyme tissue is inductive for the epithelial differentiation of odontogenic as well as nondental epithelia to become functional ameloblasts [5,19–24]. The inductive capacity of the cap stage mouse molar ectomesenchyme (circa 16-days gestation) has been demonstrated in homotypic as well as heterotypic tissue recombinations, in permissive environments, such as the anterior chamber of the eye of nude mice, intratesticular grafts, and chick chorio-allantoic membrane, and in vitro studies using embryonic chick extract and mammalian sera supplementation [see reviews 25,26]. More recently, homotypic epithelial-mesenchymal interactions have been demonstrated in vitro in the complete absence of exogenous growth factors [see review 26]. Therefore, mesenchyme inductive capacity and the responsiveness of epithelium associated with tooth development in vitro does not require hormonal mediators!

Therefore, we proposed to investigate when epithelial cell-specific amelogenin gene expression occurs in vivo using an assay based upon analysis of cytoplasmic RNAs from developing tooth organs to hybridize to the amelogenin cDNA probe pMa 5/5. Our strategy was to assay embryonic, fetal, and neonatal mouse molar tooth organs from the "inductive" cap stage through early crown stages of odontogenesis in vivo. To complement our assay for functional amelogenin transcript detection, we also assayed for immunoprecipitation of metabolically labeled amelogenins. Our results indicated that de novo expression of amelogenin mRNA as well as nascent translation of amelogenin polypeptides were first detected at Theiler stage 27 (newborn [27]. No detectable transcripts or translated polypeptides were present during Theiler stages 24–26 (16–19 days gestation in the inbred mouse strain used for those studies).

Presently, we are attempting to utilize these assays to investigate when and where mesenchyme-derived instructions mediate epithelial differentiation, and the expression of amelogenin gene products in vitro, using serumless, chemically-defined medium. The cytoplasmic dot hybridization, in situ hybridization, and immunocytological assays are extremely useful for the pursuit of these questions regarding homotypic and heterotypic tissue recombinants in vitro.

IDENTIFICATION OF THE NON-DNA OR EPIGENETIC MOLECULES PUTATIVELY RESPONSIBLE FOR EPITHELIAL CELL-SPECIFIC GENE ACTIVITY

A number of classical experimental embryological studies have demonstrated mesenchymal regional specification of epithelial tissue histogenesis as well as cytodifferentiation patterns [see reviews 2–5,13,20,23–24]. During embryogenesis, developmental regional controls for subsequent epithelial differentiation seem to be

contained within specific populations of mesenchymal cells located in discrete locations in the embryo. A number of critical questions continue to challenge developmental biology, including how putative epigenetic molecules come to be distributed during embryogenesis so as to be located in the proper cell type but not in others.

During neurulation in the rostral regions of developing vertebrate embryos, neural tube ectoderm in the rhombencephalic regions give rise to cranial neural crest cells which become displace and subsequently differentiate into a number of different phenotypes including dental ectomesenchyme cells. During mouse embryogenesis, cranial neural crest cells appear to migrate into the forming branchial arches and participate in the subsequent development of the maxillary and mandibular arches (ie, first branchial arch derivatives) at approximately 9.5–10 days gestation [see review 13]. By Theiler stage 24 (16–17 days gestation), discrete aggregates of cranial neural crest-derived dental ectomesenchyme cells form adjacent to the enamel organ epithelium (derived from oral stomodeum ectoderm)—the cap stage of the mandibular first molar tooth organ. A continuous basal lamina is associated with the inner enamel epithelia, in juxtaposition to the cuboidal sheet of aligned ectomesenchyme cells at this stage of tooth organogenesis.

Early attempts to investigate this problem enlisted the methods of epithelial-mesenchymal transfilter studies. Transfilter studies using cap stage dental mesenchyme and enamel organ epithelia reported that Millipore filters of 0.45 μm pore size and 25 μm thickness were permissive for morphogenesis, histogenesis, and cytodifferentiation in organ culture [19]. These studies suggested that dental papilla mesenchyme were critically required for inner enamel epithelial differentiation into ameloblasts and that the putative epigenetic signals derived from mesenchyme might be diffusible extracellular matrix molecules [see discussions 2–5, 20, 23]. Subsequent transfilter studies using Nuclepore filters of 0.2 μm pore size and 25 μm thickness were found to be permissive for mesenchyme-derived induction of adjacent epithelial differentiation [3, 24]. Whereas Millipore filters consist of numerous voids and channels, Nuclepore filters are manufactured with discrete and precise channels. Therefore, interpretation of Nuclepore transfilter studies have suggested that direct cell-cell contacts between dental mesenchyme and adjacent inner enamel epithelia may mediate the inductive process [3,24]. The putative mesenchyme-derived epigenetic signal may be associated with the extended mesenchymal cell processes. The signal may be allosteric information or enzymatic specificity related to integral plasma membrane glycoproteins [19–24,28–32] (Fig. 2).

Regarding inductive interactions during odontogenesis, several caveats should be emphasized: (i) the putative epigenetic molecule(s) is not known; (ii) the mode of transmission, albeit diffusible through the extracellular matrix or facilitated through direct cell-cell contacts, is not known; (iii) the requirement for information transfer has been established; (iv) odontogenic epithelial cell receptors for putative epigenetic signals have not been identified; and (v) the processes by which epithelial cells activate and express amelogenin genes have not been determined [see reviews 3–5, 13,23,29–32].

In contrast, putative epigenetic molecules have recently been reported which facilitate the production of pulmonary surfactant by fetal type II epithelial cells in vitro and possibly in vivo. These studies suggest a mechanism for epithelial-mesenchymal interactions during fetal lung morphogenesis. Mammalian lung morphogenesis has been staged according to a series of developmental phases (ie, pseudoglandular,

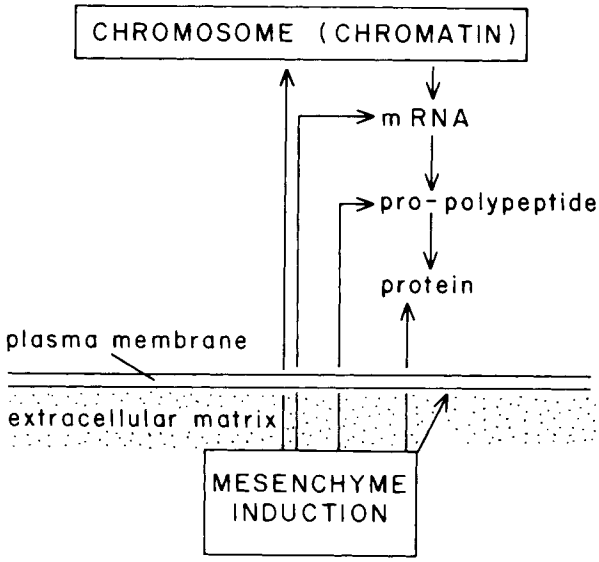

Fig. 2. General scheme for mesenchyme-derived induction for epithelial differentiation. Putative signals serve as developmental information providing regional specification for epithelial differentiation. The transmission mode enlists cell-cell direct or cell-matrix-cell indirect communication. Mesenchyme-derived signals may be received by epithelial integral plasma membrane glycoproteins which extend from the interior of the epithelial cell, through the plasma membrane, and into basal lamina and adjacent extracellular matrix microenvironment. In turn, the epithelial reception of the signal(s) effect a series of steps which provide linkages between the extracellular matrix, the cytoskeleton matrix, and the nuclear matrix.

canalicular, terminal sac, and alveolar phases). In fetal mouse lung morphogenesis, acinar tubular epithelial cells appear to differentiate into type II cells during late canalicular or early terminal sac phases of lung development (circa 16–17 days gestation). Cytologically, the type II cells can be readily identified with ultrastructure on the basis of inclusions called lamellar bodies and large storage areas of glycogen. These cells are part of the pulmonary acinus unit and produce pulmonary surfactant which lines the alveolar spaces in the mature lung. The lamellar bodies appear to contain constituents of the extracellular pulmonary surfactant material.

Evidence suggests that both glucocorticoids and thyroxin hormones regulate pulmonary surfactant synthesis and secretion by type II epithelial cells. It is also evident that lung morphogenesis and cytodifferentiation processes are regulated by epithelial-mesenchymal interactions. Throughout embryonic and fetal mouse lung development, for example, basal lamina degradation and direct cell-cell interactions between fibroblasts and adjacent acinar tubular and bronchial epithelia have been identified and described [33–35]. The basal lamina associated with the epithelial-mesenchymal interface associated with type II cell differentiation contains fibronectin, laminin, and heparan sulfate basement membrane proteoglycans [35]. In addition, analogous to the developing embryonic and fetal tooth organ, direct mesenchyme-epithelial cell-cell contacts have been described [33–35].

Recently, cortisol has been shown to induce the production of fetal pneumonocyte factor (FPF) which, in turn, enhances the production of pulmonary surfactant by responding type II cells [36]. The FPF has been postulated to mediate the significant

stimulation of saturated phosphatidylcholine synthesis and subsequent production of pulmonary surfactant (ie, neutral and phospholipids, and surfactant proteins) by type II cells. Thyroxin appears to enhance the responsiveness of epithelia to the effects of cortisol-stimulated, fibroblast production of FPF [36]. To further test the putative biological activity of FPF, inhibition of pulmonary surfactant production and lung maturation by monoclonal antibodies directed against FPF have recently been described during embryonic chick lung morphogensis [37]. A number of epithelial-mesenchymal interactions have been described which require hormonal regulation including prostate, vagina, oviduct, mammary gland, pancreas, and lung organogenesis [see review 38].

MECHANISMS FOR EPIGENETIC REGULATION OF EPITHELIAL-MESENCHYMAL INTERACTIONS: SUMMARY

During this presentation we have focused upon the question of how regional specification might regulate cell-specific gene activity. We selected examples of epithelial differentiation in the developing tooth as well as lung organ systems. In the instance of inner enamel epithelial cells differentiating into functional ameloblasts, we indicated that adjacent dental papilla ectomesenchyme cells provide regional specification for transcription and translation of amelogenin gene products. In this example, mesenchyme regional specification for epithelial-cell-specific gene activity does not appear to require hormonal cofactors when studied in vitro; these instructive epithelial-mesenchymal interactions have been demonstrated using serumless, chemically defined medium. How dental papilla ectomesenchyme cells provide regional specification for homotypic or heterotypic epithelial cell-specific amelogenin gene activity is now known.

In developing fetal mouse lung organogenesis, we described recent progress toward identifying a putative signal for the enhancement of type II epithelial cell production of pulmonary surfactant which requires cortisol and thyroxin hormone mediators. Fetal lung fibroblasts release FPF when stimulated by cortisol, which, in turn, influences type II cells to produce significant amounts of saturated phosphatidylcholine in association with pulmonary surfactant [36]. Monoclonal antibodies produced against FPF recently have been used to inhibit the process of lung maturation during late stages of chick embryogenesis (circa 15–20 days incubation) [37]. These studies suggest that mesenchyme regional specification for type II cell differentiation during fetal lung morphogenesis (late canalicular or initial terminal sac phases) appears to require glucocorticoid and thyroxin hormone mediators. The processes related to glucocorticoid influences on long maturation may be under genetic controls associated with the major histocompatibility complex in the mouse animal model system [39].

The critical problem of regional specification refers to a process by which cells in discrete regions of the developing embryo become switched to unique phenotypes. How are ameloblasts signaled to produce amelogenins, type II cells to produce pulmonary surfactant, mammary gland epithelial cells to produce casein, or chick oviduct tubular epithelial cells to produce ovalbumin? It must be emphasized that synthesis of a particular group of unique proteins clearly makes one cell's phenotype different from others (ie, cell differentiation). However, differentiation is the consequence of regional specification and not the cause. This is a key issue in the design of

experiments and in the interpretation of results regarding this important and puzzling development problem. The identification of promoter and terminator sequences in DNA does not provide answers for the problem of how mesenchyme cells instruct the commitment of epithelial cells to a unique pattern of gene activity. It is becoming apparent that regional specification for cell-specific gene activity during development perhaps resides in the asymmetrical distribution of intracytoplasmic and nuclear matrix proteins as well as the asymmetrical distribution of extracellular matrix macromolecules. It is hoped that concepts, creativity, techniques, and procedures currently in use may be sufficient to penetrate this intriguing problem in developmental biology.

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REFERENCES

1. Gurdon JB: In Brown DD (ed): "Developmental Biology Using Purified Genes." New York: Academic Press, 1981, pp 1–10.
2. Grobstein C: Natl Cancer Inst Monogr 26:279, 1967.
3. Saxen L, Karkinen-Jaaskelainen M, Lehtoneu E, Nordling S, Wartiovaara J: In Poste G, Nicolson GL (eds): "The Cell Surface In Animal Embryogenesis and Development." New York: North-Holland Publishing Co., 1976, pp 331–407.
4. Dhouailly D, Sengel P: In Sawyer RH, Fallon JF (eds): "Epithelial-Mesenchymal Interactions in Development." New York: Praeger, 1983, pp 147–162.
5. Kollar EJ: In Sawyer RH, Fallon JF (eds): "Epithelial-Mesenchymal Interactions in Development." New York: Praeger, 1983, pp 27–50.
6. Snead ML, Zeichner-David M, Chandra T, Robson KJH, Woo SLC, Slavkin HC: Proc Natl Acad Sci USA 80:7254, 1983.
7. Slavkin HC, Zeichner-David M, MacDougall M, Bringas P, Bessem C, Honig L: Differentiation 23:73, 1982.
8. Slavkin HC, Zeichner-David M, Siddiqui MAQ: Mol Aspect Med 4:125, 1981.
9. Termine JD, Belcourt AB, Christner PJ, Conn KM, Nylen MU: J Biol Chem 255: 9760, 1980.
10. Fincham AG, Belcourt AB, Termine JD, Butler WT, Cothran WC: Biosci Rep 1: 771, 1981.
11. Fincham AG, Belcourt AB, Termine JD, Butler WT, Cothran WC: Biochem J 211:149, 1983.
12. Fukae M, Tanabe T, Ijiri H, Shimizu M: Tsurunii U Dent J 6(2):87, 1980.
13. Slavkin HC, Snead ML, Zeichner-David M, Bringas P, Greenberg G: In Trelstad R (ed): "The Role of Extracellular Matrix in Development." New York: Alan R. Liss, Inc., 1984, pp 221–253.
14. Graver H, Herold R, Chung T, Christner PJ, Pappas C, Rosenbloom J: Dev Biol 63:390, 1978.
15. Herold R, Graver H, Christner PJ: Science 207:1357, 1980.
16. Slavkin HC, Zeichner-David M, Ferguson MWJ, Termine JD, Graham E, MacDougall M, Bringas P, Bessem C, Grodin M: In Riviere G, Hildemann W (eds): "Oral Immunogenetics and Tissue Transplantation." New York: Elsevier/North-Holland, 1982, pp 241–251.
17. Slavkin HC, Graham E, Zeichner-David M, Hildemann W: Evolution 37:404, 1983.

18. Slavkin HC, Zeichner-David M, MacDougall M, Bessem C, Honig LS, Lussky J, Vides J: J Dent Res 61:1457, 1982.
19. Koch W: J Exp Zool 165:155, 1967.
20. Koch W: In Slavkin HC, Bavetta LA (eds): "Developmental Aspects of Oral Biology." New York: Academic Press, 1972, pp 151–164.
21. Kollar E, Baird G: J Embryol Exp Morphol 21:131, 1969.
22. Kollar E, Baird G: J Embryol Exp Morphol 24:173, 1970.
23. Ruch JV, Karcher-Djuricic B: In Slavkin HC, Greulich RC (eds): "Extracellular Matrix Influences on Gene Expression." New York: Academic Press, 1975, pp 549–554.
24. Thesleff I: In Karkinen-Jaaskelainen M, Saxen L, Weiss L (eds): "Cell Interactions in Differentiation." London: Academic Press, 1977, pp 191–207.
25. Yamada M, Bringas P, Grodin M, MacDougall M, Slavkin HC: Calcif Tissue Int 31:161, 1980.
26. Slavkin HC, Honig LS, Bringas P: In Sarnat B, Dixon AD: "Factors and Mechanisms Influencing Bone Growth." New York: Alan R. Liss, 1982, pp 217–228.
27. Snead ML, Bringas P, Bessem C, Slavkin HC: Dev Biol 104:255, 1984.
28. Slavkin HC, Bringas P: Dev Biol 50:428, 1976.
29. Slavkin HC: J Biol Buccale 6:189, 1978.
30. Thesleff I, Hurmerinta K: Differentiation 18:75, 1981.
31. Slavkin HC, Trump GN, Brownell AG, Sorgente N: In Lash JW, Burger MM (eds): "Cell and Tissue Interactions." New York: Raven Press, 1977, pp 29–46.
32. Slavkin HC: J Craniofac Genet, Dev Biol 2:179, 1982.
33. Riso J: Cell Differ 13:309, 1983.
34. Bluemink JG, van Maurink P, Lawson KA: J Ultrastruct Res 55:257, 1976.
35. Jaskoll TF, Slavkin HC: Differentiation (in press).
36. Smith BT, Sabry K: Proc Natl Acad Sci USA 80:1951, 1983.
37. Cunha GR, Shannon JM, Taguchi O, Fujii H, Meloy BA; IN Sawyer RH, Fallon JF (eds): "Epithelial-Mesenchymal Interactions in Development." New York: Praeger, 1983, pp 51–74.
38. Post M, Floros J, Smith BT: Nature 308:284, 1984.
39. Honig LS, Smith BT, Slavkin HC, Donahue HG: Proc Exp Biol Med 176:419, 1984.