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Inductive Tissue Interactions During Inner Ear Development*

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Summary. Inductive tissue interactions were studied in the otocyst of the CBA/CBA mouse. Otocysts with surrounding mesenchyme explanted at the 12.5–13th gestational day and cultured in vitro for 4 days underwent morphogenesis with formation of semicircular canals, vestibular organs, and some cochlear coiling. Without their surrounding mesenchyme only little, if any, development was seen. However, otocysts without mesenchyme but grown in a medium precultured with mesenchyme did develop normally. A soluble and diffusible induction factor is apparently produced by mesenchyme and also by other fetal organs. Cell-cell contact may not be needed for induction.

Key words: Induction – Morphogenesis – Inner ear – Mesenchyme – Rhombencephalon

Introduction

During embryogenesis, cells differentiate in a spatially and temporally synchronized manner, implying the existence of a general controlling mechanism. Morphogenetic signals are of at least two types: (1) humoral factors acting over long distances on target cells in separate organ anlagen (e.g., morphogenetic hormones, growth-controlling factors); (2) communication between cells and cell populations in close proximity. The latter embryonic induction occurs whenever two or more tissues of different history and properties become intimately associated and alteration of the developmental course of the interactants results [6, 7, 11]. The morphogenetic information involved provides permissive signals inducing the target cells to express a

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pre-existing differentiating bias [4]. Proximal interactions in differentiation may involve two distinctly different mechanisms: direct cell contact or short-range messenger molecules. For several embryonic systems such messengers have been established and attempts at their biochemical characterization made [5, 10].

We have chosen the inner ear anlage (otocyst) of the CBA/CBA mouse to study possible morphogenetic signals during its differentiation. The experimental model is the organ culture system, which during recent years has been characterized and evaluated both ultrastructurally [1, 9] and to some extent biochemically [3].

Materials and Methods

The CBA/CBA mouse (mus musculus) was used. The gestational period was 21 days, taking the day of the vaginal plug as day 1. The inner ear anlage of the 12.5–13th gestational day was excised and the adjacent mesenchyme was removed mechanically with sharpened forceps under a stereomicroscope, leaving the otocyst epithelium and the outgrowing endolymphatic duct and sac intact ("stripped otocysts", Fig. 1). In control explants the surrounding mesenchyme was left intact (Fig. 2). Two experimental designs were employed:

1. "Stripped" otocysts were co-cultured with one other tissue (mesenchyme, fetal forebrain, fetal, and adult rhombencephalon or fetal limb) in the same culture disk.
2. A tissue with assumed influence on inner ear development was explanted and pre-cultured for 1 day to accumulate inductive factors possibly being released into the culture medium during cell growth or preservation in vitro. After 24 h the explanted tissue was removed by centrifugation (12,000 *g*; 10 min) and the "stripped" otocyst was then added to the "conditioned" medium for culture.

After 4–5 days in culture morphogenesis of the otocysts was evaluated on a 0–3 scoring system. Zero designated lack of development; 1, suspected morphogenesis with formation of parts of the semicircular canals; 2 and 3 obvious morphogenesis. Only the vestibular part of the inner ear was evaluated because complete cochlear coiling is not always achieved in the in vitro system. Evaluations were performed "blind", i.e. without knowledge of the prior treatment of the culture medium. All explants were photodocumented daily.

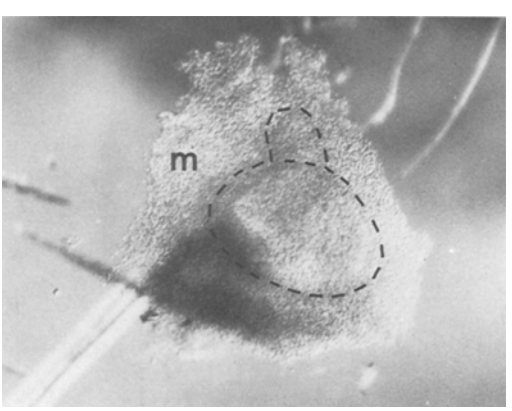


Fig. 1. Explant of the inner ear, 13th gestational day. Light micrograph. The otocyst (indicated by the *dotted line* including the outgrowth of the endolymphatic duct and sac) is surrounded by adjacent mesenchyme (*m*). To obtain a "stripped" otocyst the mesenchyme is excised as indicated by the dotted line

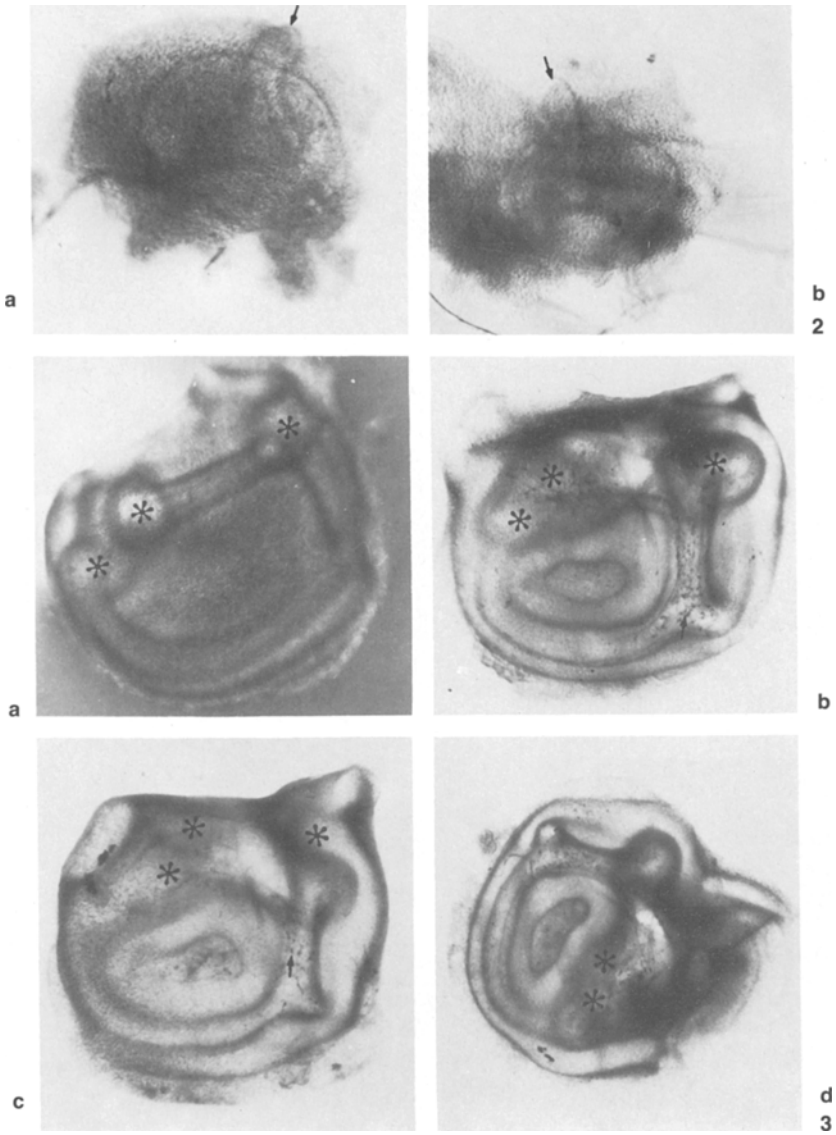
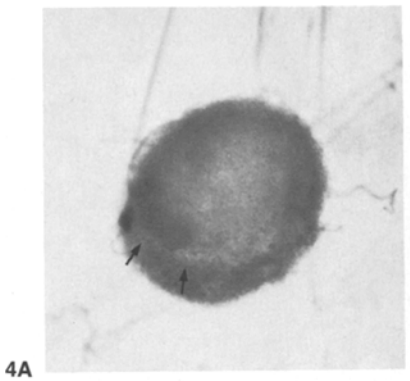
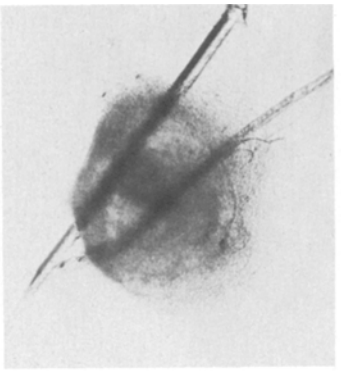
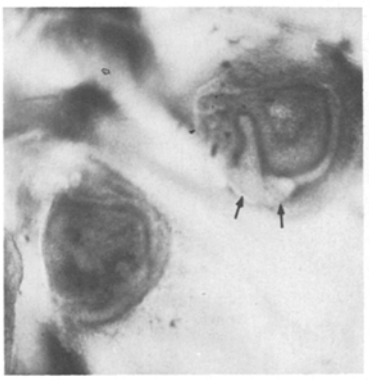


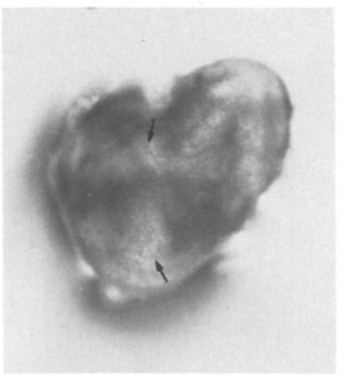
Fig. 2a–b. Otocysts, 12th gestational day, explanted with surrounding mesenchyme. Light micrograph. Day of explantation. The outgrowth of the endolymphatic duct and sac is indicated by *arrows*

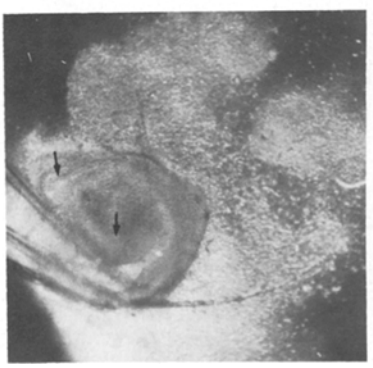
Fig. 3a–d. Otocysts, 13th gestational day, explanted with surrounding mesenchyme. Light micrograph. In vitro culture for 5 days. Semicircular canals have formed (including pigmented cells likely being future dark cells: *arrows)* and ampullar widenings (*asterisks*) are evident. The cochlear part/cochlear hook was excised at explantation. See experimental design











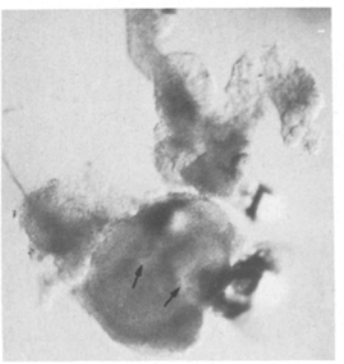


Fig. 4. "Stripped" otocyst, 12.5th gestational day, cultured for 5 days in vitro in normal culture medium. Light micrograph. **A** Lack of normal development; dysmorphogenesis is indicated (*arrows*) by formation of outgrowing extensions. **B** The "stripped" otocyst has remained without any progress in development

Fig. 5A, B. "Stripped" otocyst, 12.5th gestational day, cultured in medium conditioned with **A** mesenchyme and **B** fetal rhombencephalon. Light micrograph. Good development of gross morphology

Fig. 6A, B. Inner ear, 13th gestational day, "stripped" explant cultured in vitro for 5 days. Light micrograph. **A** Co-culture with excess of mesenchyme. **B** Co-culture with excess of fetal rhomboencephalon. Semicircular canals and ampullar widenings (*arrows*) have formed

Results

Otocysts explanted with their mesenchyme showed morphogenesis in vitro with formation of semicircular canals, vestibular organs and some cochlear coiling (Fig. 3). Lack of mesenchyme resulted in only little, if any, development and was often combined with dysmorphogenesis (Fig. 4A). Cultured "stripped" otocysts showed little increase in volume, a delayed development in vitro, if any, and frequent degeneration (Fig. 4B). Preculture of medium with mesenchyme for 24 h resulted in a "conditioned" medium. "Stripped" otocysts cultured in this conditioned medium (mesenchyme removed) showed good development (Fig. 5A). This morphogenesis was at least as good as that achieved when the "stripped" otocyst was co-cultured with excess of mesenchyme (cf. Fig. 6A). Heating of mesenchyme prior to preculture prevented conditioning of the medium and only allowed for an extremely poor morphogenesis, if any at all. Table 1 summarizes the evaluations.

The otocyst development could also be influenced by several adult and fetal tissues other than mesenchyme. Co- or preculture of fetal or adult rhomben-

Table 1. Effect of culture medium on otocyst development

Mesenchyme surrounding otocysts	Culture medium	Morphology	N_s	N_{exp}
Intact	Normal	2.8 ± 0.4	5	3
Removed	Normal	0.5 ± 0.7	4	3
Removed	Normal	Dysmorphogenesis	5	1
Removed	Conditioned[a]	2.5 ± 0.7	11	3
Removed	Conditioned[b]	0.3 ± 0.3	4	2

Otocysts of the 13th gestational day were explanted and cultured with or without adjacent mesenchyme as described in Methods

N_s = number of specimens; N_{exp} = number of independent experiments

[a] Medium conditioned for 24 h with mesenchyme (from the 13th gestational day) as described in Methods

[b] Mesenchyme heated (95° C, 15 min) prior to conditioning

Table 2. Effect of various tissues on otocysts development

Tissue	Morphology	N_s	N_{exp}
Mesenchyme	2.2 ± 0.9	18	6
Forebrain, fetal	1.8 ± 1.4[a]	9	4
Limb, fetal	1.8 ± 1.2	5	2
Rhombencephalon, fetal	2.3 ± 0.6[a]	3	2
Rhombencephalon, adult	2.0 ± 0.9	3	2

Otocysts of the 13th gestational day were explanted and cultured after removal of adjacent mesenchyme ("stripped" otocysts). Tissues were either co-cultured or used for conditioning as described in Methods

[a] Dysmorphogenesis observed in some specimens

cephalon resulted in a good inner ear morphogenesis (Figs. 5B, 6B) as did a corresponding combination with fetal forebrain or fetal limb. However, in several of these explants, in spite of formation of vestibular structures, dysmorphogenesis was evident (Table 2).

Discussion

Several principles of induction have yet to be established for the development of the inner ear: (1) the tissues interacting to trigger differentiation; (2) the mechanism(s) of transmission of inductive signals; (3) the timing of the signals; (4) characterization of induction factors.

This preliminary study indicates that cell contact with the surrounding mesenchyme is not necessary for the differentiation of the otocyst. The precultured, tissue-free "conditioned" medium allows for differentiation of "stripped" otocysts, which otherwise would not show morphogenesis. Thus, a soluble factor (or factors) appears to mediate the permissive signal for otocyst differentiation ("soluble" defined here in terms of the experimental conditions, i.e., contained in the supernatant after centrifugation). The synthesis of the factor(s) and release into the medium is dependent on the presence of intact tissue, since heated mesenchyme does not produce the effect.

The agent can be released from several different tissues, which indicates its non-specific nature, a phenomenon also observed for other organs [8]. However, whether the otocyst factor is identical to other mesenchymal factors described previously remains to be established. Another speculative aspect of inner ear development is a possible interaction with the adenylate cyclase/cAMP system. For example, cyclic AMP is known to exert a positive control on mesenchymal factor-mediated effects in the embryonic pancreas [5].

It is intriguing that adenylate cyclase levels rise sharply in the otocyst around the 13th gestational day, a period of active morphogenesis [2].

In summary, development of the inner ear anlage seems to follow patterns that are well established in other organs. While many questions still remain open, it is evident that a diffusible factor rather than cell contact mediates the permissive influence exerted by the mesenchyme on otocyst morphogenesis.

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