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# Cell and Tissue Interactions

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# Preface

Nature is nowhere wont to reveal her innermost secrets more openly than away from the beaten path, where she shows faint traces of herself.

The interphase between two liquid metals was where the alchemist searched in vain for his gold. It was the interphase between opposing "humours" where medieval physicians believed that life and death decisions were reached, for the detriment or benefit of their suffering patients. In modern science interphases are still important, it is the interphase between similar or disparate substances where we study the physical and chemical processes which give rise to new substances. In these interaction zones, chemical and physical communication determines the static and dynamic nature of all tissues. A multidisciplinary analysis of tissue interaction zones should thus lead to a better understanding of the ways and means by which tissues communicate with each other. The study of cell and tissue interactions has become very popular, and has lately been given a lot of credit, perhaps too much credit, for singlehandedly solving the principle questions in morphogenesis and embryonic development.

While a large number of biologists are seeking answers to how the genetic apparatus of cells function, an equally large number are investigating the circumstances that provoke cells to activate or regulate their genetic apparatus. One of the most fruitful areas of investigation is this rapidly growing field of cell and tissue interactions. The importance of these interactions, which invariably occur at interphases, has been recognized for many years, but only recently have technological advances made it possible to probe them at the molecular level. In just the past few years, results on cell and tissue interactions have produced exciting leads toward our understanding of many interactions, with particular emphasis on developmental events. It is now obvious that the intercellular matrix and the cell surface are implicated in most developmental and regulatory events.

When focusing with greater resolution on the zones of tissue interaction, it is realized that tissue interaction ultimately means cell interaction, and of equal importance, that these interactions are mediated through the interphase of the extracellular matrix. There is a structural and functional relation between the cell periphery, the surface membrane, and the extracellular matrix. Interactions take place directly via the plasma membrane, or via the extracellular matrix components (collagens and proteoglycans), which are intimately associated with the surface membrane.

The chapters in this volume are the result of the Thirty-second Annual Symposium of the Society of General Physiologists, which convened at the Marine Biological Laboratory in Woods Hole, September 12–16, 1976. We felt it would be fruitful to bring together a group of specialists on the extracellular matrix (Section II) with another group whose interests are directed towards the plasma membrane (Section III). As mediators, developmental biologists were helpful to form a tie between the specialists (Section I), reminding the participants that the purpose of both groups of specialists, those concerned with extracellular matrix and those concerned with plasma membranes, was to focus on developmental problems.

Tissue and cell interactions undoubtedly evoke changes in metabolic processes, thus a group of contributors was invited to deal with some basic cellular functions (cell migration and invasion, hormone response, etc.) that will have to be considered in any thorough analysis of tissue interactions (Section IV). The last two contributions illustrate how far we are from understanding the most complicated tissue organization in development, the neural network (Section V). There are very few concepts yet to explain such simple connections as those between nerve and target muscle, let alone the intricate wiring system in the brain.

It is impossible to cover all facets of the broad area of cell and tissue interactions in one volume, although the field is in its infancy and just beginning to unfold. A considerable, and some times arbitrary, selection in subject matters had to be made. Thus this volume should be useful as a primer for biomedical researchers and students who desire an introduction to the biochemistry of matrices and cell surfaces, and how they are implicated in developmental events. For those actively working in one of the many areas covered in these chapters, the bibliographies offer up to date reference material.

The meeting was a delightful educational experience for all who attended as the result of the wide variety of disciplines which were represented. In spite of the wide differences in the disciplines and training of the participants, the ultimate truth of scientific investigation was exemplified; all biological problems are related, and there are still new questions to be asked. Not only chemical, physical, and even biological interphases are necessary for the creation of new products, structures and forms, but the same principles of interphase activity also applies to the creation of new concepts and insights in science. We hope that this book will stimulate the reader to appreciate the usefulness of the interdisciplinary approach, as it did the participants.

Jay Lash
Max M. Burger

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# Directive Versus Permissive Induction: A Working Hypothesis

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Embryonic induction can be considered one of the most central and fascinating problems of developmental biology during the 1930s, when many leading schools of embryology followed in the footsteps of Hans Spemann. Soon, however, the enthusiasm died, and "induction" became almost a dirty word. The decline of this field was due to the slow progress made and the puzzling, seemingly contradictory results obtained by the different groups: Whatever the competent gastrula ectoderm, the classic target of these studies, was exposed to, neural differentiation resembling normal development resulted. Almost any heterotypic tissue, killed tissue fragments, cell-free fractions, various chemical compounds, and even traumatizing physical treatment of the target tissue triggered its neuralization, and it was not possible to find any common denominators in these artificial inducers (see ref. 21). The magic "organizine" transmitting inductive messages from the archenteron roof to the overlying ectoderm remained undiscovered, and many of the outstanding scientists involved in these studies could not resist the temptation to shift their interest to more clearly defined and more easily approachable problems in developmental biology.

Something of the ghost of the "organizine" still seems to affect our thinking, and we are confronted by the constant demand of isolating and characterizing the signal substances transmitting morphogenetic messages in various interactive events. Undoubtedly, this will be our ultimate goal as well as the clarification of the mode of action of such compounds, but in my opinion, our knowledge of the biology of most of the inductive interactions is still too fragmentary for a meaningful molecular approach. Should we search for actual informative, transmissible molecules ultimately interacting with the genome of the target cell? Or would it be more feasible to look for less specific factors which act as nutrients or growth stimulators? Or should we rather consider extracellular compounds with certain spheric configurations responsible for cell orientation and arrangement within a tissue? In most interactive situations, these questions remain unanswered, and there is no reason to believe that various morphogenetic interactions lumped together under the common epithet "induction" should operate through similar mechanisms.

Since the organizers of this section have entitled it "Evidence for Cell-Tissue Interactions" and because it will be followed by many biochemical and molecular approaches to the problem, I have felt it appropriate to open this session with a short review of some recent work on morphogenetic interactions as a biological phenomenon, and to present some evidence for their varying characteristics.

## DIRECTIVE VERSUS PERMISSIVE INFLUENCES

To emphasize the biological diversity of various interactive events during embryogenesis, I have formulated an oversimplified and perhaps naive scheme suggesting two basic types of inductive influences. When an embryonic cell possesses more than one developmental option, the choice between them is affected by extracellular factors, which thus exert a true *directive* action on differentiation. A *permissive* action, on the other hand, refers to a step of development, in which the cell has become committed to a certain pathway, but still requires an exogeneous stimulus to express its new phenotype. According to the scheme (Fig. 1), these two types of influences may alternate during progressive differentiation of the embryonic tissues associated with a gradual restriction of the number of developmental options. Whenever a cell is committed to a new pathway, a directive influence is required and the developmental options become restricted. The following step, the expression of the new phenotype, might require permissive influences, which, however, do not further canalize differentiation. For this, a new directive message is necessary and will, again, be followed by a permissive condition necessary for the expression and stabilization of the cellular phenotype, the growth and proliferation of the tissues, and the maintenance of the organotypic organization. All these interactions fulfill most of the classic definitions for "embryonic induction" or "morphogenetic tissue interactions," but are, indeed, very different in their biological consequences and most probably in their molecular mechanisms.

In what follows, I will illustrate my postulate with some examples of recent work from my own and from several other laboratories.

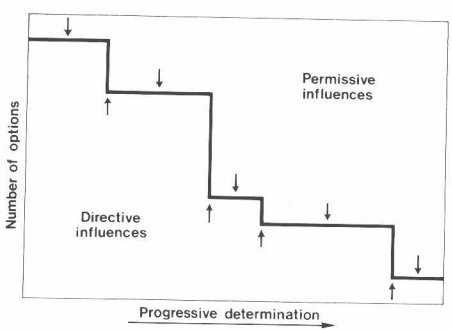


**FIG. 1.** A scheme of the relative importance of directive and permissive interactive events during progressive differentiation as a function of the number of developmental options.

## DIRECTIVE INFLUENCES

### Primary Embryonic Induction

The confusing results of the 1930s mentioned above may be taken as an example of permissive effects, since the cells of the gastrula ectoderm seem to have developed a neurogenic bias during their early development, which can be brought about by a great variety of triggers. Whether all these different treatments act by influencing the intracellular ion compartment as suggested by Barth and Barth (2) or through some other common mechanisms cannot yet be decided. Subsequent studies have, however, conclusively shown that these cells can also be experimentally converted into various cell types normally derived from mesoderm or entoderm (21), thus demonstrating their multiple developmental options. A subsequent restriction of these options during embryogenesis is shown in the following experiment: During early gastrulation, cells of the competent ectoderm can be converted into either neural or mesodermal derivatives by exposing them to various heterotypic inductors or other artificial inducers. At an early neurula stage, they have become committed toward neural elements and can no longer be geared off this pathway. However, they still have several options in the building of various regions of the central nervous system (CNS). If these cells from the anterior region of the neural plate are cultured *in vitro* without additional tissues, they will invariably follow their original destination and form forebrain structures. But if combined *in vitro* to cells of the axial mesoderm or to artificially mesodermalized cells, they develop into constituents of the caudal regions of the CNS (20,26). These options are, again, lost during neurulation and at stage 15 (Triturus), the cells of the neural plate are irreversibly regionalized (25).

Interactive processes during the later stages of neurogenesis are poorly understood, but some stages in the development of the neural crest are known to be guided by interactions of the directive type. One of the most striking examples is provided by the recent work of Le Douarin (*this volume*) showing how the mesenchymal environment directs the differentiation and functional maturation of the ganglioblasts of the autonomous nervous system.

### Determination of the Derivatives of the Integument

Embryonic and, to a lesser degree, adult epidermis represent a tissue with remarkable flexibility and many developmental options, as illustrated by experiments in which the integumental epithelium is combined to various "inductor" tissues. Four examples will be given here. The development of the *cutaneous appendages* in mammalian, avian, and reptilian embryos have been thoroughly analyzed by Sengel and his group (5,22). Using heterospecific or interclass combination techniques, he concludes that the

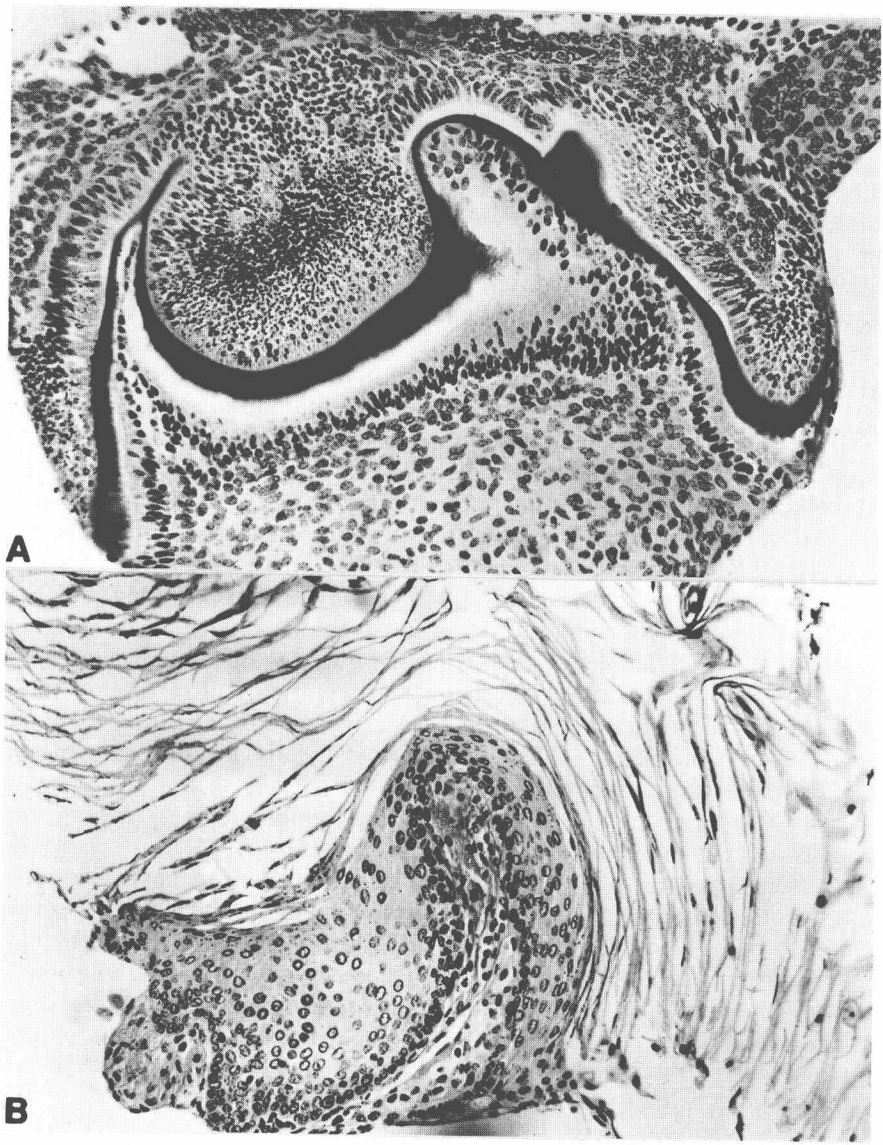


**FIG. 2.** Results of reciprocal combinations between the epithelium and mesenchyme of a tooth rudiment and of nondentogeneous gingival tissue. **A:** A combination of gingival epithelium with mesenchyme of the dental papilla results in the development of a well-shaped tooth rudiment and differentiation of both odontoblasts and ameloblasts. **B:** A combination of tooth epithelium and gingival mesenchyme shows no tooth development and the epithelium displays keratinization. (Courtesy of Dr. Irma Thesleff.)

appendages (hair, feathers, and scales) develop as a result of a two-step dermoepidermal interactive process. During the first step, a nonclass-specific inductive trigger of the dermis determines the size, shape, and distribution of the cutaneous appendages, but the ultimate class (hair, feather, scale) is determined by the genetic constitution of the epidermis. During the second step, which is class-specific, dermal influences guide the final morphogenesis of the appendages.

*Vaginal morphogenesis* is similarly guided by epitheliomesenchymal interactions in a directive way, as reported very recently by Cunha (3,4). Reciprocal combinations of uterine and vaginal tissue components demonstrated that, during the early neonatal period, the epithelial component still differentiates according to the origin of the mesenchymal component combined to it. Subsequently, the epithelia lose their competence to respond to this directive influence, but a permissive type of influence from the mesenchyme is still required for the maintenance of the structure and function of the vaginal epithelium (7).

*Lentoid bodies* developing at heterotypic sites have been detected long ago in Amphibian embryos (24). We have recently reopened this question in regard to Avian embryos and have shown that trunk epidermis of a 2-day-old chick embryo will respond *in vitro* to a directive influence of an optic cup by forming a lentoid body with advanced histodifferentiation. The epidermal origin of these lenses was confirmed by the use of biological nuclear markers and in transfilter experiments (Karkinen-Jääskeläinen, *unpublished*).

*Tooth morphogenesis* has also been shown to be guided by an epitheliomesenchymal interaction between the dental mesenchyme and the enamel epithelium. Differentiation of the latter seems to be triggered by a typical directive induction, as first shown by Kollar and Baird (11), and recently confirmed in organ culture experiments by Thesleff (23) in my laboratory. Reciprocal combinations were made of the presumptive enamel epithelium and dental mesenchyme of a bell stage tooth rudiment, and the epithelium and mesenchyme from nondentogeneous gingival tissue. Tooth epithelium combined to gingival mesenchyme failed to undergo enamel differentiation and the cultures displayed keratinizing squamous epithelium. When gingival, nondentogeneous epithelium was combined to the mesenchyme of the dental papilla, a well-differentiated enamel organ with secretion of enamel proteins was seen (Fig. 2).

## PERMISSIVE INFLUENCES

### Induction of Kidney Tubules

The interactive events behind the formation of the metanephric secretory tubules seem to represent a typical permissive influence. As shown by

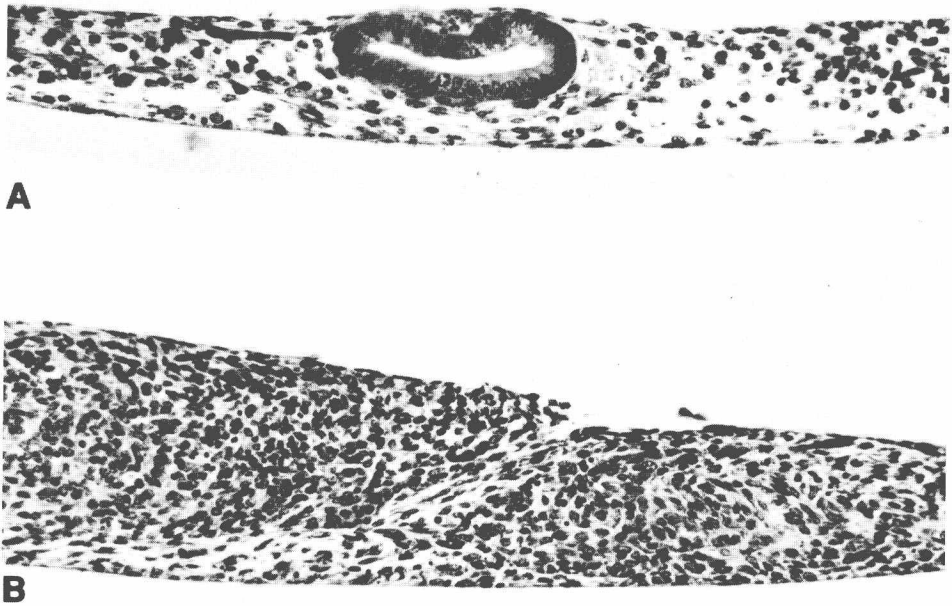


**FIG. 3.** Results of combinations of two potent tubule inductors with nonkidney mesenchyme. **A:** Isolated ureter bud combined with salivary mesenchyme for 5 days *in vitro*. **B:** Spinal cord associated with gastric mesenchyme and cultivated for 4 days. No signs of tubule formation are seen in these heterotypic mesenchymes. (From ref. 17.)

Grobstein and his school (1,10,27), a great variety of embryonal tissues can trigger this morphogenesis in the metanephric mesenchyme, which consequently can be considered "predetermined." Accordingly, other than kidney mesenchyme should not respond to these inductors by tubule formation and this has, in fact, been shown (17). Embryonic salivary, pulmonary, and gastric mesenchymes combined to either the normal tubule inductor, the ureter bud, or to another potent inducer, the spinal cord, failed to show any morphological changes suggestive of tubule formation (Fig. 3). This permissive induction is of relatively short duration and, hence, can be considered a trigger-type stimulus. Judging by our transfilter studies (14) and by recent observations of the development of cell contacts apparently required for this inductive interaction (19), an intercellular communication of 16 hours seems to be sufficient for the triggering of tubule formation, detectable only after some additional 24 hr (*unpublished*). This short interaction, however, seems to be followed by another permissive interaction between the pretubular condensates and the uninduced mesenchyme ensuring the elongation of the tubules (8).