Int. J. Dev. Biol. 33: 149-155 (1989)

The effect of neuronal cells on kidney differentiation

H.SARIOLA[1]*, K. HOLM-SAINIO[1], and S.HENKE-FAHLE[2]

[1] *Department of Pathology, University of Helsinki, Finland*
[2] *Max Planck Institut für Entwicklungsbiologie, Tübingen, Federal Republic of Germany*

ABSTRACT. During embryonic growth, tissue interactions between dissimilar cells are the driving forces of morphogenesis. Although their importance has been well known for over the past 50 years, the molecular background of these interactions has remained unelucidated. The unrecognized heterogeneity of those mesenchymal cells that are involved in the epithelio-mesenchymal tissue interactions may be one reason for this. For example, studies of kidney differentiation show that the metanephric organ rudiment contains more cell-lines than previously thought. Identification of both neural crest- and mesoderm-derived cells in the nephrogenic mesenchyme helps in re-evaluating the biology of the tubule induction. The neural crest-derived cells of the nephric rudiment differentiate into neuronal cells, and later during differentiation some of them are found in the stroma. There is also experimental evidence for the role of these neuronal cells in the morphogenetic tissue interaction.

KEY WORDS: *kidney differentiation, morphogenetic tissue interactions, neuronal development*

Introduction

Organ differentiation proceeds at two overlapping levels: cellular differentiation and tissue morphogenesis (reviews: Saxén, 1977; Wessells, 1977; Kratochwil, 1979; Gurdon, 1988). The major forces that govern tissue morphogenesis are interactions between similar and dissimilar cells. One of the latest definitions for these morphogenetic tissues interactions, or embryonic induction, is that given by John Gurdon (1987), who writes: «Embryonic induction is an interaction between one (inducing) tissue and another (responding) tissue, as a result of which the responding tissue undergoes a change in its direction of differentiation». During organogenesis inductive tissue interactions occur consecutively, but each interaction alone is insufficient to complete differentiation. Recent progress in molecular biology has reinforced the importance of the interaction between dissimilar cells (see Gurdon, 1988), and so the embryonic organizers, discovered by Hans Spemann (1936), are now more pertinent than ever.

Cellular differentiation is controlled by soluble signal substances, like hormones, neurotransmitters and growth factors, and by insoluble mediators, such as the extracellular matrix or those of direct membrane contacts (Edelman, 1983; Hay, 1983). The molecules involved in morphogenetic tissue interactions are less well known. One reason could be that the embryonic organ anlage contains a mixture of cell types and, therefore, the tissue may respond to several simultaneous inductive signals. Consequently, the differentiation «landscape» can be viewed as a complex traffic network with more or less unknown regulations governing cell decisions. To understand a cell's behavior during morphogenesis, we should first of all map its neighbors and relatives. This requires new molecular markers for specific cell types and structural components. With these tools it is then possible to define the inductive signals that a cell is exposed to during differentiation and then, at the molecular level, analyze the way a cell responds to such signals.

The first breakthrough in this line was the characterization of the role of growth factors in the mesoderm induction of *Xenopus* (Kimmelmann and Kirschner, 1987; Slack *et al.*, 1987). Fibroblast growth factor and transforming growth factor β may thus be the first characterized natural long-range morphogenetic signal substances. In mammals, however, the complexity of organogenesis has hindered such progress. With immunohistological and molecular biological tools new cell types have been found among tissues thus far considered to be homogeneous. Now the «traffic network of differentiation» is becoming more and more «jammed». One way to proceed is to ask: «What are the inducing and responding *cell types* involved in the morphogenetic *tissue* interactions?»

Epithelial and stromal differentiation

Most morphogenetic tissue interactions occur between epithelial and mesenchymal cells (see Wessells, 1977). The epithelial cells differentiate to yield cells with specialized functional duties. The mesenchymal cells are first needed to induce this differentiation, and, later as stromal cells, to control the function of the parenchymal epithelia (Kratochwil, 1979; Cunha *et al.*, 1983). During differentiation, a certain cell may have several roles. The cell may first possess a regulatory role as a mesenchymal cell, but later become an epithelial cell. Therefore, its definition is based on its present role, which may subsequently change during differentiation.

The embryonic mesenchyme or stroma may look homogeneous in histology, but can be heterogeneous both histogenetically and functionally. It is composed of cells derived from mesoderm or neural crest. Interspecies transplantation experiments have shown that in the cranial region of the embryo all mesenchymal tissues derive from the neural crest (LeLiévre and Le Douarin, 1975), whereas in other parts of the body only the peripheral nervous system represents neural crest or neural placode derivatives among mesodermal stromal cells (review: Le Douarin, 1986). Although the diverse regulatory roles of the peripheral nervous system are well established in the adult organism, much less is known about its role in morphogenetic tissue

* Address for reprints: Department of Pathology. University of Helsinki, Haartmaninkatu 3, SF -00290 Helsinki, Finland.

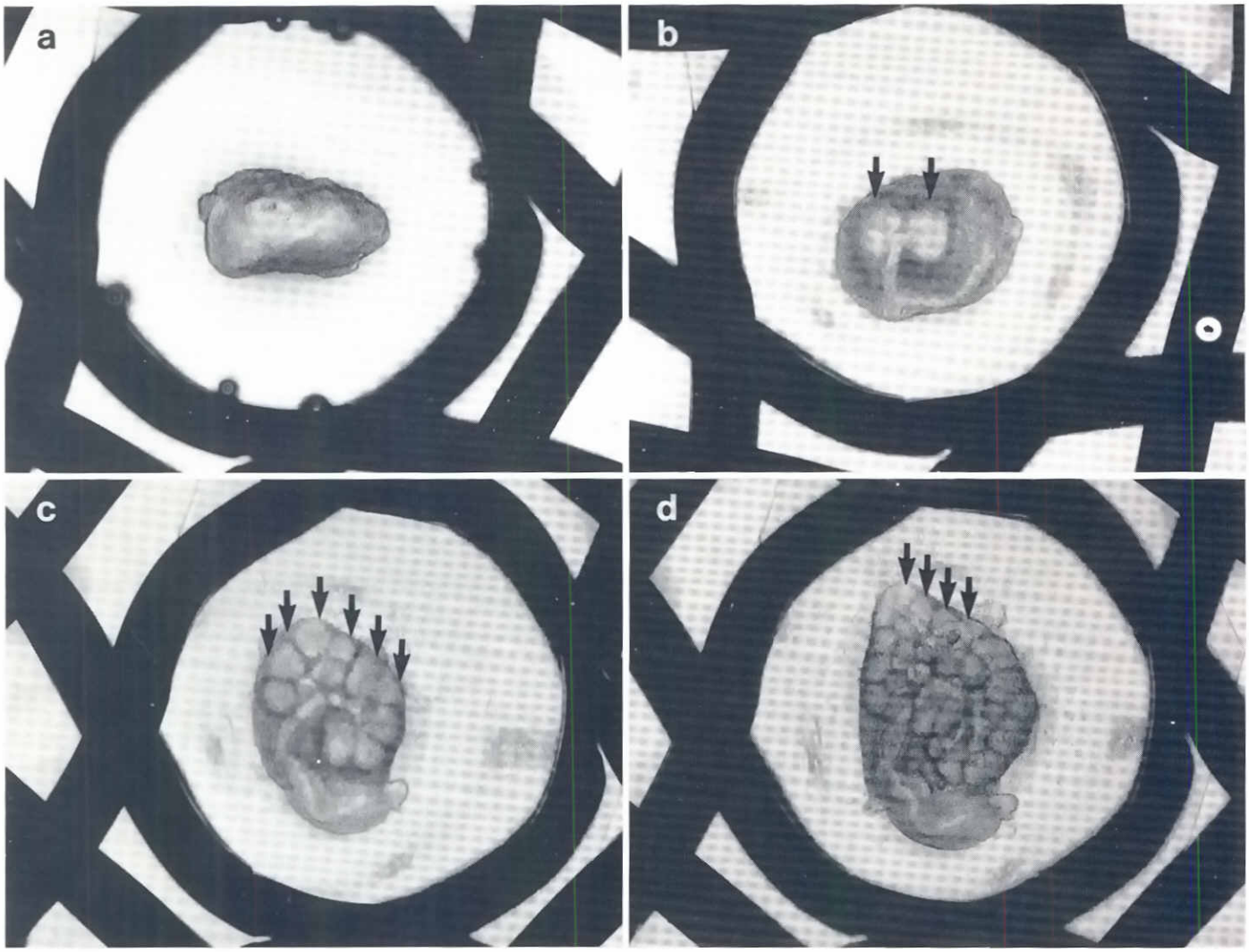

Fig. 1. In vitro *growth and differentiation of an early kidney rudiment separated from an 11-day old mouse embryo. The explant is growing on a Nuclepore filter placed on a metal grid. As with* in vivo, *the ureter bud undergoes several branchings and the nephrogenic mesenchyme splits into small aggregates, marked by arrows. Note the increasing density of the arrows, i.e. smaller size of the aggregates. After two days, the mesenchyme forms the first secretory tubules.*

a) *day 0,* b) *day 1,* c) *day 2,* d) *day 4 of culture.*

interactions. The most fascinating and concrete examples of the extensive influence that the neural crest has on embryogenesis are of course those derived from the studies of chick-quail chimeras (for a comprehensive review, see Le Douarin, 1986). In these interspecies chimeras quail neural crest has been transplanted into chicken. The birds survive long after hatching and have combined features from both chicken and quail.

The stromal control of epithelial differentiation is well established. For instance, heterotypic transplantation and recombination experiments in birds and mouse have shown that the pattern of skin appendices is governed by the dermis (Sengel and Dhouailly, 1976). The nature of the molecules involved in the embryonic induction has been analyzed in some experimental systems. A soluble growth factor synthesized by mesenchymal cells triggers differentiation of pancreatic epithelia (Ronzio and Rutter, 1973). In the developing tooth the interactive tissue components, the epithelial ameloblasts and the mesenchymal odontoblasts are separated by a continuous basement membrane and the inductive signals are contained within the extracellular matrix molecules (Thesleff and Hurmerinta, 1981).

Since the embryonic mesenchyme, as a tissue compartment, may contain several cell types, the crucial question concerns then the specific roles of different mesenchymal cells in the epithelio-mesenchymal tissue interactions. Some functional differences have already been found. For example, in mammary gland development the mesenchyme controls the branching morphogenesis of the mammary epithelium through reciprocal tissue interactions (Dürnberger and Kratochwil, 1980). Within the mesenchyme several cell types have been detected by androgen-binding studies (Wasner *et al.*, 1983). Until such specificities are better characterized in other interactive systems, there is little hope that the molecular background of embryonic induction can be elucidated.

Morphogenesis of the metanephric kidney

The development of the permanent kidney, the metanephros, is a classical example of morphogenetic tissue interactions (for a comprehensive review, see Saxén, 1987). The kidney anlagen is composed of a ureter bud, derived from the Wolffian duct, and a loose mesenchyme that polarizes into tubules. By microdissection, Clifford Grobstein (1955) showed that the presence of the ureter bud is required for tubule induction. When grown in isolation, both the mesenchyme and the ureter bud flatten out, but when recombined, the mesenchyme forms tubules and the ureter bud branches to form the renal collecting system. Grobstein further showed with heterologous recombination experiments that the ureter bud can be replaced by several other embryonic tissues that can induce tubulogenesis (Lombard and Grobstein, 1969; Unsworth and Grobstein, 1970). In contrast to the epithelial ureter bud, most of the heterologous inductors are non-epithelial tissues (spinal cord, brain, jaw, bone), and only few of them contain epithelial cells (submandibular and salivary glands). Lauri Saxén (1970) has shown that only the specific nephrogenic cells, but not mesenchymal cells from other parts of the embryo, can be converted into nephric tubules. The clues for kidney tubulogenesis seem to reside in this specific mesenchymal cell population. Transfilter culture experiments have indicated that the tubule induction requires close cell-to-cell contacts (Wartiovaara *et al.*, 1972; Lehtonen *et al.*, 1975). Unfortunately, every attempt to purify the inducing substances from extracts of the inductor tissues has so far been unsuccessful. Therefore, it was necessary to go one step backwards and re-evaluate the cell-type specificity of kidney tubule induction.

The regulatory cells for tubulogenesis

The starting point for investigation was to establish the regulatory cell type for kidney tubule induction. Since several embryonic tissues can induce tubule formation, it has been concluded that the inducing substance is «widespread and unspecific in nature» (Saxén, 1977). However, not all embryonic tissues, including for instance heart and liver, are inductors. Therefore, it could be possible that the inducing property lies within a certain cell type of the inducing tissues, i.e. that all inducing tissues possess a common cell «denominator».

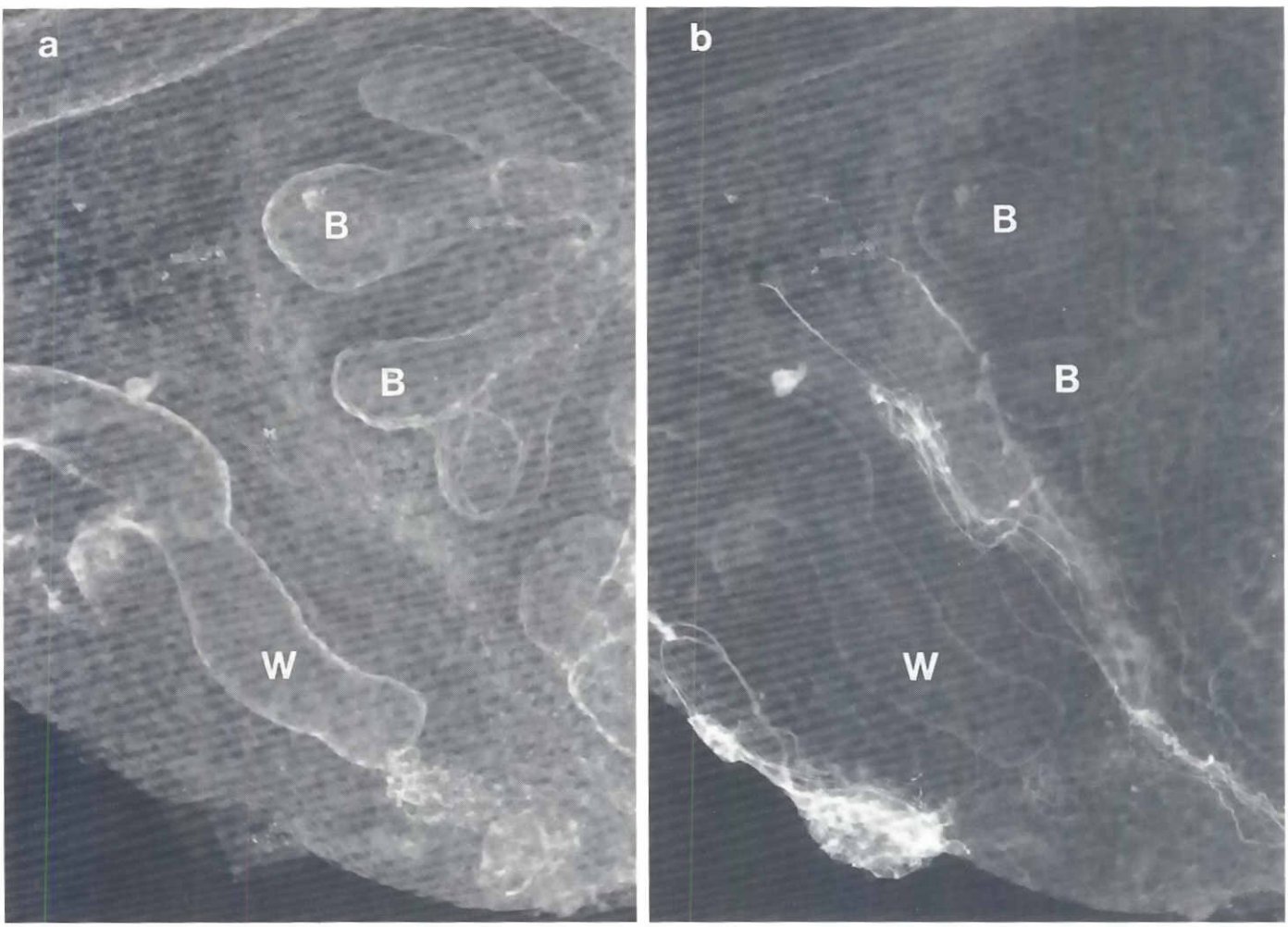

Fig. 2. *Micrograph showing the distribution of laminin (a) and neurofilaments (b) in an 11-day old kidney rudiment that has been growing for 3 days in vitro. Note the stromal localization of neurites and a cluster of neuronal cell bodies in the lower part of the picture.*
Whole mount immunofluorescence staining. Abbreviations: B: tips of the ureter bud, W: Wolffian duct.

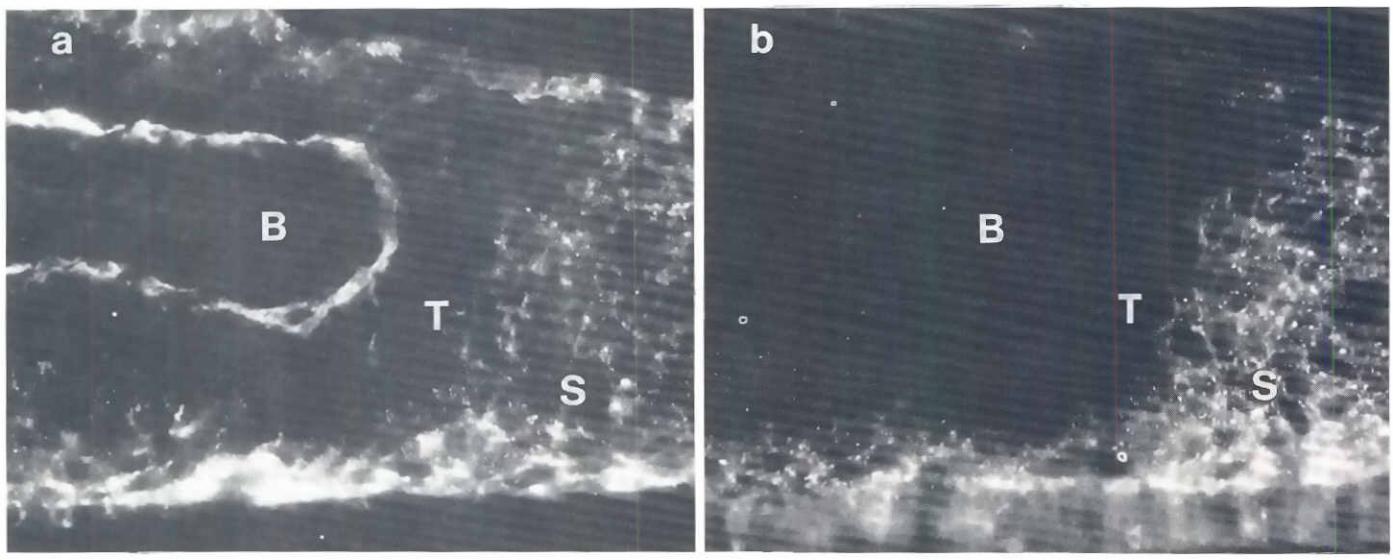

Fig. 3. *Expression of fibronectin (a) and ganglioside GD3 (b) during induction of kidney tubules. The stromal cells (S), but not the tubulogenic cells (T) or the ureter bud cells (B) are labeled by the anti-ganglioside antibody R24.*

The neuronal cells

Embryonic chicken tectum is a potent inductor of kidney tubules. To identify the inducing cell type, cells of the tectum were suspended and cultured as monolayers, on which the nephrogenic mesenchymes were pipetted (Sariola *et al.*, 1989). When grown on these monolayers, the mesenchymes formed epithelial kidney tubules. However, when the neuronal cells were lysed with a neuronal-cell-surface-specific antibody, Q211, and complement, the remaining cells failed to induce tubulogenesis. Control experiments showed that this failure was due to the lack of neurons in the lysed tectal cell cultures, but not due to the lysis system or the antibodies *per se*. Hence, in this experimental system, neurons are required for regulation of tubulogenesis. The known inductors have now been analyzed and all of them contain neuronal cells. However, there are also non-inducing embryonic tissues that also contain these cells, suggesting that not all neurons are able to induce nephrogenesis. Since kidney mesenchymes transplanted *in vivo* in the adult brain become induced, the inducing property cannot be developmentally regulated (Grobstein and Parker, 1958). Rather, it may be related to biochemical differences of neuronal cells.

The second question was whether neurons could be found in the embryonic kidneys. Neuronal elements were not found in the undifferentiated kidney mesenchymes, when sections of early kidney rudiments were analyzed with antibodies against neurofilaments and multisialated gangliosides. However, neuroblasts can not be detected with these antibodies, since both the neurofilaments and multisialated gangliosides are expressed rather late during embryogenesis (Rösner *et al.*, 1985). Therefore, another experimental system was employed. Undifferentiated murine kidney rudiments were cultivated *in vitro* for one to five days (Sariola *et al.*, 1988a). The explants were then analyzed with the neuronal markers. After one day of culture neuronal cells were found in the kidney explants. A cluster of neuronal cell bodies was always seen close to the ureter bud and the neurites grew into the kidney mesenchyme, where they surrounded cell condensates that subsequently formed kidney tubules (Fig. 2). Hence, neuronal cells are present in the kidney rudiment from the initial stage of differentiation onwards, and, therefore, they also may be involved in the *in vivo* induction of kidney tubules.

This hypothesis was supported by a series of perturbation experiments with an antibody, R24, against the cell-surface ganglioside GD 3 (Sariola *et al.*, 1988b). The R24 antibody reacts with several cell types derived from the neural crest (Dippold *et al.*, 1980, 1985; Rösner *et al.*, 1985). In the undifferentiated kidney rudiment, the antibody labels a restricted population of mesenchymal cells, and in older embryonic kidneys, stromal cells (Fig. 3). No staining was obtained in the ureter bud or in the secretory tubules. For the first time, it was possible to detect two different cell populations within the embryonic nephrogenic mesenchyme.

When added to the culture medium, 10 μg/ml of the antibody R24 inhibited kidney differentiation, without causing necrosis of the mesenchymal cells (Sariola *et al.*, 1988b). Other cellular determinants, thought to be active in the cell-to-cell interactions, like the cell-binding domain of fibronectin (Pierschbacher and Ruoslahti, 1984) or uvomorulin (Hyafil *et al.*, 1980), did not prevent tubule formation in pertubation experiments employing either synthetic peptides or antibodies (Vestweber *et al.*, 1985; Klein *et al.*, 1988; Sariola *et al.*, 1988b). The results clearly show that epithelial morphogenesis can be affected by interfering with the stromal compartment. Thus, the stromal cells expressing ganglioside GD3 are involved in regulation of the epithelio-mesenchymal tissue interactions that lead to nephrogenesis.

It is tempting to suggest that the GD3-positive mesenchymal cells of the embryonic kidney could also be derived from the neural crest. Experimental evidence for the neural crest origin of kidney stromal cells comes from the interspecies chimeras (LeDouarin and Teillet, 1974). When a piece of quail crest was transplanted into chicken, some quail cells migrated into the stroma of the metanephric kidneys. As this finding did not fit into the classic view of kidney differentiation at that time, the result was considered an experimental artefact. Our recent

results favor the alternative that this is not the case, and further show that the possibly neural crest-derived stromal cells may have a central role in governing epithelial differentiation.

The epithelial cells

Nothing is known about the early predetermination of the nephrogenic mesenchyme. The undifferentiated mesenchymal cells express interstitial matrix molecules, like fibronectin and interstitial collagens (Ekblom *et al.*, 1981), but also some molecules of the basement membrane-type matrix, namely the B chain of laminin (Holm *et al.*, 1988). The cells do not express type IV collagen or the whole laminin molecule composed of both the A and B chains. In conclusion, at least in morphology and extracellular matrix composition, the nephrogenic mesenchyme resembles loose mesenchyme in other parts of the embryo. Yet, these are the only cells in the body that can be converted into nephric tubules (Saxén, 1970).

Influenced by the inductor cells, the mesenchyme condenses and then converts into nephric tubules. The extracellular matrix shifts from the mesenchymal type towards the basement membrane type (Ekblom *et al.*, 1980, 1981a). Simultaneously, thetubules elongate and segregate into glomeruli, proximal and distal tubules, all of which express new molecular markers and functional properties (Ekblom *et al.*, 1981b). After the induction period tubular morphogenesis proceeds independent of the inductor tissue. In transfilter cultures the inductor can be removed after 12 h of co-culture and, thereafter, the first cells are irreversibly determined for tubulogenesis (Saxén and Lehtonen, 1978).

The ureter bud, traditionally regarded as the inductor of kidney differentiation, branches and elongates to form the collect-

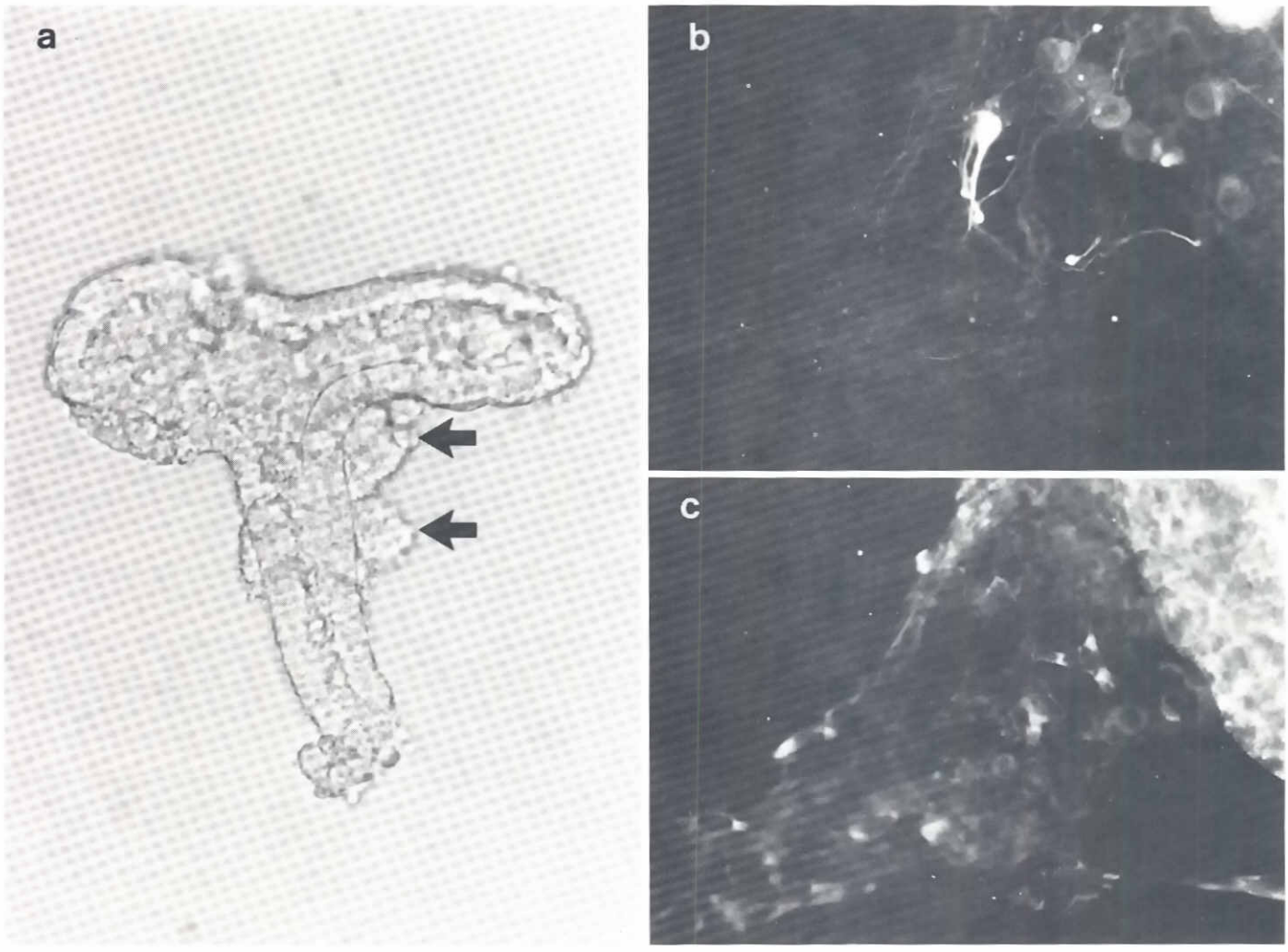

Fig. 4. *Demonstration of neuronal cells attached to the ureter bud that has been microsurgically separated from the undifferentiated kidney rudiment.*
a) *The ureter bud has been removed mechanically, and thereafter treated in a pancreatin-trypsin solution to detach the remaining mesenchymal cells. Even after this treatment, some non-epithelial cells are seen attached to the bud cells (arrows).*
b) *When the ureter bud is cultivated* in vitro *for 2 days, the cells form a monolayer, on which neuronal cells grow. Immunofluorescence staining with anti-neurofilament antibody.*
c) *Double immunofluorescence label with the anti-laminin antibody.*

ing system of the kidney (Osathanond and Potter, 1966). It is as difficult to find growth controllers for the nephrogenic mesenchyme as it is to find factors that could induce branching of the ureter bud. Ureter bud cells can be cultivated for several weeks and they grow like sheets typical of epithelial cell monolayers. In hanging drops, in which the adhesion to the substratum is excluded, the bud cells form a balloon. Thus, by an unknown mechanism, epithelial polarization and branching of the ureter bud seems to depend solely on the nephrogenic mesenchymal cells (Grobstein, 1955).

In light of our recent results, the role of the ureter bud in tubule induction should be re-defined. What is clear is that it is not indispensable for tubule induction, because the ureter bud can be removed and replaced by several other inducing tissues (Grobstein 1955; Lombard and Grobstein, 1969; Unsworth and Grobstein, 1970). However, one could still argue that, in normal kidney differentiation, the ureter bud may have an inducing function. Although this has not been disproved, we have shown that the microsurgically-isolated ureter buds always contain neuronal cells (Sariola *et al.*, 1988a; Fig. 4). Until the potential role of these neurons in kidney morphogenesis is thoroughly investigated, the effect of the ureter bud in tubule induction remains unsettled.

Meanwhile it is safe to conclude that the ureter bud has other better defined functions. Its repetitive branching leads to the splitting of the nephrogenic mesenchyme (see Fig. 1) and to the formation of the urinary collecting system (Osathanond and Potter, 1966). In experimental recombinations with heterologous inductors, the pattern of tubules remains, for the most part, disorganized (Saxén *et al.*, 1968). Hence, the two definite functions for the ureter bud are to govern the strict organization of the nephric tubules, and to form the collecting system that carries urine from the kidney. Finally, the secretory tubules and the ureter bud fuse to complete the nephron formation.

Concluding remarks

Tissue interactions are the crucial forces that govern morphogenesis. However, the attempts to purify morphogenetic signal substances have been desperately disappointing. One reason may be that the complexity and heterogeneity of the early organ rudiments have been under-estimated. There are neither homogeneous epithelial cells nor a uniform mesenchyme taking part in the inductive events. The cells that look morphologically similar may be both functionally and histogenetically different. Until these differences are better characterized, there is little hope that we shall be able to understand the control mechanisms of organogenesis or to identify the molecular background of embryonic induction.

References

CUNHA, G.R., CHUNG, L.W.K., SHANNON, J.M., TAGUCHI, O. and FUJII, H. (1983). Hormone-induced morphogenesis and growth: role of mesenchymal-epithelial interactions. *Recent Prog. Horm. Res. 39:* 559-597.

DIPPOLD, W.G., DIENES, H.P., KNUTH, A. and MEYER ZUM BUSCHENFELDE, K.H. (1985). Immunohistochemical localization of ganglioside GD3 in human malignant melanoma, epithelial tumors, and normal tissue. *Cancer Res. 45:* 3699-3705.

DIPPOLD, W.G., LLOYD, K.O., LI, L.T., IKEDA, H., OETTGEN, H.F. and OLD, L.J. (1980). Cell surface antigens of human malignant melanoma: definition of six antigenic systems with mouse monoclonal antibodies. *Proc. Natl. Acad. Sci. (USA) 77:* 6114-6118.

DURNBERG, H., and KRATOCHWIL, K. (1980). Specificity of tissue interaction and origin of mesenchymal cells in androgen response of the embryonic mammary gland. *Cell 19:* 465-471.

EDELMAN, G.M. (1983). Cell adhesion molecules. *Science 219:* 450-457.

EKBLOM, P., ALITALO, K., VAHERI, A., TIMPL, R. and SAXÉN, L. (1980). Induction of a basement membrane glycoprotein in embryonic kidney: possible role of laminin in morphogenesis. *Proc. Natl. Acad. Sci. (USA) 77:* 485-489.

EKBLOM, P., LEHTONEN, E., SAXÉN, L. and TIMPL, R. (1981a). Shift in collagen type as an early response to induction of the metanephric mesenchyme. *J. Cell. Biol. 89:* 276-283.

EKBLOM, P., MIETTINEN, A., VIRTANEN, I., WAHLSTROM, T., DAWNEY, A. and SAXÉN, L. (1981b). *In vitro* segregation of the metanephric nephron. *Dev. Biol. 84:* 88-95.

GROBSTEIN, C. (1955). Inductive interaction in the development of the mouse metanephros. *J. Exp. Zool. 130:* 319-339.

GROBSTEIN, C. and PARKER, G. (1958). Epithelial tubule formation in mouse metanephrogenic mesenchyme transplanted *in vivo*. *J. Natl. Cancer Inst. 2:* 107-119.

GURDON, J.B. (1987). Embryonic induction - molecular prospects. *Development 99:* 238-306.

GURDON, J.B. (1988). The origin of cell-type differences in early embryos. *Cell Differ. Devel. (Supp.) 25:* 1-6.

HAY, E.D. (1983). Cell and extracellular matrix: their organization and mutual dependence. *Modern Cell Biol. 2:* 509-548.

HOLM, K., RISTELI, L. and SARIOLA, H. (1988). Differential expression of the laminin A and B chains in chimeric kidneys. *Cell Differ. 24:* 223-228.

HYAFIL, F., MORELLO, D., BABINET, C. and JACOB, F. (1980). A cell-surface glycoprotein involved in the compaction of embryonal carcinoma cells and cleavage stage embryos. *Cell 21:* 927-939.

KIMMELMAN, D. and KIRSCHNER, M. (1987). Synergistic induction of mesoderm by FGF and TGF-β and the identification of an mRNA coding for FGF in the early *Xenopus* embryo. *Cell 51:* 869-877.

KLEIN, G., LANGEGGER, M., GORIDIS, C. and EKBLOM, P. (1988). Neural cell adhesion molecules during embryonic induction and morphogenesis of the kidney. *Development 102:* 749-761.

KRATOCHWIL, K. (1979). Embryonic induction. In *Cell Interaction and Development: Molecular Mechanisms* (Ed. K.M. Yamada). John Wiley Inc., New York, pp. 99-122.

LE DOUARIN, N.M. (1986). Cell lineage segregation during peripheral nervous system ontogeny. *Science 231:* 1515-1522.

LE DOUARIN, N. and TEILLET, M.-A.M. (1974). Experimental analysis of the migration and differentiation of neuroblasts of the autonomic nervous system and of neuroectodermal derivatives, using a biological cell marking technique. *Dev. Biol. 41:* 162-184.

LEHTONEN, E., WARTIOVAARA, J., NORDLING, S. and SAXÉN, L. (1975). Demonstration of cytoplasmic processes in Millipore filters permiting kidney tubule induction. *J. Embryol. Exp. Morphol. 33:* 187-203.

LELIÈVRE, C.S. and LE DOUARIN, N.M. (1975). Mesenchymal derivatives of neural crest: analysis of chimaeric quail and chick embryos. *J. Embryol. Exp. Morphol. 34:* 125-154.

LOMBARD, M.-N. and GROBSTEIN, C. (1969). Activity of various embryonic and postembryonic sources for induction of kidney tubules. *Dev. Biol. 19:* 41-45.

OSATHANONDH, V. and POTTER, E. (1966). Development of human kidney as shown by microdissection. III. Formation and interrelationships of collecting tubules and nephrons. *Arch. Pathol. 76:* 290-302.

PIERSCHBACHER, M.D. and RUOSLAHTI, E. (1984). Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature (London) 309:* 30-33.

RONZIO, R.A. and RUTTER, W.J. (1973). Effects of a partially-purified factor from chick embryos on macromolecular synthesis of embryonic pancreatic epithelia. *Dev. Biol. 30:* 307-320.

RÖSNER, H., AL-AQTUM, M. and HENKE-FAHLE, S. (1985). Developmental expression of GD3 and polysialogangliosides in embryonic chicken nervous systems reacting with monoclonal antiganglioside antibodies. *Dev. Brain Res. 18:* 85-95.

SARIOLA, H., AUFDERHEIDE, E., BERNHARD, H., HENKE-FAHLE, S., DIPPOLD, W., and EKBLOM, P. (1988b). Antibodies to cell surface ganglioside GD3 perturb inductive epithelial-mesenchymal interactions. *Cell 54:* 235-245.

SARIOLA, H., EKBLOM, P. and HENKE-FAHLE, S. (1989). Embryonic neurons as *in vitro* inducers of kidney differentiation. *Dev. Biol. In press.*

SARIOLA, H., HOLM, K. and HENKE-FAHLE, S. (1988a). Early innervation of the metanephric kidney. *Development.* 104: 589-599.

SAXÉN, L. (1970). Failure to demonstrate tubule induction in a heterologous mesenchyme. *Dev. Biol. 23:* 511-523.

SAXÉN, L. (1977). Directive versus permissive induction: a working hypothesis. In *Cell and Tissue Interaction* (Eds. J.W. Lash and M.M. Burger). Raven Press, New York, pp. 1-9.

SAXÉN, L. (1987). *Organogenesis of the Kidney.* Cambridge University Press, Cambridge.

SAXÉN, L., KOSKIMIES, O., LAHTI, A., MIETTINEN, H., RAPOLA, J. and WARTIOVAARA, J. (1968). Differentiation of kidney mesenchyme in an experimental model system. In *Advances in Morphogenesis, Vol. 7* (Eds. M. Abercombie, J. Brachet and T. King). Academic Press, London, pp. 251-193.

SAXÉN, L. and LEHTONEN, E. (1978). Transfilter induction of kidney tubules as a function of the extent and duration of intercellular contacts. *J. Embryol. Exp. Morphol. 47:* 97-109.

SENGEL, P. and DHOUAILLY, D. (1976). Tissue interactions in amniote skin development. In *Cell Interactions in Differentiation* (Eds. M. Karkinen- Jääskeläinen and L. Saxén). Academic press, London, pp 153-170.

SLACK, J.M.W., DARLINGTON, J.K., HEATH, J.K. and GODSAVE, S.F. (1987). Mesoderm induction in early *Xenopus* embryos by heparin-binding growth factors. *Nature (London) 326:* 197-200.

SPEMANN, H. (1936). *Experimentelle Beiträge zu einer Theorie der Entwicklung.* Springer, Berlin.

THESLEFF, I. and HURMERINTA, K. (1981). Tissue interactions in tooth development. *Differentiation 18:* 756-788.

UNSWORTH, B. and GROBSTEIN, C. (1970). Induction of kidney tubules in mouse metanephrogenic mesenchyme by various embryonic mesenchymal tissues. *Dev. Biol. 21:* 547-556.

VESTWEBER, D., KEMLER, R. and EKBLOM, P. (1985). Cell-adhesion molecule uvomorulin during kidney development. *Dev. Biol. 112:* 213-221.

WARTIOVAARA, J., LEHTONEN, E., NORDLING, S. and SAXÉN, L. (1972). Do membrane filters prevent cell contacts? *Nature (London) 238:* 407-408.

WASNER, G., HENNERMANN, I., and KRATOCHWIL, K. (1983). Ontogeny of mesenchymal androgen receptors in the embryonic mouse mammary glands. *Endocrinology 113:* 1771-1780.

WESSELLS, N.K. (1977). *Tissue Interactions and Development.* W.A. Benjamin, Inc., Menlo Park.