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Review

Aspects of morphogenesis and cytodifferentiation of endoderm offered by organ culture techniques

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ABSTRACT In this article, I will summarize the results obtained in my laboratory from the study of organogenesis since 1970. The results have shown region-specific directive influence of mesenchymes and reactivity of endoderms including those of the allantois, yolk sac, digestive tract and urogenital sinus. It was also shown that morphogenesis can take place without coupling cytodifferentiation in heterologous recombination experiments.

KEY WORDS: *organogenesis, morphogenesis, cytodifferentiation, mesenchymal-epithelial interactions, extraembryonic membrane*

In the early 1930s, the tissue culture method for analyzing mechanisms of cellular differentiation and proliferation was first introduced in Japan by Professor Takashi Fujii of the University of Tokyo. He noted the usefulness of this technique and studied extensively the effect of growth regulating factors on heart cells of embryonic chick *in vitro* (1934). He also devoted his time to the study of neural and mesodermal induction in *Triturus* embryos (1944). This line of study was later developed successfully by M. Asashima and coworkers and led to the discovery of activin A as a factor involved in mesodermal induction in amphibian embryos (Asashima *et al.*, 1990). Y. Kato, a student of Professor Fujii, studied in the laboratories of Professors Hamburger and Ebert in the United States, and in 1969 he demonstrated a clear scale induction in the chick chorionic ectoderm recombined and cultured with tarsometatarsal dermis (1969). In the same year as the campus dispute at the University of Tokyo came to an end, Professor Fujii sent me to Professor Wolff's laboratory in France, where I engaged in the study of metaplastic differentiation of the proamnionic ectoderm under the influence of dorsal dermis of embryonic chick using organ culture technique (Mizuno, 1970, 1972). Interested in the reactivity of the extraembryonic membranes, I returned to Tokyo with the idea that we could demonstrate the induction of digestive-tract epithelia in the allantoic endoderm or yolk-sac endoderm if the endoderm was combined and cultured with digestive-tract mesenchymes. As to the inducing action of the digestive-tract mesenchyme, Sigot (1963) demonstrated that 5-day gizzard mesenchyme can induce gizzard-type differentiation in proventricular endoderm of chick embryos.

Heterotypic differentiation of the endoderm derived from extraembryonic membranes

The endoderm isolated from the allantois of 3- to 4-day chick embryos was combined with various mesenchymes of the digestive tract of day-6 and older embryos and cultivated in vitro by the method of Wolff and Haffen (1953), or on the chorioallantoic membrane (Yasugi and Mizuno, 1974; Yasugi, 1979), or the allantoic endoderm was implanted into the presumptive digestive area of young embryos (Gumpel-Pinot *et al.*, 1978; Yasugi, 1984). The results showed that the allantoic endoderm can undergo heterotypic morphogenesis under the influence of the digestive-tract mesenchymes: with esophageal mesenchyme it became stratified squamous; with proventricular mesenchyme it formed proventricular-type complex glands; with gizzard mesenchyme it formed tubular glands with mucus; with small-intestinal mesenchyme it formed villi covered with a simple columnar epithelium. However, in terms of cytodifferentiation, the allantoic endoderm was found to be not always completely susceptible to the influence of the mesenchymes: with proventricular mesenchyme, induced complex proventricular-type glands never expressed pepsinogen (Yasugi, 1984; Yasugi *et al.*, 1987) or produced pepsinogen mRNA (Hayashi *et al.*, 1988), although, with small-intestinal mesenchyme, the induced simple columnar epithelium expressed brush-border antigens and sucrase. However, the allantoic endoderm also expressed both brush-border antigens and sucrase when it was recombined and cultured with non-intestinal digestive-tract mesenchymes (Matsushita, 1984). Goblet cells also appeared in the allantoic endoderm when it was cultured with various mesenchymes of the digestive tract (Mizuno and Yasugi, 1973; Yasugi, 1976; Gumpel-Pinot *et al.*, 1978). Moreover, a large number of allantoic endoderm cells were found to differentiate into goblet cells when the endoderm was cultured with urogenital-sinus mesenchyme of fetal rats (Mizuno, unpublished results) Thus, the

Abbreviations used in this paper: Tfm, testicular feminization mutant; TH, thyroid; OE, esophagus; PV, proventriculus; GZ, gizzard; DU, duodenum; PR, pancreas; SI, small intestine; PSBP, prostatic steroid-binding protein.

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allantoic endodermal cells were found to possess an intrinsic tendency to differentiate into intestinal epithelial cells, although the endoderm can easily undergo heterotypical morphogenesis under the influence of various digestive-tract mesenchymes. The reactivity of the isolated yolk-sac endoderm to the digestive-tract mesenchymes was then investigated. The results showed that it differentiates into yolk-sac parenchyme expressing cysteine lyase at first, but later it undergoes heterotypic morphogenesis under the influence of digestive-tract mesenchymes. However, a large number of endodermal cells differentiated into intestine-type columnar cells with the brush-border structure and its enzymes and also goblet cells. They also possess self-differentiation potency into yolk-sac parenchymal cells. This was proven when the yolk-sac endoderm was cultured alone *in vitro* (Masui, 1981, 1982; Mizuno and Masui, 1982).

These results indicate that the endoderms of the extraembryonic membranes are susceptible to the control of the digestive-tract mesenchymes with respect to morphogenesis but they are less susceptible with regard to functional cytodifferentiation. Moreover, they possess a strong tendency to differentiate into an intestine-type epithelium. In contrast, the ectoderms of the extraembryonic membranes seem "neutral" and considerably competent to react to the influence of the dermis with respect to both morphogenesis and cytodifferentiation: chorionic ectoderm forms feathers and scales and expresses keratins specific to them under the influence of dorsal and shank skin dermis, respectively (Dhouailly *et al.*, 1978); amnionic ectoderm forms typical feather filaments expressing feather-specific keratins under the influence of dorsal skin dermis (Mizuno *et al.*, 1989, 1990b). During the course of these studies, we proceeded to study the endodermal-mesodermal interaction in organogenesis of digestive organs.

Self-differentiation potency of the endoderm

First of all, we examined the stage of determination and the area possessing self-differentiation potency of the endoderm by cultivating the isolated endoderm alone *in vitro* using the method of Wolff (1961). The endodermal pieces were successfully cultured without any contamination from mesenchymal cells (Mizuno and Sumiya, 1974), and a series of experiments revealed the stage and the area of appearance of the self-differentiation potency of the endoderms of the pharynx, thyroid glands, esophagus, gizzard, small intestine and large intestine by light microscopy (Sumiya and Mizuno, 1974, 1976; Sumiya, 1976) and later by electron microscopy (Mizuno and Ishizuya, 1982; Ishizuya-Oka, 1983). As to the dorsal pancreatic endoderm, the self-differentiation potency to express glucagon appears at Hamburger Hamilton (HH) stage 11, while glucagon is first detectable in the dorsal pancreatic endoderm of normal chick embryos at HH stage 16 (Sumiya and Mizuno, 1987). However, the stage of appearance of this potency may largely depend on the culture conditions: in the presumptive hepatogenic area, the hepatogenic potency of the endoderm was detected in the anterior half of the endoderm of chick embryos after the 5-somite stage when the endoderm was cultured alone, while it could be detected at the 1-somite stage if the endodermal fragment was cultured in combination with non-hepatic heterologous mesenchymes of the splanchnopleure (Fukuda, 1979; Fukuda-Taira, 1981). The mesenchymes may offer suitable conditions permitting the committed endoderm to differentiate into hepatocytes. In any case, the results indicate that the endoderm of each region along the digestive tract is committed to possess the self-differentiation potency in the course of development. However, in the cultivation of the isolated endoderm, complex morphogenesis such as the formation of proventricular glands or intestinal villi did not take place (Fig. 1).

Then we proceeded to examine whether the self-differentiation potency of the endoderm is stable and whether the digestive-tract mesenchyme can play a directive role in morphogenesis and cytodifferentiation during digestive-tract formation.

Susceptibility of the digestive-tract endoderm to the directive influence of the mesenchymes

A large number of studies were performed in my laboratory at the University of Tokyo. Until the mid-1970s, we used exclusively morphological criteria to analyze the mesodermal-endodermal interactions, but later we also examined the expression of organ-specific digestive enzymes or detected their mRNAs. The results have been summarized in other review papers (Mizuno and Yasugi, 1990; Yasugi and Mizuno, 1990; Yasugi, 1993). Briefly, the digestive-tract mesenchymes were proven to possess region-specific inductive action for morphogenesis and cytodifferentiation of the endoderm, and the endoderm was found to be more susceptible to the mesenchymes when the endoderm was excised from younger embryos (Ishizuya-Oka and Mizuno, 1984, 1992). The degree of heterotypic differentiation of the endoderms cultured with mesenchymes varied due to the combination of tissues, but the endoderms tended to be more susceptible when they were cultured with the mesenchymes excised from a region near the one where the respective endoderms were obtained (Fig. 1). One example is that the proventricular mesenchyme could induce proventricular-type complex glands in the endoderms of not only the proventricus but also the esophagus, gizzard or small intestine (Yasugi and Mizuno, 1978; Takiguchi *et al.*, 1986, 1988a), but neither pepsinogen expression (Yasugi *et al.*, 1985) nor transcription of pepsinogen mRNA (Hayashi *et al.*, 1988) occurred in the heterotypically induced complex glands in the small-intestinal endoderm. Another example is that the gizzard mesenchyme always inhibits both proventricular gland formation and the production of pepsinogen even in the proventricular epithelium (Mizuno *et al.*, 1986b; Takiguchi *et al.*, 1988b; Urase and Yasugi, 1993). This article will not deal with the studies concerning the development of functional hepatocytes and biliary epithelial cells of the mouse (Shiojiri, 1984; Shiojiri and Mizuno, 1993), nor with the mesenchymal control of salivary gland morphogenesis (Nogawa and Mizuno, 1981).

We postulated a hypothesis that the developmental fate of the digestive-tract endoderm may be determined by both self-differentiation potency of the endoderm and the directive influence of the digestive-tract mesenchymes (Mizuno, 1975). The results obtained since then were consistent with the hypothesis, showing that the developmental fate of the endoderms can be changed to some extent under the directive influence of associated heterologous mesenchymes. The discovery of the negative control of gizzard and esophageal mesenchymes for pepsinogen expression in the proventricular endoderm was also worthy of attention. (For review, see Yasugi, 1993). A study on the characterization of the epithelia lining the junction of the stomach and intestine also offered us valuable suggestions (Matsushita, 1991).

These results were obtained mainly in the embryonic chick, but plasticity of the digestive-tract endoderm in mammalian embryos seems to be restricted: Kedinger *et al.* (1988) reported that 14-day

fetal rat intestinal endoderm cultured with skin fibroblasts still forms villi and expresses aminopeptidase, a brush-border enzyme. We also demonstrated that the developmental fates of the endoderms of forestomach and glandular stomach of 11.5-day mouse embryos are not influenced by heterologous mesenchymes (Fukamachi *et al.*, 1979). These results may indicate that the endoderms have already been previously determined by the stages when the endoderms were excised. On the other hand, duodenal fibroblasts of newborn rats can still exert inducing effects on the gizzard endoderm of the embryonic chick and the endoderm differentiates into a typical intestinal epithelium with a brush border expressing its specific enzymes (Haffen *et al.*, 1982, 1983).

Characterization of androgen-activated urogenital mesenchymes

Dr. A. Glucksmann of the Institute of Animal Physiology and Dr. I. Lasnitzki of the Strangeways Research Laboratory, Cambridge visited our laboratory for the first time in 1974, and joint research began on the effects of androgens on the development of the os penis of the mouse and of the urogenital sinus of the embryonic rat. In this article, we will not deal with the os penis, but some members of our group made further studies along this line (Glucksmann *et al.*, 1976; Murakami and Mizuno, 1984, 1986; Murakami, 1987; Uchiyama and Mizuno, 1988, 1989).

It has been well known that the differentiation of the prostate gland depends on the presence of testicular hormones, but the exact role of androgens on the induction of the gland was unknown. First, we cultivated rat urogenital sinuses *in vitro* in the presence or absence of testosterone or dihydrotestosterone, and found that the androgens could initiate prostatic bud formation in the sinuses *in vitro*, and that the exposure to androgens for 1 day was enough to elicit prostatic buds in both female and male sinuses (Lasnitzki and Mizuno, 1977). Brief androgen treatment of the fetal rat urogenital sinus showed that the rate of incorporation into the mesenchyme rose steeply during the first 12 h which then slowed down, and heavy labeling of mesenchymal cells was observed close to the epithelium (Takeda *et al.*, 1986). The results of the epithelial-mesenchymal recombinant experiments showed that it is the androgen-treated urogenital-sinus mesenchyme, and not heterotypic mesenchymes, that is essential for the initiation of prostatic buds in untreated epithelium (Cunha, 1976; Lasnitzki and Mizuno, 1979). Studies of testosterone metabolism in isolated epithelium and mesenchyme revealed that mesenchyme as well as epithelium convert testosterone to its active metabolite, 5-α-dihydrotestosterone (Bard *et al.*, 1979). These results made it unlikely that the inductive property of the mesenchyme is determined by the presence of 5-α-reductase. The requirement for androgen receptors in the mesenchyme was suggested by the inability of the mesenchyme from androgen receptor-deficient *Tfm* mice to induce prostatic bud formation in normal epithelium (Lasnitzki and Mizuno, 1980). Then, we examined binding sites of [^{3}H]testosterone and [^{3}H]dihydrotestosterone in the fetal rat urogenital sinus using steroid autoradiography. The results showed that in the fetal sinus nuclear labeling was restricted to the mesenchyme surrounding the epithelium which showed no nuclear labeling. Androgens were found to be incorporated into the epithelium only after 10 days *post partum* coinciding with the onset of its functional differentiation (Takeda and Mizuno, 1984; Takeda *et al.*, 1985). Later, Takeda and Chang (1991) reported the existence of an androgen receptor protein and its mRNA in the fetal

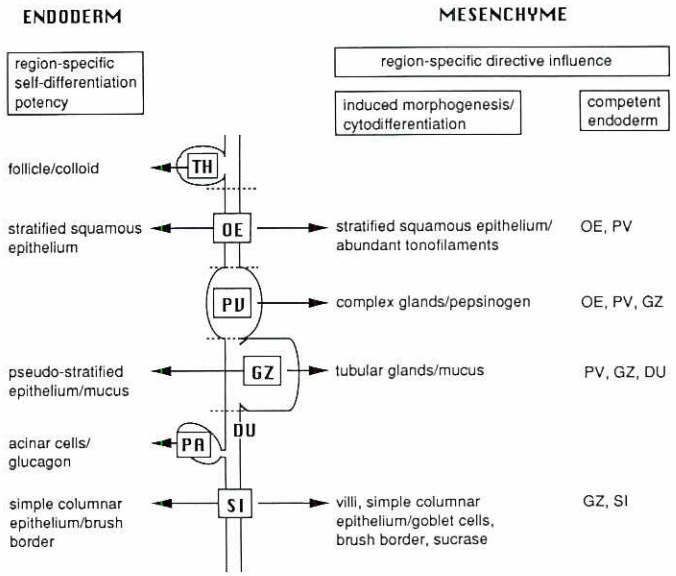

Fig. 1. Self-differentiation potency of the digestive-tract endoderms and region-specific directive influence of the digestive-tract mesenchymes.

sinus endoderm, and they suggested that the receptor protein may not be able to bind to androgens until postnatal development. Experiments using *Tfm* mouse fetuses showed that normal mesenchyme could induce the morphogenesis of prostatic glands in the *Tfm* epithelium, but the mesenchyme failed to induce androgen receptors in the epithelium (Mizuno *et al.*, 1986a, 1988). Therefore, the incorporation of androgens into the epithelium was found to be unnecessary for the morphogenesis of prostatic buds. During the course of these studies, we noted the mosaicism of the *Tfm*-gene active and inactive mesenchymal cells in the X^{Tfm}/X^{+}heterozygous female mouse fetuses. In these fetuses, only half of the mesenchymal cells of the urogenital sinus were androgen-receptor positive, and receptor-positive and -negative cells formed small irregular patches (Takeda *et al.*, 1987b). When the heterozygous sinuses were cultured *in vitro* in the presence of androgens, the sinuses formed prostatic buds projecting into the surrounding mesenchyme, in which almost all the mesenchymal cells in close vicinity to the buds were receptor-positive. In other words, the mosaicism of the mesenchymal cells disappeared around the developing prostatic buds (Takeda *et al.*, 1987a). During these morphogenetic processes, mesenchymal cells were found to contact directly with epithelial cell processes which penetrated the basal lamina (Hironaka, 1985). Thus the importance of the role of androgen-activated sinus mesenchyme on the morphogenesis of the prostatic glands has been indicated. Then, a question arises as to whether the activated mesenchyme can also direct the cytodifferentiation of the prostatic epithelium. We noticed three lobes of the prostate: ventral, lateral and dorsal lobes. Among these lobes, only the ventral lobe expresses a prostatic steroid-binding protein (PSBP). PSBP is a tetramer which consists of 2 subunits, C1-C2 and C2-C3. Northern analysis with a complementary DNA probe encoding C1 peptide revealed that the mRNAs were detected exclusively in the ventral prostate but not in the dorsal prostate or in other organs. *In situ* hybridization with a

complementary RNA probe demonstrated that the transcripts were found only in the epithelium of the ventral prostate. We then carried out tissue-recombination experiments to examine whether the transcription of the PSBP-C1 gene in the epithelium is affected by the surrounding mesenchyme. The results were as follows. The transcripts of the PSBP-C1 gene existed in the ventral or dorsal epithelium only when an epithelium was cultured with the ventral mesenchyme, but the transcripts were never found in any epithelium cultured with the dorsal mesenchyme, indicating that the ventral mesenchyme is a potent inducer for ventral prostate-specific cytodifferentiation (Takeda *et al.,* 1990). From these results, it is likely that the urogenital sinus mesenchyme exerts its directive influence not only on the morphogenesis of the prostatic buds but also on the prostatic cytodifferentiation of the urogenital sinus epithelium. However, it was also shown that the urogenital sinus mesenchyme can induce prostate-like glands in the urinary bladder epithelium of fetal and adult rats and the epithelium lost bladder-specific antigens and expressed several prostatic epithelial antigens, though it hardly expressed androgen receptors (Suematsu *et al.,* 1988; Suematsu and Mizuno, 1991). Furthermore, it showed limited ability to transcribe the PSBP-C1 gene (Takeda *et al.,* 1990). Therefore, it is conceivable that the androgen-activated urogenital sinus mesenchyme is a potent inducer for the morphogenesis of the prostatic glands and also for the cytodifferentiation of epithelia; however, the transcription of some prostate-specific genes is restricted in heterologous epithelia.

Reactivity of the urogenital sInus endoderm to the heterologous mesenchyme

The urogenital sinus endoderm itself seems to possess no self-differentiation potency, which most digestive-tract endoderms possess, irrespective of the presence or absence of androgens. The endoderm failed to undergo prostatic morphogenesis under the influence of heterotypic mesenchymes such as urinary bladder mesenchyme or dermis in the presence of androgens (Lasnitzki and Mizuno, 1979).

We examined, then, the reactivity of the urogenital sinus endoderm when it was combined and cultured with rat digestive tract mesenchymes. Studies demonstrated that the forestomach and glandular stomach mesenchymes induced morphogenesis of a typically stratified squamous epithelium and of glandular stomach-type glands in the recombinants, respectively. However, the induced glands expressed neither rat pepsinogen nor rat ventral prostatic antigen. They did not transcribe mRNA for the PSBP-C1 even in the presence of an ample concentration of androgens, although the androgens were found to stimulate mitotic activity of the sinus endodermal cells, especially in the recombinants with glandular stomach mesenchyme (Mizuno *et al.,* 1990a). The results may indicate that some mesenchymes such as those of the stomach can elicit morphogenesis in the urogenital sinus epithelium, but most genes in the epithelium remain inactivated even under the directive influence of heterologous mesenchymes.

Perspectives

In this article, I summarized the results obtained mainly from my laboratory at the Zoological Institute of the University of Tokyo, concerning the endodermal-stromal interactions in the organogenesis of the digestive tract and the prostate glands. At least 3 steps may be considered in these processes. First, the endoderms are determined and acquire certain regional specificities in early stages of the development. Consequently, a mosaicism appears in the endoderm. Secondly, mesenchymes acquire regional specificities and exert their directive, sometimes inhibitory, influence on morphogenesis and functional endodermal differentiation. Finally, endoderms respond to the mesenchymal directions or stimulations to attain the final differentiation.

The state of determination of endoderm could be recognized by the cultivation of the endoderm alone or with 'indifferent' mesenchymes. Recently, gizzard endoderm of young chick embryos was found to be determined to express pepsinogens (Urase and Yasugi, unpublished results). This was demonstrated by culturing the endoderm with lung mesenchyme. Although we do not know the basis of the determination, it should be related to the problem of gene regulation concerning the initial selection of active and inactive genes. This problem may be solved based on the research dealing with the molecular analysis of functional differentiation of the endoderms. Morphogenesis itself is a complex phenomenon, but it is certain that the primed or hormone-activated mesenchymes exert their region-specific effects on mitosis, cellular movement, or changes in the arrangement of endodermal cells. We wonder how the mesenchyme exerts such region-specific effects. It is still possible that the predetermined endoderm exerts an effect on the relevant mesenchyme, but our experiments on this possibility failed to produce sufficient results, because the mesenchyme we used often possessed a certain intrinsic activity. Some support of the concept of the determination of regional specificities may come from the expression patterns of homeobox genes, which have been brought to light by recent research. Another important finding in our experimental studies is that morphogenesis is not always followed by cytodifferentiation when a certain endoderm, such as the extraembryonic or small intestinal endoderm, is cultured with the heterologous mesenchyme possessing full inducing activities (Mizuno and Yasugi, 1990). The uncoupling of morphogenesis and cytodifferentiation which have often been observed during the course of our studies will lead us to molecular analyses of the state of the competence of the endoderm. Genes required for organogenesis will also be studied in the future. However, the "classical" analyses of developmental phenomena, such as the tissue interactions in organogenesis, may also be important in offering new targets for attack on the molecular level.

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References

ASASHIMA, M., NAKANO, H., SHIMADA, K., KINOSHITA, K., ISHII, K., SHIBAI, H. and UENO, N. (1990). Mesodermal induction in early amphibian embryos by activin A (erythroid differentiation factor). *Roux Arch. Dev. Biol. 198:* 330-335.

BARD, D.R., LASNITZKI, I. and MIZUNO, T. (1979). Metabolism of testosterone by the epithelium and mesenchyme of the rat urogenital sinus. *J. Endocrinol. 83:* 211-218.

CUNHA, G.R. (1976). Epithelial-stromal interactions in development of the urogenital tract. *Int. Rev. Cytol. 47:* 137-194.

DHOUAILLY, D., ROGERS, G.E. and SENGEL, P. (1978). The specification of feather and scale protein synthesis in epidermal-dermal recombinations. *Dev. Biol. 65:* 58-68.

FUJII, T. (1934). Action of kephalin on the growth of fibroblasts *in vitro*. *J. Fac. Sci., Tokyo Imp. Univ. Sec. IV. 3:* 491-494.

FUJII, T. (1944). Experimental studies on neural and mesodermal inductions in the early development of *Triturus*. *J. Fac. Sci., Tokyo Imp. Univ. Sec. IV 6:* 451-475.

FUKAMACHI, H., MIZUNO, T. and TAKAYAMA, S. (1979). Epithelial-mesenchymal interactions in differentiation of stomach epithelium in fetal mice. *Anat. Embryol. 157:* 151-160.

FUKUDA, S. (1979). The development of hepatogenic potency in the endoderm of quail embryos. *J. Embryol. Exp. Morphol. 52:* 49-62.

FUKUDA-TAIRA, S. (1981). Hepatic induction in the avian embryo: specificity of reactive endoderm and inductive mesoderm. *J. Embryol. Exp. Morphol. 63:* 111-125.

GLUCKSMANN, A., OOKA-SOUDA, S., MIURA-YASUGI, E. and MIZUNO, T. (1976). The effect of neonatal treatment of male mice with antiandrogens and of females with androgens on the development of the os penis and os clitoridis. *J. Anatomy 121:* 363-370.

GUMPEL-PINOT, M., YASUGI, S. and MIZUNO, T. (1978). Differenciation d'epitheliums endodermiques associés au mesoderme splanchnique. *C.R. Seances Acad. Sci. (D) Paris 286:* 117-120.

HAFFEN, K., KEDINGER, M., SIMON-ASSMANN, P.M. and LACROIX, B. (1982). Mesenchyme-dependent differentiation of intestinal brush-border enzymes in the gizzard endoderm of the chick embryo. In *Embryonic Development,* Part B. A.R. Liss, New York, pp. 261-270.

HAFFEN, K., LACROIX, B., KEDINGER, M. and SIMON-ASSMANN, P.M. (1983). Inductive properties of fibroblastic cell cultures derived from rat intestinal mucosa on epithelial differentiation. *Differentiation 23:* 226-233.

HAYASHI, K., YASUGI, S. and MIZUNO, T. (1988). Pepsinogen gene transcription induced in heterologous epithelial-mesenchymal recombinations of chicken endoderms and glandular stomach mesenchyme. *Development 103:* 725-732.

HIRONAKA, N. (1985). Direct contact between epithelial and mesenchymal cells during the development of prostatic buds in the fetal rat. *J. Fac. Sci., Univ. Tokyo, Sec. IV 16:* 13-19.

ISHIZUYA-OKA, A. (1983). Electron microscopical study of self-differentiation potency in the chick embryonic endoderm cultured *in vitro*. *Roux Arch. Dev. Biol. 192:* 171-178.

ISHIZUYA-OKA, A. and MIZUNO, T. (1984). Intestinal cytodifferentiation *in vitro* of chick stomach endoderm induced by the duodenal mesenchyme. *J. Embryol. Exp. Morphol. 82:* 163-176.

ISHIZUYA-OKA, A. and MIZUNO, T. (1992). Demonstration of sucrase immunoreactivity of the brush border induced by duodenal mesenchyme in chick stomach endoderm. *Roux Arch. Dev. Biol. 201:* 389-392.

KATO, Y. (1969). Epithelial metaplasia induced on extraembryonic membranes. I. Induction of epidermis from chick chorionic epithelium. *J. Exp. Zool. 170:* 229-252.

KEDINGER, M., SIMON-ASSMANN, P., BOUZIGES, F. and HAFFEN, K. (1988). Epithelial-mesenchymal interactions in intestinal epithelial differentiation. *Scand. J. Gastroenterol. 151(Suppl.):* 62-69.

LASNITZKI, I. and MIZUNO, T. (1977). Induction of the rat prostate gland by androgens in organ culture. *J. Endocrinol. 74:* 47-55.

LASNITZKI, I. and MIZUNO, T. (1979). Role of the mesenchyme in the induction of the rat prostate gland by androgens in organ culture. *J. Endocrinol. 82:* 171-178.

LASNITZKI, I. and MIZUNO, T. (1980). Prostatic induction: Interaction of epithelium and mesenchyme from normal wild-type mice and androgen-insensitive mice with testicular feminization. *J. Endocrinol. 85:* 423-428.

MASUI, T. (1981). Differentiation of the yolk-sac endoderm under the influence of the digestive-tract mesenchyme. *J. Embryol. Exp. Morphol. 62:* 277-289.

MASUI, T. (1982). Intestinalization of the area-vitellina endoderm cultured in association with digestive-tract mesenchymes. *J. Embryol. Exp. Morphol. 72:* 117-124.

MATSUSHITA, S. (1984). Appearance of brush border antigens and sucrase in the allantoic endoderm cultured in recombination with digestive-tract mesenchyme. *Roux Arch. Dev. Biol. 193:* 211-218.

MATSUSHITA, S. (1991). Differentiation of the epithelium lining the junctional region of the gizzard and duodenum of the chick embryo. *Zool. Sci. 8:* 521-532.

MIZUNO, T. (1970). Induction de germes plumaires, *in vitro,* dans l'épithélium proamniotique du Poulet, associé au derme de la peau dorsale. *C.R. Seances Acad. Sci. (D) Paris 271:* 2027-2030.

MIZUNO, T. (1972). Epidermal metaplasia of proamnionic epithelium induced by dorsal skin dermis in the chick embryo. *J. Embryol. Exp. Morphol. 27:* 199-213.

MIZUNO, T. (1975). Une hypothese sur l'organogenèse du tractus digestif. *C.R. Soc. Biol. 169:* 1096-1098.

MIZUNO, T. and ISHIZUYA, A. (1982). Étude au microscope electronique de la différenciation *in vitro* de l'endoderme d'intestine grêle de jeune embryon d'Oiseau, associé ou non à son mésenchyme. *C.R. Soc. Biol. 176:* 580-584.

MIZUNO, T. and MASUI, T. (1982). Différenciation hétérotypique de l'endoderme de l'aire vitelline de la Caille, associé a différents mésenchymes du tractus digestif de l'embryon de Poulet. *C.R. Soc. Biol. 176:* 885-888.

MIZUNO, T. and SUMIYA, M. (1974). Utilisation de la membrane vitelline de l'oeuf de poule pour l'étude de la différenciation de l'épithélium endodermique. *Ann. Biol. 13:* 111-115.

MIZUNO, T. and YASUGI, S. (1973). Différenciation *in vitro,* de l'épithélium de l'allantoïde associé à différents mésenchymes du tractus digestif, chez l'embryon de Poulet. *C.R. Seances Acad. Sci. (D) Paris 276:* 1606-1611.

MIZUNO, T. and YASUGI, S. (1990). Susceptibility of epithelia to directive influence of mesoderms during organogenesis: uncoupling of morphogenesis and cytodifferentiation. *Cell Differ. Dev. 31:* 151-159

MIZUNO, T., FURIHATA, C., TAKEDA, H., SUEMATSU, N. and LASNITZKI, I. (1990a). Heterotypic induction of stomach-like gland in the urogenital sinus endoderm under the influence of stomach mesenchyme of foetal rats. *Roux Arch. Dev. Biol. 198:* 483-487.

MIZUNO, T., KITAMURA,K., SAITO, M. and TANEMURA, S. (1990b). Epithelial metaplasia induced on amnionic ectoderm by the dermis of chicken embryos. *Dev. Growth Differ. 32:* 171-177.

MIZUNO, T., SAITO, M., TANEMURA, S. and KITAMURA, K. (1989). Induction des écailles dans l'ectoderme amniotique du Poulet, associé au derme de la peau tarsométatarsienne. *C.R. Soc. Biol. 183:* 551-554.

MIZUNO, T., TAKEDA, H. and SUEMATSU, N. (1986a). Récepteurs d'androgènes de l'épithélium au cours de l'induction des glandes prostatiques à partir de l'épithélium du sinus urogenital de la Souris Tfm-mutant. *C.R. Soc. Biol. 180:* 593-595.

MIZUNO, T., TAKEDA, H., SUEMATSU, N., HIRONAKA, N. and LASNITZKI, I. (1988). Absence of androgen receptors in the prostatic glandular epithelium derived from testicular feminization mutant (Tfm) mice. *Zool. Sci. 5:* 999-1006.

MIZUNO, T., YASUGI, S. and TAKIGUCHI, K. (1986b). Formation de glandes et expression de pepsinogène dans l'endoderme de gésier sous l'influence du mésenchyme proventriculaire chez l'embryon de Poulet. *C.R. Soc. Biol. 180:* 113-116.

MURAKAMI, R. (1987). Autoradiographic studies of the localization of androgen-binding cells in the genital tubercles of fetal rats. *J. Anat. 151:* 209-219.

MURAKAMI, R. and MIZUNO, T. (1984). Histogenesis of the os penis and os clitoridis in rats. *Dev. Growth Differ. 26:* 419-426.

MURAKAMI, R. and MIZUNO, T. (1986). Proximal-distal sequence of development of the skeletal tissues in the penis of rat and the inductive effect of epithelium. *J. Embryol. Exp. Morphol. 92:* 133-143.

NOGAWA, H. and MIZUNO, T. (1981). Mesenchymal control over elongating and branching morphogenesis in salivary gland development. *J. Embryol. Exp. Morphol. 66:* 209-221.

SHIOJIRI, N. (1984). The origin of intrahepatic bile duct cells in the mouse. *J. Embryol. Exp. Morphol. 79:* 25-39.

SHIOJIRI, N. and MIZUNO, T. (1993). Differentiation of functional hepatocytes and biliary epithelial cells from immature hepatocytes of the fetal mouse *in vitro*. *Anat. Embryol. 187:* 221-229.

SIGOT, M. (1963). Induction de la formation de glycogène dans l'épithélium de proventricule par le mésenchyme de gésier chez l'embryon de Poulet. *C.R. Acad. Sci. Paris, Ser. D 256:* 4970-4971.

SUEMATSU, N. and MIZUNO, T. (1991). Analysis of epithelial protein profiles of prostatic glands induced heterotypically in the bladder epithelium of rats. *Dev. Growth Differ. 33:* 379-389.

SUEMATSU, N., TAKEDA, H. and MIZUNO, T. (1988). Glandular epithelium induced from urinary bladder epithelium of the adult rat does not show full prostatic cytodifferentiation. *Zool. Sci. 5:* 385-395.

SUMIYA, M. (1976). Differentiation of the digestive tract epithelium of the chick embryo cultured *in vitro* enveloped in a fragment of the vitelline membrane, in the absence of mesenchyme. *Roux Arch. Dev. Biol. 179:* 1-17.

SUMIYA, M. and MIZUNO, T. (1974). Différenciation de l'endoderm de tractus digestif de l'embryon de Poulet cultivé *in vitro* dans une membrane vitelline, en l'absence du mésenchyme. *C.R. Seances Acad. Sci. (D) Paris 278:* 1529-1532.

SUMIYA, M. and MIZUNO, T. (1976). Development of the autodifferentiation potencies in the endoderm of the chick embryo. *Proc. Jpn. Acad. 52:* 587-590.

SUMIYA, M. and MIZUNO, T. (1987). Étude immunohistologique de l'éxpression de glucagon dans l'endoderme pancréatique dorsal chez l'embryon precoce de Poulet. *C.R. Soc. Biol. 181:* 718-721.

TAKEDA, H. and CHANG, C. (1991). Immunohistochemical and *in situ* hybridization analysis of androgen receptor expression during the development of the mouse prostate gland. *J. Endocrinol. 129:* 83-89.

TAKEDA, H. and MIZUNO, T. (1984). Incorporation des androgènes au moment de la cytodifférenciation de l'épithélium prostatique chez le Rat. *C.R. Soc. Biol. 178:* 572-575.

TAKEDA, H., LASNITZKI, I. and MIZUNO, T. (1986). Analysis of prostatic bud induction by brief androgen treatment in the fetal rat urogenital sinus. *J. Endocrinol. 110:* 467-470.

TAKEDA, H., LASNITZKI, I. and MIZUNO, T. (1987a). Change of mosaic pattern by androgens during prostatic bud formation in X^{Tfm}/X^{+} heterozygous female mice. *J. Endocrinol. 114:* 131-137.

TAKEDA, H., MIZUNO, T. and LASNITZKI, I. (1985). Autoradiographic studies of androgen-binding sites in the rat urogenital sinus and postnatal prostate. *J. Endocrinol. 104:* 87-92.

TAKEDA, H., SUEMATSU, N. and MIZUNO, T. (1990). Transcription of prostatic steroid binding protein (PSBP) gene is induced by epithelial-mesenchymal interactions. *Development 110:* 273-282.

TAKEDA, H., SUZUKI, M., LASNITZKI, I. and MIZUNO, T. (1987b). Visualization of X-chromosome inactivation mosaicism of *Tfm* gene in X^{Tfm}/X^{+} heterozygous female mice. *J. Endocrinol. 114:* 125-129.

TAKIGUCHI, K., YASUGI, S. and MIZUNO, T. (1986). Gizzard epithelium of chick embryos can express embryonic pepsinogen antigen, a marker protein of proventriculus. *Roux Arch. Dev. Biol. 195:* 475-483.

TAKIGUCHI, K., YASUGI. S. and MIZUNO, T. (1988a). Developmental changes in the ability to express embryonic pepsinogen in the stomach epithelia of chick embryos. *Roux Arch. Dev. Biol. 197:* 56-62.

TAKIGUCHI, K., YASUGI, S. and MIZUNO, T. (1988b). Pepsinogen induction in chick stomach epithelia by reaggregated proventricular mesenchymal cells *in vitro*. *Dev. Growth Differ. 30:* 241-250.

UCHIYAMA, H. and MIZUNO, T. (1988). Sexual dimorphism in the genital tubercle of the duck: studies on the normal development and histogenesis. *Zool. Sci. 5:* 823-832.

UCHIYAMA, H. and MIZUNO, T. (1989). Sexual dimorphism in the genital tubercle of the duck: analysis by sex-steroid administration and steroid autoradiography. *Zool. Sci. 6:* 71-81.

URASE, K. and YASUGI, S. (1993). The induction and inhibition of epithelial differentiation by the mixed cell aggregates of the mesenchyme from the chicken embryonic digestive tract. *Dev. Growth Differ. 35:* 33-40.

WOLFF, Et. (1961). Utilisation de la membrane vitélline de l'oeuf de poule en culture organotypique. I. Téchnique et possibilités. *Dev. Biol. 3:* 767-786.

WOLFF, Et. and HAFFEN, K. (1953). Sur une méthode de culture d'organes embryonnaires *in vitro*. *Tex. Rep. Biol. Med. 10:* 463-472.

YASUGI, S. (1976). Différenciation foncitionnelle et morphologique de l'endoderme allantoïdien sous l'influence du mésenchyme proventriculaire chez l'embryon d'oiseau. *C.R. Seances Acad. Sci. (D) Paris 283:* 179-182.

YASUGI, S. (1979). Chronological changes in the inductive ability of the mesenchyme of the digestive organs in the avian embryos. *Dev. Growth Differ. 21:* 343-348.

YASUGI, S. (1984). Differentiation of allantoic endoderm implanted into the presumptive digestive area in avian embryos. A study with organ-specific antigens. *J. Embryol. Exp. Morphol. 80:* 137-153.

YASUGI, S. (1993). Role of epithelial-mesenchymal interactions in differentiation of epithelium of vertebrate digestive organs. *Dev. Growth Differ. 35:* 1-9.

YASUGI, S. and MIZUNO, T. (1974). Heterotypic differentiation of chick allantoic endoderm under the influence of various mesenchymes of the digestive tract. *Roux Arch. Dev. Biol. 174:* 107-116.

YASUGI, S. and MIZUNO, T. (1978). Differentiation of the digestive tract epithelium under the influence of the heterologous mesenchyme of the digestive tract in the bird embryos. *Dev. Growth Differ. 20:* 261-267.

YASUGI, S. and MIZUNO, T. (1990). Mesenchymal-epithelial interactions in the organogenesis of digestive tract. *Zool. Sci. 7:* 159-170.

YASUGI, S., HAYASHI, K., TAKIGUCHI, K., MIZUNO, T., MOCHII, M., KODAMA, R., AGATA, K. and EGUCHI, G. (1987). Immunological relationships among embryonic and adult chicken pepsinogens: a study with monoclonal and polyclonal antibodies. *Dev. Growth Differ. 29:* 85-91.

YASUGI., S., MATSUSHITA, S. and MIZUNO, T. (1985). Gland formation induced in the allantoic and small-intestinal endoderm by the proventricular mesenchyme is not coupled with pepsinogen expression. *Differentiation 30:* 47-52.