Morphogenetic features in the tail region of the rat embryo

LJILJANA KOSTOVIĆ-KNEŽEVIĆ*, SREĆKO GAJOVIĆ and ANTON ŠVAJGER

Department of Histology and Embryology, Faculty of Medicine, University of Zagreb, Republic of Croatia, Yugoslavia

ABSTRACT The secondary (direct) body formation is a mechanism of development in which morphogenesis of various organs occurs directly from a mass of undifferentiated mesenchymal cells (blastema) without previous formation of germ layers. It is characteristic of the posterior end of the embryonic body, *i.e.* of the tail bud of tailless and the tail of tailed mammals. Development of the neural tube occurring by this mechanism (secondary neurulation) has been previously explained. We investigated the morphogenetic mechanism by which two other axial structures in the rat tail develop: the tail gut and the notochord. Both structures develop from an axial condensation of undifferentiated mesenchymal cells (tail cord) of tail bud origin. The tail gut forms in a similar way to the secondary neural tube: cells in the ventral part of the tail cord elongate, acquire an apicobasal polarity and form a rosette-like structure around a lumen in the centre. The notochord forms by detachment of a group of cells of the tail cord dorsally to the developing tail gut. The peculiarities of this morphogenetic mechanism in comparison with those in other parts of the embryo are discussed. Causal (including evolutionary) explanations of this mechanism are ruled out.

KEY WORDS: *secondary body formation, tail gut, notochord, neural tube, rat*

Introduction

For more than 200 years ideas concerning early organogenesis in vertebrate embryos have been based on the theory of germ layers. According to this concept the compact cell mass of the blastula rearranges during gastrulation into three definitive germ layers from which, by various morphogenetic processes, the rudiments of embryonic organs develop. However Holmdahl (1935) pointed out that this mechanism occurs only in the anterior part of the embryonic body while in the posterior part organogenesis starts directly from an undifferentiated, mesenchyme-like cell mass (the tail bud). This basic viewpoint has recently been confirmed.

According to the most recent view, early organogenesis in vertebrate embryos occurs in two different ways:
a) primary (indirect) body formation (by gastrulation, *i.e.* formation of definitive germ layers);
b) secondary (direct) body formation (from the tail bud mesenchyme, without previous formation of germ layers).

Although the secondary body formation was studied in all classes of vertebrates several decades ago (see Peter, 1941, 1951 for review) the problem of the borderline between the two morphogenetic processes still remains an enigma. Suggested positions of this borderline vary from the midlevel of the embryonic body toward the base of the tail (Holmdahl, 1935; Peter, 1951; Criley, 1969; Nakao and Ishizawa, 1984).

In tailless mammals, including humans, the tail is an abortive structure that soon undergoes regression and finally disappears (Fallon and Simandl, 1978; Ledley, 1982; Muller and O'Rahilly, 1987).

In tailed mammals the tail bud elongates and persists. In both categories, as well as in avian embryos, tail development comprises formation of several structures among which the axial ones (neural tube, notochord, tail gut) are particularly intriguing with regard to the various morphogenetic mechanisms by which they arise (Schoenwolf, 1981; Gajovic *et al.*, 1990) . The process of secondary neural tube formation (secondary neurulation) in chick and mouse embryos at the cellular level was elucidated by Schoenwolf and his colleagues (see Schoenwolf and Smith, 1990 for review). In our laboratory we compared these findings with the process of secondary neurulation in rat embryo, but we paid special attention to the morphogenesis of two other axial structures in the growing tail of the rat embryo: the notochord and the tail gut (Fig. 1, 2). This we did by analyzing serial transversal semithin sections through the tail of 10- to 15-day rat embryos (the equivalent age of the mouse embryo: 8- to 14-day). Cytological details were analyzed by electron microscopy.

Tail bud

During gastrulation the posterior part of the embryo contains, as the only axial structure, the primitive streak with the Hensen's node at its rostral end. The tail bud (French: *bourgeon tronco-caudal*, German: *Rumpfschwanzknospe)* forms beneath the remnants of

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*Address for reprints: Department of Histology and Embryology, Faculty of Medicine, Salata 3, P.O. Box 166, 41001 Zagreb, Republic of Croatia, Yugoslavia. FAX: 38-41-424.001

the primitive streak of the neurulating embryo, at the early forelimb bud stage. It is definitively formed and replaces the primitive streak when the embryo has reached hindlimb bud stage (Tam, 1984).

The tail bud is a mass of undifferentiated mesenchymal cells which originate from the remnants of the primitive streak and Hensen's node, the posterior end of the neural groove and the posterior coelomic mesodermal epithelium. The neural tube, the notochord, the hind gut and the presomitic lateral mesoderm merge with the tail bud mesenchyme at their posterior ends (Butcher, 1929; Jolly and Feréster-Tadié, 1936; Peter, 1941; Tam *et al.*, 1982; Nakao and Ishizawa, 1984). The elongation of the tail occurs by proliferation of cells at the proximal end of the tail bud (Nakao and Ishizawa, 1984; Tam, 1984). All axial and paraaxial structures within the tail are derivatives of the tail bud mesenchyme.

Secondary neurulation

In avian and mammalian embryos the neural tube develops in two distinct ways. The ectodermal neural plate represents the initial phase of the primary neurulation in which the neural tube forms by fusion of neural folds. The caudal end of the neural tube (tail region) forms by a different morphogenetic mechanism: secondary neurulation, which begins at the time of closure of the caudal neuropore (Ikeda, 1930; Klika and Jelínek, 1969; Schoenwolf, 1977, 1978, 1981, 1984; Schoenwolf and Delongo, 1980; Schoenwolf and Powers, 1987; Griffith and Wiley, 1989, 1990; Schoenwolf and Smith, 1990). This mechanism begins with the aggregation (condensation of dorsally positioned mesenchymal cells) of the tail bud which results in the appearance of an axially positioned medullary cord. The next step is the elongation and apicobasal polarisation of the medullary cord cells and their radial, rosette-like arrangement around a central cavity (cavitation). Intercellular junctions appear at apical ends of elongated cells. A basement membrane forms around the basal end of the cells and this is the final stage of transformation of a mass of mesenchymal cells into definitive epithelial tube (Schoenwolf, 1984; Schoenwolf and Powers, 1987; Schoenwolf and Smith, 1990).

The process of secondary neurulation in the rat embryo is analogous to that in mouse embryo (Gajovic *et al.*, 1990). In 11-day rat embryos a condensation of mesenchymal cells (the initial stage of the medullary cord) appears around the caudal neuropore. The caudal neuropore closes in 11.5-day embryos. At this time the secondary neurulation starts and the lumen of the future secondary neural tube appears. In 12-, 13- and 14-day embryos the process of neurulation continues in a cranio-caudal direction.

The secondary neurulation stops in 15-day embryos. First the medullary cord disappears in the tip of the tail and then the neural tube undergoes necrobiotic changes and regression in caudocranial direction (Fig. 3).

Tail cord

The tail cord is a mass of condensed mesenchymal cells situated axially in the ventral part of the embryonic tail, beneath the neural tube. Near the tip of the tail it is present before the appearance of the secondary neural tube. The nuclear-cytoplasmic ratio of its cells is reduced giving them an epithelial appearance. The tail cord is the common source of both the secondary notochord and the secondary (tail) gut (Gajovic *et al.*, 1989).

Secondary notochord formation

In the cranial part of the embryonic body the notochord arises as a cranial extension of the Hensen's node (head process). It temporarily fuses with the primitive endoderm where some of its cells are released to form the definitive endoderm, *i.e.* the epithelial lining of the gut (Jurand, 1974; Leikola, 1976; Lamers *et al.*, 1987). Afterwards the notochord separates from the gut and becomes a solid cord beneath the neural tube.

In the tail, however, the notochord develops independently from the surface ectoderm. It origins from the tail cord, which splits into two separated epitheloid rudiments: the notochord and the tail gut (Gajovic *et al.*, 1989). It was previously observed that the notochord emerges from a mass of undifferentiated cells in the tail of rat and mouse embryos (Butcher, 1929; Schoenwolf, 1984) and in the tail bud of human embryos (Muller and O'Rahilly 1987) but a detailed analysis was not made.

Tracing the full length of the tail cord of 11-, 12- and 13-day rat embryos we observed that the detachment of the notochord from the rest of the tail cord (the future tail gut) starts near the tip of the tail and progresses in rostral direction. On serial transversal sections, the fact that the onset of the notochord formation coincides with the appearance of the lumen of the tail gut is clearly visible. First a small, irregularly outlined group of cells bulges from the dorsal side of the tail cord (Fig. 1). More rostrally it detaches and acquires a sharp outline, being first flattened dorso-ventrally and then becoming round (Fig. 2).

Cells of the early secondary notochord display some ultrastructural features equivalent to those of cells of the primary notochord (Galic *et al.*, 1986; Shinohara and Tanaka, 1988; Wilson and Hendrickx, 1990). They are characterized by large accumulations of intracytoplasmic glycogen, mitochondria enveloped by rough endoplasmic reticulum, enlarged cisternae of the Golgi apparatus, large intercellular spaces and ill-defined intercellular contacts (Fig. 4).

The secondary notochord, together with the other two axial structures of the tail, is continuous with analogous structures in the caudal trunk region. The notochord is the only axial structure of the tail which does not disappear (Fig. 3). Its fate is analogous to that in the trunk region, *i.e.* its remnants form the nucleus pulposus of intervertebral discs in the tail (Butcher, 1929).

Secondary gut (tail gut) formation

The tail gut has previously been considered to develop as a cranial extension of the hind gut (Jolly and Férester-Tadié, 1936; Schoenwolf, 1977, 1978). However, our observation of rat embryos suggests that it develops from the condensed tail bud mesenchyme (tail cord) by a mechanism analogous to secondary neurulation (Svajger *et al.*, 1985).

In 10-day embryos the terminal end of the hind gut (at the level of the posterior neuropore) is lined by irregularly shaped and loosely packed cells without a sharp basal boundary. The tail gut can be observed already in 11-day embryos, and it is continuous with the hind gut. Tracing the gut of the 11-day embryo in caudal direction one can observe on serial transversal sections both the developing neural tube and the tail gut (Fig. 2). Their appearance is very similar. The wall of the tail gut consists of elongated cells in a rosette like arrangement around the central lumen filled with a flocculent,
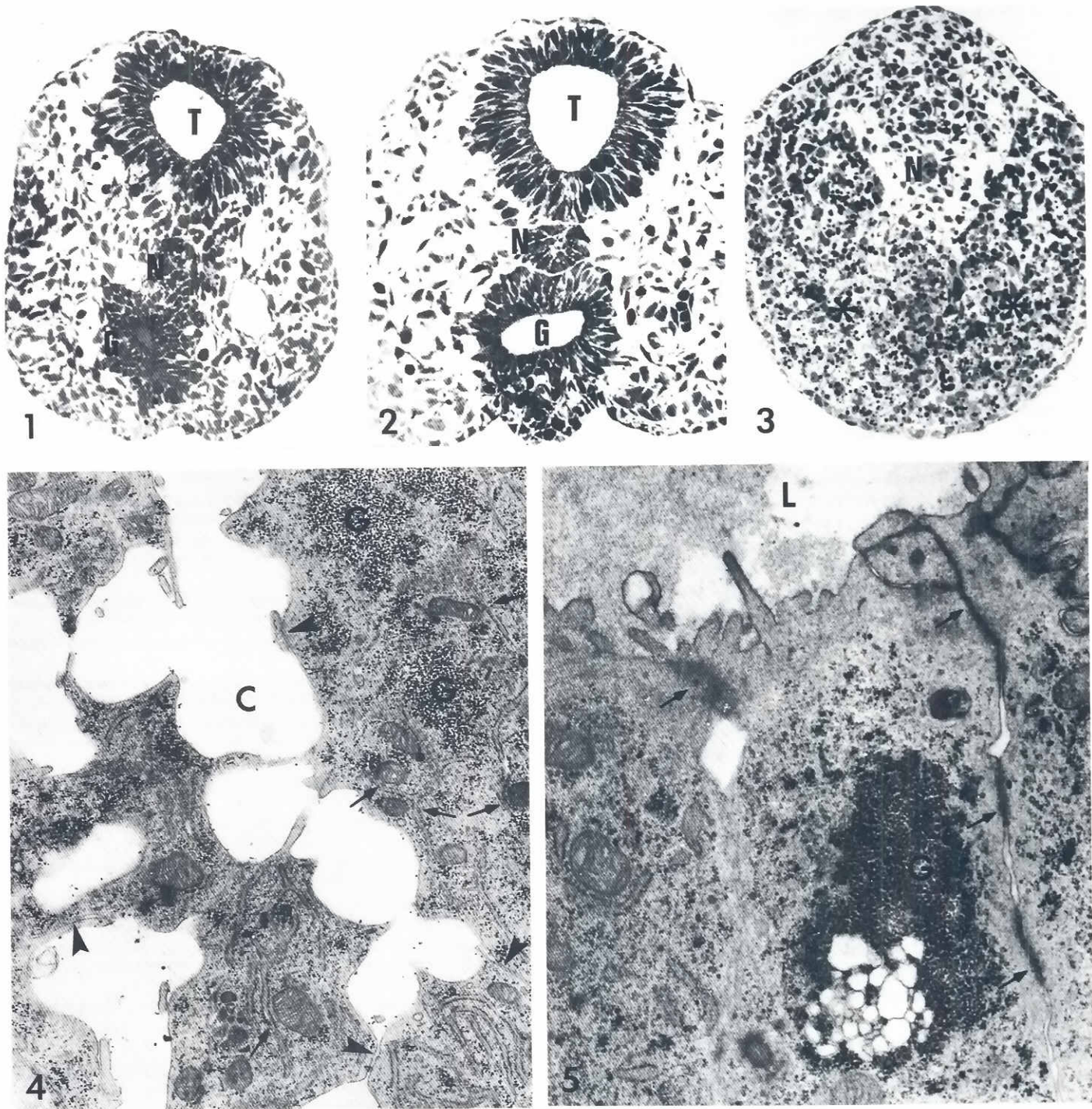
Fig. 1. 11-day embryo. Semi-thin cross section through the tip of the tail. *Newly formed notochord (N) and tail gut (G) are visible. T , neural tube. x220.*

Fig. 2. 11-day embryo. Semi-thin cross section through the tail at more anterior level. *T, neural tube; N, notochord; G, tail gut. x240.*

Fig. 3. 15-day embryo. Semi-thin cross section through the tip of the tail. *Neural tube and tail gut are missing and notochord (N) is the only axial structure present. Numerous cell death occurs in the ventral part of the tail (asterisk). x180.*

Fig. 4. 13-day embryo. Electron micrograph of secondary notochordal cells. *IC, intercellular spaces; G, glycogen; arrow , mitochondria enveloped by rough endoplasmic reticulum, arrowhead cell contacts. x16000.*

Fig. 5. 11-day embryo. Electron micrograph of adluminal side of the tail gut. *L, gut lumen; G, glycogen; arrow, cell junctions. x20000.*

electron-dense material. The apico basal polarity of these cells is peculiar. The apical (adluminal) ends are sealed (close to the lumen and at the level of lateral cytoplasmic processes) by intercellular junctions similar to the «adherent junctions» described by Takeuchi and Takeuchi (1981) in pre-primitive streak rat embryos. The apical cell surface sends out into the lumen numerous long microvilli. The cytoplasm contains granular endoplasmic reticulum, Golgi apparatus, vacuoles, coated pits and vesicles and various amounts of glycogen (Fig. 5). The opposite (abluminal) side of cells is irregularly outlined, lacks the basal lamina and contacts the neighboring cells by cytoplasmic processes. So we find the unusual feature that the same cell simultaneously bears characteristics of an absorptive epithelial cell and of a mesenchymal cell at the two opposite ends respectively. This is in agreement with the concept that the lining of the tail gut is formed by aggregation of mesenchymal cells which at the stage and level described had not yet completely lost their mesenchymal nature (Svajger et al., 1985).

The tail gut is a transitory structure. Its proximal end undergoes regression in the anterior-posterior direction, *i.e.* the distal portion continues to develop while the proximal part has disappeared and there is no more continuity between the hind gut and the tail gut (Butcher, 1929; Gajovic et al., 1990). Cell death is not restricted to the wall of the tail gut alone but can be also observed in the surrounding mesenchyme (Lanot and Bautz, 1981). The regression of the tail gut begins in 13-day rat embryos and in 15-day embryos the tail gut has completely disappeared (Fig. 3). Cell death can be observed even during formation of the tail gut when clusters of «surplus» cells detach from the tail cord and elongate (Gajovic et al., 1989).

Concluding remarks

Although we cannot confirm the traditional concept of the boundary between the primary and the secondary body formation being somewhere at the midlevel of the trunk (Holmdahl 1935), it is clear that at least within the tail of tailed mammals axial structures are formed by a mechanism different from the primary body formation with germ layers as intermediate structures. It could be defined as a mesenchymal-epithelial transformation. Instead of folding, invagination, cell migration or other «usual» morphogenetic mechanisms, the mechanism in the tail, at least formally, mimics the cell-specific aggregation of embryonic cells from mixed suspension. The nature of the selectivity of cell recognition remains obscure. Some other details of morphogenesis within the tail are peculiar when compared with those which we consider as rules in embryogenesis:
a) All three axial structures of the tail develop independently of the surface ectoderm.
b) The tail bud is the source of pluripotent cells as if it retained the capacity of the primitive streak and the Hensen's node. It gives structures considered as derivatives of all three germ layers.
c) In some cases the neural tube in the tail is longer than the other two axial structures suggesting the possibility that secondary neurulation occurs independently from the underlying notochord.
d) As during the primary body formation, there is a close relationship between the notochord and the endoderm. However, in the first case the notochord originates from the primitive ectoderm and contributes definitive endodermal cells to the gut, and in the second case both the notochord and the gut develop from the same group of condensed mesenchymal cells.

The causality of the secondary body formation is poorly understood and attempts to explain it were no more than teleological speculation (Peter, 1951). Arguments of explanation in terms of evolution are also lacking.

References

BUTCHER, E.O. (1929). The development of the somites in the white rat *(mus norvegicus albinus)* and the fate of the myotomes, neural tube, and gut in the tail. *Am. J. Anat. 44:* 381-439.

CRILEY, B.B. (1969). Analysis of the embryonic sources and mechanisms of development of posterior levels of chick neural tubes. *J. Morphol. 128:* 465-502.

FALLON, J.F. and SIMANDL, B.K. (1978). Evidence of a role for cell death in the disappearance of the embryonic human tail. *Am. J. Anat. 152:* 111-130.

GAJOVIC, S., KOSTOVIC-KNEZEVIC, LJ. and SVAJGER, A. (1989). Origin of the notochord in the rat embryo tail. *Anat. Embryol. 179:* 305-310.

GAJOVIC, S., KOSTOVIC-KNEZEVIC, LJ. and SVAJGER, A. (1990). Development of axial organs in the rat embryo tail. *Rad. Med. Fak. Zagrebu 31:* 143-150.

GALIC, M., SARAGA-BABIC, M. and SVAJGER, A. (1986). Electron microscopic observations on the notochord of human embryos and fetuses. *Rad. Jugosl. Akad. Znan. Umjet. 424:* 239-273.

GRIFFITH, C.M. and WILEY, M.J. (1989). The distribution of cell surface glycoconjugates during mouse secondary neurulation. *Anat. Embryol. 180:* 567-575.

GRIFFITH, C.M. and WILEY, M.J. (1990). Sialoconjugates and development of the tail bud. *Development 108:* 479-489.

HOLMDAHL, D.E. (1935). Primitivstreifen beziehungsweise die Rumpfschwanzknospe im Verhaltnis zur Körperentwicklung. *Z. Mikrosk. Anat. Forsch. 38:* 409-440.

IKEDA, Y. (1930). Beiträge zur normalen und abnormalen Entwicklungsgeschichte des caudalen Abschnittes des Ruckenmarks bei menschlichen Embryonen. *Z. Anat. Entw. Gesch. 92:* 380-491.

JOLLY, J. and FÉRESTER-TADIÉ, M. (1936). Récherches sur l'oeuf du rat et de la souris. *Arch. Anat. Microsc. Exp. 32:* 323-390.

JURAND, A. (1974). Some aspects of the development of the notochord in mouse embryo. *J. Embryol. Exp. Morphol. 32:* 1-33.

KLIKA, E. and JELÍNEK, R. (1969). The structure of the end and tail bud of the chick embryos. *Folia Morphol. (Praha) 17:* 29-40.

LAMERS, W.H., SPLIET, W.G.M. and LANGEMEYER, R.A.T.M. (1987). The lining of the gut in the developing rat embryo. *Anat. Embryol. 176:* 413-430.

LANOT, R. and BAUTZ, AM. (1981). La régression de l'intestin caudal chez l'embryon de poulet. Stade de détermination des dégénérescences cellulaires et rôle du mésenchyme associé. *Reprod. Nutr. Dév. 21:* 953-960.

LEDLEY, F.D. (1982). Evolution of the human tail. *New Engl. J. Med. 306:* 1212-1215.

LEIKOLA, A. (1976). Hensen's node - the «organizer» of the amniote embryo. *Experientia 32:* 269-277.

MÜLLER, F. and O'RAHILLY, R. (1987). The development of the human brain, the closure of the caudal neuropore, and the begining of secondary neurulation at stage 12. *Anat. Embryol. 176:* 413-430.

MÜLLER, F. and O'RAHILLY, R. (1988). The development of the human brain from a closed neural tube at stage 13. *Anat. Embryol. 177:* 203-224.

NAKAO, T. and ISHIZAWA A. (1984). Light- and electron- microscopic observations of the tail bud of the larval lamprey *(Lampetra japonica)*, with special reference to neural tube formation. *Am. J. Anat. 170:* 55-71.

PETER, K. (1941). Die Genese des Endoderms bei den Wirbeltieren. *Ergeb. Anat. Entw. 33:* 285-369.

PETER, K. (1951) Die zweifache Entwicklung des Wirbeltierkörpers in finaler, erhaltungs funktionellere Betrachtung. *Z. Mikrosk. Anat. Forsch. 57:* 393-401.

SCHOENWOLF, G.C. (1977). Tail (end) bud contributions to the posterior region of the chick embryo. *J. Exp. Zool. 201:* 227-246.

SCHOENWOLF, G.C. (1978). Effects of complete tail bud extirpation on early develop-ment of the posterior region of the chick embryo. *Anat. Rec. 192:* 289-296.

SCHOENWOLF, G.C. (1981). Morphogenetic processes involved in the remodeling of the tail region of the chick embryo. *Anat. Embryol. 162:* 183-197.

SCHOENWOLF, G.C. (1984). Histological and ultrastructural studies of secondary neurulation in mouse embryos. *Am. J. Anat. 169:* 361-376.

SCHOENWOLF, G.C. and DELONGO, J. (1980). Ultrastructure of secondary neurulation in the chick embryo. *Am. J. Anat. 158:* 43-63.

SCHOENWOLF, G.C. and POWERS, M.L. (1987). Shaping of the chick neuropithelium during primary and secondary neurulation: role of cell elongation. *Anat. Rec. 218:* 182-195.

SCHOENWOLF, G.C. and SMITH, J.L. (1990). Mechanisms of neurulation: traditional viewpoint and recent advances. *Development 109:* 243-270.

SHINOHARA, H. and TANAKA, O. (1988). Development of the notochord in human embryos: ultrastructural, histochemical and immunohistochemical studies. *Anat. Rec. 220:* 171-178.

SVAJGER, A., KOSTOVIC-KNEZEVIC, Lj., BRADAMANTE, Z. and WRISCHER, M. (1985). Tail gut formation in the rat embryo. *Roux Arch. Dev. Biol. 194:* 429-432.

TAKEUCHI, I.K. and TAKEUCHI, Y.K. (1981). Intercellular contacts between the embry-onic or extraembryonic ectoderm and the primitive endoderm in rat egg cylinders prior to the formation of the primitive streak. *Dev. Growth Differ. 23:* 157-164.

TAM, P.P.L. (1984). The histogenetic capacities of tissues in the caudal end of the embryonic axis of the rat embryo. *J. Embryol. Exp. Morphol. 82:* 253-266.

TAM, P.P.L., MEYER, S. and JACOBSON, A.G. (1982). Differentiation of the metameric pattern in the embryonic axos of the mouse. II. Somitomeric organization of the presomitic mesoderm. *Differentiation 21:* 109-122.

WILSON, D.B. and HENDRICKX, A.G. (1990). Cytochemical analysis of the notochord in early rhesus monkey embryos. *Anat. Rec. 228:* 431-436, 1990.