Int. J. Dev. Biol. 43: 809-815 (1999)

Original Article

Fate maps of the primitive streak in chick and quail embryo: ingression timing of progenitor cells of each rostro-caudal axial level of somites

KAICHIRO SAWADA and HIROHIKO AOYAMA*

JT Biohistory Research Hall, Osaka, Japan

ABSTRACT Developmental fates of cells emigrating from the primitive streak were traced by a fluorescent dye DiI both in chick and in quail embryos from the fully grown streak stage to 12-somite stage, focusing on the development of mesoderm and especially on the timing of ingression of each level of somitic mesoderm. The fate maps of the chick and quail streak were alike, although the chick streak was longer at all stages examined. The anterior part of the primitive streak predominantly produced somites. The thoracic and the lumbar somites were shown to begin to ingress at the 5 somite-stage and 10 somite-stage in a chick embryo, and 6 somite-stage and 9 somite-stage in a quail embryo, respectively. The posterior part of the streak served mainly as the origin of more lateral or extra embryonic mesoderm. As development proceeded, the fate of the posterior part of the streak changed from the lateral plate mesoderm to the tail bud mesoderm and then to extra embryonic, allantois mesoderm. The fate map of the primitive streak in chick and quail embryo presented here will serve as basic data for studies on mesoderm development with embryo manipulation, especially for transplantation experiments between chick and quail embryos.

KEY WORDS: *fate map, primitive streak, somite, regionalization, chick, quail*

Introduction

In avian development, gastrulation occurs at the primitive streak, through which the epiblast cells ingress to form the mesoderm and the embryonic endoderm. During gastrulation, the streak itself extensively changes its length and position (Spratt, 1947). After appearing at the posterior border of area pellucida, the streak elongates anteriorly and reaches its full length at stage 4 in chick embryo. Then Hensen's node at the anterior tip of the streak moves posteriorly and the streak regresses, progressively forming an embryonic body in the rostral to caudal direction. Such a development suggests that the developmental fate of the primitive streak might change spatially and temporarily.

The fate of the epiblast and primitive streak have been mapped by various tracing methods (Nicolet, 1971 for a review; also Schoenwolf *et al*., 1992; Bortier and Vakaet, 1992; Garcia-Martinez *et al*., 1993; Hatada and Stern, 1994; Catala *et al*., 1996; Psycoyos and Stern, 1996 for more recent works). According to these maps, the streak is subdivided into several regions being aligned in a pattern in which cells destined to form the more axial region of the embryo are placed in a more anterior part of the streak. That is, regions of prospective notochord, somites, lateral plate mesoderm, intermediate mesoderm, and extra embryonic mesoderm are aligned from anterior to posterior in the primitive streak. On the other hand, the later the developmental stage, the more posterior mesodermal tissue the streak produced (Schoenwolf *et al*., 1992; Catala *et al*., 1996; Psycoyos and Stern, 1996). Thus, we need not only a spatial but also a temporal fate map of the primitive streak to investigate its development.

One of the major issues relating to the establishment of body plan is how an animal body regionalizes rostro-caudally. The somitic mesoderm is representative of the rostro-caudal regionalization in the vertebrates. The thoracic somites give rise to the ribs as well as the vertebrae, while the cervical or lumbar somites form only the vertebrae. Although the somitic mesoderm has been shown to regionalize in the segmental plate (Kieny *et al*., 1972; Chevallier, 1975), neither the mechanism nor even the stage of the regionalization is known. Transplantation experiments between embryos of two avian species, chick and quail (Le Douarin, 1973) could be helpful in addressing this issue. So far no fate map for quail primitive streak has been reported, though a fate map for chick has been made by Psychoyos and Stern (1996), which shows beginning time of ingression of the cervical, thoracic and lumbar somitic mesoderm.

Here we traced the fates of mesodermal tissues after the ingression through the primitive streak by labeling the cells with

***Address for reprints:** JT Biohistory Research Hall, Murasakicho 1-1, Takatsuki, Osaka 569-1125, Japan. FAX: +81.726.81.9757. e-mail: Hirohiko.Aoyama@gate.brh.co.jp

0214-6282/99/$15.00

Printed in Spain
www.ehu.es/ijdb

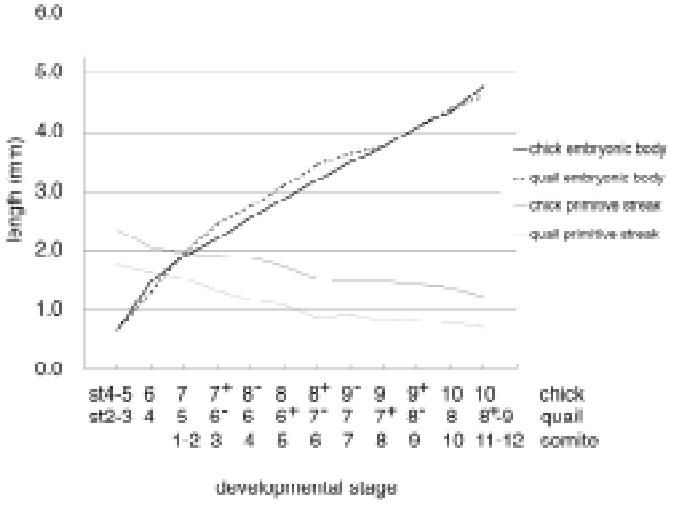

Fig. 1. Sizes of the embryonic body and primitive streak of chick and quail embryos from the fully grown streak stage to the 12-somite stage. *Lengths of embryonic body (bold line) and of the primitive streak (thin line) were measured and are the average of more than 150 chick embryos (solid line) and more than 50 quail embryos (dotted line) for each point.*

fluorescent dye, DiI, which was injected at various points along the primitive streak in a chick and a quail embryo at various developmental stages from the fully grown streak stage to the 12-somite stage. We describe the spatio-temporal origin of notochordal, somitic, lateral, tail bud and extra-embryonic mesoderm, confirming the fate maps of chick primitive streak reported previously, and construct a fate map for the quail streak. Further we showed the ingression timing of the beginning of each rostro-caudal level of paraxial mesoderm both in a chick and a quail embryo. Also, we discuss the availability of these fate maps for transplantation experiments of the primitive streak between chick and quail embryo.

Results

Growth of the embryonic body and regression of the primitive streak in the chick and quail embryo

We measured the length of the embryonic bodies and of streaks in 2046 chick embryos from stage 4 (fully grown streak stage) to stage 10^+ (12-somite stage), and in 1008 quail embryos from stage 2 (fully grown streak stage) to stage 9^-(12-somite stage). The length of embryo was measured from the rostral end of the head to the caudal end of Hensen's node or the primitive pit and the length of the primitive streak from the caudal end of Hensen's node or the primitive pit to the caudal end of the streak or the margin of the germ wall.

Comparing the chick and the quail embryos with the same number of somite pairs, the average lengths of chick and quail embryonic bodies were found to be similar. On the other hand, the primitive streak was longer in chick than in quail embryos during the stages examined (Fig. 1), while the rate of shortening against the numbers of somites was similar between these species.

Descendants of DiI labeled primitive streak cells

DiI injection at a point along the primitive streak, in one to two day old chick and quail embryos labeled several kinds of mesodermal tissues after two days of further incubation. At the time of observation, the chick embryos were at stage 20-23 (Hamburger and Hamilton, 1951) and the quail embryos were at stage 15-17 (Zacchei, 1961). We categorized the labeled tissues into five types. Figure 2 shows representative specimens of the chick embryos labeled with DiI.

1) *Notochord*. The labeled cells usually distributed throughout the notochord (Fig. 2A).
2) *Somites*. Strong labeling was usually seen in 3 to 10 sequential somites, whether they were differentiated in the dermomyotome and the sclerotome (Fig. 2A) or in the epithelial

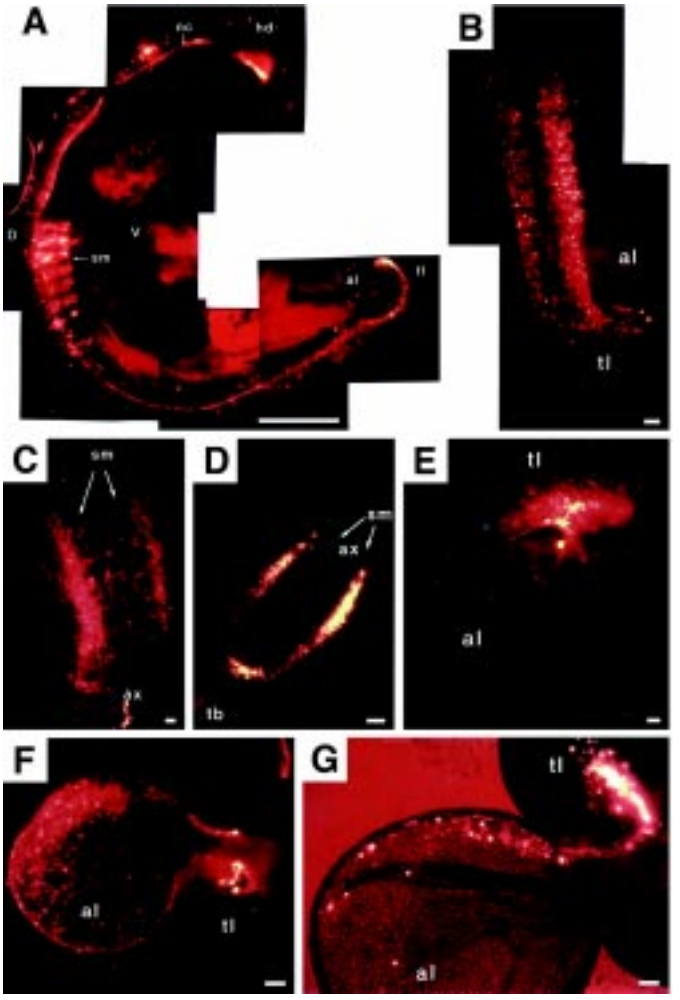

Fig. 2. Representative labeling patterns two days after the DiI injection at a point of the primitive streak in 1-day chick and quail embryos. (A) *The entire notochord (nc) from head (hd) to tail (tl) and several somite pairs (sm) are labeled in a quail embryo. The extra embryonic structure, allantois (al), is not labeled. Bar, 1 mm.* **(B)** *A series of somites are labeled at the lumbosacral region of a chick embryo. Bar, 0.1 mm.* **(C)** *The intermediate mesoderm and the lateral plate are labeled in a chick embryo. Somites (sm) and the axial structure (ax) containing the notochord are not labeled. Bar, 0.1 mm.* **(D)** *Labeled lateral plate in a chick embryo slightly younger than the embryo shown in C. Somites and the axial structure are not labeled. Bar, 0.1 mm.* **(E)** *Mesoderm on the ventral side of the tail bud (tl) is labeled and allantois (al) is not labeled in a chick embryo. Bar, 0.1 mm.* **(F)** *Labeling of allantois (al) in a chick embryo. Bar, 0.1 mm.* **(G)** *DiI labeling is seen in a line from the tail bud (tl) through allantois (al). Bar, 0.1 mm.*

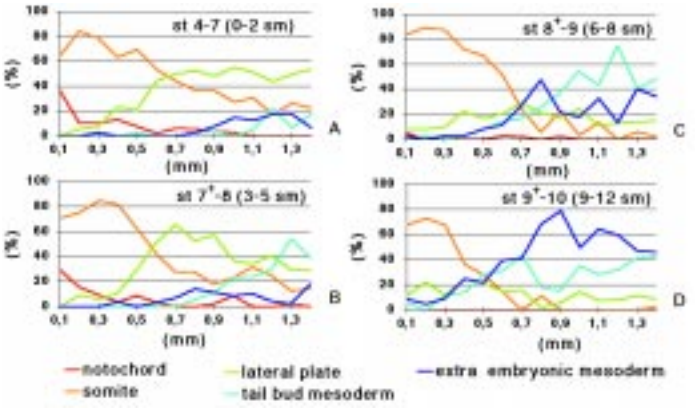

Fig. 3. Fate of each part of the primitive streak of a chick embryo from the fully grown primitive streak stage (st. 4) to the 12-somite stage (st. 10). *DiI was injected into the primitive streak at each developmental stage and then, two days later, the mesodermal tissue labeled was classified into five categories; notochord, somites, lateral plate, tail bud mesoderm and extra embryonic mesoderm. The incidence that each kind of mesoderm was labeled to total labeled tissues is plotted against the dye injection site, which is indicated as the distance from Hensen's node.*

somite stage (Fig. 2B). Sometimes the labeling extended caudally with decreasing intensity.

3) *Lateral plate mesoderm*. When the lateral plate mesoderm was labeled, the intermediate mesoderm and the lateral side of somites on the same rostro-caudal level was also often labeled (Fig. 2C). These labeled tissues were fused caudally, which was clearly evident in the younger embryos (Fig. 2D).
4) *Tail bud*. In the tail bud, labeled cells were usually observed only on the ventral side and neither the notochord nor the somitic mesoderm was labeled (Fig. 2E).
5) *Extra embryonic region*. A chain of labeled cells extended from the ventral surface of the tail into the allantoic membrane, extra embryonic mesoderm (Fig. 2F,G).

The pattern of labeling depended both on the site of the injection and on the stage of the embryo, which is described in the following sections.

Fate map of the primitive streak in a chick embryo

Origin of mesodermal tissues

Fluorescent dye, DiI was injected into the primitive streak every 0.1 mm to a distance 1.4 mm from the primitive pit or the caudal end of Hensen's node in embryos at stages 4-10. The furthest injection site, 1.4 mm, corresponds to the length of the shortest primitive streak examined. Figure 3 shows the distribution pattern of labeled cells by relative frequency of labeling of each type of mesodermal tissue, in relation to the dye injection site along the streak at each developmental stage. Although we examined the embryo at each somite stage, for convenience the data were accumulated so that about 40 embryos were examined at each injection point in Figure 3. If one injection resulted in the labeling of two or more categories of mesodermal tissue, we counted each labeling of the tissue as one case.

Dye injection into the rostral part of the primitive streak most frequently labeled somitic mesoderm among mesodermal tissues through the developmental stages examined (Fig. 3A-D). In contrast, when the dye was injected into the caudal part of the streak, the predominantly labeled tissues were lateral plate (Fig. 3A,B), tail bud (Fig. 3C) and extra embryonic mesoderm (Fig. 3D), according to the developmental stage of the embryo at the time of dye injection. Before the 4-somite stage (HH stage 8$^-$), the lateral plate mesoderm was predominantly labeled. The tail bud and the extra embryonic mesoderm began to be labeled predominantly at the 5-somite stage (HH stage 8) and 9-somite stage (HH stage 9$^+$), respectively. When the dye was injected at the most rostral part of the streak in the chick embryo earlier than at 5-somite stage (HH stage 8), the notochord was labeled, though less frequently than the somite.

Thus the primitive streak could be divided into two parts in terms of the developmental fate, the rostral, somite forming region with the notochord forming region at its rostral end and the caudal, non-somitic mesoderm forming region. At first, the border of these two regions was not strict because the posterior streak produced the somitic mesoderm with considerable frequency although the lateral plate mesoderm was produced in the posterior region of the streak (Fig. 3A). When the posterior streak began to produce the tail bud mesoderm, the region producing somitic mesoderm gradually constricted rostrally (Fig. 3B,C), and then finally became shorter (Fig. 3D).

Origin of cervical/thoracic/lumbosacral somites

To know when and where the cervical, thoracic and lumbosacral somites ingress through the primitive streak, we analyzed the

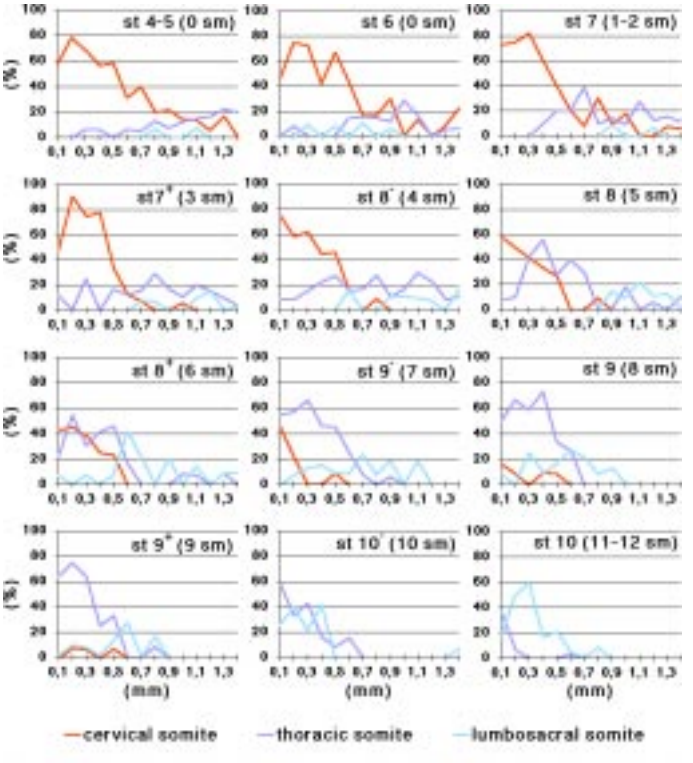

Fig. 4. Fate of each part of the primitive streak of a chick embryo from the fully grown primitive streak stage (st. 4) to the 12-somite stage showing somite regionalization. *The incidence of the cervical, the thoracic and the lumbosacral somites labeled with DiI is plotted against the dye injection site. The incidence of each region of labeled somites is plotted against the total number of all types of labeled mesodermal tissues. The labeled somite region was determined as the region containing the most rostral labeled somites.*

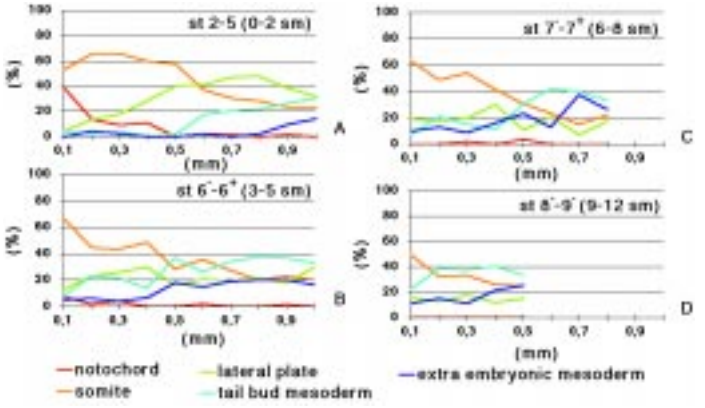

Fig. 5. Fate of each part of the primitive streak of a quail embryo from the fully grown primitive streak stage (st. 2) to the 12-somite stage (st. 9⁻). *See legend to Figure 3 for details.*

same embryos using the above sections, focusing on the level of somites labeled with DiI. The labeling always extended over several somites, and we classified the labeling pattern according to the region where the most rostral labeled somite was seen. Consequently the cervical, thoracic and lumbosacral somites mentioned in Figure 4 could include a more posterior region also. In other words, the emergence of each line indicates the cessation of production of the more anterior region of the somitic mesoderm in that area of the streak.

Figure 4 shows that the streak at a certain developmental stage produced somites of several levels and the more rostral part of the streak gives rise to the more rostral somites and that the later the dye was injected into the streak, the more caudal somites were labeled. Exclusively, cervical somites (somite 5 to 18) were labeled in the chick embryo when the dye was injected earlier than 5-somite stage (st. 8 by HH). Thoracic somites (somite 19 to 26) began to be labeled by the injections at the 5-somite stage (st. 8) and were predominantly labeled by the injections after the 7-somite stage (st. 9⁻). Finally lumbosacral somites (somite 27 and more caudal somites) became predominantly labeled after the 10-somite stage (st. 10⁻) injections. Thus the primitive streak produces somites in a rostro-caudal sequence during the development of the embryo.

Transition of the fate of the streak stated above was found to progress in a caudal to rostral direction. At the 5-somite stage (HH st. 8), a transition from cervical to thoracic somite production was clearly seen in the region posterior to 0.3 mm from Hensen's node, while the more rostral part of the streak predominantly produced the cervical somitic mesoderm. At the 6-somite stage, only the most rostral part of the streak gave rise to the cervical somite more frequently than the thoracic somite. Then at the 7-somite stage (st. 9⁻), the entire streak produced predominantly the thoracic somite, while the cervical somite was still produced with considerable frequency at the most rostral part of the streak. Finally, at the 8-somite stage (st. 9), the streak almost exclusively gave rise to the thoracic somite. Transition of the fate of the streak from producing the thoracic somitic mesoderm to the lumbosacral one was shown to occur similarly between the 9- and 12-somite stage (st. 9⁺-10).

Fate map of the primitive streak in developing quail embryo

Origin of mesodermal tissues

We mapped the fate of the primitive streak of quail embryos in the same way as for chick embryos. We injected DiI into the streak every 0.1 mm to a distance 1.0 mm from the primitive pit or the caudal end of Hensen's node in quail embryos at Zacchei's stages 2-9⁻. Since the streak regresses and shortens to less than 1.0 mm, the furthest site of the dye injection was less than 1.0 mm in embryos at stage 10⁺ or later.

As shown in Figure 5, labeling patterns of mesoderm after injection of DiI into the primitive streak were similar to those of chick embryos. The notochord was labeled only by an injection at the most rostral part of the streak and around the full streak stage (Fig. 5A). The predominantly labeled mesoderm was somite when the dye was injected in the rostral part of the streak (Fig. 5A-D). The lateral plate (Fig. 5A) and then the tail bud (Fig. 5B-D) were labeled by the injection in the caudal part of the streak. Although the extra embryonic mesoderm, allantois, was also labeled, it never predominated during the stages we examined.

Although the border of the somite forming region and the non-somite forming region progressively shifted rostrally in the course of development, it was not as evident as that in chick embryos, suggesting that the same fate map as in the chick primitive streak is compressed in the shorter streak of quail embryo.

Origin of cervical/thoracic/lumbosacral somites

We determined the stage at which cervical, thoracic and lumbosacral somitic mesoderm ingressed through the primitive

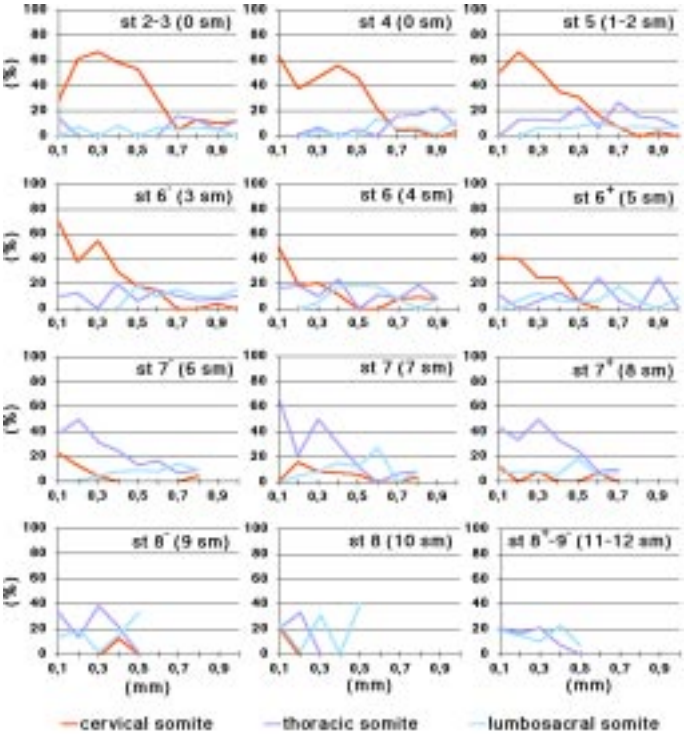

Fig. 6. Fate of each part of the primitive streak of a quail embryo from the fully grown primitive streak stage (st. 2) to the 12-somite stage (st. 9⁻) showing somite regionalization. *See legend to Figure 4 for details.*

streak (Fig. 6) in the same way as for the chick embryos stated above. The streak producing the cervical and the posterior somitic mesoderm changed to produce the thoracic and the posterior somitic mesoderm during the 5-6-somite stages. Then the fate of the streak changed to give rise to the lumbosacral somitic mesoderm during the 10-12-somite stages. This is similar in chick embryos, in which these changes of the streak fate occurred during the 5-7-somite stage and 10 and more somite stage. Thus the ancestor of each somite ingresses at a similar developmental stage as represented by the number of somite pairs in chick and in quail embryos.

Discussion

The fate maps of the primitive streak serve for transplantation experiments between chick and quail embryo

To date, many studies on various issues of developmental biology have been done using inter specific chimeras, including chimera inter classes (Fontaine-Perus *et al.*, 1995). Among them, chick quail chimera has been used widely because of the natural cell marker of quail nucleoli coupled with the availability of micro surgical techniques (Le Douarin, 1973; Le Douarin *et al.*, 1996). Although chick and quail embryos develop in a similar manner, they differ in size and in incubation period, suggesting that care should be taken when determining boundary and developmental stages of a graft corresponding to those of host embryo in transplantation experiments.

Our fate maps of chick and quail primitive streak presented here show the fate of each part of the primitive streak in the embryos at the 0 to 12 somite stage, which allows us to design various combinations of transplantation experiments, iso- or hetero-topic and iso- or hetero-chronic transplantation of part of the primitive streak, between chick and quail embryos to study the development of the streak.

That the time and site of the ingression of cervical, thoracic and lumbo-sacral levels of the prospective somitic mesoderm were determined is especially useful to the study of the rostro-caudal regionalization of paraxial mesoderm, which gives rise to axial skeleton constituted of vertebrae and ribs. To elucidate the mechanisms of the regionalization of the paraxial mesoderm, it is necessary to know when it is determined. While thoracic somites or segmental plate were shown to be determined to form ribs (Kieny *et al.*, 1972; Chevallier, 1975), we do not know whether the fate of the somitic mesoderm is determined so as to form a certain unit of axial skeleton at the primitive streak or not. To address this issue, it is necessary to do heterotopical and heterochronical transplantation experiments replacing a part of the primitive streak in the chick embryo with that of a quail embryo. For such experiments, the fate maps presented here provide necessary and useful data.

Fate map of the primitive streak of chick and quail embryo

The fate map of the primitive streak of chick and quail embryos were shown to be essentially identical, except in total length of the streak and in the lengths the primordium of each mesoderm. Our findings on chick embryos confirm previous extensive studies constructing fate maps of the primitive streak of chick embryos (Nicolet, 1971 for a review; also Bortier and Vakaet, 1992; Schoenwolf *et al.*, 1992; Garcia-Martinez *et al.*, 1993; Hatada and Stern, 1994; Catala *et al.*, 1996; Psycoyos and Stern, 1996 for more recent works). The migration and destination of the presumptive mesoderm in the primitive streak of chick embryos are schematically summarized in Figure 7 based on our results (Figs. 3-6) and on those of Schoenwolf *et al.* (1992) and Psychoyos and Stern (1996) concerning migration routes. Briefly, our confirmation was as follows. First, the presumptive notochord, somites and lateral plate are aligned rostro-caudally in the primitive streak, while these mesoderm arranged medio-laterally at their destination. Second, the earlier the presumptive somitic mesoderm ingressed at the primitive streak, the more rostral its derivative are situated. Thus, the rostro-caudal axis and the age of the streak is converted into the medio-lateral axis and the rostro-caudal axis of the embryonal body, respectively.

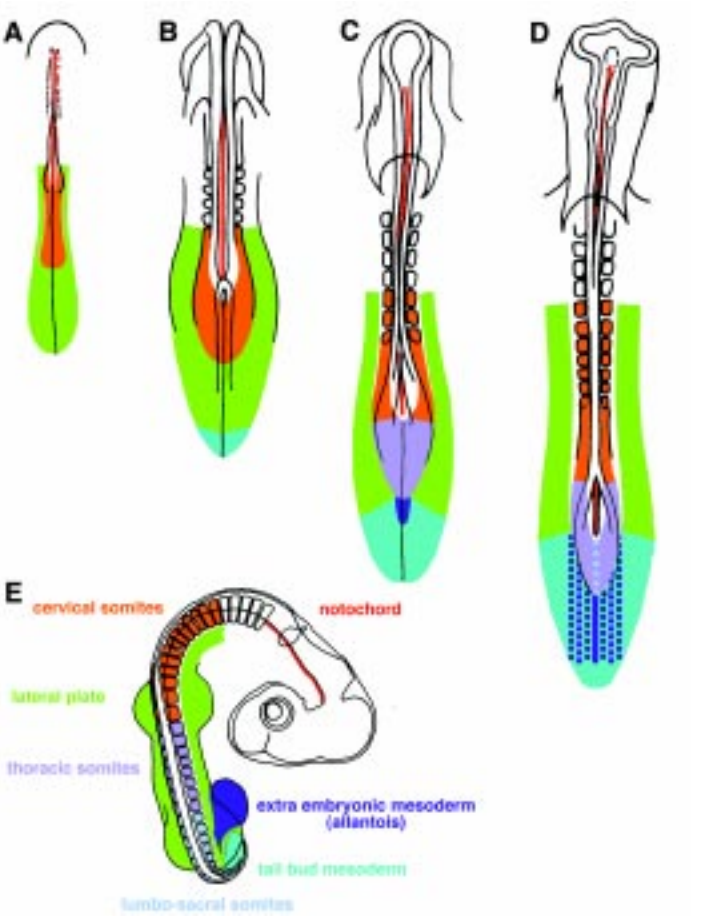

Fig. 7. Schematic drawing of the migration and destination pattern of the mesodermal cells from the primitive streak in the chick embryo at Hamburger and Hamilton stage 6 (A), stage 8⁻, or 4 somite stage (B), stage 9⁻, or 7 somite stage (C), stage 10⁻, or 10 somite stage (D), and stage 20 (E). *We labeled chick embryos with DiI at stages 4-10* **(A-E)** *and labeling pattern was observed around stage 20 (E). This scheme was constructed based on our results (Figs. 3-6) and those of Schoenwolf et al. (1992) and Psychoyos and Stern (1996). Each color in the figures representing the categories of mesodermal tissues corresponds to that in Figures 3-6.*

The boundary between the regions of the streak with different fates is not as strict as that shown in Figure 7 but ambiguous because of considerable overlapping of different fates as shown in Figures 3 and 5. Psychoyos and Stern (1996) also showed that

cells at the same position in the streak migrate along various routes and differentiate into different types of cells. Such overlapping was more apparent in the quail than in the chick embryo. This may be due to the low resolution of our labeling, suggesting that the presumptive mesoderms are arranged in a more compressed manner in quail than in chick embryos, though their overall arrangement is similar.

Origin of somitic mesoderm of different levels along the rostro-caudal axis of the embryo

Although the rostral limit of DiI labeling of the series of somites was clear, it distributed caudally with decreasing intensity of fluorescence, so that we could not determine the caudal end derived from the injection site of the streak. This may be caused by precipitation of DiI labeling cells passing the injection site later on, as mentioned by Garcia-Martinez and Schoenwolf (1993), or alternatively, as Psychoyos and Stern (1996) claimed, by a resident population of presumptive somite cells in the regressing primitive streak, as demonstrated by the labeled cells remaining at the injection site. On the other hand, in transplantation experiments between chick and quail embryos (Schoenwolf *et al.*, 1992; Catala *et al.*, 1996), limited pairs of somites were shown to be derived from the transplants, suggesting a portion of the streak produced certain levels of somites.

In either case, our fate maps show the ingression timing of various regions of the somitic mesoderm by indicating the cessation of production of the more rostral region of somites as stated in the Results, since they are constructed based on the level of the most anterior somite labeled by DiI injection.

Origin of the notochord

It has been reported that the origin of the notochord is Hensen's node (Wolff, 1935a,b; Grabowski, 1956,; Smith and Schoenwolf, 1989; Hirano *et al.*, 1991; Smith *et al.,* 1994; Yuan *et al.*, 1995; Catala *et al.*, 1996). When we labeled the most anterior portion of the streak, 0.1 mm caudal to the primitive pit, in the early phase of gastrulation, up until the five somite stage in chick and four somite stage in quail embryo, the whole length of the notochord was usually labeled. Later, DiI injection at the same point seldom labeled the notochord.

These findings suggest that the notochord forming region first spread around the primitive pit, or Hensen's node and then concentrated around the pit after the five somite stage. This result concurs with that of Catala *et al.* (1996). They demonstrated that the superficial layer immediately caudal to the primitive pit of avian embryos at the six somite stage gave rise to the baso-lateral spinal cord and not the notochord, while Hensen's node itself produced the entire notochord and floor plate, by transplantation experiments between quail and chick embryos.

Materials and Methods

Fertilized White Leghorn and Japanese quail eggs were purchased from local farms. The eggs were incubated at 38°C in a humidified incubator. All operations on embryos were carried out through a window opened on the egg shell (Aoyama and Asamoto, 1998). After injecting Chinese ink diluted with Tyrode's solution beneath the embryo to facilitate observation, developmental stages were determined according to Hamburger and Hamilton (1951) for chick and to Zacchei (1961) for quail embryos. The length of the embryo and of the primitive streak was measured under a dissecting microscope with the aid of a micrometer mounted on the eyepiece.

Fluorescent dye, DiI was injected into the primitive streak of chick and quail embryo *in ovo* according to Selleck and Stern (1991) and Ruiz i Altaba *et al.* (1993). Injection points were on the median line and at various distances from the primitive pit or the caudal end of Hensen's node. After DiI injection, the windows on the shell were sealed with adhesive tape. The embryos were further incubated for two days and then fixed with 3.7% formaldehyde in phosphate buffer (pH 7.4) and observed under a fluorescent microscope.

Acknowledgments

We would like to thank Dr. T.S. Okada for his encouragement and critical reading of the manuscript. We also thank Drs. T.C. Takahashi and K. Kato for helpful discussions.

References

AOYAMA, H. and ASAMOTO, K. (1988). Determination of somite cells: independence of cell differentiation and morphogenesis. *Development 104:* 15-28.

BORTIER, H. and VAKAET, C.A. (1992). Fate mapping the neural plate and the intraembryonic mesoblast in the upper layer of the chicken blastoderm with xenografting and time-lapse videography. *Development (Suppl.) 116:* 93-97.

CATALA, M., TEILLET, M-A., DE ROBERTIS, E.M. and LE DOUARIN, N.M. (1996). A spinal cord fate map in the avian embryo: while regressing, Hensen's node lays down the notochord and floor plate thus joining the spinal cord lateral walls. *Development 122:* 2599-2610.

CHEVALLIER, A. (1975). Rôle du mésoderm somitique dans le développment de la cage thorcique de l'embryon d'oiseau. I. Origine du segment sternal et mécanismes de la differenciation des côtes. *J. Embryol. Exp. Morphol. 33:* 291-311.

FONTAINE-PERUS, J., JARNO, V., FOURINIER LE RAY, C. and PAULIN, D. (1995). Mouse chick chimera: a new model to study the in ovo developmental potentialities of mammalian somites. *Development 121:* 1705-1718.

GARCIA-MARTINEZ, V. and SCHOENWOLF, G. (1993). Primitive-streak origin of the cardiovascular system in avian embryos. *Dev. Biol. 159:* 706-719.

GARCIA-MARTINEZ, V., ALVAREZ, I.S. and SCHOENWOLF, G. (1993). Locations of ectodermal and nonectodermal subdivisions of the epiblast at stages 3 and 4 of avian gastrulation and neurulation. *J. Exp. Zool. 267:* 431-446.

GRABOWSKI, C.T. (1956). The effects of the excision of Hensen's node on the early development of the chick embryo. *J. Exp. Zool. 133:* 301-343.

HAMBURGER, V. and HAMILTON, H.L. (1951). A series of normal stages in the development of the chick embryo. *J. Morphol. 88:* 49-92.

HATADA, Y. and STERN, C.D. (1994). A fate map of the epiblast of the early chick embryo. *Development 120:* 2879-2889.

HIRANO, S., FUSE, S. and SOHAL, G.S. (1991). The effect of the floor plate on pattern and polarity in the developing central nervous system. *Science 251:* 310-313.

KIENY, M., MAUGER, A. and SENGEL, P. (1972). Early regionalization of the somitic mesoderm as studied by the development of the axial skeleton of the chick embryo. *Dev. Biol. 28:* 142-161.

LE DOUARIN, N.M. (1973). A biological cell labeling technique and its use in experimental embryology. *Dev. Biol. 30:* 217-222.

LE DOUARIN, N.M., DIETERLEN-LIEVRE, F. and TEILLET, M.-A. (1996). Quail-chick transplantations. *Methods Cell Biol. 51:* 23-59.

NICOLET, G. (1970a). Analyze autoradiographique de la localisation des differentes ebauches presomptives dans la ligne primitive de l'embryon de poulet. *J. Embryol. Exp. Morphol. 23:* 79-108.

NICOLET, G. (1970b). Is the presumptive notochord responsible for somite genesis in the chick? *J. Embryol. Exp. Morphol. 24:* 467-478.

NICOLET, G. (1971). Avian gastrulation. *Adv. Morphogen. 9:* 231-262.

PSYCHOYOS, D. and STERN, C.D. (1996). Fates and migratory routes of primitive streak cells in the chick embryo. *Development 122:* 1523-1534.

RUIZ I ALATABA, A., WARGA, R.M. and STERN, C.D. (1993). Fate maps and cell lineage analysis. In *Essential Developmental Biology: A Practical Approach* (Ed. C.D. Stern and P.W.H. Holland). Oxford: IRL Press at Oxford University Press.

SCHOENWOLF, G.C., GARCIA-MARTINEZ, V. and DIAS, M.S. (1992). Mesoderm movement and fate during avian gastrulation and neurulation. *Dev. Dyn. 193:* 235-248.

SELLECK, M.A. and STERN, C.D. (1991). Fate mapping and cell lineage analysis of Hensen's node in the chick embryo. *Development 112:* 615-626.

SMITH, J.L. and SCHOENWOLF, G.C. (1989). Notochordal induction of cell wedging in the chick neural plate and its role in neural tube formation. *J. Exp. Zool. 250:* 49-62.

SMITH, J.L., GESTELAND, K.M. and SCHOENWOLF, G.C. (1994). Prospective fate map of the mouse primitive streak at 7.5 days of gestation. *Dev. Dyn. 201:* 279-289.

SPRATT, N.T.Jr. (1947). Regression and shortening of the primitive streak in the explanted chick blastoderm. *J. Exp. Zool. 104:* 69-100.

WOLFF, E. (1935a). Les conséquences de la lésion de la région du noeud de Hensen sur le développement du poulet. *C.R. Soc. Biol. 118:* 77-80.

WOLFF, E. (1935b). Sur la formation d'une rangée axiale de somites chez l'embryon de poulet après irradiation du noeud de Hensen. *C.R. Soc. Biol. 118:* 77-80.

YUAN, S., DARNELL, D.K. and SCHOENWOLF, G.C. (1995). Identification of inducing, responding, and suppressing regions in an experimental model of notochord formation in avian embryos. *Dev. Biol. 172:* 567-584

ZACCHEI, A.M. (1961). Lo sviluppo embrionale della quaglia giapponese (Coturunix coturnix japonica). *Arch. Anat. 66:* 36-62.

Received: December 1997
Accepted for publication: October 1999