Dual mode of paraxial mesoderm formation during chick gastrulation

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The skeletal muscles and axial skeleton of vertebrates derive from the embryonic paraxial mesoderm. In amniotes, paraxial mesoderm is formed bilaterally to the nerve cord as a result of primitive streak and tail-bud regression during body axis formation. In chick and mouse embryos, paraxial mesoderm was proposed to derive from a population of resident cells located in the regressing primitive streak and tail bud. In contrast, in lower vertebrates, paraxial mesoderm is formed as a result of the continuation of ingression movements of gastrulation. Here, we reinvestigate paraxial mesoderm formation in the chicken embryo and demonstrate that these two modes are concomitantly at work to set up the paraxial mesoderm. Although the medial part of somites derives from stem cells resident in the primitive streak/tail bud, the lateral part derives from continuous ingression of epiblastic material. Our fate mapping further shows that the paraxial mesoderm territory in the epiblast is regionalized along the anteroposterior axis as in lower vertebrates. These observations suggest that the mechanisms responsible for paraxial mesoderm formation are largely conserved across vertebrates.

vertebrate | epiblast | primitive streak | tail bud | body axis formation

At the body level, the posterior unsegmented paraxial mesoderm is called presomitic mesoderm (PSM). At the anterior tip of the PSM, cells become periodically organized into epithelial blocks called somites, which form sequentially in an anteroposterior (AP) fashion. Somites later differentiate into vertebrae, muscles, and dermis, which become regionalized along the AP axis to form structures characteristic of their axial level. Each amniote somite can be subdivided into medial and lateral compartments that contribute to distinct sets of derivatives (1). The medial compartment gives rise to the epaxial muscles, vertebral column, and dermis of the back, whereas the lateral compartment produces essentially the hypaxial muscles, which include intercostals and limb muscles (1, 2). The medial and lateral parts of amniote somites arise from largely distinct territories in the primitive streak (3–6). In the chicken embryo, medial somites derive from a population of precursors exhibiting stem cell behavior located in the anteriormost primitive streak and Hensen's node, whereas no such stem cells have been reported for the lateral somite precursors, which derive from a more posterior part of the primitive streak (6). In contrast, it has been proposed that mouse somites derive from a regionalized stem cell pool located in the primitive streak (7) in which an anterior domain contributes to medial somitic cells, whereas more posterior cells contribute to the lateral somites (8). The self-renewal capacity of these stem cells was established either by lineage analysis using various cell-labeling strategies or by serial transplants (6, 7, 9). These stem-like properties persist during axis formation. This proposed mode of paraxial mesoderm formation from a resident cell population in the anterior streak is quite different from the one observed in frog and fish, in which continuation of the gastrulation movements was proposed to account for the formation of the paraxial mesoderm (10, 11).

In this report, we have reinvestigated the origin of paraxial mesoderm in the primitive streak of chick embryos. Using 1,1′-dioctadecyl-3,3,3′-tetramethylindocarbocyanine perchlorate (DiI)- or GFP-based labeling methods and time-lapse analysis, we observed that cells forming the medial and lateral parts of a given somite are produced at distinct times by the primitive streak. We then investigated in detail the origin and fate of paraxial mesoderm precursors by grafting fluorescently labeled epiblast fragments of the primitive streak or the adjacent epiblast. Our data support the existence of stem cells contributing to the medial part of the somites and, additionally, that primitive streak cells contributing to the lateral somites do not exhibit such a stem cell behavior. We demonstrate that cells of the posterior territory of the paraxial mesoderm in the primitive streak are constantly generated through ingression of the lateral epiblast even after the beginning of regression of the primitive streak. We also show that the epiblast territory generating the lateral paraxial mesoderm is regionalized along the AP axis, such that cells fated to contribute to more anterior somitic regions are localized more anteriorly in the epiblast. Therefore, these observations indicate a dual mode of somite precursor production: the medial somite portion derives from a stem cell population, whereas the lateral somitic cells derive from continuous ingression of epiblast cells. Together, these data indicate that the epiblast adjacent to the primitive streak in the chicken embryo presents an organization highly similar to that of the marginal zone in frog and fish, suggesting that gastrulation mechanisms are more conserved between lower vertebrates and amniotes than currently thought.

Results

To study the establishment of AP identity in the paraxial mesoderm, we first set out to test whether cells produced at the same time by the anterior and posterior parts of the paraxial mesoderm territory in the primitive streak end up in the same somites. We labeled primitive streak cells with both 3,3′-dilinoleyloxacarbocyanine perchlorate (DiO) at the 90% level (see *Materials and Methods*) and DiI at the 70% level (Fig. 1 *a* and *b*) and monitored the position of the descendants of the labeled cells along the AP axis. Descendants of the DiO-labeled cells were found in the medial part of somites, whereas DiI-labeled cells were found in the lateral part of somites (Fig. 1*c*). Strikingly, however, the anteriormost descendants of DiO-labeled cells were always observed to be located up to two to five somites anterior to DiI-labeled cells (Fig. 1*c*, $n = 12$). Such labeled embryos were also analyzed in time-lapse movies [supporting information (SI) Movies 1 and 2]. The analysis of these movies suggests that the difference in the final positioning of

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Abbreviations: AP, anteroposterior; PSM, presomitic mesoderm; HH, Hamburger and Hamilton; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; DiO, 3,3′-dilinoleyloxacarbocyanine perchlorate.

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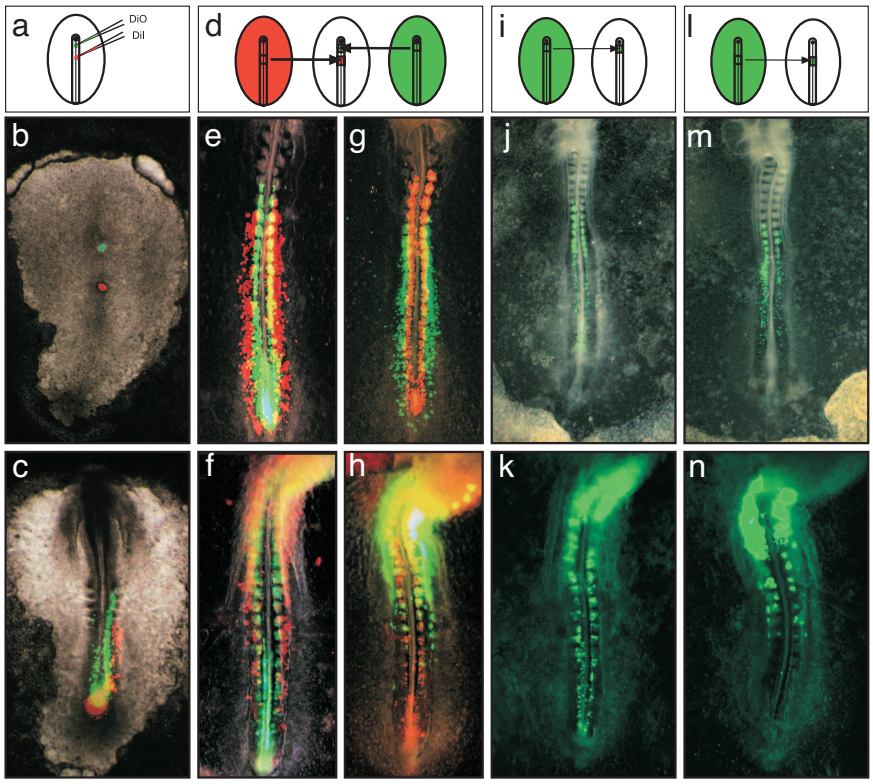

Fig. 1. Medial and lateral somites are produced at different times by the anterior primitive streak. (*a*) Schematic representation of the DiI-/DiO-labeling procedure. (*b*) Stage 4+HH chicken embryo immediately after DiI/DiO labeling showing the position of labeled cells along the primitive streak. (*c*) Same embryo as in *b* after 12 h of reincubation. (*d*) Double-homotopic and -homochronic grafts of different primitive streak levels labeled by electroporation with EGFP and DsRed. (*e* and *f*) Embryo grafted at the 90% streak level with an EGFP-labeled fragment and at the 70% streak level with a DsRed-labeled fragment 24 h (*e*) and 48 h (*f*) after reincubation. (*g* and *h*) Embryo grafted at the 90% streak level with a DsRed-labeled fragment and at the 70% streak level with an EGFP-labeled fragment 24 h (*g*) and 48 h (*h*) after reincubation. (*i* and *l*) Homotopic and homochronic graft of a fragment of EGFP-electroporated anterior primitive streak at 90% level (*i*) and at 70% level (*l*). (*j* and *k*) EGFP-labeled descendants of the primitive streak fragment in an embryo grafted at the 90% level of the streak after reincubation times of 24 h (*j*) and 48 h (*k*). (*m* and *n*) EGFP-labeled descendants of the primitive streak fragment in an embryo grafted at the 70% level of the streak after reincubation times of 24 h (*m*) and 48 h (*n*). Ventral views, anterior to the top.

labeled cells in the medial and lateral somites essentially reflects the initial AP difference in the primitive streak, and that the path of posterior cells to reach the lateral paraxial mesoderm is longer than that of the anterior streak cells to reach the medial column. To confirm this observation, we used an alternative labeling technique, in which we replaced a fragment from the 90% level of the primitive streak with an EGFP-labeled equivalent piece from a stage-matched donor embryo and performed in the same embryo a second homotopic graft of a DsRed-labeled streak fragment from the 70% to the 80% level (Fig. 1*d*). This electroporation-based labeling technique made it possible to track the fate of the labeled cells after longer incubation periods than the vital dye-based labeling, in which labeling, especially in the caudal part of an embryo, largely faded out because of rapid dilution of the dye 2 days after incubation. In these embryos, descendants of the EGFP-labeled cells were observed mostly in the medial somites, whereas the descendants of the DsRed-labeled cells were observed in the lateral somites (Fig. 1*e*). As observed in the DiI- and DiO-labeling experiments, the anteriormost medial derivatives were always found to lie two to five somites anterior to the lateral cells (Fig. 1*e*, $n = 4$). The same result was observed when DsRed was used to label anterior streak cells and EGFP to label the posterior streak graft (Fig. 1*g*, $n = 4$). Thus, these experiments are consistent with previous fate maps (ref. 12 and refs. therein) and demonstrate that the medial and the lateral parts of a single somite are produced at distinct times by the primitive streak.

We have investigated further the contribution of different regions of the primitive streak to the different axial levels of the paraxial mesoderm. In double- (Fig. 1*d*) or single-graft (Fig. 1 *i* and *l*) experiments analyzed after 24 h, cells from the 90% level of the streak mostly gave rise to cells in medial somites from the anterior level of the somitic region to the tail bud (SI Table 1; Fig. 1 *e*, *g*, and *j*). In contrast, grafts of more posterior regions of the primitive streak (70–80% level) essentially produced descendants in the lateral part of the somites extending over more posterior levels (SI Table 1; Fig. 1 *e*, *g*, and *m*). As reported (12), however, labeled cells were not always entirely restricted either to the medial or to the lateral somitic compartments, suggesting some intermingling between the two populations. When grafted embryos were incubated for 48 h, derivatives of the 90% streak level continued to contribute to more posterior paraxial mesoderm and tail bud (SI Table 1; Fig. 1 *f*, *h*, and *k*). In contrast, derivatives of grafts from more posterior regions of the streak were not seen to contribute to the posterior part of the embryo (Fig. 1 *f*, *h*, and *n*). Therefore, these experiments support the existence of stem cells contributing to the medial somites located in the anteriormost part of the primitive streak, caudal to the notochord precursors in Hensen's node (6). These stem cells contribute to all axial levels of the paraxial mesoderm. These observations also demonstrate that lateral somite precursors in the anterior streak do not behave as stem cells, indicating that at these stages, the precursors of the posterior lateral somites are not located in the primitive streak.

These experiments raised the question of the origin of cells contributing to the lateral somites in more posterior regions of the AP axis. Available fate maps indicate that until stage 4 Hamburger and Hamilton (HH), the paraxial mesoderm territory is located in

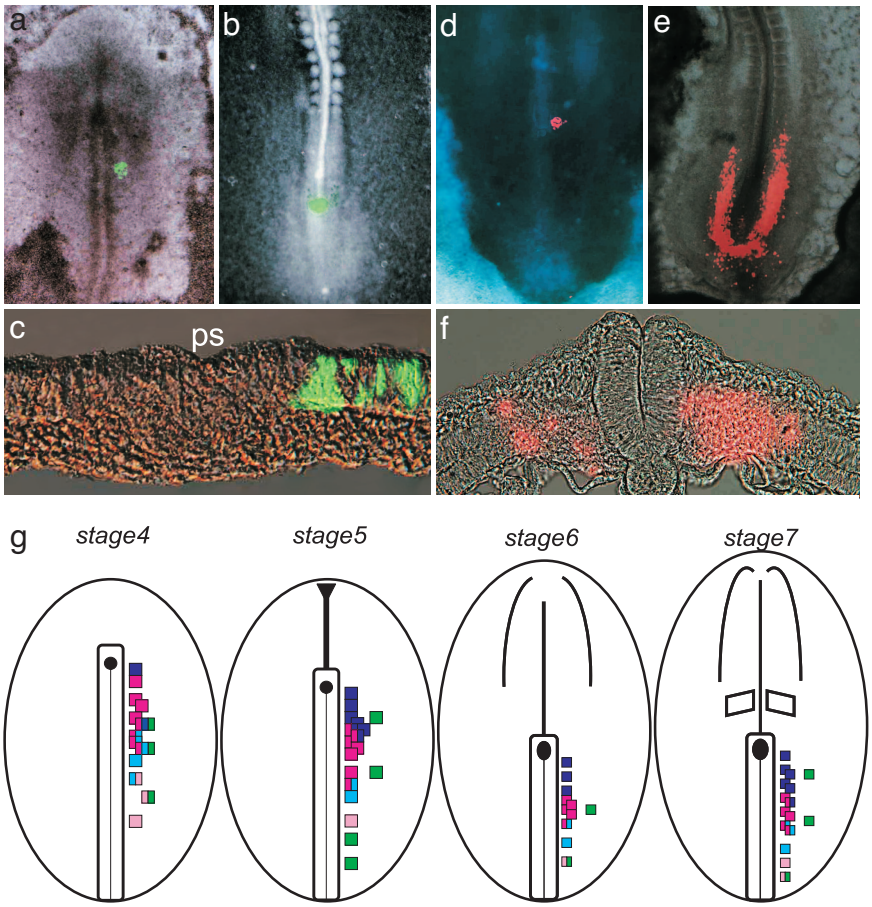

Fig. 2. Fate mapping of the epiblast. (*a–c*) Fate mapping of the epiblast using EGFP labeling followed by grafting. Epiblast cells adjacent to the anterior streak were labeled by electroporation with EGFP at stage 5HH (*a*), grafted into an unlabeled host, and reincubated for 12 h (*b*). (*c*) Transverse section at anterior 70% level of the primitive streak level of the embryo shown in *a*. PS, primitive streak. (*d–f*) Epiblast fate mapping using DiI labeling *in ovo*. Epiblast cells adjacent to the anterior 70% of the streak were labeled with a DiI crystal at stage 5HH (*d*) and reincubated for 20 h (*e*). (*f*) Transverse sections at the posterior PSM level of the embryo shown in *e*. (*g*) Summary of the results of the epiblast fate mapping. Each square indicates the level of individual epiblast graft, and the colors indicate the fate of the grafted tissue. Dark blue, neural tube; red, paraxial mesoderm; blue, lateral plate mesoderm; pink, extraembryonic mesoderm; green, surface ectoderm.

the epiblast lateral to the primitive streak and that between stages 4HH and 7HH, somitic cells arise from a region corresponding to the 70–90% level of the primitive streak (12–15). After stage 4HH, ingression of the epiblast territory adjacent to the anterior streak was proposed to be completed (16). Thus, at the beginning of primitive streak regression, the contribution of the epiblast adjacent to the anterior streak was essentially thought to be restricted to the neural plate (17). However, this view is in contrast to studies in the chicken embryo that indicate paraxial mesoderm progenitors can be found in the epiblast adjacent to the anterior streak even after the beginning of streak regression (18, 19). Because the fate of the epiblast adjacent to the streak between stages 4HH and 7HH has been poorly characterized, we have undertaken an extensive fate mapping of this region. To that end, we performed a series of homotopic grafts of EGFP-labeled fragments of different levels of this epiblast (SI Table 2; Fig. 2 *a–c* and *g*). Our fate map of the epiblast adjacent to the primitive streak indicates that after the beginning of Hensen's node regression, the anteriormost epiblast that lies adjacent to the node and the anterior primitive streak (90% level) essentially gives rise to neural and ectodermal derivatives (Fig. 2*g*, dark blue). More posteriorly, extending from the 90–60% level of the primitive streak, a territory essentially contributing to the lateral somites is found (Fig. 2*g*, red). Posterior to this territory, the epiblast contributes to the lateral plate and extraembryonic mesoderm (Fig. 2*g*, blue and pink, respectively). To ensure that the results were not biased by our grafting method, we used an alternative labeling strategy in which a small DiI crystal was transiently applied on the epiblast *in ovo* to label only cells in the superficial layer of the embryo (SI Table 3; Fig. 2 *d–f*). A similar contribution to the somites was observed from the DiI-labeled epiblast (Fig. 2 *e* and *f*). Using both labeling procedures, the contribution of the epiblast to the posterior somites was not exclusively restricted to the lateral compartment and included some medial derivatives, which must, therefore, intermix with derivatives of the anterior primitive streak. Thus, these data indicate that the paraxial mesoderm precursors of the lateral epiblast mostly contribute to the lateral somites.

We then analyzed in more detail the fate of cells of the epiblastic paraxial mesoderm territory in terms of its contribution along the AP body axis. We noticed that grafts of more anterior regions of the somitic domain of the epiblast gave rise to descendants contributing to more anterior regions of the AP axis than grafts of more posterior regions (SI Table 2; compare Fig. 3 *a–c* and *d–f*). Therefore, the presumptive territory of the somites in the epiblast is regionalized along the AP axis.

Discussion

In amniotes such as chick and mouse, gastrulation is usually defined as the period corresponding to the formation of the primitive streak. During this phase, the territories located in the superficial layer, the epiblast, converge to form the primitive streak where they become internalized. The presumptive territory of the paraxial mesoderm is

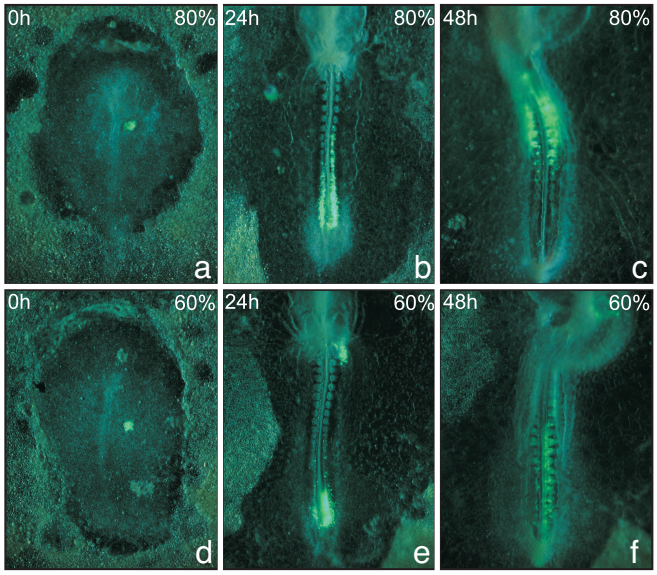

Fig. 3. Regionalization of PSM precursor cells in the epiblast. (*a–c*) Embryo grafted at the 80% streak level before reincubation (*a*), after 24 h (*b*), and after 48 h (*c*). (*d–f*) Embryo grafted at 60% streak level before reincubation (*d*), after 24 h (*e*), and after 48 h (*f*). Ventral views, anterior to the top.

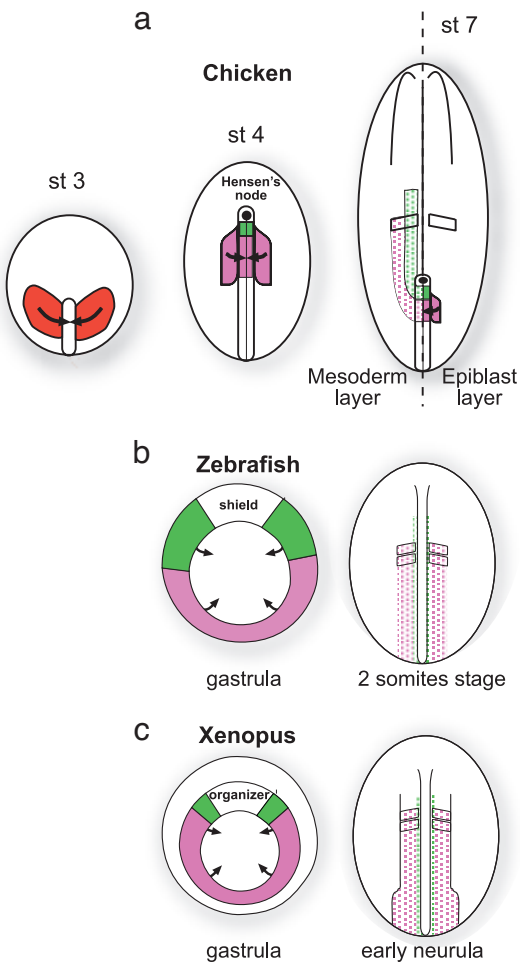

Fig. 4. Schematic representation of paraxial mesoderm formation in chick (*a*), zebrafish (*b*), and *Xenopus* (*c*). (*a*) Chicken embryo. Red, presumptive territory of the paraxial mesoderm in the superficial epiblast. Green, presumptive territory of medial somite containing the stem cells. Pink, presumptive territory of paraxial mesoderm in the epiblast. Stippled green, medial somite precursors. Stippled pink, lateral somite precursors. Arrows indicate gastrulation movement of epiblast cells into the primitive streak. Dorsal views, anterior to the top. (*b*) Zebrafish gastrula (vegetal view) and two-somite stage embryo (dorsal view). Green territory adjacent to the shield in the gastrula will form adaxial cells (stippled green in the two-somite stage), whereas the pink territory yields the lateral PSM (stippled pink in the two-somite stage). Arrows indicate involution movement of cells through the blastopore. (*c*) *Xenopus* gastrula (vegetal view) and early neurula (dorsal view) depicted as if the neural plate were transparent. Hypothetical presumptive territories of medial somite precursors in the area adjacent to the organizer and of lateral somites in the marginal zone are marked in green and pink, respectively, in the gastrula. Hypothetical territories of medial somites and lateral somites are marked in stippled green and stippled pink, respectively, in the early neurula. Arrows indicate involution movement of cells through the blastopore.

initially located in the epiblast lateral to the forming primitive streak (13, 14). At the end of primitive streak extension in the chicken embryo (stage 4HH), ingression of the paraxial mesoderm territory was considered to be complete, and precursor cells of the paraxial mesoderm were proposed to localize in the anterior primitive streak as a resident population of cells (12). At this stage, the epiblast lateral to the anterior streak was proposed to correspond to the posteriormost neural territory (17). After the beginning of primitive streak regression, the resident population of paraxial mesoderm precursors would regress in concert with the primitive streak and lay the paraxial mesoderm in its wake. Using grafts of labeled epiblast fragments, we observed that, in contrast to this widely accepted scenario, ingression movements resulting in paraxial mesoderm formation continue well after the beginning of primitive streak regression, contributing to the lateral part of somites (Fig. 2). Together with the late ingression movements reported in the chick tail bud (20, 21), this observation strongly supports the idea that, as in lower vertebrates (10, 11), gastrulation movements continue throughout axis extension to produce posterior mesoderm. Such a situation is also seen in mouse, where involution of material from the posterior neural plate gives rise to posterior mesoderm, suggesting that this territory corresponds to a remnant of the epiblast that continues to undergo gastrulation movements to generate mesoderm (9) (Val Wilson, personal communication). These data strongly support the notion that continuation of the gastrulation movements resulting in epiblast ingression during axis extension is involved in posterior mesoderm formation across all vertebrates (Fig. 4).

Our data, nevertheless, confirm the existence of resident stem cells participating in medial somite formation in the anterior primitive streak (Fig. 1) (6, 7). Whereas existence of such a resident stem cell population in lower vertebrates is unclear, there are a number of arguments suggesting that such cells might exist. In *Xenopus*, injection of GFP in single blastomeres at the 32- to 64-cell stage often results in labeling a few muscle fibers in a large number of myotomes, which led to the idea of the existence of stem cells at the origin of somitic muscles (22). Fate maps of the 32-cell-stage frog embryo indicate that the somitic contribution of dorsalmost blastomeres extends along the entire AP axis, whereas the more ventral ones only contribute to posterior somites (23). In fish, the adaxial and lateral presomitic cells define a medial and a lateral population that gives rise to the slow and fast muscle lineages, respectively, and derives from cells located correspondingly both close and more distant to the organizer (24, 25). The correspondence of this medial and lateral subdivision in the amniote somites, and whether adaxial cells derive from a resident stem cell population, however, remain to be established (Fig. 4*b*).

The primitive streak of amniotes is generally considered homologous to the *Brachyury*-expressing marginal zone of lower vertebrates such as amphibians (26, 27). Its AP organization essentially reflects the future fate of its mesodermal derivatives along the mediolateral axis (the dorsoventral axis of amphibians). Namely,

the most anterior part of the primitive streak gives rise to notochord, and then, moving progressively posteriorly, are located the territories of the medial somite, lateral somite, intermediate mesoderm, lateral plate, and extraembryonic mesoderm (5, 12, 14, 28). Our fate-mapping data in the chicken indicate this organization is also seen at the level of the epiblast adjacent to the streak, suggesting that the region homologous to the marginal zone of lower vertebrates in fact not only corresponds to the primitive streak but also includes the adjacent epiblast. We also observed that epiblast cells located closer to Hensen's node contribute to more anterior somites than cells located more posteriorly, indicating that the arrangement of lateral somite precursor cells along the AP axis in the epiblast also correlates with their definitive distribution along the embryonic AP axis (Fig. 3). In the chicken embryo, cells of the anterior streak tend to contribute to more anterior somites than cells of more posterior regions of the streak (12, 15, 29) (SI Table 1). Fate maps in frog and fish indicate that cells of the marginal zone closer to the organizer contribute to more anterior somites than cells located more distantly (23, 30–34). Therefore, our data show that in chick, the presumptive territory of the lateral somite in the epiblast presents a very similar organization to the presumptive somitic territory in the marginal zone of lower vertebrates. Thus, the territory mapped as somitic in the frog marginal zone could, in fact, be homologous to the chick lateral somite presumptive territory, and that the *Xenopus* homologue of the chick medial somites presumptive territory could derive from a small stem cell population located close to the organizer (Fig. 4*c*). Taken together, in higher vertebrates, the AP axis of the primitive streak and adjacent epiblast reflect both the future mediolateral and AP fates of the mesodermal tissue, as observed in lower vertebrates along the proximodistal axis of the blastopore (Fig. 4).

This revised fate map suggests a dual mechanism for paraxial mesoderm production that is likely conserved throughout vertebrates. Unexpectedly, it reveals that in the chicken embryo, *Hox* gene expression (18) is initiated not in somite stem cells but rather in somite precursors in the epiblast before their ingression into the streak (Fig. 4). Thus, although medial somite precursors impose their segmentation pattern onto the lateral ones (35), our results indicate that *Hox* identity is first acquired in the lateral somite precursors. The spatial dissociation of these two patterning processes suggests they can be independently regulated, potentially accounting for the extraordinary diversity of vertebral formulae observed in vertebrates.

Materials and Methods

Embryos and Nomenclature. Fertilized chick eggs were obtained from commercial sources. Eggs were incubated at 38°C in a humidified incubator. Embryos were staged according to Hamburger and Hamilton (HH) (36) and by counting the somites.

Electroporation and Fate Mapping of the Primitive Streak and Epiblast. Embryos ranging from stage 3HH to stage 7HH were prepared for EC culture (37). A DNA solution containing the fluorescent protein expression vectors (*pCAGGS-EGFP* or *pCAGGS-DsRed*) (1.0 μg/μl) (18) was microinjected in the space between the vitelline membrane and the groove of the primitive streak or epiblast lateral to the streak. *In vitro* electroporations were carried out with five successive square pulses of 8 V for 50 msec, keeping a 4-mm distance between anode and cathode using Petri dish-type electrodes (CUY701P2; Nepa Gene, Tokyo, Japan) and a CUY21 electroporator (Nepa Gene). This procedure strictly labels the superficial epiblast layer (18). Electroporated embryos were cultured for 2–3 h at 38°C in a humidified incubator until EGFP fluorescence was observed in the electroporated embryo. EGFP-positive anterior streak fragments or EGFP-positive lateral epiblast fragments with the underlying, unlabeled mesoderm and endoderm ($\approx$100 $\times$ 100 μm) were excised and grafted into the same location in stage-matched unlabeled host embryos. After a 15- to 48-h reincubation time, the AP position of the labeled cells in the somitic series was scored.

DiI and DiO Mapping of the PSM and Epiblast. Chick embryos ranging from stage 3HH to stage 7HH were injected with both DiI and DiO (Molecular Probes, Eugene, OR; dissolved at a final concentration of 1 mg/ml) using pulled-glass capillaries. A small focal injection of each vital dye was made at different AP levels along the primitive streak. Using an eyepiece reticule, the relative position of these marks in the streak at the time of injection was determined by measuring the distance between the injection site and the posterior tip of the streak (i.e., the 100% level corresponds to the anterior tip of Hensen's node) (12). After a 15- to 24-h reincubation time to allow the PSM to segment, the position of the descendants of the injected cells in the somitic series was scored. For epiblast fate mapping, a small DiI crystal (Molecular Probes) was gently placed *in ovo* at different AP levels of epiblast. The crystal was left in place for a few minutes and subsequently removed. Quality of the staining was confirmed under fluorescent microscope. Embryos were then reincubated for 15–24 h, photographed, and the fate of the stained cells was analyzed in cryosections.

Time-Lapse Imaging. To follow the cell movements *in vivo*, DiI-, DiO-, and EGFP-labeled embryos were incubated in a custom-made air-heated microscope chamber maintained at 38°C with a heater (AIR-THERMZ; World Precision Instruments, Hertfordshire, U.K.) and 90% humidity. For each experiment, we chose a representative embryo for time-lapse filming from $\approx$10 prepared embryos. All embryos were photographed at the beginning and end of the experiment. Each experiment was repeated at least four times to verify that similar experimental results were obtained. Both bright-field and fluorescence images were taken every 3 min during 15 h of overnight incubation using a Zeiss (Oberkochen, Germany) Axiovert100 inverted microscope through a plan-NEOFLUAR 2.5×/0.075 NA objective (Zeiss). Images were recorded by using a Hamamatsu (Hamamatsu City, Japan) ORCA-II-ER cooled CCD camera driven as described (38).

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1. Ordahl CP, Le Douarin NM (1992) *Development (Cambridge, UK)* 114:339–353.
2. Olivera-Martinez I, Coltey M, Dhouailly D, Pourquié O (2000) *Development (Cambridge, UK)* 127:4611–4617.
3. Bellairs R (1986) in *Somitogenesis in Developing Embryos*, eds Bellairs R, Ede D, Lash J (Plenum, New York), pp 161–178.
4. Nicolet G (1970) *J Embryol Exp Morphol* 23:79–108.
5. Rosenquist GC (1966) *Contr Embryol Carnegie Inst Wash* 38:71–110.
6. Selleck MA, Stern CD (1991) *Development (Cambridge, UK)* 112:615–626.
7. Nicolas JF, Mathis L, Bonnerot C, Saurin W (1996) *Development (Cambridge, UK)* 122:2933–2946.
8. Eloy-Trinquet S, Nicolas JF (2002) *Development (Cambridge, UK)* 129:3609–3619.
9. Cambray N, Wilson V (2002) *Development (Cambridge, UK)* 129:4855–4866.
10. Kanki JP, Ho RK (1997) *Development (Cambridge, UK)* 124:881–893.
11. Gont LK, Steinbeisser H, Blumberg B, de Robertis EM (1993) *Development (Cambridge, UK)* 119:991–1004.
12. Psychoyos D, Stern CD (1996) *Development (Cambridge, UK)* 122:1523–1534.
13. Hatada Y, Stern CD (1994) *Development (Cambridge, UK)* 120:2879–2889.
14. Nicolet G (1971) *Adv Morphogenet* 9:231–262.
15. Schoenwolf GC, Garcia-Martinez V, Dias MS (1992) *Dev Dyn* 193:235–248.
16. Stern CD (2004) in *Gastrulation: From Cells to Embryos*, ed Stern CD (Cold Spring Harbor Lab Press, Valencia, CA), pp 219–232.
17. Sheng G, dos Reis M, Stern CD (2003) *Cell* 115:603–613.
18. Iimura T, Pourquié O (2006) *Nature* 442:568–571.

19. Brown JM, Storey KG (2000) *Curr Biol* 10:869–872.
20. Knezevic V, De Santo R, Mackem S (1998) *Development (Cambridge, UK)* 125:1791–1801.
21. Catala M, Teillet MA, Le Douarin NM (1995) *Mech Dev* 51:51–65.
22. Zernicka-Goetz M, Pines J, Ryan K, Siemering KR, Haseloff J, Evans MJ, Gurdon JB (1996) *Development (Cambridge, UK)* 122:3719–3724.
23. Dale JK, Slack JM (1987) *Development (Cambridge, UK)* 99:527–551.
24. Devoto SH, Melancon E, Eisen JS, Westerfield M (1996) *Development (Cambridge, UK)* 122:3371–3380.
25. Hirsinger E, Stellabotte F, Devoto SH, Westerfield M (2004) *Dev Biol* 275:143–157.
26. Technau U (2001) *BioEssays* 23:788–794.
27. De Robertis EM (2004) in *Gastrulation: From Cells to Embryos*, ed Stern CD (Cold Spring Harbor Lab Press, Valencia, CA), pp 581–589.
28. Garcia-Martinez V, Schoenwolf GC (1992) *Dev Dyn* 193:249–256.
29. Pasteels J (1937) *Arch Biol* 48:381–488.
30. Vogt W (1929) *Wilhelm Roux' Arch Entwicklungsmech Org* 120:384–706.
31. Moody SA (1987) *Dev Biol* 122:300–319.
32. Keller R (1991) *Methods Cell Biol* 36:61–113.
33. Kumano G, Smith WC (2002) *Dev Dyn* 225:409–421.
34. Agathon A, Thisse C, Thisse B (2003) *Nature* 424:448–452.
35. Freitas C, Rodrigues S, Charrier JB, Teillet MA, Palmeirim I (2001) *Development (Cambridge, UK)* 128:5139–5147.
36. Hamburger V, Hamilton HL (1992) *Dev Dyn* 195:231–272.
37. Chapman SC, Collignon J, Schoenwolf GC, Lumsden A (2001) *Dev Dyn* 220:284–289.
38. Yang X, Dormann D, Munsterberg AE, Weijer CJ (2002) *Dev Cell* 3:425–437.