The somitogenetic potential of cells in the primitive streak and the tail bud of the organogenesis-stage mouse embryo

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Summary

The developmental potency of cells isolated from the primitive streak and the tail bud of 8.5- to 13.5-day-old mouse embryos was examined by analyzing the pattern of tissue colonization after transplanting these cells to the primitive streak of 8.5-day embryos. Cells derived from these progenitor tissues contributed predominantly to tissues of the paraxial and lateral mesoderm. Cells isolated from older embryos could alter their segmental fate and participated in the formation of anterior somites after transplantation to the primitive streak of 8.5-day host embryo. There was, however, a developmental lag in the recruitment of the transplanted cells to the paraxial mesoderm and this lag increased with the extent of mismatch of developmental ages between donor and host embryos. It is postulated that certain forms of cell-cell or cell-matrix interaction are involved in the specification of segmental units and that there may be age-related variations in the interactive capability of the somitic progenitor cells during development. Tail bud mesenchyme isolated from 13.5-day embryos, in which somite formation will shortly cease, was still capable of somite formation after transplantation to 8.5-day embryos. The cessation of somite formation is therefore likely to result from a change in the tissue environment in the tail bud rather than a loss of cellular somitogenetic potency.

Key words: primitive streak, tail bud, lacZ transgene, somitogenesis, mouse embryo, in vitro culture, transplantation.

Introduction

Recent studies on the regionalization of cell fates in the epiblast of the mouse embryo have shown that precursors for the various definitive germ layers are already installed in the epiblast shortly before the onset of gastrulation (Lawson and Pedersen, 1992; Lawson et al., 1991). According to the fate maps, the progenitor cells for the somitic mesoderm are initially localized in the lateral region of the epiblast but are progressively relocated towards the primitive streak as gastrulation proceeds. This is brought about by the anisotropic growth of the epiblast and the departure via the primitive streak of other progenitor populations that are localized in the posterior regions of the epiblast (Lawson et al., 1991). Ultimately by the late-primitive streak stage, cells destined for the somitic and lateral mesoderm are confined to the anterior portion of the primitive streak and the adjacent epiblast (Tam, 1989; Tam and Beddington, 1987). Analyses of the developmental fate of cells in the primitive streak of the organogenesis-stage mouse and avian embryos reveal that some cells which reside permanently in the primitive streak are acting as a major and constant source of somitic mesoderm during axis development (Tam and Beddington, 1987; Ooi et al., 1986; Selleck and Stern, 1991). Extirpation and grafting experiments performed in the avian embryos have shown that the somites are composed of cells of dual origin. Each somite receives, in addition to the cells recruited from the primitive streak, contributions from a population of cells in the presomitic mesoderm (=segmental plate). The latter population of cells also acts as the focal point for organizing the cells which have recently ingressed through the primitive streak into somitomeres (Bellairs, 1986; Bellairs and Veini, 1984; Tam, 1988).

In the early-organogenesis stage (8.5- to 9.5-day) mouse embryos, an epithelial-mesenchymal interface which is reminiscent of the primitive streak is always found in the caudal aspect of the posterior neuropore. This remnant of the primitive streak eventually disappears when the neural tube closes and it is replaced by a core of mesenchymal cells in the tail bud (Rugh, 1968; Tam et al., 1982; Gajovic et al., 1989). In rodent and avian embryos, the remnant of the primitive streak and the tail bud mesenchyme both abut directly against the epithelium of the posterior neuropore (or the medullary neural tube), the paraxial mesoderm, the notochord and the hindgut (Gajovic et al., 1989; Sanders et al., 1986; Svaiger et al., 1985; Schoenwolf, 1984; Tam, 1981). This histological contiguity of tissues, particularly of the primitive streak or the tail bud and the paraxial mesoderm,
indicates that tissues at the caudal end of the embryonic axis may be important sources of the somitic mesoderm. Two studies in the mouse embryo have provided indirect evidence in support of this contention. First, embryo fragments containing the presomatic mesoderm of 9.5- to 11.5-day mouse embryos can only generate a limited number of somites in the absence of the primitive streak or the tail bud. The number of somites that is added to the explant correlates directly with the number of pre-existing somitomeres in the presomatic mesoderm. However, if either the primitive streak or the tail bud is included in the embryo fragment, somite formation will proceed uninterrupted (Tam, 1986). Secondly, fragments of the primitive streak and the tail bud when grown as teratomas in ectopic sites display extensive chondrogenesis and myogenesis (Tam, 1984) which is indicative of the ability of cells contained in these fragments to differentiate into somitic tissues (Griffith and Sanders, 1991). The present study was undertaken in order to examine more directly the somitogenetic potency of the cells in the primitive streak and tail bud of the mouse embryo as development proceeds. The strategy was to transplant small groups of cells isolated from the primitive streak or the tail bud mesenchyme of 8.5- to 13.5-day embryos to the primitive streak of the 8.5-day early-somite-stage host embryos. An additional experiment was also performed by transplanting 9.5-day primitive streak cells orthotopically to host embryos of the same age. These cells were labelled genetically by the β-galactosidase transgene (HMG-IcZ), or by exogenous cell marker: [3H]thymidine (3H-TdR) and wheat germ agglutinin-gold conjugate (WGA-Au). The distribution of the labelled cells in the paraxial mesoderm and other embryonic tissues was examined in the host embryo after 22-24 hours of in vitro development. Because of the inability to support good in vitro development of advanced postimplantation embryos and the difficulty in grafting cells precisely to the tail bud, mesenchyme of 10.5- to 13.5-day tail buds cannot be assessed for somitogenetic activity by orthotopic transplantation. They were transplanted instead to the primitive streak of 8.5-day embryos which may provide the best alternative embryonic environment for inducing somitogenesis.

During normal development, the total number of somites formed in the vertebrate embryo is stringently controlled and does not vary for more than 3-5% in individuals of the same species (Maynard-Smith, 1960). A proper regulation of somite number is achieved even when the developing embryo has experienced an altered tissue mass and underwent compensatory growth (Cooke, 1977; Tam, 1981). Somite formation ceases abruptly when a determinate number of somites have been formed in the embryonic axis. This termination of somite formation could be brought about either by the loss of somitogenetic potency of tail bud mesenchyme or by certain changes in the tissue environment in the tail bud which inhibit somite differentiation. Tail buds isolated from avian embryos of different developmental stages when grafted to the chorioallantoic membrane will continue to form somites until the 50th-53rd pair of somites of the equivalent intact embryos have been formed (Sanders et al., 1986). Ultrastructural examination of the terminal mesoderm in the tail bud of embryos that have ceased to form somites reveals the presence of somitomeres (Bellairs, 1986), suggesting that the tail bud mesenchyme is capable of undergoing the initial events of somitogenesis. Somite segmentation, however, never occurs, probably as a result of inadequate cell-matrix interaction (Mills et al., 1990) or excessive cell death (Mills and Bellairs, 1989). In the present study, transplantation experiments were performed to test whether the cessation of somitogenesis in the mouse embryo is the result of either a restriction in the somitogenic potency of the tail bud mesenchyme or an inhibitory tissue environment. Tail bud mesenchyme of 13.5-day mouse embryos, which nearly have completed somitogenesis, was transplanted to the primitive streak of 8.5-day host embryos for a direct assessment of the developmental potency in an environment which is favourable for somitogenesis. For a comparison, somite formation was also studied in explants of intact tail buds of 13.5-day embryos cultured under similar in vitro conditions.

Materials and methods

Recovery and in vitro culture of embryos

Host embryos for the transplant experiments were obtained from pregnant female mice of ARC/S or ICR strains at 8.5 and 9.5 days p.c. (plug day = 0.5 day p.c.). The embryos were dissected out of the decidua and kept in warm PB1 or M2 medium supplemented with 10% heat-inactivated rat or fetal calf serum. The parietal yolk sac was removed and the embryos were transferred to fresh medium. The somite number of 8.5-day embryos was scored and only embryos having 4-8 pairs of somites were used as host embryos. The somite number of 9.5-day embryos could not be scored accurately through the investing extraembryonic membranes, which have to be left intact to ensure normal in vitro development. Instead, the somite number was counted in several (4-5 per experiment) randomly selected embryos which were not intended for in vitro culture. embryos were cultured in a 1:1 or 1:3 mixture of Dulbecco’s modified Eagles medium (containing 4 g l⁻¹ glucose; Flow Laboratory) and heat-inactivated rat serum (DMRS medium), supplemented with 100 mM L-glutamine. 8.5-day embryos were cultured in groups of 4-5 in 50 ml glass bottles (Wheaton) containing 3-4 ml of DMRS medium. The culture bottle was kept at 37°C on a roller apparatus (30 revs minute⁻¹) and gassed intermittently for the first 16-18 hours with 5% CO₂, 5% O₂ and 90% N₂ followed by 5% CO₂, 20% O₂ and 75% N₂ for the rest of the 22-24 hours culture period. 9.5-day embryos were cultured at 37°C in the same DMRS medium in 20 ml bottles which were gassed continuously with 5% CO₂, 20% O₂ and 75% N₂ on a rotating drum (30 revs minute⁻¹; BTC Engineering, Cambridge).

In vitro culture of posterior fragments

Somite formation was studied in explanted posterior fragments of 9.5-day and 13.5-day embryos. The embryo fragments were isolated by transecting the trunk of 9.5-day embryos immediately posterior to somite 19 and the tail of 13.5-day embryos at somite 55. Each fragment thus contained a known number of somites, a complete presomatic mesoderm and the caudal tissue consisting of either the primitive streak (9.5-day embryo) or the tail bud (13.5-day embryo). These fragments were cultured at 37°C for 22-24 hours in DMRS medium in rotating bottles gassed with 5% CO₂, 20% O₂ and 75% N₂ as for whole embryo culture. The total number of somites found in the explants after culture was counted and the fragments were fixed in Sanfelice solution for histology.
**Preparation of cells for transplantation**

Cells marked with HMG-lacZ transgene - pHMG CoA-lacZ plasmid

HMG-CoA reductase (3-hydroxy-3-methylglutaryl coenzyme A reductase) catalyzes a key step in the mevalonate pathway for the synthesis of cholesterol and other non-sterol compounds and plays a housekeeping role in cholesterol homeostasis and cell proliferation (Goldstein and Brown, 1990). The 5′-regulatory region of the mouse HMG-CoA reductase gene was therefore used to provide the regulatory signal for directing ubiquitous lacZ gene expression in the embryo (Mehtali et al., 1988). A 5.6 kb BamHI fragment consisting of 1.4 kb promoter sequences, 0.7 kb of the untranslated exon 1 and 3.5 kb of intron 1 segment was subcloned into the polylinker site of pBluescript KS+ vector (Stratagene) to produce pHMG-BS. Restriction enzymes EcoRI and KpnI were used to cut pHMG-BS to yield 5′-blunt and 3′-overhanging ends, respectively. The resulting fragment was ligated to a 3.3 kb SmaI-KpnI fragment of the reporter lacZ gene (derived from plasmid SRL-30L7-βGal, Kalderon et al., 1984) which has at its 5′ end a nuclear-location signal (NLS) coding for amino acid residues 127-147 of SV40 large T antigen and at its 3′ end the SV40 polyadenylation signal (Tan, 1991). The final construct was cut with NorI and KpnI to give a 8.9 kb linear fragment (Fig. 1) for microinjection.

**Generating transgenic mice**

Transgenic mice were generated by microinjection of DNA into pronuclei of fertilized oocytes. Prior to injection, the DNA was purified on agarose gels and then by ion-exchange chromatography on NACS Prepac columns (BRL). The DNA solution was injected in picolitre volumes at a concentration of 2 ng µl⁻¹ into fertilized oocytes obtained from superovulated (C57BL6 X DBA/2)F1 mice. The pups were weaned at 3 weeks and positive founder animals were identified from Southern blotting of tail DNA as previously described (Tan, 1991).

**Detection of β-galactosidase activity**

Specimens were fixed for 1 hour in 4% paraformaldehyde in fixative buffer (0.1 M pH 7.4 Sorensen’s phosphate buffer containing 10 mg F⁻¹ MgCl₂ and 5 mM EGTA). After fixation, the embryos were washed in 2-3 changes of washing buffer (fixing buffer containing 0.01% sodium deoxycholate and 0.02% Noridet P-40). Specimens were stained overnight at 37°C with X-gal solution, which was made up of 0.1% 4-chloro-5-bromo-3-indolyl-β-D-galactopyranoside (X-gal, Sigma), 2 mM MgCl₂, 5 mM EGTA, 0.01% (w/v) sodium deoxycholate, 0.02% (w/v) Noridet P-40, 5 mM KFe(CN)₆ and 5 mM K₃Fe(CN)₆.6H₂O in 0.1 M pH 7.4 Sorensen’s phosphate buffer. The X-gal was prepared as a 4% stock solution in dimethylformamide and was added to the mixture immediately before use.

**Isolating cells for transplantation**

8.5- to 13.5-day embryos were obtained from pregnant transgenic animals by dissecting the decidua. The somite number of embryos was scored after the fetal membranes were removed. For 8.5- and 9.5-day embryos, the cephalic region was separated from the rest of the embryo and used for X-gal staining for an assessment of the transgenic status of the embryo. The bodies of embryos were cultured individually in a 96-well culture plate in DMRS medium at 37°C under 5% CO₂ in air. Cephalic fragments were incubated in the X-gal staining solution at 37°C for identifying the transgenic embryos in the litter. For 10.5- to 13.5-day embryos, half of the head was removed for X-gal staining and the trunk was then cut at somite 30. The posterior fragments were cultured individually in DMRS medium in a 24-well culture plate. Transgenic embryos were selected on the basis of an intense blue coloration (see Results) of the cephalic fragments after staining for 1-1.5 hours in X-gal solution. The posterior fragments of the transgenic embryos were then removed from the culture plates and washed in several changes of M2 or PB1 medium supplemented with 10% heat-inactivated rat serum.

Cells were isolated from the primitive streak (PS) located at the caudal aspect of the posterior neuropore of 8.5- and 9.5-day embryos. A small wedge of tissue fragment containing the PS was excised. The surface ectoderm adjacent to the PS and underlying gut endoderm was surgically removed with finely polished alloy needles and the PS was dissected into small clumps of 10-20 cells for transplantation. For 10.5- to 13.5-day embryos, the tail bud (TB) was isolated from the terminal portion of the tail by a transverse cut made at 150-200 µm from tail tip. The neural tube and the hind gut were first removed and the mesodermal cells were obtained by scraping the mesenchyme off the basal aspect of the surface ectoderm. Clumps of 10-20 mesenchymal cells were isolated by further dissection with fine glass needles (Tam, 1991) and were transferred to a 10 µl microdrop of PB1 solution under paraffin oil for transplantation. Embryo fragments left behind after cells were isolated were then incubated in X-gal solution to cross-check the transgenic status of the donor embryo.

**Cells labelled with ³H]thymidine**

8.5-, 10.5- and 13.5-day ARC/S or ICR strain embryos were explanted from the decidua into PB1 medium. The ectoplacental cone, yolk sac and amnion of the 8.5-day embryos were left intact. For 10.5- and 13.5-day embryos, all the extraembryonic membranes were removed and, after scoring the somite number, the tail region of the embryo was cut off from the trunk and transferred to fresh DMRS medium in a 24-well culture plate. Transgenic embryos were selected on the basis of an intense blue coloration (see Results) of the cephalic fragments after staining for 1-1.5 hours in X-gal solution. The posterior fragments of the transgenic embryos were then removed from the culture plates and washed in several changes of M2 or PB1 medium supplemented with 10% heat-inactivated rat serum.

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**Fig. 1. HMG CoA-lacZ fusion gene structure.** The 8.9 kb NorI-KpnI linear fragment excised from the vector consists of 5.6 kb of the mouse 3-hydroxy-3-methylglutaryl coenzyme A reductase gene sequence and 3.3 kb of lacZ gene. A nuclear location signal (NLS) coding for amino acid residues 127-147 of SV40 large T antigen is fused to the 5′-terminal of the lacZ gene to provide a β-galactosidase hybrid protein with nuclear localization.
PB1 medium. Whole 8.5-day embryos and the tail fragment of older embryos were transferred to a labelling solution of DMRS medium containing 0.4 μM cold thymidine and 0.37 mBq ml\(^{-1}\) of radioactive [methyl-\(^3\)H]thymidine (Amersham TRK 637, specific activity 1.48 TBq mmol\(^{-1}\)). The embryos were cultured for 2 hours at 37°C in rotating bottles and gassed with 5% CO\(_2\) in air. After labelling, embryos were washed with PB1 medium containing 8 μM cold thymidine. The yolksac and amnion were removed and the embryonic fragments containing the primitive streak and the tail bud were excised and then dissected with glass needles to obtain clumps of 10-20 cells for transplantation.

**Wheat germ agglutinin-gold conjugate labelling**

8.5-, 10.5- and 13.5-day ARC/S or ICR strain embryos were dissected from the yolksac and amnion and the posterior portion of the embryos were isolated. Small clumps of cells were isolated from the primitive streak or the tail bud mesenchyme. These cell clumps were then transferred to 50 μl microdrops of M2 or PB1 medium containing about 50 nl of a concentrated WGA-Au (containing 1-2 mg ml\(^{-1}\) of WGA) preparation (Tam and Beddington, 1987) and incubated on a warm plate at 35-37°C for 60 minutes. After labelling, the cell clumps were washed in microdrops of PB1 or M2 medium and subdivided for transplantation.

**Transplantation experiments**

The following transplantation experiments were performed.

1. **Orthotopic transplantations of 8.5-day and 9.5-day PS cells to synchoric hosts**

These experiments were designated as 8→8 and 9→9 transplants, which represent, respectively, the transplantation of 8.5-day PS cells to 8.5-day host embryos and 9.5-day PS cells to 9.5-day host embryos. The 8→8 transplant was done by microinjecting PS cells marked with HMG-lacZ, \(^3\)H-TdR or WGA-Au into the primitive streak of the host embryo through the visceral endoderm, as described by Tam and Beddington (1987). Double labelling of HMG-lacZ and WGA-Au (4 experiments) or \(^3\)H-TdR and WGA-Au (2 experiments) was also done to ascertain the precision of grafting and the co-distribution of different cell labels among the progeny of the transplanted cells.

For 9→9 transplants, the yolksac and amnion of the host embryo were first slit open with watchmaker’s forceps to gain access to the posterior part of the embryo. The lateral aspect of the tail was held by suction with a flame-polished pipette which was inserted through the opening in the membranes. The injection pipette containing the 9.5-day PS cells was then inserted into the primitive streak through the neuroepithelium of the posterior neuropore. PS cells were expelled gently from the pipette as it was slowly withdrawn. After transplantation, the opening was closed by pinching the yolksac together with a pair of forceps.

2. **Transplantation of PS and TB cells to asynchronic hosts**

In these experiments, PS cells from 9.5-day embryos and TB mesenchyme from 10.5-, 11.5- and 13.5-day embryos were transplanted to the primitive streak of 8.5-day host embryos. The transplantation procedure was similar to that of the 8→8 experiment but the experiments were designated differently as 9→8, 10→8 11→8 13→8 transplants according to the age of the donor embryos.

**Analysis of the result of transplantation experiments**

Host embryos were harvested after 22-24 hours of in vitro culture and the morphology assessed under a dissecting microscope. Grossly abnormal embryos were discarded. Embryos receiving transplants of \(^3\)H-TdR- or WGA-Au-labelled cells were fixed in Carnoy’s fixative and processed for wax histology. For autoradiography, dewaxed sections were coated with Ilford K2 Nuclear Research Emulsion or Kodak NTB-2 emulsion and exposed for 14 days at 4°C. The autoradiographic preparation was developed with Kodak D19 developer, fixed in Kodak rapid fixer and counterstained with haematoxylin and eosin. For visualisation of the WGA-Au label, the dewaxed sections were silver impregnated and counterstained with light green. Embryos containing HMG-lacZ cells were stained for β-Gal activity as previously described. After overnight staining, embryos were washed with 2-3 changes of buffer and examined for the presence of lacZ-expressing cells. Embryos with blue X-gal-stained cells were counterstained with eosin and processed for wax histology.

The distribution of labelled cells in various tissue types was determined from sections of the embryos. Specific attention was given to the location of labelled cells in specific somites and somite-meres in the paraxial mesoderm. The segmental position of labelled cells in the neural tube, the notochord and the lateral mesoderm was determined with reference to the equivalent somitic address. In order to estimate the clonal size of graft-derived cells, the total number of lacZ-expressing cells was counted in embryos from which serial sections were obtained.

**Results**

**HMG-lacZ transgenic mice**

Seven transgenic lines of mice were established from 41 founder animals obtained after microinjection of 370 fertilized oocytes. Among these lines, line 253 was found to carry at least 14 copies of the injected DNA and showed the highest expression of the lacZ gene. Breeding results of the mice suggested strongly that the transgene may have integrated into a single locus on the X chromosome. Homozygous females were usually under-represented in the litters (Tan and Tam, unpublished). Embryos used for this study were therefore derived mostly from matings of hemizygous males and females which produced litters with a mixture of wild-type, hemizygous and homozygous embryos. Ubiquitous and uniform expression of the transgene as revealed by X-gal staining was observed in all cells (Fig. 2A,B) of the hemizygous male and homozygous female embryos from the morula stage (2.5-day) onwards, and remained strong at least until 13.5 days p.c. Non-transgenic embryos showed no staining, and hemizygous female embryos could readily be distinguished from others by the patchy staining of tissues after the onset of X-chromosome inactivation (Fig. 2C). Only embryos that showed an intense and uniform blue coloration during the initial screening by X-gal reactivity were used for isolating cells for transplantation experiments. Eight 7.5-day embryos obtained from a transgenic HMG-lacZ female were cultured for 40 hours in DMRS medium. Six developed normally and all stained blue with X-gal reagent. Fragments of 8.5- and 9.5-day embryos obtained after isolation of cells and cultured for 6-20 hours in vitro did not show any diminution of X-gal staining, suggesting that the expression of the transgene was not altered by in vitro manipulations.

**Somite formation in embryo fragments**

Posterior fragments isolated from 9.5- and 13.5-day embryos...
Fig. 2. (A) A transgenic 8.5-day early-somite-stage embryo showing ubiquitous expression of lacZ in all tissues including the paraxial mesoderm (somites, sm) and the primitive streak (ps). (B) Uniform expression of lacZ in the dermamyotome and sclerotome of the somites (sm) of a 9.5-day transgenic embryo showing nuclear localization of the enzyme reaction. Strong expression is seen in the notochord (nd) and the gut endoderm (en). (C) Patchy expression of lacZ in cells of the neural tube (nt), somite (sm), the lateral plate mesoderm (lm) and the endoderm (en) of a 8.5-day transgenic embryo. (D) In situ X-gal staining of the lacZ-expressing cells in the last 6 somites and the anterior portion of the presomitic mesoderm of a chimaera 8→9 embryo (the distribution is marked by a dashed line) 24 hours after transplantation. The X-gal-positive cells appear brown due to the mismatching of colour temperature of the light source and the film. (E) Longitudinal section of the paraxial mesoderm of an embryo of the 8→8 transplant showing the distribution of lacZ-expressing (blue) cells in the last 2 somites and the first 3 somitomeres in the presomitic mesoderm (boundaries of segmental units are marked by arrowheads). (F) WGA-Au-labelled cells (arrowheads) in the somites, the presomitic mesoderm and the lateral mesoderm (10→8 transplant). (G) The presence of WGA-Au-labelled cells (arrowheads) in the somites and presomitic mesoderm (8→8 transplant; silver grains appear as bright blue spots under dark-field illumination). (H) Colonization of the somites and the first somitomere in the presomitic mesoderm by lacZ-expressing cells (9→9 transplant, dark-field illumination). Most labelled cells (whose nuclei appeared bright red under dark-field illumination) are found in the ventromedial aspect of the somites. (I) Colonization of the presomitic mesoderm (pm) and the hindgut (hg) endoderm by lacZ-expressing cells (9→9 transplant, dark-field illumination). The labelled cells are localized in the medial portion of the somitomeres. (J) LacZ-expressing cells in the notochord (nd) of a 9→9 embryo (oblique section through the floor of the caudal spinal cord (sc) and the dorsal aorta (da), dark-field illumination). (K) LacZ-expressing cells in the ventral region of the spinal cord(sc) (tangential section of the neural tube of a 9→9 embryo, dark-field illumination). Magnification: bar = 100 µm (A,B,C,D, E, F, G, J and K), bar = 50 µm (H and I).

continued to form new somites in vitro. The 9.5-day embryo has an average of 20.6 somites at the time of dissection and an additional 9.4 somites were formed in the posterior fragment after 24 hours of culture, thus giving a total of 30.1 somites if the embryo has remained intact (Table 1). In separate experiments, intact 9.5-day embryos cultured for 24 hours under similar conditions formed 31.4 pairs of somites (Table 1). Somite formation therefore proceeded normally even in surgically manipulated explants. 13.5-day embryos used for this experiment had an average of 63.1 somites (Table 1). An additional 2.1 pairs of somites were formed after 7 hours in culture and when the explants were re-examined at 24 hours, there was no further increase in somite numbers. Somite number of intact embryos in vitro increased to an average of 65.2 pairs of somites over the same time period. Explantation of the posterior TB fragment, therefore, did not promote the formation of more somites than expected. Histological examination of the explants revealed the presence of bona fide epithelial somites and well-defined intersomitic fissures. The somites were however more closely packed together because of the reduced axial elongation of the fragments in vitro.

Development of experimental embryos
Table 2 gives the mean somite number of 8.5-day embryos that were used as hosts. There was no significant difference in the somite number (range of means: 5.28-6.03) between different transplant experiments. Over 75% of the embryos developed normally after experimental manipulation and in vitro culture. Between 16.3 and 19.5 pairs of somites were present in the cultured embryos and these together with another 6 somitomeres in the presomitic mesoderm gave at least 23 to 26 segmental units in which cell distribution could be analyzed. Some embryos were cultured for up to 44 hours in vitro and they formed 28-30 somites (n=8). However, these embryos began to show morphological anomalies and therefore were not included in this study. 9.5-day host embryos had 20.7 somites at the time of transplantation and contained 29.2 somites after 24 hours of culture (Table 2).

Pattern of tissue colonization
Cells used for transplantation were obtained from the primitive streak (PS) or tail bud (TB) of 8.5- to 13.5-day embryos whose somite numbers are shown in Table 2. Transgenic cells were used in all types of transplants, but ³H-TdR- and WGA-Au-labelled cells were used only in 8→8 10→8 and 13→8 experiments.

Altogether 448 chimaeric embryos were analyzed histologically for the spatial distribution of transplanted cells in various embryonic tissues (Table 3). The following observations were common to all groups of embryos: (1) PS or TB cells placed in the primitive streak of 8.5-day embryos colonized only those embryonic tissues that were formed after transplantation. (2) In most host embryos, PS or TB cells contributed predominantly to the paraxial mesoderm (Fig. 2D-I) and the lateral mesoderm (Fig. 2F), but in some cases also to the hindgut endoderm (Fig. 2I), the notochord (Fig. 2J) and the neural tube (Fig. 2K). (3) Colonization of the paraxial mesoderm in most cases spanned a considerable

Table 1. Formation of somites in intact 9.5-day embryos and in posterior fragments isolated from 9.5-day and 13.5-day embryos

<table>
<thead>
<tr>
<th>Embryos</th>
<th>Initial somite number</th>
<th>Number of somites added in the cultured fragments</th>
<th>Equivalent somite number of intact embryos in vitro</th>
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<tbody>
<tr>
<td>9.5-day intact embryo</td>
<td>20.7±2.8 (23)</td>
<td>ND</td>
<td>31.4±1.9 (48)</td>
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<tr>
<td>9.5-day fragments</td>
<td>20.6±1.4 (35)</td>
<td>ND</td>
<td>30.1±2.2 (35)</td>
</tr>
<tr>
<td>13.5-day fragments</td>
<td>63.1±1.2 (36)</td>
<td>9.4±2.0 (35)</td>
<td>65.2±1.0 (33)</td>
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<tr>
<td></td>
<td>2.1±1.0 (34)</td>
<td>2.2±0.9 (25)</td>
<td></td>
</tr>
</tbody>
</table>

ND, not determined. Data as mean ± s.e.m. Number of embryos analyzed in parentheses.
axial distance (Fig. 2D-G). (4) Cells derived from the transplanted population tended to colonize the ventromedial tissues of the somites and the presomitic mesoderm (Fig. 2H, I). (5) Labelled cells were not restricted to any lineage within the somites and were found in both the sclerotome and dermamyotome of the somites (Fig. 2E, F, H). (6) The colonization of the lateral mesoderm, the notochord and the neural tube often occurred at the same segmental levels as the paraxial mesoderm in the host embryo. (7) In spite of the extensive contribution to newly formed embryonic tissues, some transplanted cells always remained within the primitive streak of the host embryos.

Results obtained from the transplantation of three differently marked cell populations did not differ significantly from one another with respect to colonization of the paraxial mesoderm, the lateral mesoderm and the caudal structures (PS/TB). The only disparity between the differently marked cells was the more frequent colonization of the hindgut endoderm by the WGA-Au-labelled cells. In four experiments where cells of the transgenic embryos were secondarily labelled with WGA-Au prior to transplantation, about 70% of the β-Gal-positive cells found later in the paraxial mesoderm and the hindgut endoderm also carried the WGA-Au marker. Similar transplant experiments involving cells labelled with ³H-TdR and WGA-Au revealed that about 70-80% of transplanted cells in the paraxial mesoderm contained both labels after 24 hours of in vitro development. The lack of co-localisation of transgenic label and the exogenous markers in 20-30% of the transplanted cells suggested that either some indiscriminate transfer of the non-genetic markers may have occurred.

Table 2. Somite number (mean±s.e.m.) of donor and host embryos at the time of grafting and in embryos analyzed after 20-22 hours of in vitro culture

<table>
<thead>
<tr>
<th>Embryos</th>
<th>Grafting experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donors</td>
<td></td>
</tr>
<tr>
<td>HMG-lacZ</td>
<td>6.4±0.5 (51)</td>
</tr>
<tr>
<td>³H-TdR and WGA-Au</td>
<td>7.4±1.0 (46)</td>
</tr>
<tr>
<td>Hosts</td>
<td></td>
</tr>
<tr>
<td>HMG-lacZ</td>
<td>5.58±0.16 (108)</td>
</tr>
<tr>
<td>³H-TdR</td>
<td>5.87±0.08 (24)</td>
</tr>
<tr>
<td>WGA-Au</td>
<td>5.57±0.18 (42)</td>
</tr>
<tr>
<td>Cultured and analysed</td>
<td></td>
</tr>
<tr>
<td>HMG-lacZ</td>
<td>18.6±0.1 (77)</td>
</tr>
<tr>
<td>³H-TdR</td>
<td>17.2±0.2 (24)</td>
</tr>
<tr>
<td>WGA-Au</td>
<td>17.2±0.4 (42)</td>
</tr>
</tbody>
</table>

Number of embryos in parentheses.

Cells for grafting were obtained from transgenic (HMG-lacZ), tritiated thymidine-labelled (³H-TdR) or wheat germ agglutinin-gold conjugate (WGA-Au)-labelled embryos.

Table 3. Distribution of the donor cells in the host embryos 22-24 hours after the transplantation of primitive streak cells or tail bud mesenchyme

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Number of embryos showing colonization of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graft</td>
<td>Para-Meso</td>
</tr>
<tr>
<td>8→8 HMG-lacZ</td>
<td>76</td>
</tr>
<tr>
<td>³H-TdR</td>
<td>24</td>
</tr>
<tr>
<td>WGA-Au</td>
<td>42</td>
</tr>
<tr>
<td>9→8 HMG-lacZ</td>
<td>50</td>
</tr>
<tr>
<td>³H-TdR</td>
<td>33</td>
</tr>
<tr>
<td>WGA-Au</td>
<td>92</td>
</tr>
<tr>
<td>10→8 HMG-lacZ</td>
<td>33</td>
</tr>
<tr>
<td>³H-TdR</td>
<td>27</td>
</tr>
<tr>
<td>WGA-Au</td>
<td>32</td>
</tr>
<tr>
<td>11→8 HMG-lacZ</td>
<td>20</td>
</tr>
<tr>
<td>13→8 HMG-lacZ</td>
<td>17</td>
</tr>
<tr>
<td>³H-TdR</td>
<td>38</td>
</tr>
<tr>
<td>WGA-Au</td>
<td>56</td>
</tr>
<tr>
<td>9→9 HMG-lacZ</td>
<td>33</td>
</tr>
</tbody>
</table>

Cell markers: ³H-TdR, tritiated thymidine; WGA-Au, wheat germ agglutinin-gold conjugate; HMG-lacZ, transgenic

Tissue types: Para-Meso, paraxial mesoderm; Sm, somite; Pm, presomitic mesoderm; Lat-Meso, lateral mesoderm; Som, somatopleure, Sp, splanchnopleure; PS-TB, tail bud and primitive streak; NP/NT, neural plate and neural tube; HGN, hindgut endoderm; ND, notochord; SurEct, surface ectoderm.
embryos examined histologically, 364 (87.7%) showed a spreading of labelled cells over many segmental units in the paraxial mesoderm which was consistent with the continuous recruitment of cells from the transplanted population in the primitive streak. In the remaining 51 embryos, clumps of labelled cells were found distributed to only 2-3 adjacent somites or somitomeres, and therefore may indicate a failure of incorporation of transplanted cells into the PS of the host embryo which resulted in an en masse allocation of cells to only a few somites.

The distribution of labelled cells in the paraxial mesoderm was studied in those 364 embryos that showed widespread segmental colonization. Table 5 summarizes the data on the localization of the transplanted cells in the somites and the somitomeres of the host embryos. The assignment of cells to specific somitomeres was made by relating the position of the labelled cells to a previously established map of somitomeres in the presomitic mesoderm (Tam, 1986). Results of statistical analyses of the data from the various transplant experiments using different cell markers are summarized in Table 6. Within-experiment comparisons revealed that there were significant differences in the distribution pattern of cells with respect to the type of markers carried by the cells but none of the markers was consistently different from the others in every experiment. Such differences were thus more likely to be due to sample sizes and experimental variations. For a general comparison of results of the different transplantation experiments, data obtained by using different markers were thus pooled. An analysis using the non-parametric Friedman’s test based on $\chi^2$ analysis revealed significant differences among all 5 transplant experiments (8→8, 9→8, 10→8, 11→8 and 13→8) and among the 3 transplant experi-

Table 4. Number (mean±s.e.m.) of lacZ-expressing cells in the mesodermal tissues of host embryos cultured for 22-24 hours following the transplantation of primitive streak or tail bud cells

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Paraxial mesoderm</th>
<th>Lateral mesoderm</th>
<th>Primitive streak</th>
</tr>
</thead>
<tbody>
<tr>
<td>8→8</td>
<td>27.7±4.8 (25)</td>
<td>30.7±9.4 (4)</td>
<td>27.5 (2)</td>
</tr>
<tr>
<td>9→8</td>
<td>20.1±3.4 (18)</td>
<td>9 (1)</td>
<td>6.8±1.7 (4)</td>
</tr>
<tr>
<td>10→8</td>
<td>28.1±2.0 (17)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11→8</td>
<td>17.1±3.4 (13)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13→8</td>
<td>11.1±3.3 (15)</td>
<td>0</td>
<td>5 (1)</td>
</tr>
</tbody>
</table>

Number of embryos analyzed in parentheses.

between the transplanted cells and the host cells, or that the transplanted cells may have been isolated from a hemizygous female transgenic carrying a mixed population of expressing and non-expressing cells due to random inactivation of the X chromosome. However, in contrast to the non-genetic markers, the use of the supposedly non-perishable HMG-lacZ marker is more likely to give a conservative but more realistic quantitation of the size of the population derived from transplanted cells at the end of the experiment. Cell counting was therefore performed on mesodermal tissues in serial sections of 88 embryos receiving HMG-lacZ cells (Table 4). The total number of labelled cells present in these embryos varied from about 15 to as many as 75. Most labelled cells were found in the paraxial mesoderm (range of the means: 11.1-27.7).

Within the paraxial mesoderm of the chimaeric embryos, transplanted cells were found more frequently in the pre-somitic mesoderm than in the somites (Table 3). Of the 415

Table 5. Distribution of donor cells in segments of the paraxial mesoderm in host embryos following the transplantation of primitive streak or tail bud cells and cultured for 22-24 hours in vitro

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Cell-marker</th>
<th>Number of embryos showing colonization of the following somites or somitomeres:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graft</td>
<td>Number of embryos</td>
<td>11</td>
</tr>
<tr>
<td>8→8</td>
<td>HMG-lacZ</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>$^3$H-TdR</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>WGA-Au</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>127</td>
</tr>
<tr>
<td>9→8</td>
<td>HMG-lacZ</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>$^3$H-TdR</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>WGA-Au</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>79</td>
</tr>
<tr>
<td>10→8</td>
<td>HMG-lacZ</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>$^3$H-TdR</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>WGA-Au</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>103</td>
</tr>
</tbody>
</table>


Numbers in bold type represent the total for all cell marker types in each experiment.
percentage of cases for each specific segmental unit (somite or somitomere) that showed colonization by the transplanted PS or TB cells. Data of the 11 experiments in which all three types of markers were used (8→8, 10→8 and 13→8) were derived from fewer samples and therefore were not included in the graphs. Fig. 3A and B show that following the orthotopic transplantation of 8.5- and 9.5-day PS cells, colonization of the paraxial mesoderm by the transplanted PS cells continued during the 24 hours period of the experiment. An analysis of the frequency at which specific segmental units were colonized revealed that a progressive increase in the contribution of labelled cells occurred as far as somite 20-21 followed by a decline in the colonization of more posterior somites. PS cells of 9.5-day embryos that were transplanted orthotopically to the PS of 9.5-day embryo behaved similarly to 8.5-day PS cells grafted into 8.5-day embryos with respect to their immediate recruitment to the most recently formed somitomeres which correspond to 25-26th somites (Table 5). However, an entirely different pattern of colonization was observed when 9.5-day PS cells were transplanted to 8.5-day embryos, somites located much more anteriorly than somites 25-38 were now colonized (Fig. 3B, Tables 5 and 6). TB cells of older embryos when transplanted to 8.5-day host embryos tended to colonize more posterior segmental units than PS cells (Fig. 3C,D) and this posterior bias seemed to increase with the mismatch of developmental ages between TB cells and the PS of the host embryo. There was also a distinctive lag in the onset of recruitment of the TB cells to the paraxial mesoderm with respect to the first segmental unit which was colonized after transplantation. PS cells of 8.5-day and 9.5-day donor embryos colonized on average the 11th somites which were formed after transplanted (Table 7). However, in some cases, colonization began as early as in the 6th-7th newly formed somites, which is equivalent to the first 2 somitomeres established after transplantation (Tam and Beddington, 1986). By contrast, TB cells of 10.5-, 11.5- and 13.5-day donor embryos started to colonize the paraxial mesoderm about 2-4 somites later than the PS cells and this delay was more pronounced when TB cells of oldest (13.5-day) donor embryos were transplanted (Table 7).

In this study, somite formation in whole posterior fragments of 13.5-day (63-somite) embryos stopped when the correct number of somites for the embryonic axis had been generated. This is unlikely due to the limitation of the in vitro culture environment because 9.5-day posterior fragments cultured under similar conditions did continue to form the normal number of somites as expected in intact embryos. However, when 13.5-day TB cells were transplanted to the PS of 8.5-day embryo, they were found to participate in the formation of at least another 10-11 somites in the host embryo during a 24-hour period of in vitro development (Fig. 3D).

### Discussion

#### Summary of results

The development fate of the primitive streak (PS) cells and the tail bud (TB) mesenchyme of organogenesis-stage mouse embryos has been examined. Orthotopic transplantation of the PS cells of 8.5-day (early-somite stage) and 9.5-day (forelimb bud stage) embryos resulted in an extensive colonization of the paraxial mesoderm of the host embryo. In both cases, contributions to the paraxial mesoderm, began immediately after transplantation with the most recently formed somitomeres of the presomatic mesoderm and progressively increased with somites that formed subsequently.
Somitogenesis in mouse embryos during a 22-24 hours period of in vitro development. Other tissues such as the lateral mesoderm, the notochord, the neural tube and the hind gut were also colonised, but to a lesser extent, by the transplanted PS cells. Significantly, a small population of transplanted cells always remained in the primitive streak or the early tail bud of some host embryos after 24 hours of in vitro development. Primitive streak cells of 9.5-day embryos and tail bud mesenchyme taken from more advanced (10.5- to 13.5-day) embryos showed similar ranges of tissue colonization when transplanted asynchronously to the primitive streak of the early-somite stage embryo. However, in contrast to the behaviour of PS cells in the 8→8 and 9→8 transplant experiments, there was a significant delay in the initiation of colonization of the paraxial mesoderm by the transplanted TB cells in the 10→8, 11→8 and 13→8 embryos. TB cells obtained from more advanced embryos also tended to colonise more posterior somites. Nevertheless, transplanted 9.5-day PS cells and TB cells of older embryos were found to colonize somites that were much more anterior than those to which they would normally contribute in the undisturbed embryo. Most interestingly, TB cells of 13.5-day embryos, which were unable to sustain somitogenesis in the intact tail bud, were capable of participating in further somite formation well beyond their normal developmental life span following transplantation to the PS of the early-somite-stage embryo.

The caudal tissues as multi-progenitor cell populations
Results of the transplant experiments extend the observations previously made on gastrulating and early-somite-stage embryos (Tam and Beddington, 1987; Tam, 1989) and provide direct evidence that cells of the primitive streak and the tail bud mesenchyme of advanced organogenesis-stage embryos are important sources of somitic mesoderm as well as other embryonic tissues such as the lateral mesoderm, the neural tube, the notochord and the gut endoderm. Removing these reservoirs of cells by surgical extirpation of the primi-
The ability to contribute cells to somites at different segmental levels of the embryonic axis. The 8.5-day and 9.5-day PS contribution to the somitic mesoderm by cells located specifically at the anterior part of the primitive streak strongly suggest a distinct heterogeneity in the developmental fate of PS cells. The present study has demonstrated unequivocally that the allocation of cells from the caudal progenitor population to the segmental units in the paraxial mesoderm follows a strict cranio-caudal order. In line with previous observations, the allocation of PS cells that were transplanted to orthotopic sites (e.g. 8→8 and 9→9 transplants) began first with the most recently established somitomeres in the posterior part of the presomitic mesoderm (Tam et al., 1982; Tam and Beddington, 1986, 1987). There was a slight tendency for the PS cells to colonize the medial aspect of the somitomere and the somite, the ventromedial tissues of the neural tube and the adjacent midline notochord. There is however no restriction to any somitic tissue lineages since cells were found in both the dermamyotome and the sclerotome of the somites. In the chick embryo, cells originating from the node and the streak are allocated almost exclusively to the medial and lateral portion of the somites respectively (Selleck and Stern, 1991) but it is not clear how such medio-lateral segregation of cell populations can be maintained when there is extensive mixing of cells during the maturation of the somitomeres in the presomitic mesoderm (Stern et al., 1988; Tam, 1988). Tissue heterogeneity, which may be associated with lineage- or region-specific gene expression, is usually established after somite segmentation when cell movement is more restricted, e.g. Pax-3 in the dorsolateral region of the somite (Gaunt et al., 1991), col2a-1 in the sclerotome (Cheah et al., 1991), α-actins in the myotome (Sassoon et al., 1988) and cytactin in the anterior half of the sclerotome (Tan et al., 1987).

Specification of the segmental fate

Primitive streak cells of 9.5-day embryos and tail bud mesenchyme of 10.5- to 13.5-day embryos are capable of colonising a multitude of tissues in the host embryo and therefore display no obvious restriction of developmental potency. That embryonic cells destined for posterior somites can participate in the formation of somites belonging to the anterior part of the body pattern clearly necessitates a re-specification of the segmental fate of the somitic precursors. Studies in the mouse embryo have shown that different homeobox-containing genes are expressed in well-defined subsets of somites and their derivatives (Gaunt et al., 1988; Holland and Hogan, 1988). The expression of a unique combination of Hox genes in groups of somites has been postulated to be instrumental in the specification of vertebral identity of the somites. The evidence in support of this notion

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comes from a detailed study on the homeotic transformation of the mouse vertebrae induced by retinoic acid. The adoption of more anterior or posterior vertebral characteristics by specific somites in the treated embryo is accompanied by an alteration of the expression of the *Hox* genes (Kessel and Gruss, 1991) and the specification of segmental characteristics is likely to occur as early as the stage of recruitment of cells to the specific somitomeres (Sofaer, 1985; Tam and Beddington, 1986). Transplantation studies in the chick embryo have indicated that segmental specification at least for the chondrogenic tissues may have already taken place when the somitic cells are still in the segmental plate. Thus somitic mesoderm of the prospective thoracic region when transplanted to the cervical regions will develop into the thoracic type of rib-carrying vertebrae (Kieny et al., 1972). By contrast, the myogenic population has escaped from this early segmental specification because muscles derived from the prospective thoracic somites will differentiate into muscles consisting of the fibre types appropriate for the brachial muscles (Cauwenbergs et al., 1986; Butler et al., 1988). The apparent lability in the myogenic fate of the somitic mesoderm may be explained by the greater extent of tissue mixing and interaction involved during the differentiation of the myotome to muscle. The sclerotomal population, which migrates en masse and does not mix with the host tissue, is perhaps less compelled to alter its developmental fate. In the mouse, transplantation of *Hox*-1.1 expressing somites to ectopic positions in the embryonic axis does not alter the expression of the *Hox* gene (Beddington et al., 1992). If *Hox* gene expression is related to segmental specification, then this result would suggest that such specification is both irreversible and stable. It is not known from this experiment and the avian experiments, which also involve the relocation of whole tissues so that the original tissue environment is still preserved, whether gene expression and cell fate are indeed regulated in a cell-autonomous fashion.

In the present study, PS and TB cells were transplanted in small clumps to new host sites and their descendants were widely scattered and mixed extensively with the host cells. Their participation in the formation of somites inappropriate to their expected fate must therefore require a major re- specification of their original developmental program. It remains to be elucidated whether the expression of segment-specific genes such as the *Hox* gene is also altered under such circumstances.

Cells derived from the PS and the TB of older embryos tended to colonize more posterior somites when transplanted to the early-somite-stage host embryo. This posterior bias in tissue contribution is more pronounced as the mismatch in developmental status increases between the transplanted cells and the host tissue. This behaviour of PS and TB is consistent with the observation that the progenitor population in the PS and TB becomes progressively restricted in its histogenetic potency with age: the younger tissues has the potential to contribute to tissues of both the cranial and caudal body parts but the older tissue generates tissues predominantly in the caudal region of the embryo (Tam, 1984). In the asynchronous transplant, somitic precursor cells derived from the PS and TB cells of older embryos are confronted by the host tissue of a different developmental status, and somite formation would have to be accomplished by the compliance and responsiveness of the transplanted cells to the interactive signals emanating from the host cells or the new tissue environment. Several studies have identified a regulatory role in somite morphogenesis of extracellular matrix components such as fibronectin and laminin (Cheney and Lash, 1984; Mills et al., 1990), adhesion molecules such as N-CAM and N-cadherin (Duband et al., 1987) and cell surface glycoconjugates and carbohydrate antigens (Griffith and Wiley, 1989, 1990; Loveless et al., 1990; Thorpe et al., 1988). Most of these molecules are expressed during the maturation of the somitomere or at somite segmentation. There is no systematic study of the expression of the cell surface molecules, growth-promoting factors and the extracellular components or their cellular receptors in the developing primitive streak or tail bud of the mouse embryo. Results of the present study would suggest that the mismatch in developmental status might be related to the incompatibility of the transplanted cells with their immediate cellular or tissue environment. An effective interaction between the somitic precursor of the primitive streak and the resident cell population in the presomatic mesoderm has been proposed to be one of the key steps in somite formation: cells in the presomitic mesoderm act as the focal point for primitive streak derived cells during the assembly of the somitomere (Bellairs and Veini, 1984; Ooi et al., 1986; Tam, 1988). The initial lag and the posterior bias in the segmental allocation of advanced PS and TB cells might reflect the time and effort taken by the transplanted cell to comply and to interact properly with the native PS cells and the presomitic mesoderm.

### Developmental potency of the tail bud cells

Whole tail fragments of 13.5-day embryos containing the tail bud failed to sustain somitogenesis beyond the stage that the expected number of somites are formed (this study, Sanders et al., 1986 for the chick embryo). Ultrastructural examination of the terminal regions of the paraxial mesoderm in the tail of the chick embryo (Bellairs and Sanders, 1986) and the mouse embryo (Tam, unpublished) has revealed the presence of fully organized somitomeres that should potentially differentiate into somites. However, somites are never formed from these somitomeres either in situ (Bellairs, 1986) or after grafting the tail bud to ectopic sites in the wing bud (Krenn et al., 1990). By contrast, when the tail buds are grown as teratomas under the kidney capsule (mouse: Tam, 1984) or as disaggregated cells in vitro (chick: Griffith and Sanders, 1991), the tail bud cells differentiate extensively into a diverse multitude of tissues including some typical somitic derivatives such as muscle, bone and cartilage. It has also been shown that the differentiative capacity of the tail bud mesenchyme in vitro is influenced significantly by the types of extracellular matrix substratum (Griffith and Sanders, 1991). These observations seem to suggest that the developmental potency (particularly the somitogenetic potency) of the tail bud mesenchyme is profoundly influenced by the tissue environment in the tail bud. The failure of somitogenesis may be brought about by the mismatching of the types of matrix molecules and their receptors (Mills et al., 1990; Griffith and Wiley, 1989; Griffith and Sanders, 1991) or the induction of excessive cell death in the tail bud mesenchyme (Mills and Bellairs, 1989). The removal of environmental
constraints by culturing TB cells in vitro or by breaking up the normal tissue relationship in the teratoma may therefore allow the expression of the somitogenetic potency still retained by the tail bud mesenchyme. This contention is further supported by the results obtained by transplanting TB cells from 13.5-day embryos to an embryonic environment which is still actively committed to somite formation. The TB cells continue to participate in somitogenesis well beyond their expected developmental life span and by specifying their fate, can contribute to embryonic structures in an entirely different part of the embryonic body. Although programmed cell death may also be involved with the withdrawal of the tail bud mesenchyme from somitogenesis, this certainly does not involve the entire tail bud population (Griffith and Sanders, 1991). It is more likely therefore that the abrupt cessation of somitogenesis during development is not due to a loss of the somitogenetic potency of the progenitor population but instead to the withdrawal of cells from somitogenesis as the environment in the tail bud becomes unfavourable. In order to investigate further the relative importance of the environment versus cell potency, it may be worthwhile extending the present study to examine the somitogenetic potency of cells of mutant embryos that fail to form somite normally (Hogan et al., 1985). A particularly interesting case is the amputated mouse embryos, which fail to form somites of the lower trunk and the tail (Flint et al., 1978). The onset of this developmental defect seems to coincide with the changeover of the recruitment of somitic mesoderm from the primitive streak to the tail bud (Schoenwolf, 1977), suggesting that, in these mutant embryos, the somitic progenitors in the tail bud may have a more reduced somitogenetic potency compared to their predecessors in the primitive streak.

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