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ORIGINAL ARTICLE

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The fate of the first avian somite

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Abstract We have studied the derivatives of the first somite using the quail-chick marking technique. After transplantation of the somite, the chick embryos were reincubated for periods ranging from 4 h to 11 days. Coronal and sagittal sections of the embryos were prepared for parallel staining with Feulgen-reaction, anti-quail antibody, anti-desmin antibody and QH-1 antibody. The first somite consists of an epithelial envelope surrounding somitocoele cells. Like other somites, it forms sclerotome, dermatome and myotome. Cells contribute to the occipital and parasphenoid bone, the meninges, the dermis in the occipital region and the pharyngeal connective tissue. The contribution of the first somite to bones, meninges, dermis and pharyngeal connective tissue is characterised by sharp anterior and posterior boundaries. In contrast, other derivatives such as connective tissue surrounding the vagus nerve, the carotid artery, and jugular vein exceed 10 to 18 segments. This is also true for myogenic cells participating in the formation of the cucullaris capitis muscle that extends from the temporal bone to the shoulder. In one third of the embryos, myocytes of the intrinsic laryngeal muscles are derived from the grafted first somite. Moreover, endothelial cells originate from this somite and migrate into the head (hindbrain, meninges, dermis), neck (pharynx, connective tissue surrounding the vagus nerve, carotid artery and jugular vein) and thorax. With respect to differentiation and derivatives the first somite is similar to other somites.

Key words First somite · Head · Occipital bone · Muscle · Quail-chick chimeras

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Introduction

Somites develop from the unsegmented paraxial mesoderm and are the first distinct metameric units of the vertebrate embryo. The process of somite formation proceeds from cranial to caudal. The newly formed somite consists of an epithelial ball of columnar cells enveloping mesenchymal cells within a central cavity, the somitocoel. During early maturation, the ventral wall of the somite and the somitocoel cells form the sclerotome, which gives rise to the vertebral column and ribs. The dorsal half of the somite remains epithelial and develops into the dermomyotome from which the dermis of the back and the skeletal musculature originate.

The unsegmented paraxial mesoderm is characterized by the expression of the *Pax-3* and *Pax-7* genes (Bober et al. 1994; Goulding et al. 1994; Williams and Ordahl 1994). Epithelialization of the segmental plate and formation of somites depends on the expression of *paraxis*, a basic helix-loop-helix transcription factor (Burgess et al. 1996). Hox-gene expression is involved in regional specification of the paraxial mesoderm (Kessel 1991; Kessel and Gruss 1991). Pax-genes play a role in the early somite compartmentalization. *Pax-1* and *Pax-9* are expressed in the ventral part of the epithelial somite, whereas its dorsal part expresses *Pax-3* and *Pax-7* (Brand-Saberi et al. 1993; Goulding et al. 1993, 1994; Love and Tuan 1993; Williams and Ordahl 1994; Ebensperger et al. 1995).

The development of single somites in the neck (Bagnall et al. 1988), brachial (Beresford 1983; Zhi et al. 1996) and thoracic (Huang et al. 1996) regions was followed by means of isotopic grafting experiments between quail and chick embryos that have been introduced by Le Douarin (1969, 1973). It was found that somite cells differentiate into epaxial and hypaxial muscles, cartilage and connective tissue of the vertebral column, dermis of the back, and endothelial cells. In addition, the brachial somites give rise to the wing muscles. However, it is not known whether the first somite develops like other somites. The formation, maintenance, and

differentiation of the first somite has been of interest for a long time (von Baer 1828; His 1868; Kupffer and Benecke 1879; Balfour 1885; Platt 1889; Rex 1905; Patterson 1907; Hubbard 1908; Gräper 1926; Jager 1926; De Beer and Barington 1934; Arey 1938; Hinsch and Hamilton 1956; Noden 1983; Couly et al. 1993).

How can the first somite be identified? According to Hamburger and Hamilton (1951), the first somite to appear in time is actually the second somite in its position in the entire series because another smaller somite with an indefinite anterior boundary appears just anterior to it within the next hour or two. This "first positioned" somite is called "rudimentary" or incomplete, due to the lack of a fissure between this somite and the paraxial head mesoderm. In contrast, the second somite is clearly bounded anteriorly and posteriorly by sharp intersomitic clefts. Thus, the first intersomitic cleft delimits the anterior edge of the second somite from the posterior edge of the first somite.

This first somite lies behind the otic placode and is directly connected with the paraxial head mesoderm (Fig. 1A). According to Hinsch and Hamilton (1956), the first somite of the chick embryo can be seen up to stage 17 and then disperses. The disappearance of the first somite can also be observed in the mouse (Butcher 1929) and human embryo (Arey 1938). Rex (1905) has shown by means of light microscopical observations that the first somite of avian embryos develops into dermomyotome and sclerotome. After labeling the first somite with particles of powdered carbon, carbon particles were found in the basis cranii cartilage between the glossopharyngeus and the vagus nerve (Hinsch and Hamilton 1956). After transplantation of the first two somites from quail into chick embryos, graft-derived cells were found in the cucullaris and intrinsic laryngeal muscles (Noden 1983). Couly et al. (1993) described the contribution of first somite cells to the following tissues: bones (basioccipital bone between the first and second roots of the hypoglossal nerve and exoccipital part of the chondro-occipital arch), and muscles (several glossal muscles, intrinsic and external laryngeal muscles as well as several neck muscles). Thus, the development of the first somite remains unresolved and a subject of controversy.

Pax-1 (Ebensperger et al. 1995) and *Pax-9* (Müller et al. 1996) are expressed in the sclerotome, whereas *Pax-3* transcripts (Goulding et al. 1993; Dietrich et al. 1993) are found in the dermomyotome of the first somite. According to Kessel and Gruss (1991), *Hox-a1* and *Hox-b1* are expressed in occipital somites. However, there is no Hox-gene that is special for the first somite.

To be able to understand molecular data of axis development and somite differentiation, it is important to find out the detailed distribution of single somites. In the present work we have studied the derivatives of the first somite using the quail-chick marking technique (Le Douarin 1969, 1973). Our results show that the development of the first somite is similar to other somites. It gives rise like them to different cell lineages such as muscle, cartilage, bone, and connective tissue as well as endothelial cells. The derivatives are described in detail.

Materials and methods

Embryos

Fertilized eggs of the Japanese quail (*Coturnix coturnix*) and the White Leghorn chick (*Gallus gallus*) were incubated for 30–40 h at 80% humidity and 37.8°C. Due to inherent variations of developmental stage the embryos were usually operated in HH-stages 8–10 (Hamburger and Hamilton 1951). The majority of embryos used had 5–8 pairs of somites. No embryos was operated beyond HH-stage 10 (10-somite stage). According to Tosney (1982) and Kuratani and Kirby (1991), at the stages used for operation (HH stages 8–10) neural crest cell migration has not yet occurred at otic level.

Grafting procedure

The operations in chick and quail embryos were performed both in ovo and at the same time. At first a window was made in the egg. Locke solution containing antibiotics (penicillin 1000 IU/ml, Sigma, Deisenhofen, Germany) was added to prevent the embryo from drying out.

To permit visualization of the somites a small amount of black drawing ink (Pelikan, Hannover, Germany) diluted 1:10 with Locke solution was injected subendodermally. The somites were counted from cranial to caudal. The second visible somite has clear fissures between it and the anterior and posterior paraxial mesoderm. The somite anterior to this second one has no anterior fissure and was regarded as the first somite. The somite is made up of densely packed epithelial cells and can be distinguished from the loosely structured and transparent paraxial head mesoderm. After counting of the somites, the vitelline membrane covering the region of the first somite was opened.

Grafting of the first somite was performed as described below.

Quail embryo (donor): The first somite was isolated with the help of a small amount of 0.4%–0.6% trypsin solution (Sigma, Deisenhofen, Germany). The enzymatic reaction was stopped with horse serum (Gibco, Eggenstein, Germany) after 5–10 min. Then the dorso-lateral part of the isolated somite was stained with Nile blue sulfate for correct orientation. The grafted somite was always still epithelial.

Chick embryo (host): Firstly, the ectoderm covering the first somite was opened. The dorsal part of the first somite was removed with a tungsten needle and the ventral part with a micropipette.

The quail somite was transferred to the chick host operation site using a micropipette. It was then moved into the position where the first somite was located. The opened ectoderm was closed with tweezers. The window in the egg shell was sealed with adhesive tape and the eggs were reincubated.

After reincubation for 4 h–10.5 days, the embryos were fixed in Serras' fluid (Serra 1946), dehydrated, embedded in paraffin and serially sectioned at 8 μm. The distribution of quail cells was assessed by means of the Feulgen-reaction (Feulgen and Rossenbeck 1924) and the anti-quail antibody (Developmental Studies Hybridoma Bank, Iowa) on parallel sections. The immunostaining with anti-quail antibody has the advantage of making it possible to identify quail cells at lower magnifications (Huang et al. 1996; Zhi et al. 1996).

Immunohistochemistry

Anti-quail-antibody was diluted 1:1500. Unspecific binding was blocked with 1% BSA (Serva, Heidelberg, Germany). To block endogenous peroxidase activity 0.3% H_2O_2 in methanol was used.

Sections were incubated with the first antibody for 60 min at room temperature. As second antibody an alkaline phosphatase conjugated goat-anti-mouse antibody (DAKO, Hamburg, Germany) was used, diluted 1:400 and incubated for 60 min. NBT and X-phosphate (Boehringer, Mannheim, Germany) were used as chromogen to reveal a blue signal. Quail cells were identified as muscle cells in Feulgen-stained sections by morphological criteria and in double-labelled sections by anti-desmin (dilution 1:400) and anti-quail (dilution 1:500) staining. The desmin antibody was detected by a second antibody coupled with peroxidase (dilution 1:300, Sigma) and DAB as chromogen.

In order to recognize quail hemangiopoietic cells and their derivatives some parallel sections were processed for immunhistochemical studies with the QH-1 antibody (Pardanaud et al. 1987). QH-1 antibody (Developmental Studies Hybridoma Bank, Iowa) was diluted 1:2500. Peroxidase-conjugated goat-anti-mouse IgG (Sigma) was used as second antibody, diluted 1:250. DAB was used as chromogen.

Results

Sixty-four microsurgical operations were performed and 27 embryos were subsequently evaluated. The embryos were reincubated for periods from 4 h to 11 days after operation and sectioned in coronal or sagittal planes (see Table 1). Twenty-six evaluated specimens used for histological examination exhibited normal morphology of all of the structures and tissues in the operated region. One embryo was abnormal, with its eye absent on the unoperated side.

In order to evaluate the grafting procedure 5 embryos were fixed after 4–5 h reincubation. The first somite of the chimeric embryos is of quail origin (Fig. 1B). A few cells of the chick first somite are not removed, adjacent to aorta, endoderm, and the ventral part of neural tube. After 4 h of reincubation, the defect in the ectoderm has healed. The surrounding tissue of the first somite was not damaged. The first somite of the chick embryo was removed and replaced with the complete first somite of the quail embryo. Semithin sagittal sections of an unoperated chick embryo with 8 pairs of somites show that the first somite is epithelially structured at this stage. The first somite is directly connected with the head mesoderm and thus has no anterior fissure (Fig. 1A). The first intersomitic cleft and intersomitic blood vessel are between the first and the second somite. The first somite is situated behind the otic placode and close to the heart anlage (Fig. 1B).

Table 1 Experimental data (*coronal*, *sagittal* planes of section)

HH stage at fixation	Reincubation periods	Number of embryos	
		Coronal *n*	Sagittal *n*
9–10	2–4 h		4
14–15	1 day	1	6
20	2 days	1	2
24	3 days	2	1
28	4 days	1	1
30	5 days	1	1
32	6 days		1
34	7 days	1	1
38	11 days	2	1

The first somite gives rise to dermotome, myotome and sclerotome

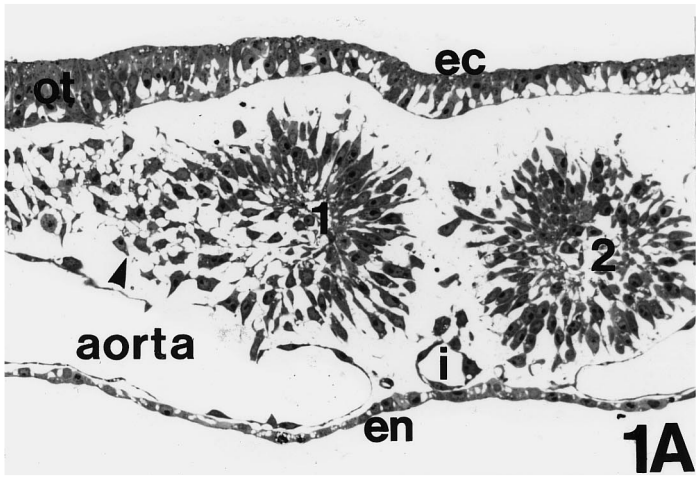
After 1 day of reincubation the ventral part of the first somite de-epithelializes and gives rise to the sclerotome. Quail cells were also found in the mesenchyme rostrally from the first dermomyotome and posterior to the otic vesicle. Mesenchymal cells surrounding the primary pharynx at the segment level of the first somite are of quail origin. The dorsal part of the first somite forms a dermomyotome. No quail cells are found in the heart anlage (Fig. 2).

After 2–3 days of reincubation 3 embryos were sectioned sagittally and 3 coronally (Figs. 3–6). In these stages the axons of the glossopharyngeal nerve have left the base of the brain just behind the otic vesicle. The vagus nerve arises behind the glossopharyngeal nerve but rostrally to the first myotome (Fig. 3B). Quail cells are located along the longitudinal axis within one segment situated between the otic vesicle and the anterior boundary of the second myotome. Within this segment, all cells except for neural crest cells (glossopharyngeal and vagus nerve) are of quail origin. Quail cells are found in the dermis of the occipital region. The first myotome is located in the caudal half of this segment. This myotome has a sharp boundary between it and the second myotome. In contrast, the rostral boundary of

Fig. 1 **A** Semithin sagittal section of an unoperated chick embryo with 8 pairs of somites (*arrowhead* transition zone between the first somite and the head mesoderm). **B** Sagittal section of a chimaeric embryo after replacement of the first somite by a corresponding quail somite. Reincubation period: 4 h. The nuclei of the quail cells (*dark points*) are stained with anti-quail antibody (*arrow* first intersomitic cleft, *ec* ectoderm, *en* endoderm, *hm* head mesoderm, *i* intersomitic blood vessel, *ot* otic placode, *1* first somite, *2* second somite). ×290 ▶

Fig. 2 Sagittal section through a chimaeric embryo stained with anti-quail antibody. Reincubation period: 1 day. Quail cells (*dark points*) found in the first dermomyotome (*d*), myotome (*m*) and sclerotome (*s*). A few quail cells (*arrows*) extend rostrally to the otic vesicle (*ot*; *asterisks* primary pharynx). ×290

Fig. 3A–C Sagittal sections through the head of a chick embryo; anti-quail and anti-desmin antibody; Reincubation period: 2 days. **A** The first myotome (*1*) which is stained with anti-desmin antibody, and the sclerotome at this segment level are formed by quail cells (*dark points*). Cells surrounding the glossopharyngeal (*IX*) and vagus (*X*) nerve as well as the cardinal vein (*v*) contain also labeled nuclei. A few cells (*arrows*) migrate from the first sclerotome into the third branchial arch (*b3*; *2* second myotome). ×50. Line shows section plane for **Fig. 4**. **B** Higher magnification of the frame in (*dashed line*) **Fig. 3A**. ×120. **C** The same plane but more medial to **Fig. 3A**. Distribution of quail cells (*dark points*) shows clear boundaries (*arrows*). A few quail cells (*arrowheads*) are found in the fourth branchial arch. The subdivision into cranial and caudal half-sclerotome can be seen from the second segment downwards (*b1, 2, 3, 4* first, second, third, fourth branchial arch, *hb* hindbrain, *2* second myotome, *VIII* vestibulocochlear nerve, *X* vagus nerve). ×50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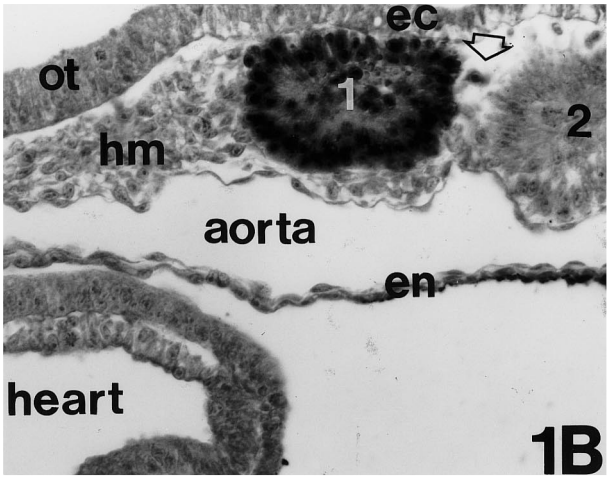
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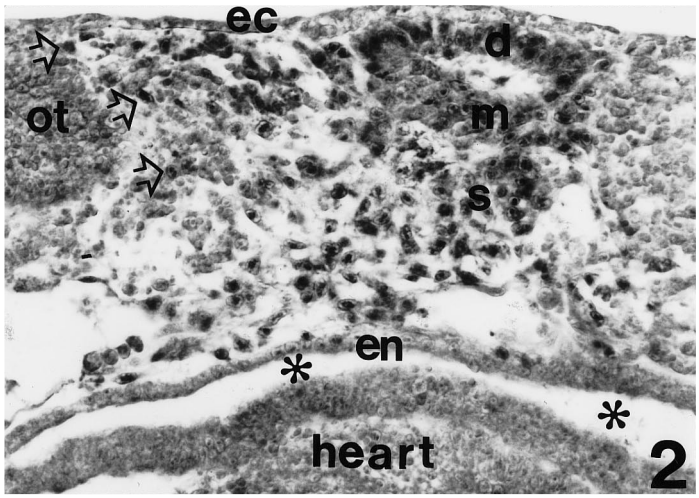
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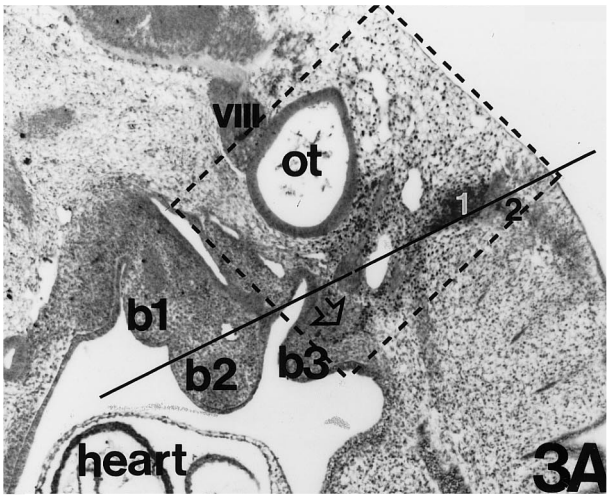
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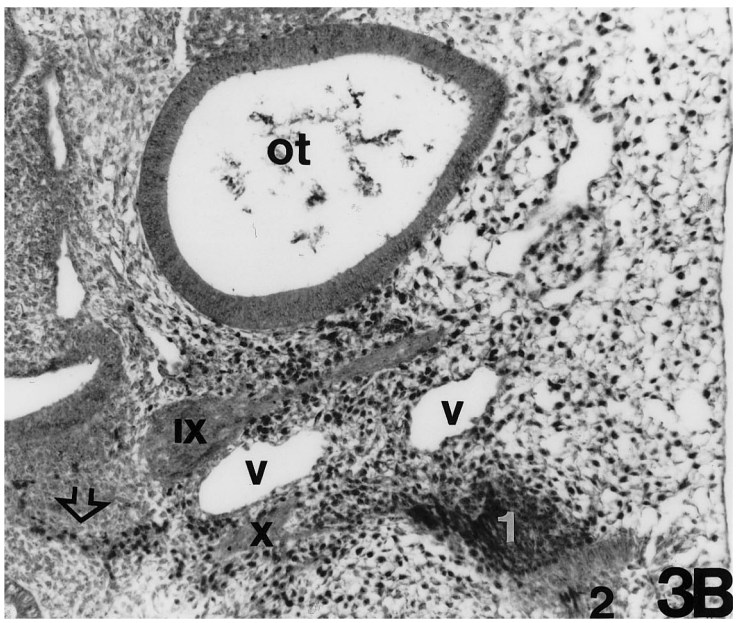
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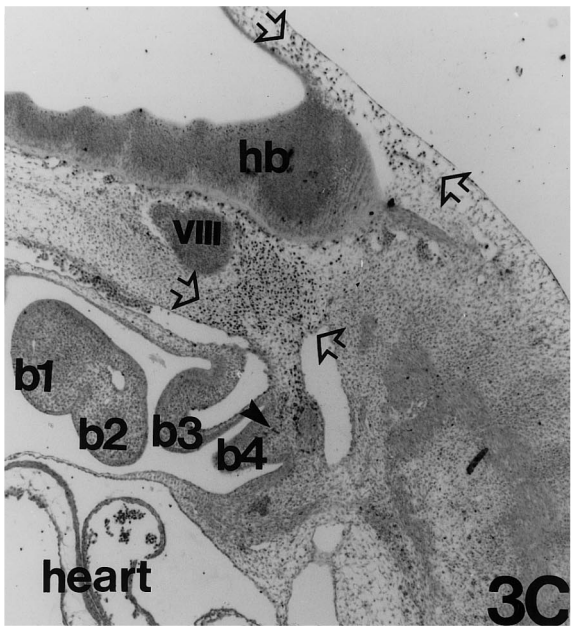
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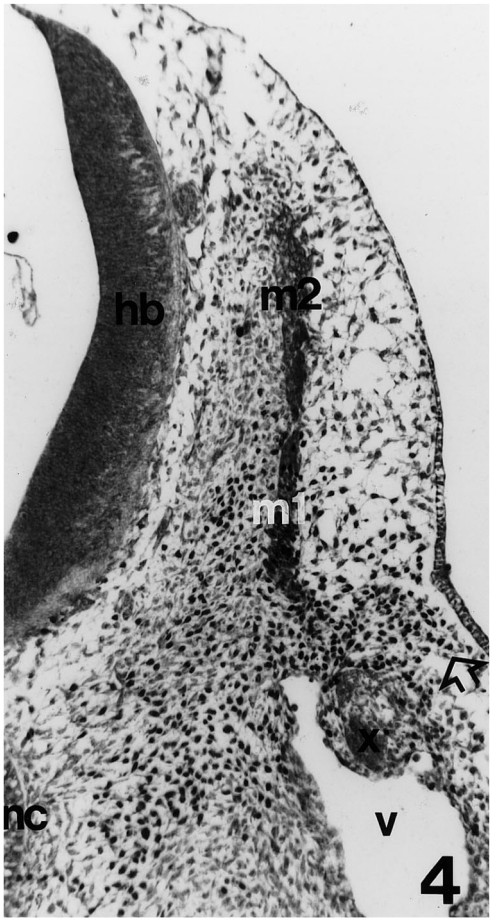
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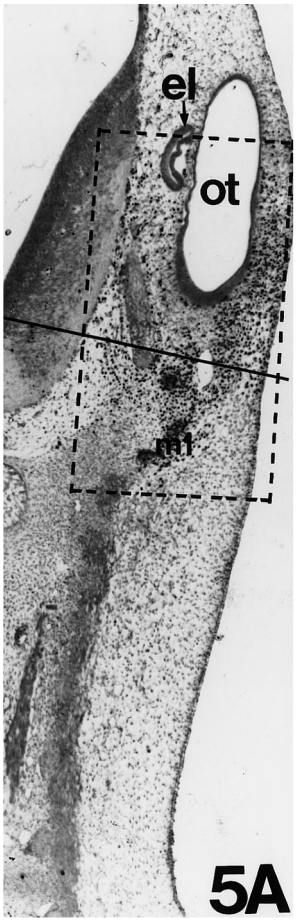
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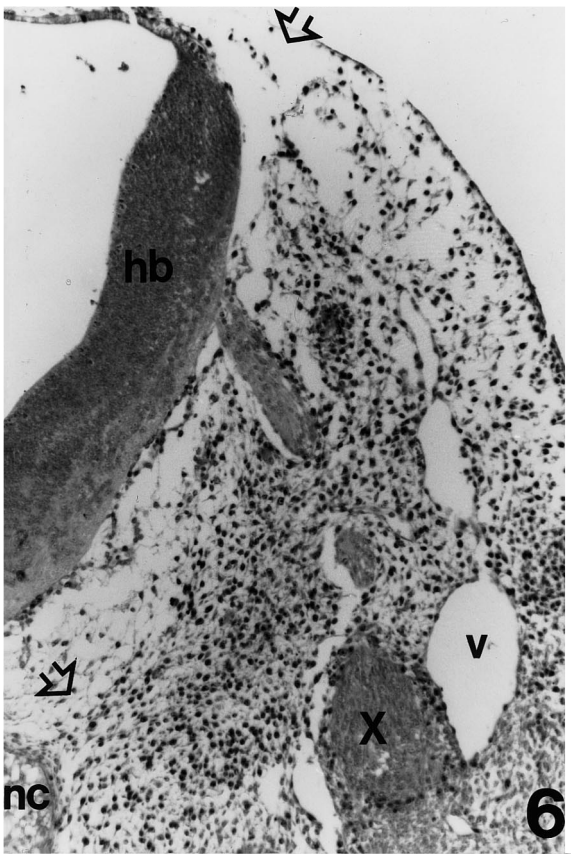
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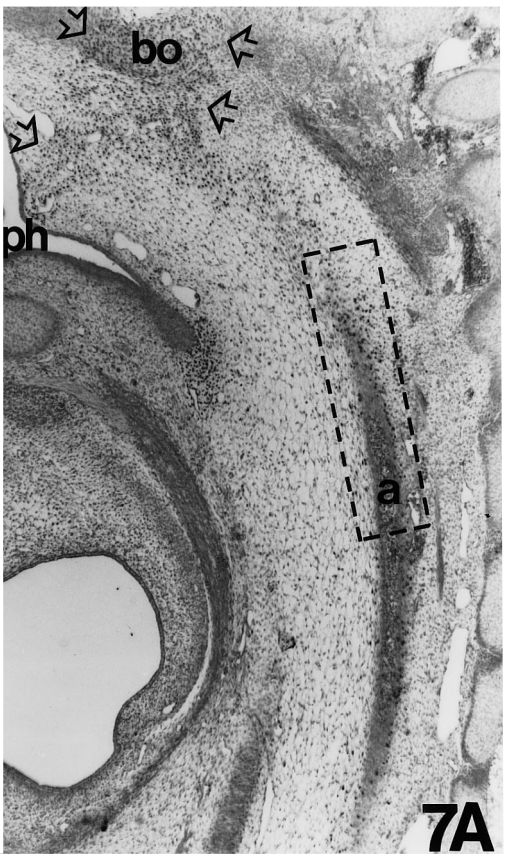
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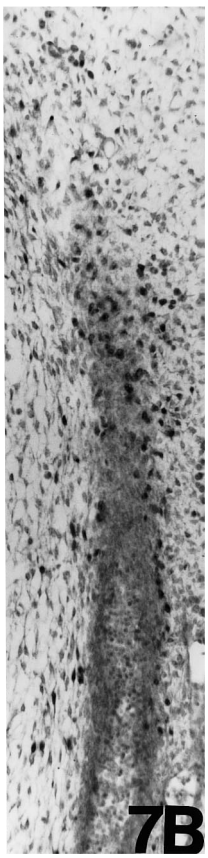
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Table 2 The contribution of the first somite (*Sag* sagittal plane of section, *Cor* coronal plane of section, + grafted quail cells present, – grafted quail cells not present, the supraoccipital (*so*) and parasphenoid (*pa*) bone do not form before day 12, *bo* basioccipital bone, *cu* cucullaris capitis muscle, *d* days, Derm Dermis, *eo* exoccipital bone, *la* laryngeal muscles, me meninges, *n. a. v.* glossopharyngeal and vagus nerves carotid artery and jugular vein, *opi* opisthotic bone, *pa* parasphenoid bone, *ph* pharynx, *so* supraoccipital bone)

Embryos (*n*)	Stage at operation	Age at fixation (days)	Plane of section	Skeleton					Muscle		Dermis	Connective tissue		
				bo.	eo.	so.	opi.	pa.	cu.	la.		me.	ph.	n.a.v.
392	9–	5.5d	Sag	+	+		+		+	–	+	+	+	+
414	9	5.5d	Cor	+	+		+		+	–	+	+	+	+
353	9+	6.5d	Sag	+	+		+		+	+	+	+	+	+
412	9–	6.5d	Cor	+	+		+		+	–	+	+	+	+
358	10–	7.5d	Sag	+	+		+		+	–	+	+	+	+
352	9+	8.5d	Sag	+	+		+		+	+	+	+	+	+
404	10	8.5d	Cor	+	+		+		+	–	+	+	+	+
400	9+	12d	Cor	+	+	+	+	+	+	–	+	+	+	+
403	10	12d	Sag	+	+	+	+	+	+	+	+	+	+	+
413	10–	12d	Cor	+	+	+	+	+	+	–	+	+	+	+

the first myotome is like a saw (Fig. 3B). Compared with other somites the first sclerotome is not divided into a cranial and a caudal half (Figs. 3C, 5B). No intersegmental blood vessel can be found between the first sclerotome and the paraxial head mesoderm. A few quail cells have moved to the third and fourth visceral arch (Fig. 3).

Quail cells have migrated dorsomedially to the midline above the fourth ventricle and ventromedially to the notochord. The dermis formed by grafted quail cells forms a sharp boundary with the dermis derived from other sources (Figs. 4, 6). The distribution of grafted quail cells after 4–11 days of reincubation is summarized in Table 2. All evaluated specimens operated at the stages we used (HH stages 8–10, 6–10 somites) show identical distribution of the first somite cells.

◀ **Fig. 4** Transverse section at the level of the line in Fig. 3A. Reincubation period: 2 days. The first myotome (*m1*) is formed by transplanted quail cells (*dark points*). The sclerotome cells from the first somite migrate ventrally to the notochord (*nc*). The dermis formed by the transplanted first somite shows a boundary (*arrow*) to the dermis in the lateral head region (*m2* second myotome, *v* cardinal vein). ×120

Fig. 5 Low (**A**) and high (**B**) magnification of cross section at the level of the otic vesicle (*ot*) in a chimaeric embryo after 3 days reincubation. The first myotome (*m1*) and the mesenchyme cells at the segment level behind to the otic vesicle (*ot*) are formed by grafted quail somite cells (*dark points*). *Line* shows section plane for Fig. 6. **A** ×50; **B** ×120

Fig. 6 Transverse section at the axial level shown by the line in **Fig. 5A**. Reincubation period: 3 days. The labeled nuclei (*dark points*) are found both in dorsomedial and ventromedial areas (*arrows*). (*nc* notochord). ×120

Fig. 7 Low (**A**) and high (**B**) magnification of sagittal section through a chimaeric embryo. Reincubation period: 5 days. The contribution of quail cells (*dark points*) to the basiooccipital bone (*bo*) and the connective tissue of the pharynx (*ph*) is restricted to the segment boundaries (*arrows*). A few labeled cells (*dark points*) are found in the connective tissue surrounding the carotid artery (*a*). **A** ×50; **B** ×120

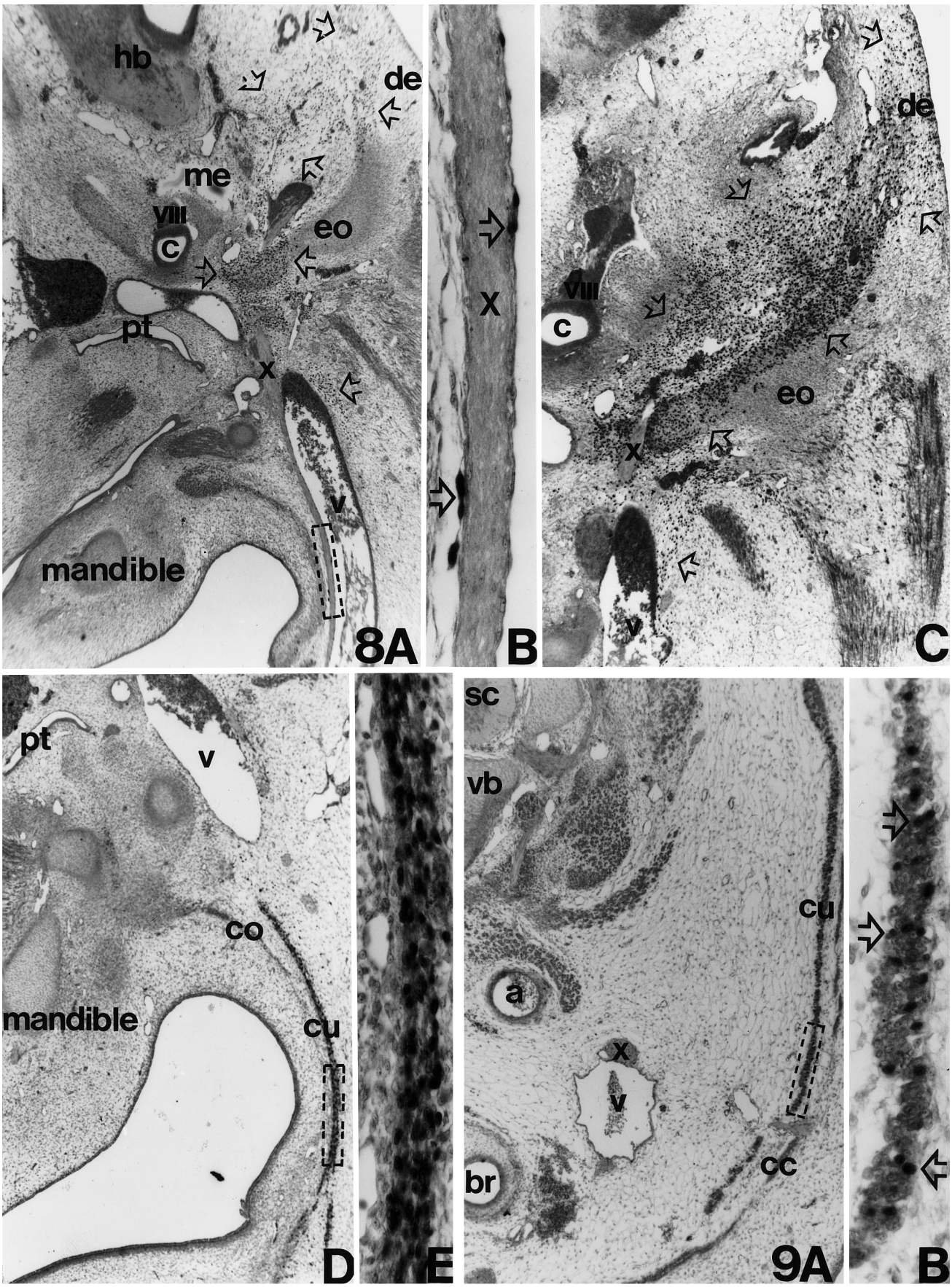
Contribution to the bones

To understand the distribution of the first somite cells we describe at first two reference marks (notochord and otic vesicle) in the development of the neurocranium. Due to the extension of the notochord, the head can be subdivided into a notochordal and a prechordal region. The otic vesicle is also used as a reference mark, because of its fixed position. With reference to the otic vesicle, preotic and postotic regions can be distinguished. The quail cells (first somite-derived cells) are always located in the postotic region.

After 4 days of reincubation the mesenchymal cells derived from the grafted somite are located around the notochord and take part in the formation of the parachordal cartilaginous tissue. Chondrification begins in the midline on each side and extends laterally on either side. After 5–7 days of reincubation somite-derived quail cells participate in the formation of the basioccipital cartilage on the operated side (Figs. 7A, 10A, B). In the dorsolateral direction the quail cells contribute to the exoccipital cartilage, which extends dorsolaterally and fuses with the rear portion of the otic capsule (opisthotic).

Fig. 8A–E Sagittal sections through the same embryo shown in Fig. 7 (The plane is more lateral to Fig. 7). **A** Low magnification illustrating the localization of grafted quail cells (*arrows*) in dermis (*de*), meninges (*me*), exoccipital bone (*eo*) and in the connective tissues surrounding the jugular vein (*v*) and vagus nerve (*X*). ×30. **B** Higher magnification of the frame in **A**. ×120. **C** The plane slightly lateral to **A**. The localization of grafted quail cells shows very clear segmental boundaries (*arrows*). ×50. **D** Low magnification of a sagittal section (slightly lateral to **C**) showing the cucullaris capitis muscle (*cu*) containing quail nuclei (*dark points*). ×50. **E** Higher magnification of the frame in **D**. ×120. (*c* cochlear duct, *co* constrictor colli muscle, *pt* pharyngotympanic tube) ▶

Fig. 9 Low (**A**) and high (**B**) magnification of transverse section of the caudal neck of a host embryo. Reincubation period: 7 days. Quail nuclei (*arrows*) within myocytes of the cucullaris capitis muscle (*cu*; *a* carotid artery, *br* right principal bronchus, *cc* cucullaris cervicalis muscle, *sc* spinal cord, *v* jugular vein, *vb* vertebral body). **A** ×50; **B** ×120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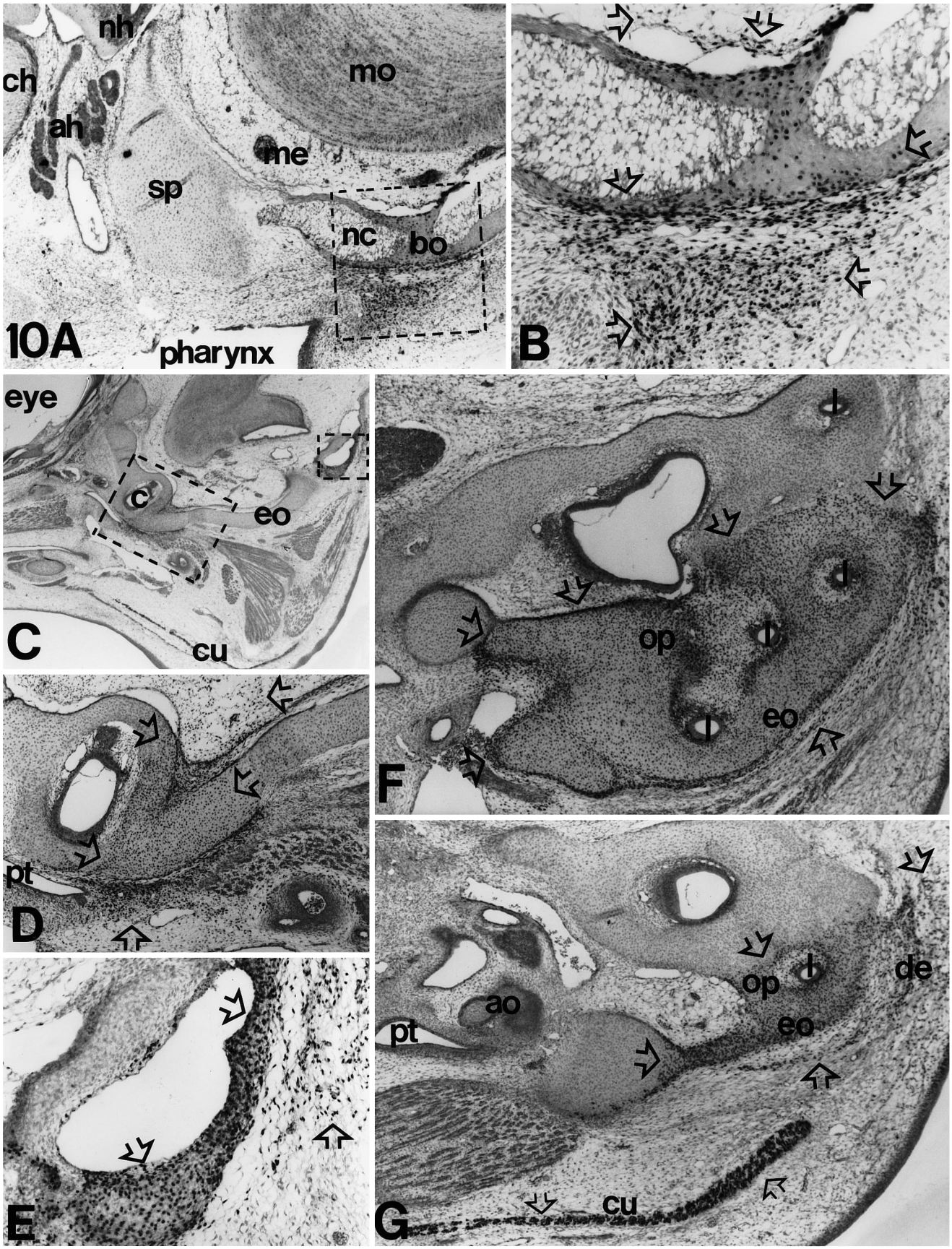
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The otic region expands by the enlargement of the membranous labyrinth. The cochlear process grows ventrally and towards the midline and thus invades the original parachordal region. The posterior region of the otic capsule expands dorsally above the hindbrain. Quail cells take part in the formation of the opisthotic capsule and are always situated behind the cochlear duct (Figs. 8A, C; 10C, D, F, G).

After 10.5 days of reincubation the supraoccipital bone appears as a pair of ossifications on either side of the dorsal midline. The perichondrium of the supraoccipital cartilage is formed by grafted quail cells. The caudal part of the supraoccipital cartilage is mainly of quail origin, whereas in the cranial part only few quail cells are found (Figs. 11A, B, G). At the same time the membranous parasphenoid bone beneath the basioccipital bone is arising from the connective tissue containing predominantly quail cells. In the bone spicules of the parasphenoid bone some of the osteoblasts and osteocytes contain quail nuclei (Figs. 11E, F). It is interesting to note that the contribution of the first somite-derived cells to the occipital bones, opisthotic bone and parasphenoid bone is restricted to the segment boundaries (Figs. 7A; 8A, C; 10A, B, C, F, G; 11G). The first somite-derived occipital bones look like an arch embracing the hindbrain.

Contribution to the laryngeal muscle

The chick embryo has two intrinsic laryngeal muscles attaching to the cricoid and arytenoid cartilages. These are a deep transversely orientated constrictor glottidis muscle and a pair of larger, more superficial dilator glottidis muscles (Fig. 13B). The horseshoe-shaped constrictor glottidis muscle surrounds the glottidis caudally and is almost covered by the dilator glottidis. The constrictor glottidis muscle originates from the procricoid cartilage and inserts on the cricoid cartilage and on the arytenoid cartilage. The dilator glottidis muscle extends from the cricoid cartilage to the arytenoid cartilage.

In 3 of 10 chimeric embryos myocytes derived from the grafted quail cells were found in the intrinsic laryngeal muscles. The connective tissue associated with these muscles is not of quail origin (Fig. 11H).

◀ **Fig. 10A–G** Sagittal sections through the head of a host embryo after a reincubation period of 7 days. **A** Median section. ×50. **B** Higher magnification of the frame in **A**. ×120. **C** Paramedian section. Quail cells (*arrows*) are found in the otic bone caudal to the cochlear duct (higher magnification in **D**) and in the exoccipital bone (higher magnification in **Fig. 10E**). **F** Paramedial section at the level of labyrinth (*l*). Only the opisthotic bone (*op*) contains quail cells (*arrows*). ×50. **G** Paramedian section at the level of the auditory ossicles (*ao*) (*ah* adenohypophysis, *bo* basioccipital bone, *ch* chiasma opticum, *cu* cucullaris muscle, *de* dermis, *eo* exoccipital bone, *l* labyrinth, *me* meninges, *mo* medulla oblongata, *nh* neurohypophysis, *op* opisthotic bone, *sp* sphenoidal bone, *arrows* quail cells)

Contribution to the cucullaris capitis muscle

The cucullaris capitis muscle, which is considered to be homologous with the mammalian trapezius and sternocleidomastiodeus muscles (Bütschli 1921), is a subcutaneus muscle of the bird (Nickel et al. 1992). The muscle originates on the temporal bone over the external acoustic meatus and extends obliquely caudoventrally to the shoulder region (Fig. 13C). This muscle is flat and large. Its cranial part is covered superficially by the constrictor colli (Fig. 8D) and its caudal part by the cucullaris cervicis muscle (Fig. 9A). Labeled myocytes are found throughout the whole length of this muscle. All the connective tissue associated with the cucullaris muscle is of chick origin (Figs. 8D, E; 9A, B; 10G; 11A, C, D) with the exception of its occipital part that is located in the same segment in which the exoccipital bone originates from quail cells.

Contribution to the meninges of the hindbrain

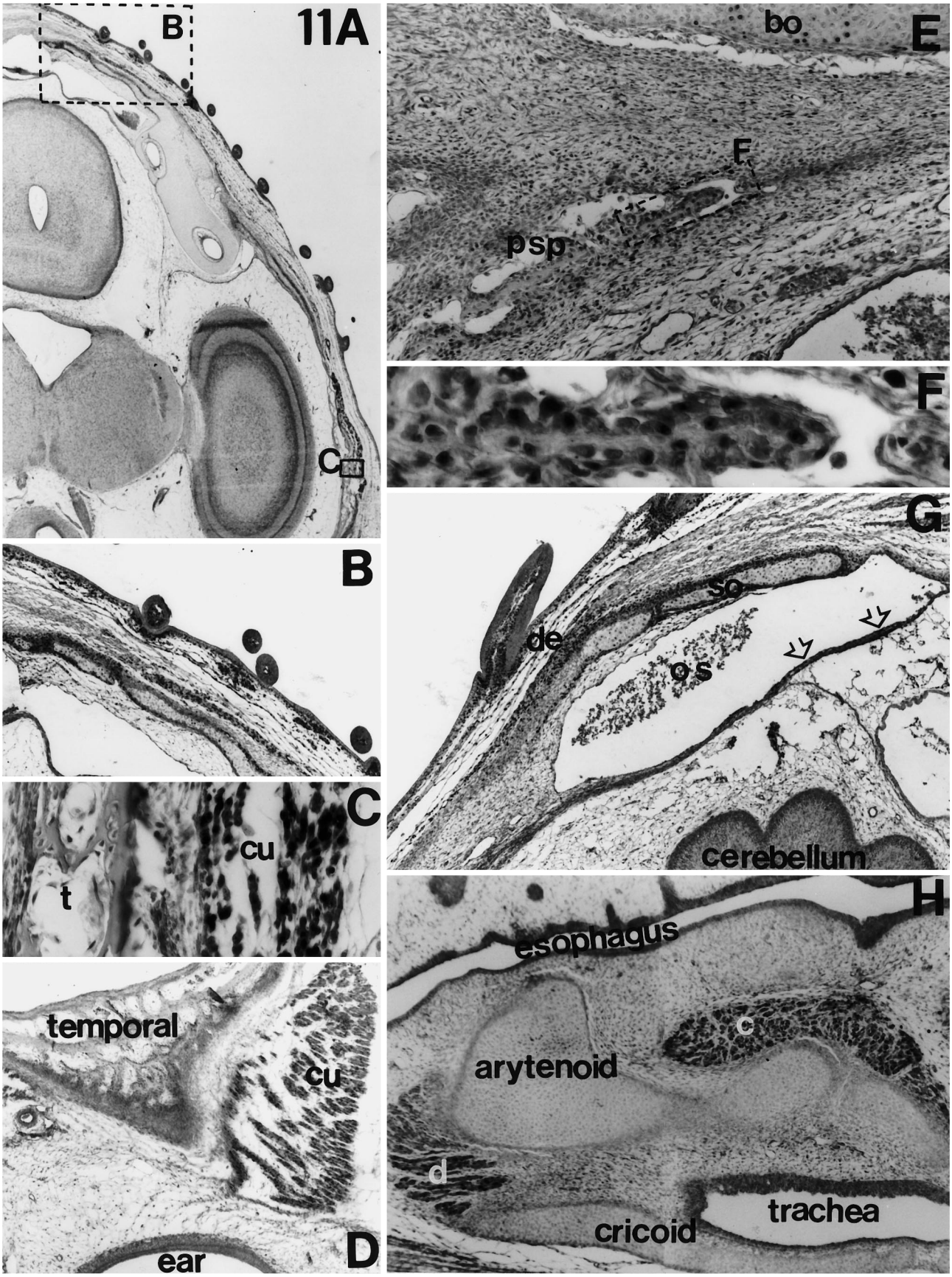
Grafted quail cells participate in the formation of the meninges of the hindbrain. They are distributed at the level of the basioccipital and exoccipital bones, which are formed by quail cells, and are restricted to the segmental boundaries (Figs. 8A; 10A, B, D).

Contribution to the connective tissue surrounding the pharynx, vagus nerve, carotid artery and jugular vein

Grafted quail cells contribute to the connective tissue surrounding that part of the pharynx that is located immediately beneath the quail-derived basioccipital bone. The distribution of cells within the pharynx connective tissue shows a sharp cranial and caudal segment boundary (Figs. 7A; 10A, B).

In contrast, the first somite-derived connective tissue bordering on the vagus nerve, carotid artery and jugular vein is not restricted to the head region; it also extends to the caudal neck (Figs. 7A, B; 8A, B, C).

Fig. 11 Coronal (**A**, **B**, **C**) and sagittal (**D**, **E**, **F**, **G**, **H**) sections through the head of chimaeric embryos. Reincubation period: 10.5 days. **A** Coronal section at the level of the supraoccipital and temporal bones. ×20. **B** Higher magnification of the supraoccipital bone where the quail cells (*dark points*) are located. ×50. **C** Higher magnification of the origin on temporal bone (*t*) of the cucullaris capitis muscle (*cu*) containing grafted quail cells (*dark points*). ×290. **D** Low magnification of paramedian section at the level of external acoustic meatus (*ear*) illustrating the origin of cucullaris capitis muscle (*cu*) containing quail cells (*dark points*). ×50. **E** Paramedian section showing basioccipital bone (*bo*) and membranous parasphenoidale bone (*psp*). ×120. **F** Higher magnification of one bone spicule in the formation of parasphenoidal bone. Some of osteoblasts contain labeled nuclei (*dark points*). ×467. **G** Paramedian section showing the supraoccipital bone (*so*) and dermis (*de*) with quail cells (*dark points*). Some endothelial cells (*arrows*) of the occipital sinus are also of quail origin. ×50. **H** Median section through the larynx. The grafted quail cells (*dark points*) participate in the formation of intrinsic laryngeal muscles (*c* constrictor glottidis muscle, *d* dilator glottidis muscle) ▶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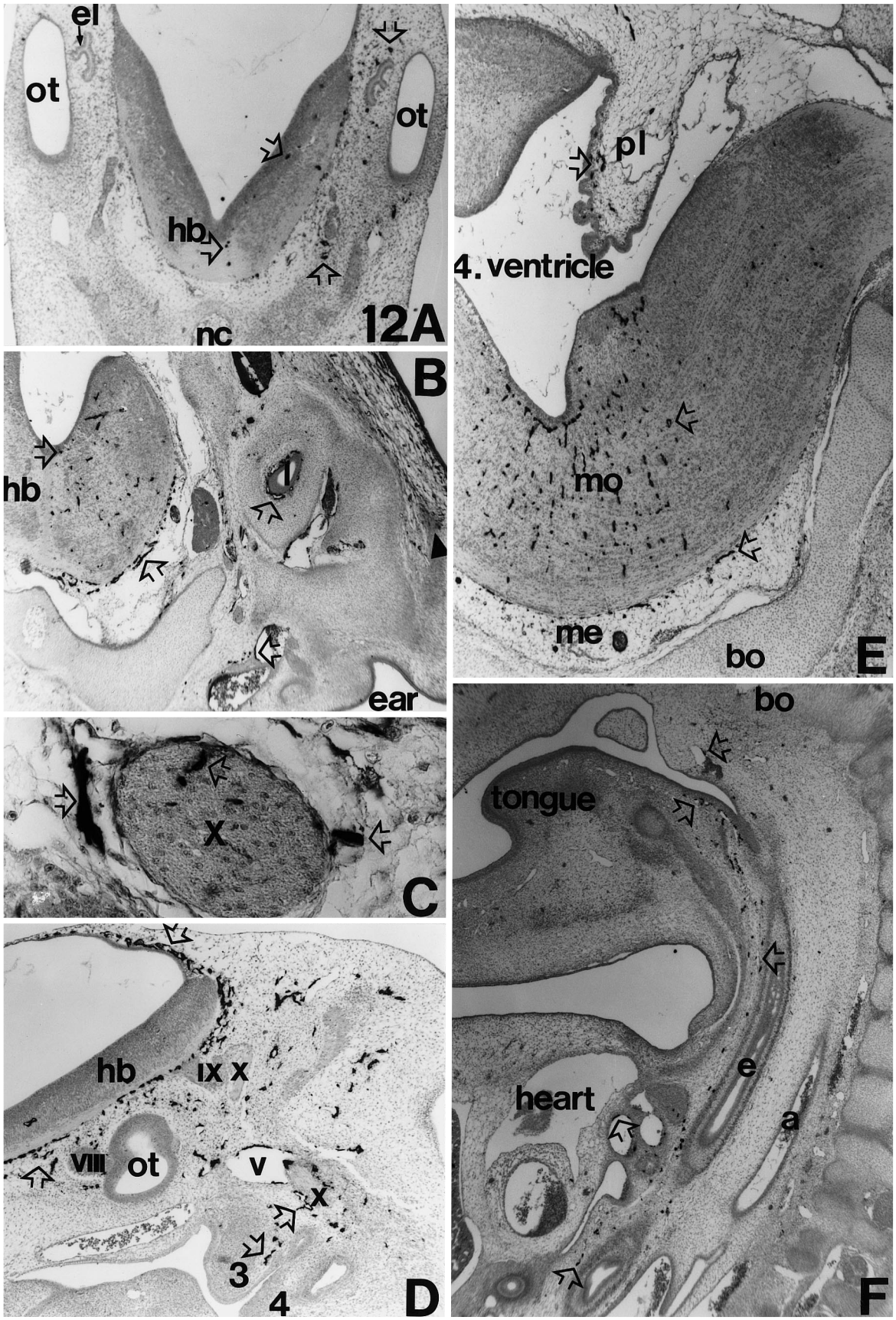
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Contribution to the dermis

The dermatome derived from the first somite gives rise to the dermis in the occipital region (Figs. 3, 4, 8, 10, 11). Its distribution is restricted along the longitudinal axis to the cranial and caudal segment boundary (Figs. 3A, B; 8C). The dermis formed by quail somite cells also maintains a boundary with the dermis located in the lateral head region (Fig. 4).

Contribution to blood vessels

The angiogenic potential of the first somite was analyzed using the QH1 antibody. After 3 days of reincubation, the endothelial cells derived from the first somite are found only in the head region (Figs. 12A, D). The endothelial cells extend rostrally over the otic vesicle and caudally to the level of the fourth branchial arch. The endothelial cells participate in the formation of the blood vessels in the hindbrain, the meninges, and the occipital region as well as in the connective tissue adjacent to the otic vesicle and the 8th, 9th and 10th cranial nerves. After 5 days of reincubation the endothelial cells derived from the first somite are not restricted in the head region but also extend through the entire length of the neck down to the thorax region (Fig. 12F). Endothelial cells are found in blood vessels surrounding the pharynx and the esophagus, carotid artery, jugular vein and vagus nerve (Figs. 12B, C, D, F). However, the endothelial cells in the dermis are restricted only to the occipital region (Fig. 11B). The tongue does not contain endothelial cells of quail origin (Fig. 12F). In addition, endothelial cells derived from the first somite are found in the hindbrain, meninges and choroid plexus. The distribution of these endothelial cells also shows cranial and caudal segment boundaries (Fig. 12E). Endothelial cells are located in the labyrinth (Fig. 12B). The occipital sinus formed on day 12 includes endothelial cells of quail origin (Fig. 11G). Most of the endothelial cells associated with the cucullaris capitis muscle are localized in its cranial part.

Unilateral distribution of grafted quail cells

Grafted quail cells always showed a unilateral distribution. After an incubation period of 2 days, mesenchymal cells from the grafted quail somite migrate ventromedially to the notochord and dorsomedially above the fourth ventricle (Figs. 4–6). However, these somitic cells never crossed the midline of the head. In the further head development the quail somite-derived cells within all tissues are always restricted to the operated side. In the midline of the head a sharp boundary is found between the part of quail origin and the part of chick origin (Figs. 11A, B; 12A, B). The myocytes containing quail nuclei are only located in the cucullaris (Fig. 9) and laryngeal muscle of the operated side. The quail-derived endothelial cells migrate over many segments (Fig. 12F); nevertheless they never crossed the midline of the body (Figs. 12A, B).

◀ **Fig. 12** Cross (**A**, **B**, **C**) and sagittal (**D**, **E**, **F**) sections of chimaeric embryos stained with the QH1 antibody showing distribution of first somite-derived endothelial cells (*dark points*) marked by *arrows* after a reincubation period of 3 days (**A**, **D**), 5 days (**F**) and 7 days (**B**, **C**, **E**). *Arrowhead* (**B**) lateral boundary of the first somite-derived endothelial cells within the dermis (*a* carotid artery, *e* esophagus, *el* endolymphatic diverticulum, *pl* choroid plexus, *IX* glossopharyngeal nerve, *3, 4* third, fourth branchial arch)

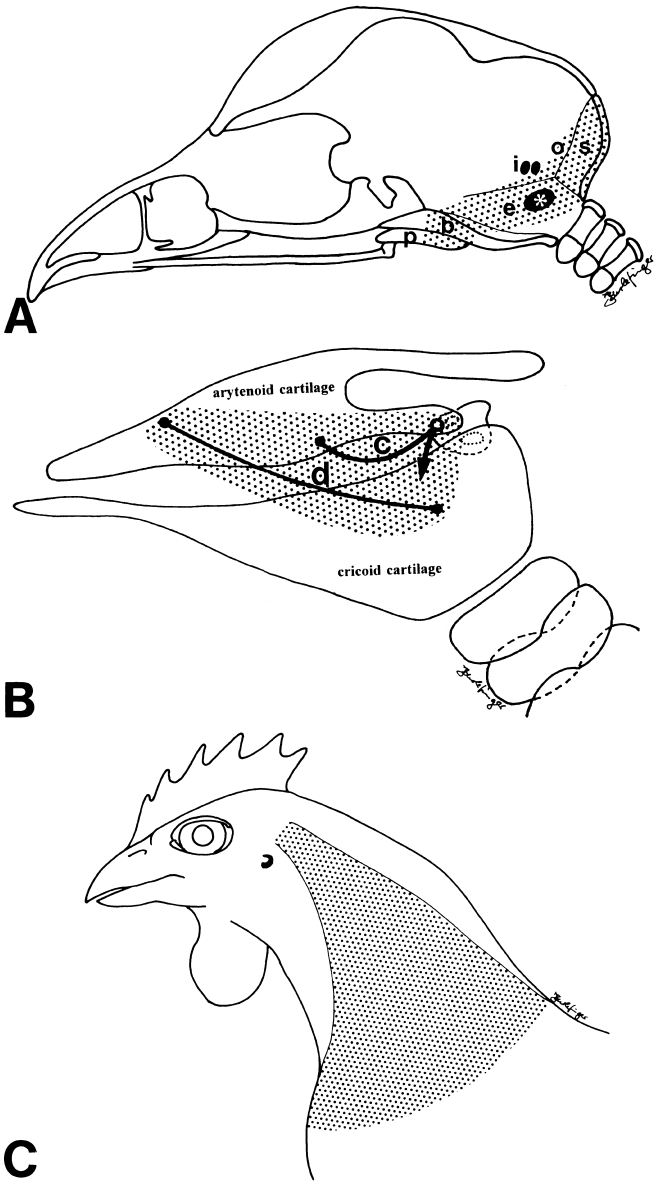

Fig. 13 Schematic illustrations showing the distribution of the first somite-derived cells (*dark points*) to neurocranium (**A**), intrinsic laryngeal muscle (**B**) and cucullaris capitis muscle (**C**); *b* basioccipital bone, *e* exoccipital bone, *i* internal acoustic meatus, *o* opisthotic bone, *s* supraoccipital bone, *asterisk* foramen of the glossopharyngeal and vagus nerve

Discussion

We have grafted the first somite between early quail and chick embryos. The first somite that appears in front of the first intersomitic cleft is not sharply separated from the head mesoderm. It is considered to be a rudimentary (incomplete) somite (Jager 1926; Hinsch and Hamilton 1956). According to Rex (1905), it corresponds to the second "metotic" somite. The second somite is located posteriorly to the first intersomitic cleft. Because it has sharp anterior and posterior intersomitic boundaries, it was considered to be the first complete somite. In whole-mount preparations the first somite is smaller than the second one. In a few cases we observed a small cluster of cells located anteriorly to the first somite in HH stage 9 (6-somite stage). This often unilaterally located and transitory cluster was considered to be the second rudimentary somite (Jager 1926; Hinsch and Hamilton 1956) or the first "metotic" somite (Rex 1905). The most important prerequisite of our experiments was to be sure to identify the first real somite in both the donor and host embryo.

It was found that the first somite becomes compartmentalized into dermomyotome, myotome and sclerotome on day 2. The dermomyotome loses its epithelial structure on day 3 to 4 and gives rise to the dermis of the occipital region. The myotome develops in its original segment; however, later, myoblasts migrate caudally and cranially to form the polysegmental cucullaris muscle on day 5. Compared with other somites the first sclerotome is not divided into a cranial and a caudal half. A few mesenchyme cells from the first somite extend to the otic vesicle. We have never found a boundary between the otic capsule and the derivatives of the first somite. There is no intersegmental blood vessel anterior to the first myotome.

Hinsch and Hamilton (1956) held that the first somite becomes dispersed after the 3rd day. The reason might be the absence of a anterior boundary of the first sclerotome. Rex (1905) has observed that the first somite forms a dermomyotome and a sclerotome. This is supported by the expression domains of the *Pax*-gene. *Pax-3* transcripts are found in the dermomyotome (Goulding et al. 1993; Dietrich et al. 1993) whereas *Pax-1* (Ebensperger et al. 1995) and *Pax-9* (Müller et al. 1996) are expressed in the sclerotome of the first somite.

According to Lim et al. (1987), HNK-1-positive cells are seen within the first segment, immediately caudal to the otic vesicle. These authors also showed that the cranial half-sclerotome of segments 2–6 are populated by HNK-1-positive cells, while the caudal halves are not. These findings are in line with our observation showing that the subdivision of the sclerotome into a cranial and caudal half begins with the second segment.

According to our findings, the first somite contributes not only to the formation of cartilaginous bones (basi-, ex-, supraoccipital and opisthotic bone) but also to a membranous bone (parasphenoid bone). The 9th and 10th cranial nerves penetrate that part of the exoccipital bone that is formed by the first somite. Hinsch and Hamilton (1956) have found that the first somite contributes to the basis cranii cartilage between the 9th and 10th cranial nerve on the basis of carbon particles labeling. However, the distribution of single cells cannot be followed with carbon particles. According to Couly et al. (1993), who grafted the first somite from quail to chick, chondrocytes of the first somite contribute to the basioccipital bone between the first and second root of the hypoglossal nerve and to the exoccipital part of the chondro-occipital arch as well as to the pars canalicularis of the otic capsule. The supraoccipital bone has been described by these authors as arising only from the paraxial head mesoderm at the metacephalic level. Our observations show that the first somite cells are always distributed in that part of the exoccipital bone in which the foramina of the glossopharyngeal and vagus nerve are found. The other controversial point is the origin of the supraoccipital bone. We have found that the first somite cells participate in the formation of the supraoccipital bone. Additionally, other tissue must contribute to the supraoccipital bone because chick cells were found in its cranial part. It is likely that the caudal part of supraoccipital bone derives mainly from the first somite cells and the cranial part mainly from the paraxial head mesoderm. The conclusion made by Couly et al. (1993), that the first somite cells do not participate in the formation of the supraoccipital bone, might come from identifying the supraoccipital bone only in coronal sections. Another possibility could be a variable inclusion of the ventromedial part of the first somite that is still covered by the neural tube and not well separated from the paraxial head mesoderm at the 3-somite stage they used. In addition, the observations by Couly et al. (1993) showed that the parasphenoid bone is formed by the paraxial head mesoderm and neural crest cells. We found that a part of the parasphenoidal bone is made up of cells derived from the grafted quail somite. One can be sure that the grafted somite did not include neural crest cells, because the operations were always carried out before the onset of neural crest cell migration at the otic level (Tosney 1982; Kuratani and Kirby 1991). From this it can be concluded that the cells contributing to membranous ossification in the head must have originated not only from the paraxial head mesoderm and neural crest cells, but also from the first somite. Our results also show that first somite-derived chondrocytes are situated immediately behind the cochlear duct in the opisthotic bone. This reflects the close relationship of the first somite to the otic placode in early stages. It is conceivable that the ear rudiment prevents the rostral movement of the cells of the first somite.

It is worth mentioning that the contribution of the first somite-derived cells to the bones (occipital, opisthotic and parasphenoidal), meninges, pharynx and dermis is bounded by sharp anterior and posterior boundaries that may reflect segmental boundaries. The part of the occipital bone derived from the first somite embraces the hindbrain like an arch. Therefore, the first somite-derived part of the occipital bone as a whole can be considered to

resemble a large vertebra in which the basioccipital bone represents the vertebral body and the ex- and supraoccipital bone represents the neural arch. This segment pattern of the somite has already been observed in the cervical region by Bagnall et al. (1988, 1989) and in the thoracic region by Huang et al. (1996).

Our results show that myocytes originating from the first somite participate in the formation of the cucullaris capitis muscle and the intrinsic laryngeal muscles. The former has been considered to be homologous with the mammalian trapezius and sternocleidomastoideus muscles (Bütschli 1921). Couly et al. (1993) concluded that the cells of the first somite contribute to glossal muscles, intrinsic and external laryngeal muscles and several neck muscles. Our results confirm some but not all of these findings. We never observed a contribution of graft-derived cells to glossal, external laryngeal and various neck muscles. These discrepancies may be due to different grafting procedures.

In our experiments, only one third of the operated embryos show a participation of the first somite in the formation of the intrinsic laryngeal muscles. This indicates that the first somite cannot be the only source of these muscles and is in line with observations made by Noden (1983) showing that the laryngeal muscles are mainly derived from the second somite. The myocytes derived from the first somite are found throughout the whole length of the cucullaris capitis muscle, but the first somite-derived connective tissue associated with this muscle is restricted to the occipital region. This indicates that the myocytes of the first somite must have migrated longitudinally at least over 18 body segments. In contrast, the cells of the connective tissue do not leave the segment of their origin. The myocytes derived from a single thoracic somite extend also over at least two segments (Huang et al. 1996).

Endothelial cells in the ventral part of the neck migrate caudally to the upper thorax. The “caudal migration” of the thyroid gland during development, bringing it down the upper thorax, is thought to correlate with the descensus cordis (Hilfer and Brown 1984). It is possible that the long migration of the endothelial cells in the ventral part of the neck is under the influence of the descending heart.

Our results show that grafted cells never cross the midline of the embryo; they contribute only to tissues on the operation side. This finding supports the results after unilateral replacement of single somites of the thoracic and brachial level (Huang et al. 1996; Zhi et al. 1996) and demonstrates the laterality of cell distribution. The laterality in the distribution pattern of endothelial cells has been shown by Christ et al. (1990), Wilting et al. (1992), Brand-Saberi et al. (1994), Wilting et al. (1995), Wilting and Christ (1996) and Klessinger and Christ (1996). Klessinger and Christ (1996) were able to show that the notochord exerts a barrier function for the endothelial cells. In the absence of the notochord and floor plate; the endothelial cells regularly cross the midline to invade the contralateral body side. Our results confirm and extend these findings with respect to the occipital region and other cell types.

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