

Mouse-chick neural chimeras

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ABSTRACT Embryonic chimera production was used to study the developmental processes of the mouse nervous system. The difficulty of performing *in situ* transplantation experiments of neural primordium of mouse embryo was overcome by isotopic and isochronic grafting of mouse neural tube fragments into chick embryo. Mouse neural tube cells differentiated perfectly *in ovo* and neural crest cells associated with the grafted neural tube were able to migrate and reach the normal arrest sites of host neural crests. Cranial neural crest cells penetrated into chick facial areas and entered into the development of dental bud structures, participating in vibrissa formation. Depending on graft level, *in ovo* implanted mouse neural crest cells formed different components of the peripheral nervous system. At trunk level, they located in spinal ganglia and orthosympathetic chains and gave rise to Schwann cells lining the nerves. When implanted into the lumbosacral region, they penetrated into the enteric nervous system. At the precise 18-24 somite level, they colonized host adrenal gland. Mouse neural tube was involved in the mechanisms required to maintain myogenesis in host somites. Furthermore *in ovo* grafts of mouse cells from genetically modified embryos, in which many mutations induce early death, are particularly useful to investigate cellular events involved in the development of the nervous system and to identify molecular events of embryogenesis.

KEY WORDS: *neural crest, neural tube, mouse embryo, chick embryo, chimera*

Introduction

Embryonic development involves not only decisions concerning cell fate (proliferation, differentiation or death), but also a series of strictly organized cellular migrations. The most compelling methods for tracing migratory patterns often imply experimental manipulations between two different embryos. As avian embryos are easily accessible, the avian system has often been studied. In particular, the chimera obtained by combining quail and chick cells has been elegantly exploited (for reviews, see Le Douarin 1982; Le Douarin and Kalcheim 1999). While the avian embryo is particularly suitable for grafting experiments after opening of the egg, this type of investigation is difficult to perform in the mammalian fetus *in utero*. To circumvent this difficulty, the mouse embryos were removed from the mother, microinjected with a solution of DiI (1.1', dioctadecyl-3,3,3',3',-tetramethylindo-carbocyanine perchlorate, Molecular Probes, Eugene, OR) or DiO (3,3'-dioctadecyloxa-carbocyanine perchlorate, Molecular Probes), or fluorescent dyes (Serbedzija *et al.*, 1990; Lee and Sze, 1993; Trainor and Tam, 1995; Tam *et al.*, 1997), or lysinated rhodamine dextran (Beddington and Martin, 1989; Tam and Tan, 1992; Lee and Sze, 1993; Serbedzija *et al.*, 1994; Sze *et al.*, 1995) and subsequently cultured. As whole mouse embryo cultures can be maintained for only 40 h, this

technique is too restrictive for efficient study of developmental mechanisms in mammalian embryogenesis. This limitation can be overcome by using a procedure first developed in our laboratory, which consists in implanting xenografts of fetal mouse tissue into the avian embryo (Fontaine-Pérus *et al.*, 1995). This allows murine fetal cells to be studied by the classical methods of experimental embryology applicable to avian embryo cells. The combination of tools used in avian embryology and murine transgenesis lead us to determine how genetic control of cellular migration and differentiation occurs in embryonic development (Houzelstein *et al.*, 1999, 2000).

The work presented here, inspired by that of Professor Nicole Le Douarin, allows the development of neuroepithelial cell derivatives to be monitored by transplanting fragments of neural primordium from 8- to 8.5- and 9-day postcoital mouse embryos into 1.5- and 2-day-old chick embryos (i.e., homologous stages) at different levels of the neuraxis. We find that grafted mouse cells differentiated *in ovo* and organized to form the different cellular compartments normally contributing to the central nervous system.

Abbreviations used in this paper: DiI, 1.1', dioctadecyl-3,3,3',3',-tetramethylindo-carbocyanine perchlorate; DiO, 3,3'-dioctadecyloxa-carbocyanine perchlorate.

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The graft also contributed to the development of the peripheral nervous system through the migration of neural crests associated with mouse neuroepithelium. Depending on graft level, mouse neural crest participated in the various derivatives, such as head components, parasympathetic and orthosympathetic ganglionic chains and neuroendocrine glands (Fontaine-Pérus *et al.*, 1997; Fontaine-Pérus, 2000). Recently published results about tooth formation (Mitsiadis *et al.*, 2003) will be reviewed here, as well as unpublished findings concerning vibrissa formation in relation to the species from which implanted neural crests are derived.

Results

Fate of mouse prosencephalic-mesencephalic-metencephalic neural crest cells transplanted in ovo

Macroscopic examination of each chimera showed that heads differed morphologically according to the level of origin of mouse explants and that the features were strikingly different from those of chick heads. Transplantation of the prosencephalic-mesencephalic-metencephalic region suppressed beak formation and the eyes were smaller than in wild type chick embryo (Fig. 2A). Unexpectedly the chimeras took on a rodent-like shape, with a snout and forehead bulges. Histological studies showed that cells from implanted mouse neural crest reached the facial aspect of the head readily invading the maxillary and mandibular processes of the chick host (Fig. 2B) and contributing to the formation of tooth-like germ structures. Seven to nine days after grafting, chick oral epithelium invaginated into underlying mouse ectomesenchymal cells and acquired bud configurations (Fig. 2C). Mouse *Msx1*, *Pax9*, *MK* and *Barx1* genes were expressed in mesenchymal cells surrounding epithelial bud structures, whereas chick *Shh* gene expression was limited to areas of stomatodeal epithelium corresponding to sites reached by mouse neural crests. Clear morphological evidence of tooth formation was observed 14 days postgrafting. The presence of dentin, sialoprotein and intermediate filament nestin strongly suggested that odontoblasts differentiated in chick host embryos. Lateral neural

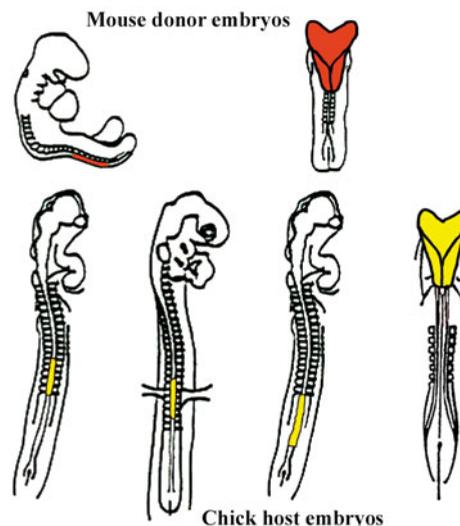


Fig. 1. Transplantation of mouse neural tube into chick host embryos.

Levels from which mouse donor tissues were obtained are shown in red and the different implantation levels in chick host embryos in yellow.

ridges derived from the mouse graft gave rise to superficial ectoderm lining the nasal cavity. In one case examined 17 days after prosencephalon-mesencephalon-metencephalon transplantation, sagittal sections of the grafted area showed vibrissa-like structures underneath mouse ectoderm. However elongation of hair germ occurred without hair shaft production (Fig. 3).

Fate of mouse trunk neural crest cells transplanted in ovo

Mouse neural crest cells contributed to the development of the chick host peripheral nervous system. At 18 h postsurgery, the mouse cells left the donor neural tube and invaded cell-free spaces between the graft and the inner side of host somites. As in quail/chick chimeric embryos, most of the cells that migrated

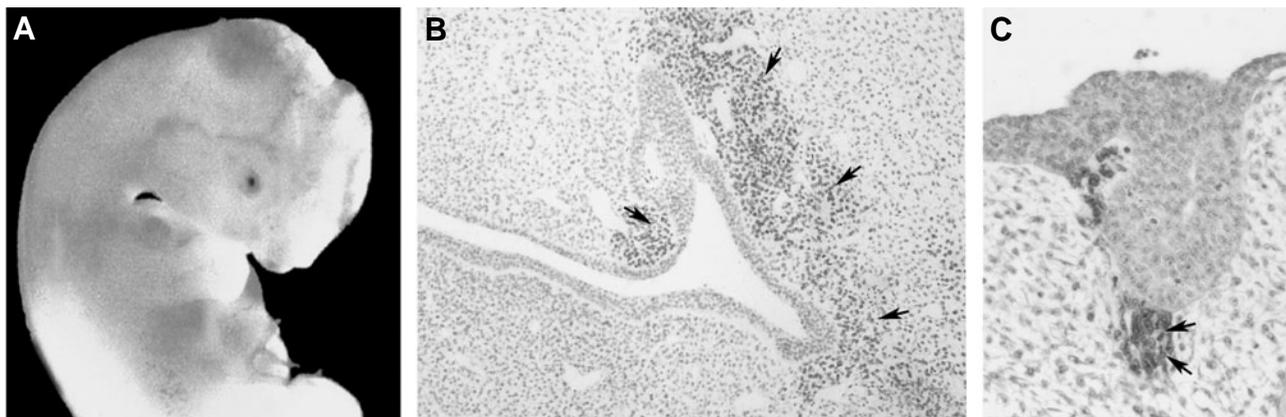


Fig. 2. Head region of a mouse/chick chimera. (A) Examination 5 days after implantation (6-somite stage) at the prosencephalon-mesencephalon level of the equivalent zone from an 8 dpc mouse embryo. (B) Visualization of mouse neural crest cells in a transverse section of the head region of the mouse/chick chimera viewed in A. Mouse cells are identified in the oral region after Feulgen-Rossenbeck staining (arrows). (C) Migration and localization of mouse neural crest cells in the mandibular process, as visualized after Feulgen-Rossenbeck staining in a transverse section of a mouse/chick chimera operated on as in A and examined 9 days postsurgery. Chick oral epithelium has invaginated into underlying mouse crest cells (arrows) and acquired a tooth-bud configuration.

between the neural tube and the somites aggregated to form dorsal root ganglia (Fig. 4A), whereas those that passed between two adjacent somites migrated further ventrally and were quite probably involved in building sympathetic trunk structures (sympathetic chains and plexus and adrenal medulla). Spinal ganglia developing *in vivo* were comparable to 12-day mouse spinal ganglia 4 days after surgery and reached maximum size at 7 days. At the ventral level, the cells of the grafted neural tube, through their processes, combined ventral roots into ramus communicans that penetrated the axial muscle primordia by dorsal and ventral rami (Fig. 4B). At the grafting site, mouse neural crest-derived glial cells (Fig. 4C) accompanied growing nerve rami and later developed into myelinating Schwann cells. At the time when mouse neural crest cells would normally condense to form the primordia of spinal ganglia, mouse-derived neural crest cells appeared ventrally in the chick, indicating the first condensation of primary sympathetic ganglia. The authenticity of their differentiation into adrenergic derivatives was demonstrated by their anti-tyrosine hydroxylase immunoreactivity. Four days after mouse neural tube implantation, graft-derived neural crest cells aggregated close to ramus ventralis axons and ventrally to host vertebra, helping to form the secondary sympathetic ganglia that developed subsequently, whereas primary sympathetic ganglia disappeared progressively.

When chick embryos were grafted at the 18-24 somite level, some mouse neural crest cells near adrenal gland primordium infiltrated between host cortical cells where they expressed somatostatin and could be considered as adrenal cells. Mouse neural crest cells located laterally to the aorta formed two dense masses that reacted strongly with antineurofilament antibody and participated in the formation of adrenal plexus. When the mouse neural tube graft was implanted into 25-somite chick embryos at a level including the two posterior-most somites and the segmental plate, mouse cells were identified in Remak's ganglion, which originates from the neural crest posterior to the level of somite 28 and develops in the dorsal mesentery (Le Douarin, 1982). This process provides indirect proof that mouse crest cells can participate in the formation of enteric ganglia, since at least part

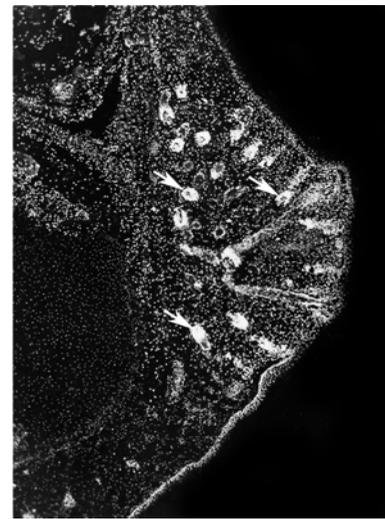


Fig. 3. Nasal region of a mouse/chick chimera. Sagittal section examined 16 days after implantation (6-somite stage) at the prosencephalon-mesencephalon-metencephalon level with the equivalent zone from 8 dpc mouse embryo. Vibrissa-like structures are visible after Hoechst staining (arrows).

of the lumbosacral intramural ganglionic supply migrated through Remak's ganglion.

The quail/chick chimeric system showed that prospective melanocytes migrate essentially through the mesenchyme and that those which seed the skin are derived from neural crest cells that pass between the dermomyotome and the ectoderm (Teillet, 1971). The most active migration in skin occurs in 6-day-old chick embryo, whereas some cells remain in the dermal mesenchyme. As in the avian embryo, the migratory pathways used by melanoblasts in mouse embryo lied within the dermal mesenchyme. Some were found within the epidermis and others were apparently inserted into feather germs. In view of the sites involved, all these cells presumably belonged to the melanoblast lineage.

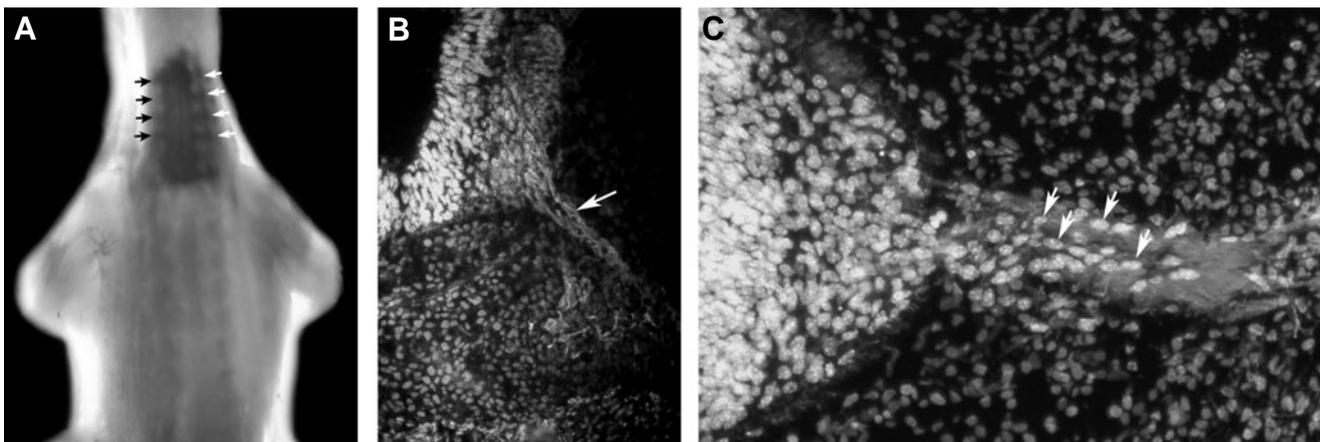


Fig. 4. Fate of mouse crest cells transplanted *in ovo*. (A) Macroscopic view of a mouse/chick chimera examined 3.5 days after implantation (10-somite stage) at unsegmented plate level with 9 dpc Rosa 26 mouse caudal neural tube. Mouse neural tube cells and their derivatives (arrows) expressing β -galactosidase are identified by LacZ staining. Transverse section of a mouse/chick chimera examined 4 days after implantation (17-somite stage) at the 13-17 somite level with 9 dpc mouse caudal neural tube. (B) Neurofilament immunostaining. Note the positive nerves emerging from the implanted tube (arrow). (C) Mouse neural crest-derived cells are observed along neurofilament positive nerves (arrows).

Somite myogenesis occurred normally after *in ovo* mouse neural tube graft

In chimeras with neural tube implanted at the segmental plate level, expression of MyoD transcripts occurred in somites differentiated at surgical level. MyoD expression was equivalent all along the paraxial zone in chimeras observed one day post-implantation. In other words, expression was similar in the myotomes of chimeras and control embryos (Fontaine-Pérus *et al.*, 1997). These results indicate that mouse neural tube was able to send signals conducive to chick myogenesis (presumably the same signals as that of the chick). Later on, muscles differentiated normally at surgical level. Motor axons originating from the ventral roots of the mouse spinal cord were first observed entering into chick somitic myotome and ultimately forming neuromuscular contacts with embryonic chick muscle cells (results not shown).

Materials and Methods

Preparation of mouse/chick chimeras

JA657 chick embryos between 1 (5-7 somites) and 2 (15-21 somites) days of incubation and mouse embryos between 8 (5-7 somites) and 9 (15-21 somites) days post-coitus (dpc), were used for microsurgery. Rosa 26 mice expressing β -galactosidase in all their cell progeny were also used as donors. Neural primordium substitution was performed at neuraxis level at different stages of development. In one series, mouse prosencephalon-mesencephalon was implanted into a 5-7 somite chick host embryo (homotopic and homochronic graft). In another series, grafts were performed at different neuraxis levels (increasingly posterior with embryo age) (Fig. 1).

Visualization of mouse cells

Chimeras killed 1-2-days after surgery were prepared for whole mount *in situ* hybridization with mouse probes to check for grafted cells in chick host embryos. Older chimeras were either prepared for histological examination or *in situ* hybridization on sections. The chimerism of cryostat-cut frozen sections was analyzed after Hoechst or Feulgen-Rossenbeck staining to distinguish between DNA distribution in mouse and chick nuclei (Fontaine-Pérus *et al.*, 1995).

In situ hybridization – Immunocytochemistry

Dioxygenin-labeled specific probes were used for *in situ* hybridization (chick *FGF8*, chick *BMP4*, Chick *MyoD*, chick *Shh*, chick *Pitx2*, mouse *Msx1*, mouse *Pax9*, mouse *MK* and mouse *Barx 1*). According to the type of tissue analyzed, affinity-purified antibodies against different epitopes (dentin, nestin, substance P, somatostatin, neurofilament, islet-1 and tyrosine hydroxylase) were used.

Conclusion

In vivo transplantation provides the most definitive test of the developmental capacities of a progenitor population, but the lack of an accessible host system has made it difficult to perform such tests on rodent neural crest cells. The *in ovo* technique described here, which allows analysis of the fate of mouse neural tube and its associated neural crest, consists in grafting fragments of neural primordium. This technique is derived from that of Le Douarin (for a review, see Le Douarin, 1982), who transplanted quail neural tube segments into chick embryos. As in the quail/chick chimera, one of the techniques used to identify mouse grafted cells among chick host cells was based on differences in DNA nuclear distribution in the mouse and chick species, which made it easy to follow the fate of mouse cells in serial sections obtained at all stages of

incubation. Although mouse neural tube was able to develop in a chick environment, the neural crest cells that escaped had the capacity to differentiate into sensory, sympathetic and parasympathetic neurons as well as into satellite glial and Schwann cells. The capacities of mouse neural crest cells to differentiate into medullary cells in adrenal gland were tested, but paradoxically their differentiation into enteric neurons of the host guts could not be tested directly. This may relate to the difficulty of delivering transplanted cells into a crest migration pathway providing access to the gut or to competition with endogenous crest cells for limited space. However, it is reasonable to assume that mouse grafted crest cells possess enteric potential, since they are able to colonize chick host Remak's ganglia (Teillet 1978). When migrating into the subcutaneous mesenchyme and the epidermis, they participate in the melanocyte lineage (Teillet 1971). Cells from mouse implanted prosencephalic neural primordium congregate in the chick host face and contribute to the frontonasal process by forming the rostral end of the maxillary process.

Evidence of tooth formation has been noted after *in ovo* transplantation of the mouse mesencephalic-metencephalic region (Mitsiadis *et al.*, 2003). Practically all molecular markers involved in tooth development were expressed and the deposition of mineralized matrix was observed. Our results indicate that the disappearance of teeth in birds is due to a lack of the appropriate neural crest signaling molecules involved in epithelial-mesenchymal interactions. However, interactions created experimentally between mouse neural crest cells and localized regions of chick oral epithelium led to tooth formation. In equivalent experimental conditions (prosencephalic – mesencephalic - metencephalic homotopic implantation) vibrissa-like structures derived from the implant were observed in the nasal cavity. These results indicate that differences in facial development between avian and mammalian embryos depend on species-specific neural crest cell-derived signals.

Briefly, regardless of the level selected, mouse crest cells migrated into chick host and finally colonized the sites corresponding to crest graft levels. Interestingly, our mouse-chick graft system, when combined with genetic mutations, ensures that the fate of the cell type expressing a particular gene can be monitored (Houzelstein *et al.*, 1999, 2000). Mouse-chick grafts are currently being developed in our laboratory to study the implications of *Pax3* gene, a transcription factor that severely affects the behavior of neural crest cells in the mutant *Spotch* mouse.

Acknowledgments

This research was supported by grants from the Association Française contre les Myopathies, the Centre National de la Recherche Scientifique and the University of Nantes.

References

- BEDDINGTON, R.S.P. and MARTIN, P. (1989). An *in situ* transgenic enzyme marker to monitor migration of cells in the mid-gestation mouse embryo: somite contribution to the limb. *Mol. Biol. Med.* 6, 263-274.
- FONTAINE-PERUS, J., JARNO, V., FOURNIER LE RAY, C., LI, Z. and PAULIN, D. (1995). Mouse chick chimera: a new model to study the *in ovo* developmental potentialities of mammalian somites. *Development* 121, 1705-1718.
- FONTAINE-PERUS, J., HALGAND, P., CHERAUD, Y., ROUAUD, T., VELASCO, M.E., C. CIFUENTES-DIAZ, C. and RIEGER, F. (1997). Mouse chick chimera: a developmental model of murine neurogenic cells. *Development*, 124, 3025-3036.

- FONTAINE-PERUS, J. (2000). Mouse Chick Chimera: An Experimental System for Study of Somite Development. *Somitogenesis*, Developmental Biology Vol. 48 (269-299) Eds Ch. Ordahl (Academic Press).
- HOUZELSTEIN, D., AUDA-BOUCHER, G., CHERAUD, Y., ROUAUD, T., BLANC, I., TAJBAKHSH, S., BUCKINGHAM, M.E., FONTAINE-PERUS, J. and ROBERT, B. (1999). The homeobox gene *Msx1* is expressed in a subset of somites and in muscle progenitor cells migrating into the forelimb. *Development*, 126, 2689-2701.
- HOUZELSTEIN, D., AUDA-BOUCHER, G., CHERAUD, FONTAINE-PERUS, J. and ROBERT, B. (2000). The homeobox gene *Msx1* reveals two populations of dermal progenitor cells originating from the somites. *Development*, 127, 2155-2164.
- LE DOUARIN, N. (1982). The Neural Crest (ed. P.W. Barlow, P.B. Green and C.C. Wylie). *Cambridge University Press, London*.
- LE DOUARIN, N. and KALCHEIM, C. (1999). The neural crest. *Developmental and Cell Biology Series*.
- LEE, K.H. and SZE, L.Y. (1993). Role of the brachial somites in the development of appendicular musculature in rat embryos. *Dev. Dyn.* 198, 86-93.
- MITSIADIS, T.A., CHERAUD, Y., SHARPE, P., FONTAINE-PERUS, J. (2003). Development of teeth in chick embryos after mouse neural crest transplantations. *Proc. Natl. Acad. Sci. USA* 100, 6541-6545.
- SERBEDZIJA, G.N., FRASER, S.E. and BRONNER-FRASER, M. (1990). Pathways of trunk neural crest cell migration in the mouse embryo as revealed by vital dye labelling. *Development* 108, 605-612.
- SERBEDZIJA, G.N., BRONNER-FRASER, M. and FRASER, S.E. (1994). Developmental potential of trunk neural crest in the mouse. *Development* 120, 1709-1718.
- SZE, L.Y., LEE, K., WEEB, S.E., LI, Z. and PAULIN D. (1995). Migration of myogenic cells from the somites to the fore-limb buds of developing mouse embryos. *Dev. Dyn.* 203, 324-336.
- TAM, P.P.L. and TAN, S.S. (1992). The somitic potential of cells in the primitive streak and the tail bud of the organogenesis stage mouse embryo. *Development* 115, 703-715.
- TAM, P.P.L., PARAMESWARAN, M., KINDER, S.J., WEINBERGER, R.P. (1997). The allocation of epiblast cells to the embryonic heart and other mesodermal lineages: The role of ingression and tissue movement during gastrulation. *Development*. 124(9), 1631-1643.
- TEILLET, M.A. (1971). Recherche sur le mode de migration et la différenciation des mélanoblastes cutanés chez l'embryon d'oiseau: Etude expérimentale par la méthode des greffes hétérosécifiques entre embryons de caille et de poulet. *Annales d'embryologie et de morphogénèse* 4, 95-109.
- TEILLET, M.A. (1978). Evolution of the lumbo-sacral neural crest in avian embryo: origin and differentiation of the ganglionated nerve or Remak studied in interspecific quail-chick chimaerae. *Wilhelm Roux's Arch.* 184, 251, 268.
- TRAINOR, P.A. and TAM, P.P.L. (1995). Cranial paraxial mesoderm and neural crest cells of the mouse embryo: co-distribution in the craniofacial mesenchyme but distinct segregation in branchial arches. *Development* 121, 2569-2582.