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Expression of the *SOX10* gene during human development

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Abstract SOX10, a new member of the *SOX* gene family, is a transcription factor defective in the *Dom* (*Dominant megacolon*) mouse and in the human Shah-Waardenburg syndrome. To help unravel its physiological role during human development, we studied *SOX10* gene expression in embryonic, fetal, and adult human tissues by Northern blot and in situ hybridization. As in mice, the human *SOX10* gene was essentially expressed in the neural crest derivatives that contribute to the formation of the peripheral nervous system, and in the adult central nervous system. Nevertheless, it was more widely expressed in humans than in rodents. The spatial and temporal pattern of *SOX10* expression supports an important function in neural crest development.

Key words: *SOX10*; In situ hybridization; Northern blot; Human; Neural crest

1. Introduction

The identification of the mammalian testis determination factor SRY (*sex determining region on the Y chromosome*) led to the description of the *SOX* (SRY box-containing) gene family, a new class of genes coding for transcription factors [1–3]. These genes are characterized by a DNA binding domain similar to the high-mobility group (HMG) domain of SRY [1,4,5]. The *SOX* genes are highly conserved through evolution, and show diverse and changing patterns of expression throughout development. Some members play important roles during embryonic stages, in segmentation, chondrogenesis, haematopoiesis and sex determination, and in the CNS (central nervous system) development. They appear to govern cell fate during embryogenesis by functioning both as classical (co)transcription factors and as architectural components of the chromatin [6]. The functional role of *SOX* genes in human genetic diseases was demonstrated by the involvement of *SRY* in XY sex reversal [7,8], and of *SOX9* in campomelic dysplasia [9–11], a dominantly inherited congenital osteochondrodysplasia associated in some instances with male to female autosomal sex reversal.

Recently, a new member of this family, *Sox10*, was cloned [12]. In mouse and rat, this gene seems to be selectively expressed in neural crest cells during early stages of development, and in glial cells of the peripheral and central nervous systems later in development and in adulthood. We and others established that the defects observed in the spontaneous mouse mutant *Dominant megacolon* (*Dom*), which displays intestinal aganglionosis and depigmentation, are caused by a *Sox10* gene mutation [13,14]. We also showed that its human homologue *SOX10* is defective in some cases of the Shah-Waardenburg syndrome (WS4) [15], a disease in which the neurosensory deafness and pigmentation abnormalities usually seen in the Waardenburg syndrome are combined with the aganglionic colon characteristic of Hirschsprung disease [16]. These two syndromes have been causatively linked to deficient migration/proliferation/differentiation of neural crest cells (neurocristopathies) [17].

To help unravel the physiological role of SOX10 during human development, we studied *SOX10* gene expression in embryonic, fetal, and adult human tissues by Northern blot and in situ hybridization.

2. Materials and methods

2.1. Northern blot analysis

Multiple tissue Northern blots (Clontech) were sequentially hybridized with a γ-^{32}P (Amersham) labelled *SOX10*c oligonucleotide probe (see Section 2.3) and a human β actin cDNA probe according to the suppliers' instructions. A 1.7-kb fragment corresponding to the 3′ end of the *SOX10* cDNA devoid of the HMG box (from nucleotide 870, GenBank accession number AJ001183), was also used as a probe.

2.2. In situ hybridization

Twelve morphologically normal human embryos (4 to 6 weeks of development) were obtained from legal abortions induced by mifepristone (RU486) at Hôpital Broussais in Paris. Complete independence was respected between the medical staff and the research group. The women's consent was obtained after thorough explanation of the planned research. All procedures were approved by the ethics committee of Hôpital Necker-Enfants-Malades in Paris.

Embryo and slide preparations, and subsequent steps of in situ hybridization, were performed as previously described [18]. The brain of a 25-weeks-old fetus was obtained and treated in the same way. Ten-micrometer-thick cryostat sections mounted on ProbeOn Plus slides (Microm) were used. The probes were labelled with α-^{35}S-dATP (NEN) and purified on Biospin columns (Biorad) before hybridization. Finally, slides were exposed to Hyperfilm β max X-ray films (Amersham) for 7 days and then to Kodak NTB2 photographic emulsion for 8 weeks at 4°C.

2.3. In situ hybridization probes

60-mers oligonucleotide probes (Genset) were chosen in the 3′ untranslated region of human *SOX10* cDNA (GenBank accession number AJ001183). The antisense probes SOX10c, SOX10d and SOX10j have the following sequences: 5′-TCTTTCAGTGTGGGTGCAACA-GTCAACCTCCTTCTCCTCTGTCCAGCCTGTTCTCCTGGG-3′

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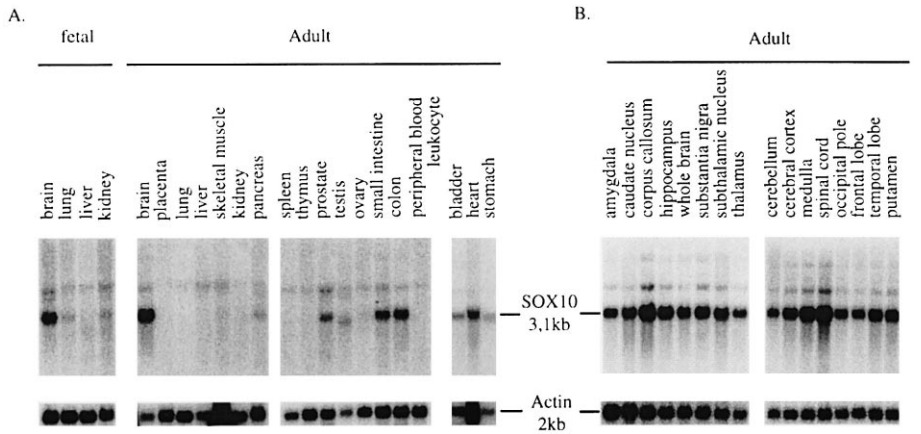

Fig. 1. Tissue distribution of *SOX10* mRNA. Upper panel: Northern blots (Clontech) containing various fetal and adults tissues (A) or adult human brain areas (B) hybridized with the oligonucleotide probe *SOX10*c. Lower panel: Integrity and equal loading of the RNAs were checked by rehybridization with a β actin probe.

(nucleotides 1776–1717), 5′-GATGAGAACTCCACTAAGTCCCTC-GAACCCCCCACTCCCCAATGAGGCTCCTCAAAGCTA-3′ (nucleotides 2090–2031) and 5′-GATGCGTCTCAAGGTCATGGAG-GTTGTAGTGGAGGAGGACTGGGGGCTGTTTCTCAGACA-3′ (nucleotides 2237–2178), respectively. Sense probes were used as negative controls.

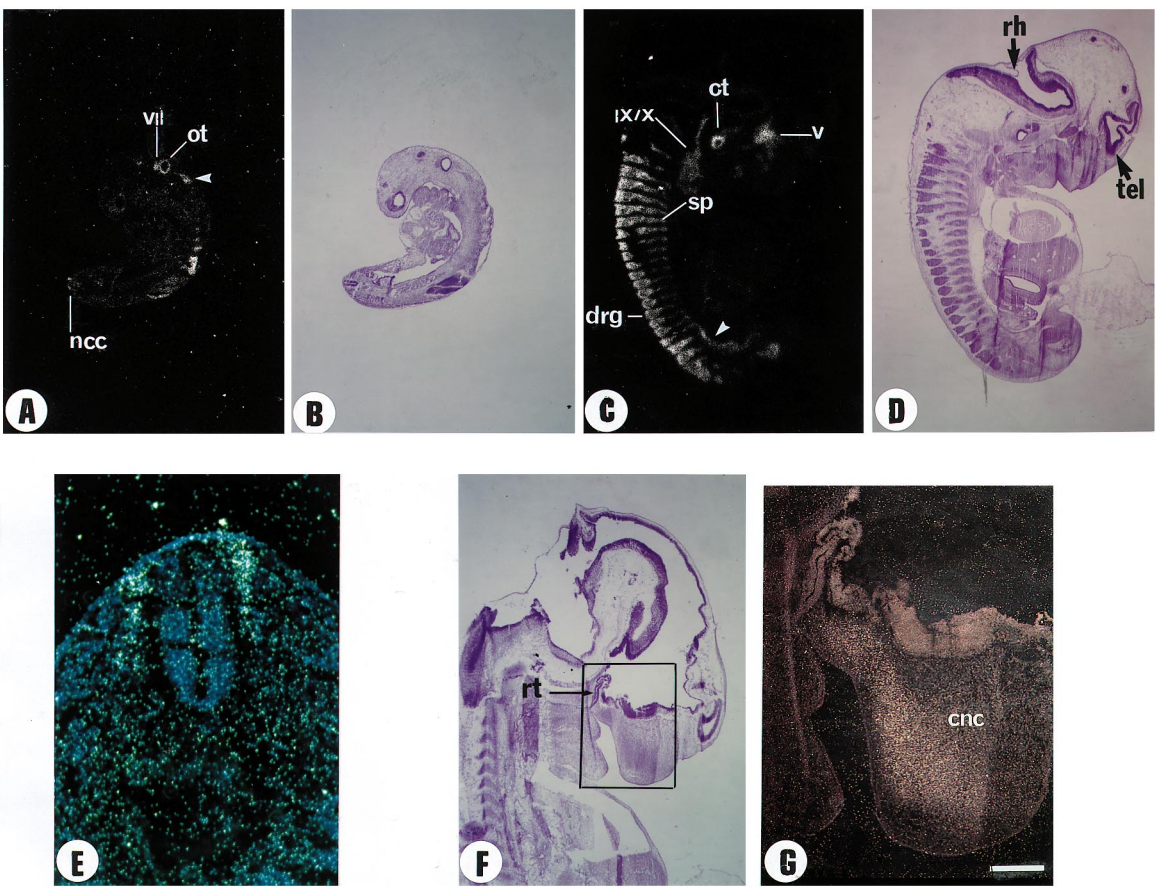

Fig. 2. Distribution of *SOX10* mRNA in the human embryo. Parasagittal section of a 4-weeks-old (A, B) and sagittal section of a 6-weeks-old (C, D) human embryo. A, C: Autoradiographic labelling after in situ hybridization using *SOX10* oligonucleotide probes. B, D: Histological aspect of the same embryo sections (bright field). In each case, after observation of the in situ hybridization signals, sections were counterstained again with hematoxylin and eosin. E: Putative caudal neural crest cells of a 4-weeks-old embryo (dark field). F: Sagittal section of a 6-weeks-old embryo (bright field). G: Enlargement of box in F (dark field) showing no signal in the Rathke pouch and a diffuse signal identified as the cephalic neural crest (mesectoderm). Cranial nerves and ganglia are shown in Roman numerals: V, trigeminal; VII, acoustico facial; IX, glossopharyngeal; X, vagus. cnc, cephalic neural crest; drg, dorsal root ganglia; ncc, neural crest cells; ot, otic vesicle; rt, rathke pouch; rh, rhombencephalon; sp, spinal nerves; tel, telencephalon. Arrowhead in A shows hybridization signal in migrating cells at the origin of the glossopharyngeal ganglia. Arrowhead in C shows spinal nerves pathway. Scale bar: A, B: 1.3 mm; C, D: 2 mm; E: 125 μm; F: 1.4 mm; G: 446 μm.

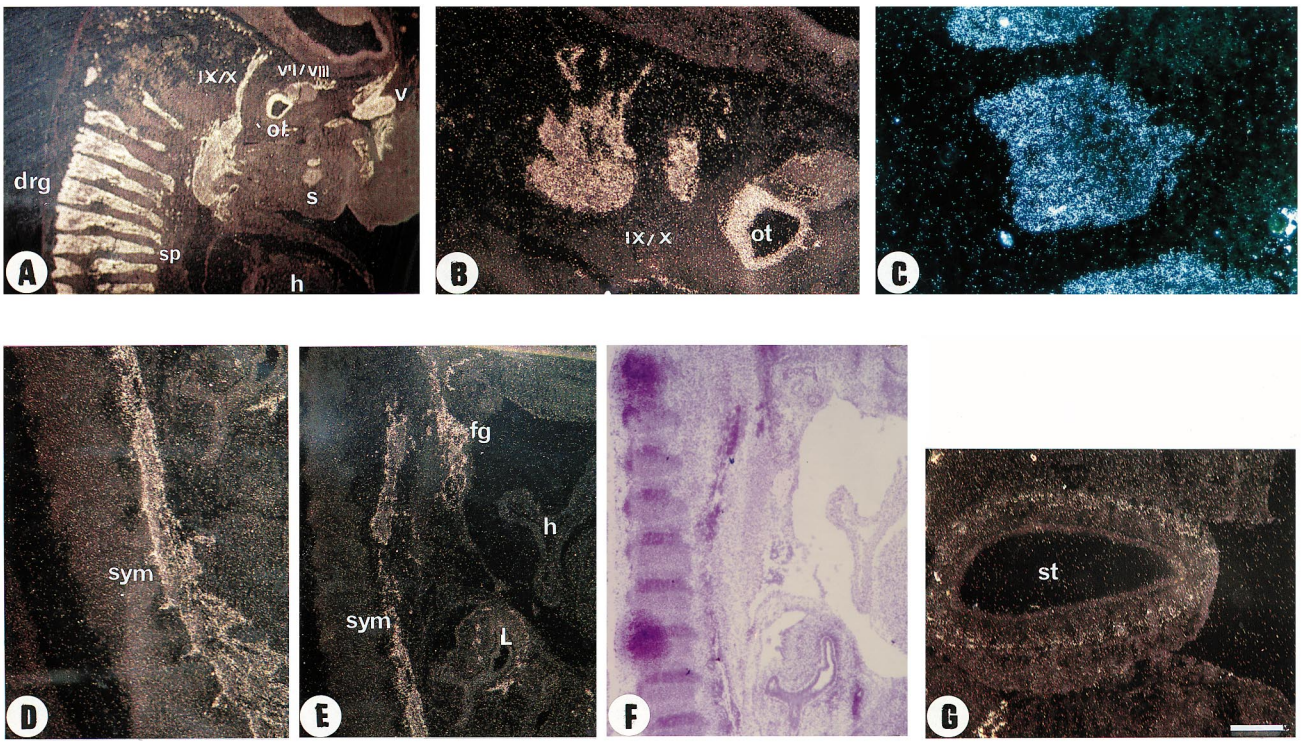

Fig. 3. Expression of *SOX10* in the peripheral nervous system. Dark-field (A–E and G) and bright-field (F) examination of sagittal sections of 6-weeks-old embryos. A, B: *SOX10* expression was detected in the cranial ganglia and otic vesicle. A shows also signals in the dorsal root ganglia and spinal nerves. C: Dorsal root ganglia. D: Labelling of the sympathetic trunk. E, F: Corresponding dark- and bright-field photomicrographs showing labelling of the sympathetic trunk and cranial foregut derivatives. G: Putative enteric ganglia in a stomach section. Note the *SOX10* expression in the submandilar gland (A) and lung (E). Cranial nerves and ganglia are shown in Roman numerals: V, trigeminal; VII, facial; VIII, acoustic; IX, glossopharyngeal; X, vagus. drg, dorsal root ganglia; fg, foregut; h, heart; L, lung; ot, otic vesicle; s; submandibular gland and lingual nerve; sp, spinal nerves; st, stomach; sym, sympathetic chain. Scale bar: A: 960 μm; B: 320 μm; C: 125 μm; D: 384 μm; E, F: 446 μm; G: 320 μm.

3. Results

3.1. General tissue distribution of SOX10 mRNAs

SOX10 mRNA expression was first analyzed with Northern blots containing a panel of fetal and adult tissues. Similar results were obtained with the oligonucleotide probe *SOX10*c (Fig. 1A) and the 1.7-kb partial cDNA probe (data not shown). A major transcript of 3.1 kb was detected in several tissues. In fetal tissues from 17 to 25 weeks of development, *SOX10* expression was essentially found in brain and, to a lesser extent, in lung and kidney. In adult tissues, *SOX10* was preferentially expressed in brain, colon, small intestine and heart and, to a lesser extent, in prostate, bladder, pancreas and stomach. Interestingly, we detected a major transcript of approximately 2.7 kb in testis. In addition, a faint 5.3-kb band, the identity of which remains to be determined, was present in most lanes.

3.2. SOX10 gene expression in adult and developing CNS

The detection of a strong *SOX10* signal in whole fetal and adult brain prompted us to undertake a detailed evaluation of *SOX10* gene expression in various adult brain areas by Northern blotting (Fig. 1B). *SOX10* was expressed in each brain region analyzed. However, the intensity of the signals varied from one area to another. A high level of *SOX10* gene transcripts was seen in the spinal cord, medulla and corpus callosum, while it was weaker in the cerebellum, occipital pole, frontal lobe, amygdala and thalamus. Interestingly, *SOX10* transcripts were detected in major brain nuclei such as the subthalamic and caudate nuclei.

To gain more insight into *SOX10* expression during development, we performed in situ hybridization on human embryos and fetal brain tissue sections. No signal was detected with any of the sense probes used as negative controls. At 4 weeks of development, *SOX10* hybridization signals were first detected both at the autoradiographic (Fig. 2A, B) and cellular levels (Fig. 2E) between the neural tube and the somites. This distribution is consistent with the presence of *SOX10* transcripts in putative neural crest cells. As embryonic development proceeds this signal tended to fade. Throughout the first 6 weeks of development, no *SOX10* gene expression was found in the central nervous system. In a 6-weeks-old human embryo, no hybridization signal was detected in the prosencephalon, mesencephalon, rhombencephalon, Rathke's pouch and posthypophysis (Fig. 2C, D, F, G).

During the fetal period, *SOX10* autoradiographic in situ hybridization signals started to be detected in the CNS. The *SOX10* gene was expressed in the brain at 25 weeks of development. The strongest autoradiographic signals were observed in the cerebral cortex and germinative zone. The hippocampal formation and the thalamic nuclei showed weaker positive hybridization signals (data not shown).

3.3. SOX10 gene expression in embryonic neural crest cell derivatives

As previously mentioned, during early human development,

SOX10 gene is expressed in neural crest cells. As human development progresses, *SOX10* becomes preferentially expressed in the neural crest derivatives which contribute to the formation of the peripheral nervous system. All cranial and spinal ganglia yielded a strong *SOX10* in situ hybridization signal as soon as they were formed. *SOX10* signals were also detected in some migrating cells giving rise to the glossopharyngeal ganglia (Fig. 2A, B). Between the fifth and sixth week of development all cranial ganglia and nerves, and the otic vesicle, were intensely labelled at the autoradiographic (Fig. 2C, D) and cellular levels (Fig. 3A, B). *SOX10* was strongly expressed in spinal nerves linked to dorsal root ganglia, and in dorsal root ganglia themselves (Fig. 3A, C). The labelling of these nerves was detected not only along their pathways but also in their terminal branches (Fig. 2C, D). Interestingly, the entire sympathetic ganglia chain was strongly labelled (Fig. 3D–F). *SOX10* gene expression was also observed in parasympathetic ganglia (data not shown).

Hybridization signals were detected in the digestive tract. *SOX10*-positive cells were observed in the cranial foregut (Fig. 3E, F). In the oesophagus and stomach, positive cells were found at the site of the putative enteric ganglia (Fig. 3G).

Diffuse positive signals corresponding to cephalic neural crest derivatives (mesectoderm) were also detected (Fig. 2F, G). These cephalic neural crest derivatives form cartilaginous rudiments of several nasal bones.

SOX10 gene expression was found in the submandibulary gland and lingual nerve (Fig. 3A), the foramen caecum at the origin of the thyroid, the pancreas (data not shown) and the lung (Fig. 3E, F). Up to 6 weeks of development, no signal was detected in the heart, the future ectoderm or the pigmentary epithelium of the retina.

4. Discussion

The identification of a new member of the *SOX* gene family, *Sox10* [12], and its involvement in the Shah-Waardenburg syndrome [15] prompted us to study its expression pattern in humans by Northern blotting and in situ hybridization.

In situ hybridization was performed on human embryos from 4 to 6 weeks of development. This period encompasses crucial processes of morphogenesis and organogenesis. During this period, neural crest cells emerge from the neural tube, migrate extensively, and form most elements of the peripheral nervous system, as well as facial cartilage, pigment cells, and neuroendocrine cells [19,20].

Our study clearly shows that, in the human embryo, the *SOX10* gene is expressed in neural crest cells, and as development progresses, in their derivatives that give rise to the peripheral nervous system and cephalic mesectoderm. No *SOX10* in situ hybridization signal was observed in the embryonic central nervous system.

SOX10 gene expression was first described in rodents [12]. During embryogenesis the expression pattern seems to be conserved through evolution. However, a detailed analysis of *SOX10* signals shows slight differences between humans and rodents. Indeed, a key finding of our work was the labelling of cephalic neural crest cell derivatives (mesectoderm) giving rise to cartilaginous rudiments of nasal bones.

Moreover *SOX10* is more widely expressed in human fetal and adult tissues than in rodents. In the CNS, strong *SOX10* gene expression characterized several adult brain areas such as the corpus callosum, medulla and spinal cord but, interestingly, contrary to rodents, consistent *SOX10* gene expression was found in the human fetal and adult cerebral cortex and major brain nuclei. It thus seems that *SOX10* gene expression is not restricted to glial cells in humans. Additional double-labelling experiments are required to establish whether *SOX10* is expressed in glial cells and/or neuronal cells.

Other noteworthy differences in *SOX10* gene expression between rodents and human tissues were observed. We detected *SOX10* gene transcripts in human adult heart, prostate and testis by Northern blot analysis. The precise function of *SOX10* in these tissues is unknown, but neural crest cells contribute to the organization of the outflow septum and form the cholinergic cardiac ganglia of the parasympathetic plexus [21]. Interestingly, a smaller transcript was detected in human adult testis. The variable size of the transcripts could result from alternative splicing or from the use of different promoters and/or polyadenylation signals. Alternative splicing has been shown to generate *SOX17* transcripts of various sizes in adult mouse testis tissue [22], and a similar mechanism might account for the shorter *SOX10* transcripts observed here.

Because of their transient and highly restricted tissue distribution, several SOX factors are thought to function as genetic switches that determine cell fate during embryogenesis [6]. In contrast, the continuous expression of *SOX10* during development argues for a role in defining and maintaining the cell phenotype. As suggested in the case of the *SOX22* gene, which also shows a large spectrum of expression [23], *SOX10* might trans-regulate distinct pools of targets according to the tissues in which it is expressed, most likely in conjunction with additional tissue-specific factors.

SOX10 gene mutations have been identified in patients with the Shah-Waardenburg syndrome [15], a disorder in which two neural crest-derived cell lineages, melanocytes and intestinal ganglia cells, are affected. Our analysis of *SOX10* gene expression in the embryonic digestive tract (by in situ hybridization) and adult colon and small intestine (by Northern blotting) is in keeping with the presence of *SOX10* transcripts in enteric ganglia. We observed no *SOX10* hybridization signal in the epidermis throughout the first 6 weeks of development. However, given that several human *SOX10* ESTs derive from skin melanocytes and that melanocytes enter the epidermis at 6 weeks of development, it is possible that the low level of *SOX10* transcripts precluded their detection at the stages analyzed.

Two other genes, the Endothelin 3 (*EDN3*) and Endothelin B receptor (*EDNRB*) genes, are involved in the Shah-Waardenburg syndrome [24,25]. It is noteworthy that the *SOX10* and *EDNRB* genes have an overlapping and concomitant expression pattern in the peripheral nervous system during human embryogenesis [26], suggesting that they may act via a common regulatory pathway.

In conclusion, the results reported here further support the important function of *SOX10* in neural crest development. The observation that *SOX10* is more widely expressed in humans than in rodents infers that SOX10 may have additional roles in human development and physiology. If this is indeed the case, some *SOX10* mutations may give rise to other phenotypic features distinct from those of the Shah-Waardenburg syndrome. Thus, detection of *SOX10* defects may help decipher the molecular basis of other neurocristopathies.

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References

[1] Gubbay, J. et al. (1990) Nature 346, 245–250.
[2] Wright, E.M., Snopek, B. and Koopman, P. (1993) Nucleic Acids Res. 21, 744.
[3] Denny, P., Swift, S., Brand, N., Dabhade, N., Barton, P. and Ashworth, A. (1992) Nucleic Acids Res. 20, 2887.
[4] Sinclair, A.H. et al. (1990) Nature 346, 240–244.
[5] Laudet, V., Stehelin, D. and Clevers, H. (1993) Nucleic Acids Res. 21, 2493–2501.
[6] Pevny, L.H. and Lovell-Badge, R. (1997) Curr. Opin. Genet. Dev. 7, 338–344.
[7] Goodfellow, P.N. and Lovell-Badge, R. (1993) Annu. Rev. Genet. 27, 71–92.
[8] Berta, P., Hawkins, J.R., Sinclair, A.H., Taylor, A., Griffiths, B.L., Goodfellow, P.N. and Fellous, M. (1990) Nature 348, 448–450.
[9] Foster, J.W. et al. (1994) Nature 372, 525–530.
[10] Kwok, C. et al. (1995) Am. J. Hum. Genet. 57, 1028–1036.
[11] Wagner, T. et al. (1994) Cell 79, 1111–1120.
[12] Kuhlbrodt, K., Herbarth, B., Sock, E., Hermans-Borgmeyer, I. and Wegner, M. (1998) J. Neurosci. 18, 237–250.
[13] Southard-Smith, E.M., Kos, L. and Pavan, W.J. (1998) Nat. Genet. 18, 60–64.
[14] Herbarth, B. et al. (1998) Proc. Natl. Acad. Sci. USA 95, 5161–5165.
[15] Pingault, V. et al. (1998) Nat. Genet. 18, 171–173.
[16] Read, A.P. and Newton, V.E. (1997) J. Med. Genet. 34, 656–665.
[17] Bolande, R.P. (1974) Hum. Pathol. 5, 409–429.
[18] Abitbol, M., Menini, C., Delezoide, A.L., Rhyner, T., Vekemans, M. and Mallet, J. (1993) Nat. Genet. 4, 147–153.
[19] Bronner-Fraser, M. (1994) FASEB J. 8, 699–706.
[20] Le Douarin, N. (1982) The Neural Crest, Cambridge University Press, Cambridge.
[21] Creazzo, T.L., Godt, R.E., Leatherbury, L., Conway, S.J. and Kirby, M.L. (1998) Annu. Rev. Physiol. 60, 267–286.
[22] Kanai, Y., Kanai-Azuma, M., Noce, T., Saido, T.C., Shiroishi, T., Hayashi, Y. and Yazaki, K. (1996) J. Cell Biol. 133, 667–681.
[23] Jay, P., Sahly, I., Goze, C., Taviaux, S., Poulat, F., Couly, G., Abitbol, M. and Berta, P. (1997) Hum. Mol. Genet. 6, 1069–1077.
[24] Chakravarti, A. (1996) Hum. Mol. Genet. 5, 303–307.
[25] Hofstra, R.M., Osinga, J. and Buys, C.H. (1997) Eur. J. Hum. Genet. 5, 180–185.
[26] Brand, M., Le Moullec, J.M., Corvol, P. and Gasc, J.M. (1998) J. Clin. Invest. 101, 549–559.