EXPRESSION OF THE p75 NEUROTROPHIN RECEPTOR IN THE DEVELOPING AND ADULT TESTIS OF THE RAT

Mario A. RUSSO, Maria L. GIUSTIZIERI, Donatella FARINI,
Luisa CAMPAGNOLO, Massimo DE FELICI and Gregorio SIRACUSA.
Department of Public Health and Cell Biology,
Section of Histology and Embryology, University of Rome "Tor Vergata"
00173 Rome, Italy

Introduction. Neurotrophic factors are primarly known for their essential role in neuron development and function. Several studies have shown, however, that thay may also have important effects on various types of non-neuronal tissues. Neurotrophins' effects are initiated by their binding to two types of cell surface receptors: p75, or low affinity receptor, which binds all neurotrophins, and a neurotrophin-specific high-affinity tirosine kinase receptor belonging to the *trk* proto-oncogene family (Hempstead et al., 1991; Chao et al., 1992). Like nerve growth factor (NGF), p75 appear to be expressed and developmentally regulated in a broad range of adult and embryonic non-nervous tissues (Yan et al., 1988). The extensive expression of p75 in the mesenchyme during development suggests that such receptor is involved in events that control morphogenesis of mesodermal tissues. NGF and p75 have been detected in the adult mouse (Russo et al., 1994), rat (Parvinen et al.,1992) and human (Seidl et al., 1990) testis. In order to define the role of NGF or other neurotrophins in testicular differentiation and during spermatogenesis we have analyzed the location and timing of appearance of p75 mRNA and protein during embryonic and postnatal development of the rat testis.

We show here a mesenchymal cell-specific expression of p75 during testicular morphogenesis as well as a specific location of p75 in the adult testis.

Materials and Methods. Embryos were obtained by mating Wistar rats. Noon of the day of vaginal plug was considered as 0.5 day-postcoitum (dpc) and the day of birth was designed as postnatal day 1. The sex of the embryos was identified by the morphological aspect of the gonads. Immunolocalization was performed on methanol-fixed cryostat sections prepared from rat testes at different stages of development. Sections were stained using a mouse monoclonal antibody anti-rat p75 (Chandler et al., 1984) and a monoclonal antibody anti-α–smooth muscle actin. The primary antibodies were revealed with the approriate FITC or rhodamine-labelled second antibodies. Control sections were treated with non-immune IgG or by omitting the primary antibodies. In situ hybridization was performed according to Wilkinson et al. 1987, using a riboprobe prepared from a fragment of the 5' region of the rat 5a p75 cDNA (Buck et al., 1988) subcloned into pGEM 3Z (f+) (Promega) and transcribed in the presence of ^{35}S-labeled UTP (Amersham).

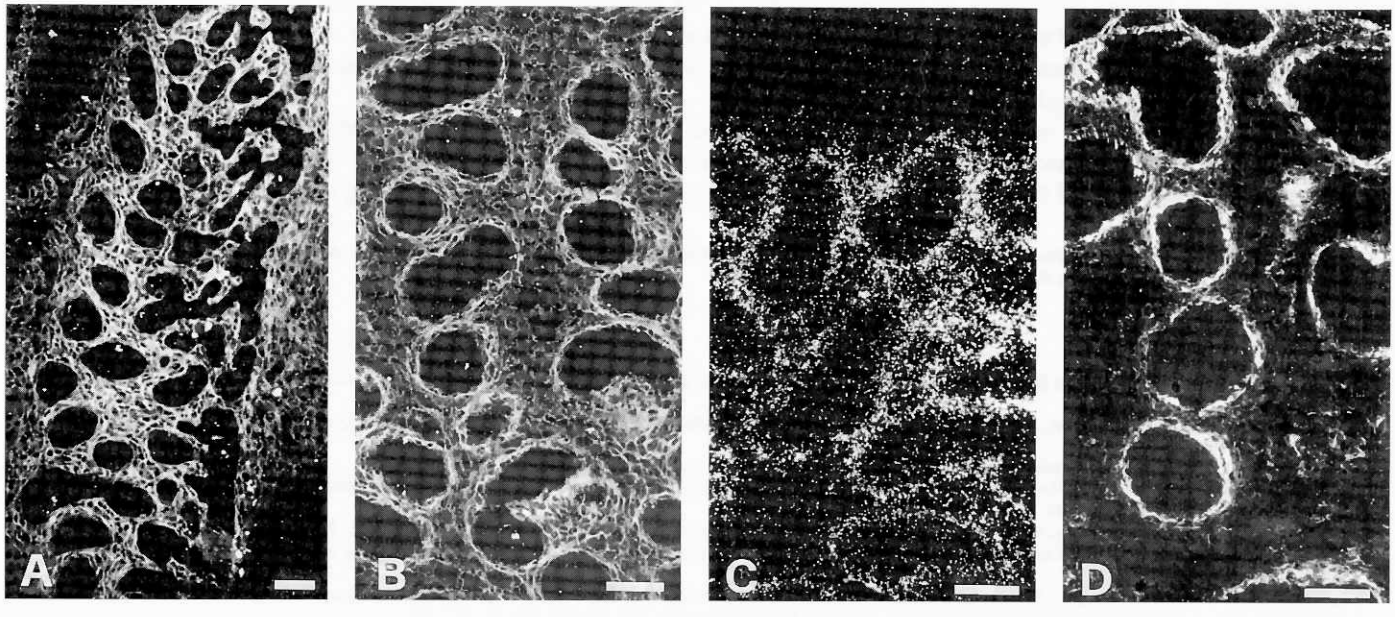

Fig.1. Immunoflorescence micrographs of cryosections of embryonic rat testis of 14.5 (A) and 19.5 (B and **D**) dpc stained for p75 (A and B) and α-smooth muscle actin (**D**); and *in situ* hybridization analysis of p75 expression in the developing testis of a 19.5 dpc rat embryo (**C**). Bar: 50 μm.

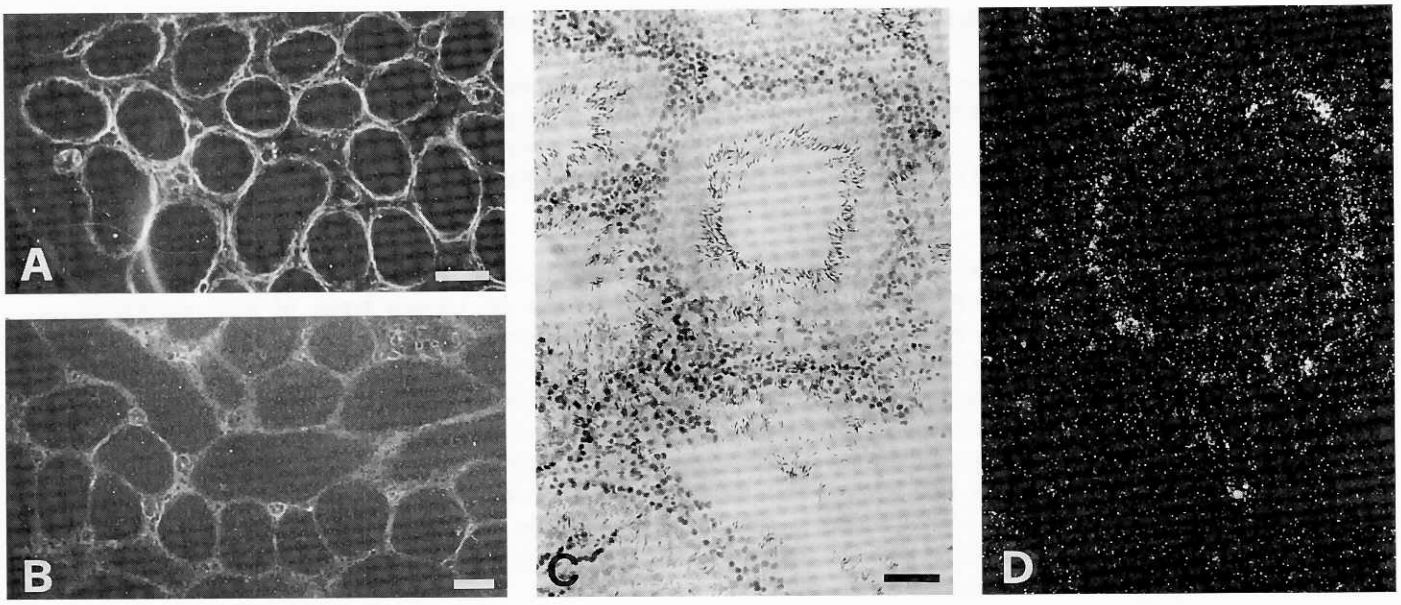

Fig.2. Immunoflorescence staining of rat testis of 8 (A) and 19 (B) days of postnatal development by anti-p75 antibody; and p75 mRNA localization in the adult testis analyzed by in situ hybridization (C: bright field; D: dark field) Bar: 50 μm.

Results and discussion. Immunohistochemical analysis using anti-p75 monoclonal antibody showed intense specific staining on numerous mesenchymal cells spread through the interstitial compartment of the embryonic testis and absence of immunoreactivity in the testis cords (Fig.1A and 1B). At 19.5 dpc, p75 staining was mainly localized to a subpopulation of mesenchymal cells surrounding each testicular cords (Fig.1B). *In situ* hybridization analysis of p75 mRNA location showed a strong specific signal over the mesenchymal cells surrounding the testicular cords (Fig.1C). Immunofluorescence analysis also showed that p75-positive mesenchymal cells that surround testicular cords express α-smooth muscle actin as well (Fig.1D). Co-expression of p75 and α-smooth muscle actin suggest that this cell type is the precursor of myoid cells.

Receptor staining intensity gradually decreases postnatally (Fig.2A and 2B); in the adult a very faint signal is still visible around a few seminiferous tubules (not show). *In situ* hybridization in the adult testis shows the presence of a specific signal located close to the periphery of a limited number of seminiferous tubules (Fig.2C and 2D). Of particular interest is the observation that all labeled tubules were at stages VII to IX of the seminiferous epithelium cycle, thus suggesting a specific function of the p75 receptor during spermatogenesis.

The results indicate that p75 neurotrophin receptor is involved in the differentation of mesenchymal tissue during testicular morphogenesis and is expressed in a developmentally regulated pattern in the differentating myoid cells. Furthermore, the stage-dependent expression of the p75 in the adult suggests a specific role of this receptor during spermatogenesis.

Acknowledgments. We thank Dr. Moses Chao for providing rat p75 cDNA and Mr. Graziano Bonelli for preparation of the photographs. Research supported by CNR Grant 95.00912.PF41 (Progetto Finalizzato FAT-MA)

References

Buck, C.R., Martinez, H.J., Chao, M. and Black, I.B. (1988). Differential expression of the nerve growth factor receptor gene in multiple brain areas. Dev. Brain Res. 44: 259-268.

Chandler, C.E., Parson, L.M., Hosang, M. and Shooter E.M. (1984). A monoclonal antibody modulates the interaction of nerve growth factor with PC12 cells. J. Biol. Chem. 259: 6882-6889.

Chao, M.V. (1992). Neurotrophins receptors. A window into neuronal differentiation. Neuron 9:583-593.

Hempstead, B.L., Martin Zanca, D., Kaplan, D.R., Parada, L.F. and Chao, M.V. (1991). High-affinity NGF binding requires coexpression of the trk proto-oncogene product and the low-affinity NGF receptor. Nature 350: 678-683.

Parvinen, M., PeltoHuikko, M., Soder, O., Schultz, R., Kaipia, A., Mali, P., Toppari, J., Hakovirta, H., Lonneberg, P., Ritzen, E.M., Ebendal, T., Olson, L., Hokfelt,T. and Parsson H. (1992). Expression of β-nerve growth factor and its receptor in rat seminiferous epithelium: specific function at the onset of meiosis. J. Cell Biol. 117: 629-641.

Russo, M.A., Odorisio, T., Fradeani, A., Rienzi, L., De Felici, M., Cattaneo, A. and Siracusa G. (1994). Low-affinity nerve growth factor receptor is expressed during testicular morphogenesis and in germ cells at specific stages of spermatogenesis. Mol. Reprod. Dev 37:157-166.

Seidl, K. and Holstein, A.F. (1990). Evidence for the presence of nerve growth factor (NGF) and NGF receptors in human testis. Cell. Tissue Res. 261:549-554.

Wilkinson, D., Bailes, J., Champion, J. and McMahon, A. (1987). A molecular analysis of mouse development from 8 to 10 days post coitum detects changes only in embryonic globin expression. Development 99: 493-500.

Yan, Q. and Johnson, E.M. Jr. (1988). An immunohistochemical study of the Nerve Growth Factor receptor in developing rats. J Neurosci 8:3481-3498.