Dependence on p75 for Innervation of Some Sympathetic Targets

Kuo-Fen Lee, Kathy Bachman, Story Landis, Rudolf Jaenisch*

The low-affinity neurotrophin receptor p75 binds all neurotrophins with similar affinity. For elucidation of its function, mice bearing a null mutation in the p75 locus were generated. Examination of sympathetic innervation of target tissues revealed that pineal glands lacked innervation and sweat gland innervation was absent or reduced in particular footpads. The absence of adult innervation reflects the failure of axons to reach these targets during development rather than a target deficit. These results indicate that p75 facilitates development of specific populations of sympathetic neurons, for which it may support axon growth.

Members of the neurotrophin family, such as nerve growth factor (NGF), brain-derived neurotrophic factor, neurotrophin-3 (NT-3), NT-4, and NT-5, have diverse effects on distinct populations of developing neurons, such as promoting survival and axon growth (*1*). Neurotrophins bind two classes of receptors: tyrosine receptor kinases (trkA, trkB, and trkC) and p75 (*2*). The trks contribute to high-affinity binding, can subserve signal transduction, and appear to mediate the biological actions of neurotrophins. In contrast, the role of p75, which was first identified as the low-affinity receptor for NGF, is incompletely understood. Although p75 was initially assumed to participate in forming the functional NGF receptor, p75 does not appear to be required for neurotrophin signal transduction, though it may alter the binding affinity of trkA for NGF (*2*). Finally, during development, p75, but not trkA, is widely expressed by nonneuronal peripheral tissues, including Schwann cells.

To elucidate the role of p75 in neural development, we studied mice carrying a null mutation in the p75 locus (*3*). The sensory ganglia of these mice are smaller, and the mice exhibit decreased pain sensitivity and cutaneous innervation. In contrast, the superior cervical ganglia (SCG) and stellate ganglia appear normal in size, and sympathetic innervation of the iris and salivary glands, targets of the SCG, is not affected (*3*). These results were surprising because they suggested that p75 was required for development of one population of NGF-dependent neurons—cutaneous sensory neurons—but not for another, sympathetic neurons.

To determine whether the p75 mutation affected the development of any sympathetic neurons, we examined additional target tissues. Whereas sympathetic innervation of many tissues, including hairy skin, the thymus, heart, lung, spleen, kidney, vas deferens, iris, and salivary glands, appeared qualitatively normal, there were striking abnormalities in two targets—the pineal gland and the sweat glands. In adult wild-type mice, the pineal gland, which receives catecholaminergic innervation from the SCG, contained numerous tyrosine hydroxylase–immunoreactive (TH-IR) fibers (Fig. 1A). In contrast, pineal glands of p75-deficient animals contained few TH-IR fibers (Fig. 1B). The TH-IR fibers in pineal glands of wild-type mice were thick and intensely immunoreactive, but fibers in pineal glands of p75-deficient mice were thin and moderately immunofluorescent. These axons may be central and not sympathetic in origin (*4*). As was consistent with the absence of TH-IR fibers, no catecholamine-containing fibers or vesicle-filled axonal varicosities were observed in pineal glands of p75-deficient mice, whereas both were common in wild-type mice. Sympathetic axons were also not detected in pineal glands and sweat glands when stained for L1, a cell adhesion molecule that does not depend on transmitter function (*5*). Pineal glands in p75-deficient mice were smaller than those in wild-type mice. This difference could reflect the lack of an anterograde trophic effect normally exerted by sympathetic innervation (*6*).

Sympathetic innervation of sweat glands was also affected. Because cholinergic sympathetic innervation induces and maintains secretory responsiveness, sweat secretion is a reliable indicator of innervation (*7*). When sweating was assayed in p75-deficient mice, a subset of footpads exhibited substantially reduced responses. Pilocarpine activated many glands on medial and lateral interdigital hind footpads of wild-type mice (Fig. 2A). In p75-deficient mice, however, the medial in-

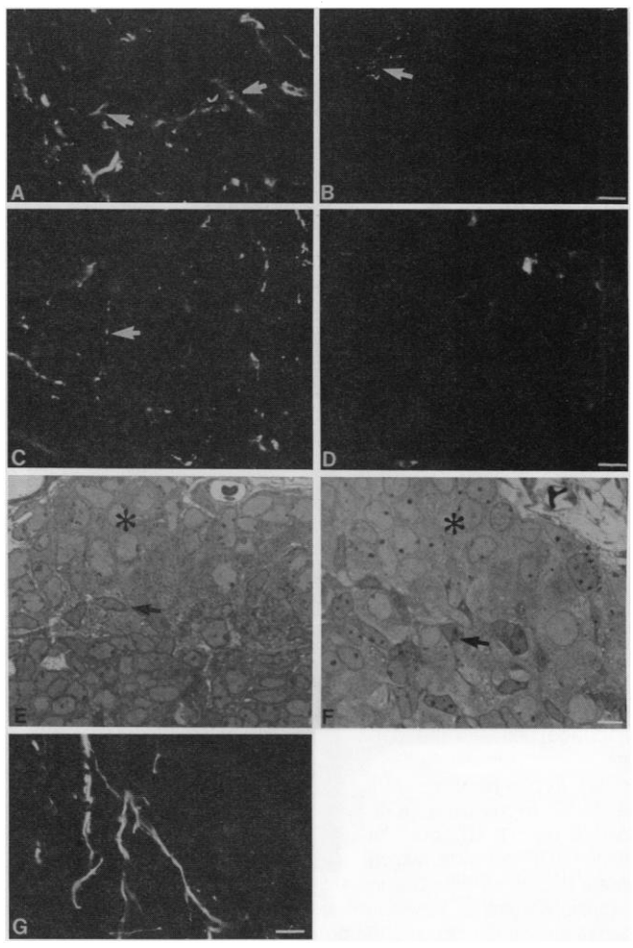

Fig. 1. Pineal glands of p75-deficient mice lack sympathetic innervation. Pineal glands from wild-type (**A, C, E**, and **G**) and p75-deficient (**B, D**, and **F**) mice were examined (*16, 17*). Pineal glands of adult wild-type mice contain TH-IR fibers (A, arrows), in contrast to those of p75-deficient mice. Arrow in (B) indicates occasional TH-IR fibers. Pineal glands of P4 wild-type mice possess a sparse plexus of TH-IR fibers (C, arrow). The TH-IR fibers are absent from pineal glands of P4 p75-deficient mice (D). Plastic sections of P4 wild-type (E) and p75-deficient (F) pineal glands revealed no differences. In both, large differentiated pinealocytes (asterisks) and small darkly stained cells (arrows) were present. In the pineal gland, p75 immunoreactivity is associated with thick fibers (whose distribution resembles that of TH-IR fibers) but not with pinealocytes (G). Scale bar: (A), (B), and (G), 25 μm; (C) and (D), 20 μm; (E) and (F), 10 μm.

K.-F. Lee and R. Jaenisch, Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology, Nine Cambridge Center, Cambridge, MA 02142, USA.
K. Bachman and S. Landis, Department of Neurosciences, School of Medicine, Case Western Reserve University, Cleveland, OH 44106, USA.

*To whom correspondence should be addressed.

terdigital pad possessed a normal complement of active glands but the lateral pad did not. In some mice, a small number of glands was activated (Fig. 2B); in others, none were. The difference in secretory responsiveness correlated with a difference in innervation. Vasoactive intestinal peptide (VIP), a neuropeptide in sweat gland fibers (*8*), was present in both interdigital footpads of wild-type mice (Fig. 2C) and in medial (Fig. 2D), but not lateral (Fig. 2E), footpads of p75-deficient mice. All seven animals showed deficits in this footpad.

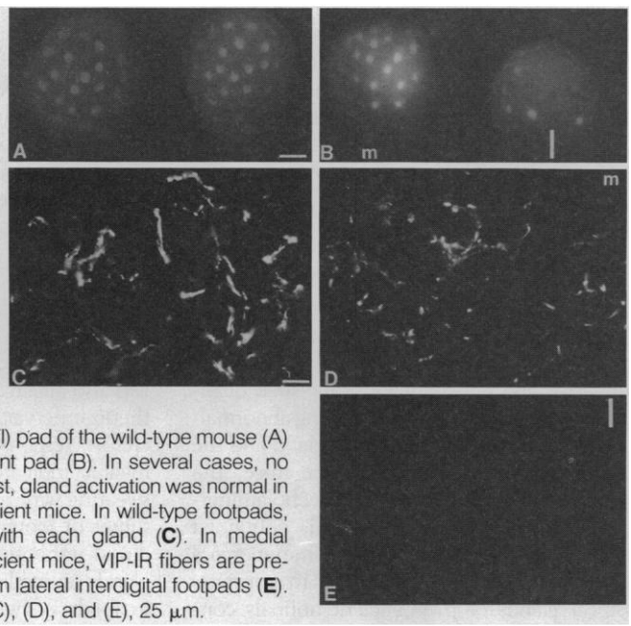

Fig. 2. Sweat glands in adjacent footpads of p75-deficient mice differ in sympathetic innervation. When agonist-evoked sweating was assayed (*18*), all pads of wild-type mice possessed active glands, whereas specific pads of p75-deficient mice showed reduced or absent sweating. (**A**) (control) and (**B**) (p75-deficient) are photomicrographs of molds of the two interdigital footpads after stimulation. Each spot represents a sweat droplet from a single gland. Twenty-three glands were active in the lateral (l) pad of the wild-type mouse (A) but only eight in the p75-deficient pad (B). In several cases, no glands were activated. In contrast, gland activation was normal in the medial (m) pad of p75-deficient mice. In wild-type footpads, VIP-IR fibers are associated with each gland (**C**). In medial interdigital footpads of p75-deficient mice, VIP-IR fibers are present (**D**), but they are absent from lateral interdigital footpads (**E**). Scale bar: (A) and (B), 1 mm; (C), (D), and (E), 25 μm.

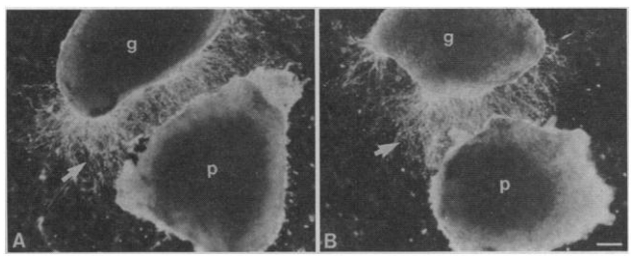

Fig. 3. Neurite extension is indistinguishable when SCG are co-cultured with pineal gland cells from wild-type and p75-deficient mice. Wild-type SCG (g) from P4 mice were grown in collagen gels with pineal gland (p) from either adult wild-type (**A**) or adult p75-deficient (**B**) mice (*19*). In both, pineal gland elicited robust, directed neurite extension (arrows). Scale bar: 150 μm.

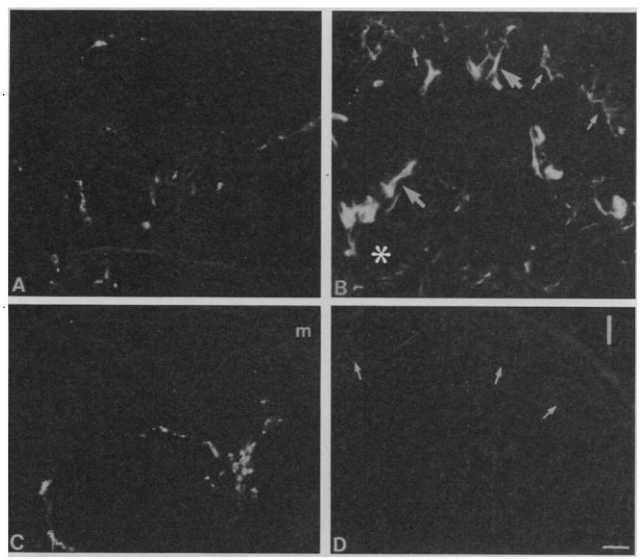

Fig. 4. Deficiency in p75 alters development of sweat gland innervation. At P4, TH-IR fibers have associated with glands forming in the interdigital footpads of wild-type mice (**A**). Their distribution partially overlaps with p75 in the gland region (**B**, asterisk). p75 is also present in nerve bundles (large arrows) that extend through the dermis to the epidermis and have the same distribution as sensory fibers (*3*) as well as at the interface between the dermis and epidermis (small arrows). In p75-deficient mice at P4, TH-IR axons are present in medial (**C**) but not lateral (**D**) footpads, which presages the difference in adult innervation. Each micrograph is oriented with epidermis up and sweat gland primordia down. Small arrows (D) indicate the border between epidermis and dermis. Scale bar: 25 μm.

The absence of innervation in pineal glands and lateral footpads of adult p75-deficient mice could reflect either the failure of these targets to be innervated during development or successful innervation during development, followed by loss. We therefore examined pineal glands and sweat glands at postnatal day 4 (P4), when innervation is beginning (*9*, *10*). At P4, pineal glands of wild-type mice contained TH-IR fibers (Fig. 1C) but pineal glands of p75-deficient mice did not (Fig. 1D), even though the cytology of pineal glands of wild-type (Fig. 1E) and p75-deficient (Fig. 1F) mice was similar. To determine how the absence of p75 contributes to failed innervation, we examined its localization in P4 wild-type pineal glands. We found that p75 was not associated with pinealocytes but with nerves whose distribution resembled that of the TH-IR terminals (Fig. 1G), which suggests a direct effect on growing axons.

To determine whether failure of pineal innervation reflected the inability of p75-deficient pineal glands to exert a tropic effect, we tested their ability to elicit neurite outgrowth from SCG. We observed no difference in directed outgrowth from SCG of mice at P3 when co-cultured with pineal glands from adult p75-deficient mice or from wild-type mice (Fig. 3). Similarly, no difference was observed in neurite outgrowth from p75-deficient SCG or wild-type SCG when elicited by wild-type pineal glands. Thus, failure of innervation is not caused by a lack of tropic influence from the pineal gland or of SCG responsiveness.

The absence of sympathetic innervation in sweat glands of lateral footpads of adult p75-deficient mice also resulted from failure of innervation during development. At P4, TH-IR fibers were associated with developing glands in the interdigital footpads of wild-type mice (Fig. 4A) and in the medial interdigital footpad of p75-deficient mice (Fig. 4C) and were positive for p75 (Fig. 4B). However, TH-IR fibers were absent from the lateral footpad (Fig. 4D).

The failure of innervation in p75-deficient animals is not the consequence of a direct deleterious effect on target tissue development. Pinealocytes do not express p75, the cytology of p75-deficient pineal glands is normal, and pineal glands from p75-deficient mice elicit neurite outgrowth. Because sweat glands in most footpads are innervated, their development is not perturbed by the loss of p75. Further, sympathetic axons that succeed in innervating sweat glands undergo the target-directed switch from noradrenergic to cholinergic (*10*).

We suggest that p75 supports the growth of

axons toward their targets. p75 is found not only on axons but also on pathways along which axons grow (*11*), including Schwann cells, where it could sequester and present neurotrophins to growing axons (*12*). The specificity of the lesion could arise from developmental differences between the abnormally innervated pineal and sweat glands and the other, normally innervated, sympathetic targets. The pineal gland and footpads are relatively distant from the ganglia and are not innervated until after birth (*9*, *10*), in contrast to the heart, salivary glands, and iris, which are innervated prenatally (*13*). This may account for the absence of pineal gland innervation in p75-deficient mice, but it is less easy to explain why sweat glands in adjacent footpads are innervated differently. Sympathetic innervation to the hind footpads originates from distinct distal branches of the sciatic nerve (*7*): The medial footpad is innervated predominantly by the medial plantar nerve, whereas the lateral pad is innervated predominantly by the lateral plantar nerve. Thus, successful innervation in p75-deficient mice is correlated with one nerve and unsuccessful innervation with another.

How p75 functions to support axon growth remains unclear. It could guide sympathetic axons. If all sympathetic neurons, however, use similar pathfinding mechanisms to reach their targets, the selective effect of the p75 mutation argues for p75 homologs that are differentially expressed in subsets of neurons, Schwann cells, or targets and that compensate for the lack of p75. Alternatively, p75, by sequestering and presenting NGF, could provide trophic support for neurons as they extend axons to targets. Finally, p75 may increase NGF sensitivity either independently or in concert with trkA (*14*). Consistent with this hypothesis, SCG neuron survival from p75-deficient mice at P3 is normal at saturating levels of NGF, but survival is reduced at subsaturating levels (*15*). If the concentration of NGF produced by Schwann cells and fibroblasts along axonal pathways is limiting, then by sequestering and presenting NGF or by increasing sensitivity to NGF (or both), p75 would contribute to neuronal survival. Accordingly, late-growing neurons with distant targets, such as pineal and sweat glands, would be uniquely affected.

REFERENCES AND NOTES

1. E. Barde *et al.*, *EMBO J.* **1**, 549 (1982); R. Levi-Montalcini, *Science* **237**, 1154 (1987); A. Hohn *et al.*, *Nature* **344**, 339 (1990); K. Jones and L. Reichardt, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8060 (1990); A. Rosenthal *et al.*, *Neuron* **4**, 767 (1990); P. C. Maisonpierre *et al.*, *Science* **247**, 1446 (1990); L. R. Berkemeier *et al.*, *Neuron* **7**, 857 (1991); F. Hallbook, C. F. Ibanez, H. Persson, *ibid.* **6**, 845 (1991); N. Ip *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 3060 (1992).
2. M. V. Chao, *Neuron* **9**, 583 (1992); S. Meakin and E. Shooter, *Trends Neurosci.* **15**, 323 (1992); D. Glass and G. Yancopoulos, *Trends Cell Biol.* **3**, 262 (1993); M. Barbacid, *Oncogene* **8**, 2033 (1993).
3. K.-F. Lee *et al.*, *Cell* **69**, 737 (1992).
4. E. Zhang, J. Mikkelesen, M. Moller, *Cell Tissue Res.* **264**, 64 (1991).
5. K.-F. Lee *et al.*, unpublished data.
6. T. A. Slotkin *et al.*, *J. Pharmacol. Exp. Ther.* **243**, 101 (1987); D. Tucker and C. Gauthier, *Ann. N.Y. Acad. Sci.* **588**, 120 (1990).
7. W. Kennedy, M. Sakuta, D. Quick, *Neuroscience* **11**, 741 (1984); L. Stevens and S. Landis, *Dev. Biol.* **130**, 703 (1988); M. Grant and S. Landis, unpublished data.
8. M. Schwab, R. Siegel, S. Landis, *Dev. Biol.* **126**, 129 (1988).
9. K. Li and M. Welsh, *Cell Tissue Res.* **264**, 515 (1991).
10. S. Landis and D. Keefe, *Dev. Biol.* **98**, 349 (1983); G. Leblanc and S. Landis, *J. Neurosci.* **6**, 260 (1986).
11. Q. Yan and E. Johnson, *J. Neurosci.* **8**, 3481 (1988); R. Heumann *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8735 (1987).
12. E. Johnson, M. Taiuchi, P. DiStefano, *Trends Neurosci.* **11**, 299 (1988).
13. J. de Champlain *et al.*, *Acta Physiol. Scand.* **80**, 276 (1970); C. Sachs *et al.*, *Eur. J. Pharmacol.* **9**, 67 (1970); E. Rubin, *J. Neurosci.* **5**, 673 (1985).
14. A. Davies, K.-F. Lee, R. Jaenisch, *Neuron* **11**, 565 (1993); M. Benedetti *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 7859 (1993).
15. K.-F. Lee, A. Davies, R. Jaenisch, *Development*, in press.
16. To localize TH, VIP, and p75, we perfused animals with 4% paraformaldehyde (*8*). Tissue was rinsed and equilibrated with 30% sucrose. Cryostat sections were thawed onto coated slides, labeled by incubating overnight with primary antiserum in dilution buffer (0.5 M NaCl, 0.01 M phosphate buffer, 0.01% sodium azide, and 5% bovine serum albumin), and rinsed and incubated in secondary antiserum. The VIP antiserum was generated in guinea pigs by use of porcine VIP. The p75 antiserum was generated in rabbits by use of peptide encoded by the third exon. The TH antiserum was purchased from Pel-Freez (Rogers, AR). All research on experimental animals was conducted according to NIH guidelines and in compliance with Massachusetts Institute of Technology Institutional Animal Care and Use Committee regulations, under protocol 89-054-3.
17. Pineal glands were dissected from P4 and adult mice that were perfused with 2% paraformaldehyde and 2% glutaraldehyde in phosphate buffer. Tissues were postfixed in osmium, stained with uranyl acetate, and embedded in Epon (Polysciences, Warrington, PA). Sections of 1 μm were stained with toluidine blue.
18. We assayed sweating by making a mold of the plantar surface with silicone elastic material (Denture Elasticon, Kerr, Romulus, MI) (*7*). Mice were anesthetized with Avertin (Aldrich, Milwaukee, WI) and injected with the muscarinic agonist pilocarpine (3 mg per kilogram of body weight, intraperitoneally; Sigma). The base material, mixed with hardener, was applied and allowed to polymerize. As the impression material hardens, sweat droplets form pores.
19. To establish co-cultures, we dissected pineal glands from adult mice and SCG from P3 mice. Pineal gland cells and SCG were embedded 5 mm apart in a collagen gel, and the cultures were incubated at 37°C for 15 min. We added 0.5 ml L15-CO_2 of medium (Specialty Media, Lovallette, NJ) supplemented with 5% fetal calf serum (Hyclone, Logan, UT) to each well, and the cultures were incubated at 37°C. Neurite outgrowth was scored 16 hours after medium addition.

20. We thank M. Chao and J. Huber for p75 antiserum and J. Loring, R. Curry, and C. Marks for assistance. Supported by NIH grants NS 023678 (S.L.) and 5 R35 CA44339 (R.J.) and an Amgen fellowship (K.-F.L.).

28 October 1993; accepted 21 January 1994

Autonomy and Nonautonomy in Cell Fate Specification of Muscle in the *Caenorhabditis elegans* Embryo: A Reciprocal Induction

Ralf Schnabel

EMS, a blastomere of the *Caenorhabditis elegans* embryo, produces body wall muscle cell-autonomously in isolation. Within the embryonic context, however, the specification of body wall muscle derived from EMS depends on inductive interactions between its daughter MS and ABa descendants that are required to overcome inhibitory interactions with other cells. The inductive events between the MS and ABa descendants are reciprocal, specifying subsequent fates in both lineages. Both induction events are blocked by mutations in the gene *glp-1*, known to encode a Notch-like transmembrane receptor protein.

Several criteria are used in developmental biology to determine the mechanisms by which cells become committed to a fate. If cells in isolation from the embryo do not acquire the fate expected from the fate map, it is assumed that the normal fate of these cells is specified nonautonomously by means of an induction from other cells. If the cells acquire the fate expected from the fate map in isolation from the embryo, they are considered autonomously specified. Classical developmental biology introduced another criterion to distinguish the state of specification of cells. When grafted to ectopic positions in the embryo, only cells that are not reprogrammed to follow a new fate reflecting their new position in the embryo are considered to be terminally determined. The biological meaning of this test, however, remains obscure when the fate of cells can be changed even though they are already restricted to their

Max-Planck-Institut für Biochemie, D-82152 Martinsried, bei München, Germany.