Development 129, 3739-3750 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
DEV4630

Diverse dependencies of developing Merkel innervation on the trkA and both full-length and truncated isoforms of trkC

Katharine M. Cronk[1], George A. Wilkinson[2], Rachel Grimes[3], Esther F. Wheeler[3], Sonal Jhaveri[4], Bengt T. Fundin[5], Immaculada Silos-Santiago[6], Lino Tessarollo[7], Louis F. Reichardt[8] and Frank L. Rice[1,*]

[1]Center for Neuropharmacology and Neuroscience, Albany Medical College, Albany, NY 12208, USA
[2]Neuroscience Unit, Howard Hughes Medical Center, University of California, San Francisco, CA 94143-0724, USA
[3]Division of Life Sciences, University of Texas, San Antonio, TX 78249, USA
[4]Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[5]Astra Zeneca R&D Södertälje, Department of Molecular Sciences, SE-141 57 Huddinge, Sweden
[6]Department of Neurobiology, Millennium Pharmaceuticals Inc., Cambridge, MA 02139-4815, USA
[7]Neural Development Group, NCI-FCRDC, Frederick, MD 21702, USA
*Author for correspondence (e-mail: ricef@mail.amc.edu)

Accepted 30 April 2002

SUMMARY

This study demonstrates that innervation dependent on two different neurotrophin tyrosine kinase (trk) receptors can form the same types of sensory endings (Merkel endings) in the same target (Merkel cells of vibrissa follicles). Some endings transiently express trkA during their initial development, whereas others express trkC throughout their development. Consequently, elimination of kinase domains of either trkA or trkC each result in a partial loss of Merkel endings, whereas absence of kinase domains of both receptors results in a total loss. At the onset of Merkel ending development, at least one kinase-lacking trkC isoform is transiently expressed on all the follicle cells, while neurotrophin 3 is transiently expressed only in the cells at the middle third of the follicle where the Merkel endings and cells develop. This transient non-neuronal expression of truncated trkC is essential for development of any Merkel endings, whereas some Merkel endings and cells still begin to develop in the absence of neurotrophin 3. Therefore, truncated trkC plays a more important role in the development of this innervation than kinase forms of trkA or trkC or of NT3, the only known ligand for trkC receptors.

Key words: Neurotrophins, Cutaneous innervation, Trigeminal, Mechanoreceptors, Mouse

INTRODUCTION

During development of the peripheral nervous system, neurotrophins contribute to such diverse functions as cell proliferation, neuronal lineage differentiation, neuronal survival, neurite outgrowth and synapse formation. In mammals, nerve growth factor (NGF) and three related neurotrophins [brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3) and neurotrophin 4 (NT4)] signal through three related tyrosine kinase receptors (trkA, trkB and trkC) that can exist in multiple isoforms (reviewed by Reichardt and Fariñas, 1997; Huang and Reichardt, 2001). Full-length kinase isoforms ($trkA_K$, $trkB_K$ and $trkC_K$) have tyrosine kinase signaling domains, while truncated isoforms of trkB and trkC ($trkB_{Tr}$ and $trkC_{Tr}$) lack kinase domains. All four neurotrophins also bind to a non-kinase receptor, p75, that has a small signaling domain similar to that of tumor necrosis factor receptors.

Phenotypes of mice with targeted null mutations or transgene enhancements indicate that development and maintenance of all cutaneous innervation is dependent on one or more of the four neurotrophins and four receptors (Ip et al., 1993; Albers et al., 1996; Pinon et al., 1996; Davies, 1997; Fundin et al., 1997; Rice et al., 1998; LeMaster et al., 1999). Dependency of some innervation on more than one neurotrophin or receptor (Fundin et al., 1997; Rice et al., 1998) is consistent with observations that many neurons in dorsal root and trigeminal ganglia can express more than one receptor either simultaneously or sequentially during development [reviewed by Reichardt and Fariñas (Reichardt and Fariñas, 1997)] (Buchman et al., 1994; Snider and Wright, 1996; Huang et al., 1999). Dependence on more than one neurotrophin-receptor interaction is supported by the observation that knockouts of the kinase domain in either $trkA_K$ or $trkC_K$ ($trkA_K^{-/-}$ or $trkC_K^{-/-}$; *Ntrk1*$^{-/-}$ and *Ntrk3*$^{-/-}$ – Mouse Genome Informatics) each result in a loss of substantially more than half of the neurons in dorsal root or trigeminal ganglia. In addition, absence of NT3 (*Ntf3*$^{-/-}$) results in a greater loss of neurons than absence of $trkC_K$ whose only known ligand is NT3. Presumably, the excess loss is due to possible interactions of NT3 with $trkA_K$ and $trkB_K$.

Based on such results, hypotheses have been proposed about

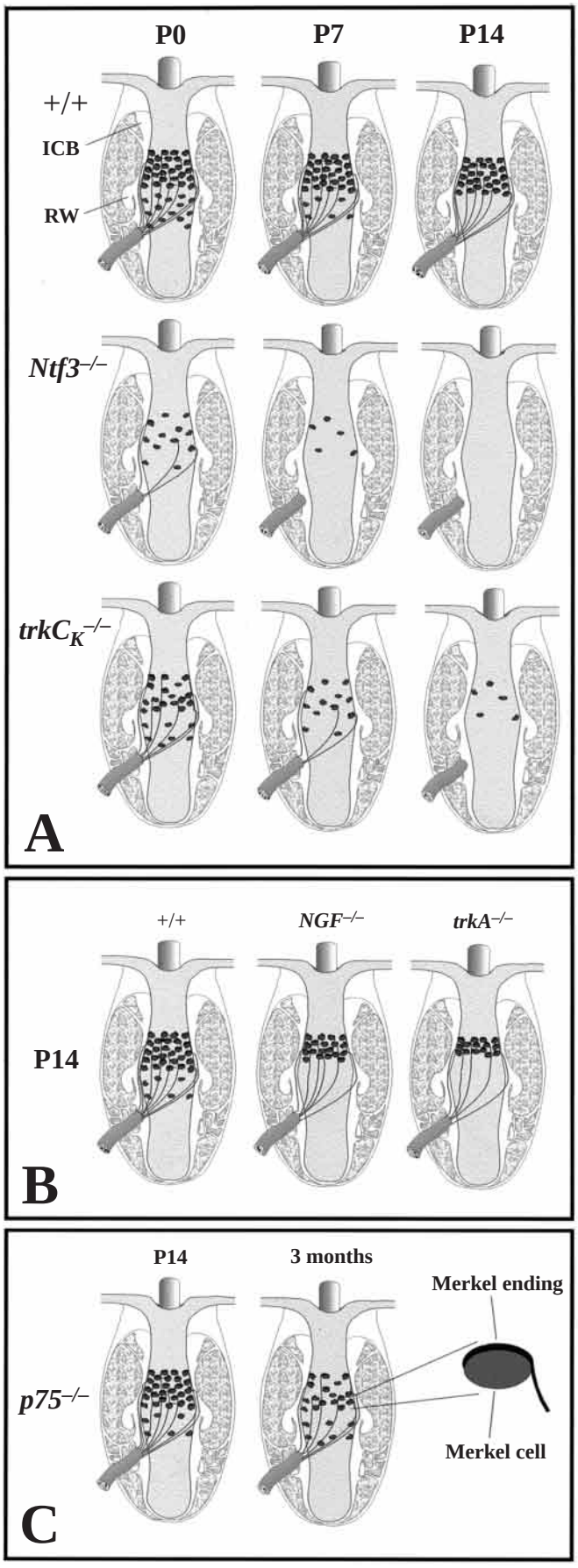

Fig. 1. Summary of impacts of various previous knockouts on innervation (black fibers) of mid-vibrissa follicle Merkel cells (dark gray ovals) (Fundin et al., 1997). An enlarged schematic of a Merkel ending and cell is shown in C. For illustrative purposes, the follicles are all shown at the same size and maturity for all ages. (A) In newborn *Ntf3*$^{-/-}$, only a few Merkel cells are present and only a few of these are innervated (Fig. 6A,B). Only a few uninnervated Merkel cells remain on P7 and these disappear by P14. Although reduced compared with normal, more Merkel cells and innervation are present in newborn *trkC*$_K$$^{-/-}$ than in *Ntf3*$^{-/-}$ (Fig. 6C,D, Fig. 7E). These Merkel cells and innervation are further reduced but still present by P7. Only a few uninnervated Merkel cells remain by P14. (B) In *Ngf*$^{-/-}$ or *trkA*$^{-/-}$ animals, Merkel cells and innervation from P0 to P14 are reduced and tightly packed to a level near the ICB (Fig. 7A). The impact was slightly less severe in the *Ngf*$^{-/-}$. (C) Merkel innervation seems normal from P0 to P14 in *p75*$^{-/-}$ and gradually declines over the next few months.

the types and sequences of receptors that should be expressed during the development of specific types of cutaneous innervation (Fundin et al., 1997; Rice et al., 1998). However, direct evidence for expression of these receptors on specific types of definitive cutaneous sensory endings has not been shown during their development. An especially interesting model for exploring complexities of neurotrophin and related receptor dynamics during development is the Merkel innervation located at the mid-follicle level of rodent mystacial vibrissae (Fundin et al., 1997; Rice et al., 1997). This innervation consists of large caliber myelinated axons, whose endings penetrate the basement membrane of the follicles and terminate on Merkel cells located among the keratinocytes in the outer root sheath. As shown schematically in Fig. 1, either *trkA*$_K$$^{-/-}$ or *trkC*$_K$$^{-/-}$ substantially reduced the number of Merkel endings and cells present at birth (P0). However, many endings and cells remain for at least four weeks postnatally in *trkA*$_K$$^{-/-}$ mice, whereas in *trkC*$_K$$^{-/-}$ virtually all of the Merkel endings disappear by P7 and all of the Merkel endings and cells are absent by P14. By contrast, *Ntf3*$^{-/-}$ is relatively more severe with only a few Merkel endings and cells present at P0. All of the Merkel endings and nearly all of the Merkel cells disappear by P7. *Ngf*$^{-/-}$ also had a partial reduction of Merkel innervation that was less severe than that in *trkA*$_K$$^{-/-}$ mice. These observations led to the hypothesis that the mid-follicle Merkel innervation initially depends upon trkA$_K$ and trkC$_K$ prenatally, but becomes entirely dependent on trkC$_K$ postnatally (Fundin et al., 1997). NT3 appears to be the primary ligand involved in the development and maintenance. Although the Merkel innervation appeared to be intact at P0 in *p75*$^{-/-}$ (*Ntfr*$^{-/-}$ – Mouse Genome Informatics) mice, a gradual loss over several weeks suggested that p75 contributes to long term maintenance perhaps by facilitating NT3 signaling through trkC$_K$.

One objective of our study was to determine if trkA, trkC and p75 receptors are expressed on this mid-follicle Merkel innervation in mice in a sequence consistent with these hypotheses and if development of this Merkel innervation is indeed totally dependent on both trkA$_K$ and trkC$_K$. As rats are more amenable for experimental studies involving nerve manipulations, behavioral assessments and neurophysiology, expression of receptors was also assessed in rats to see if their normal receptor expressions are comparable with those in mice where the genetic manipulations have been performed.

An especially puzzling aspect of neurotrophin research is the functional role of the truncated isoforms (Tr) of the trk receptors (Klein et al., 1990; Middlemas et al., 1991; Valenzuela et al., 1993; Tsouflas et al., 1993; Garner and Large, 1994). Mice containing null mutations targeted at the ligand-binding extracellular domain locus (*trkC*$_E^{-/-}$) appeared to eliminate both the trkC$_K$ and trkC$_{Tr}$ isoforms, and resulted in a more severe neuronal losses than *trkC*$_K^{-/-}$ (Tessarollo et al., 1997). However, the specific impact of the *trkC*$_E^{-/-}$ on cutaneous innervation was not explored in detail. Interestingly, in situ hybridization results indicate that trkC$_{Tr}$ isoforms are expressed primarily on non-neuronal tissues of fetal mystacial pads instead of trigeminal neurons (Wheeler et al., 1998). These observations indicate that expression of trkC$_{Tr}$ isoforms in non-neuronal tissues may contribute to development of the innervation. Therefore, the second objective of our study was to determine whether trkC$_{Tr}$ also contributes to development of the Merkel innervation.

MATERIALS AND METHODS

Animals

Normal fetal and postnatal Swiss-Webster mice were obtained after overnight matings (Taconic Farms). At least five specimens from three different pregnancies were obtained at consecutive daily intervals ranging from embryonic day E9.5 to postnatal day P14. E0.5 was regarded as noon on the first day after the mating, P0 was the morning of the day of birth. Fetal and postnatal Sprague Dawley rats were also obtained from overnight matings (Taconic Farms). At least five specimens from three different pregnancies were obtained at consecutive daily intervals ranging from embryonic day E10.5 to postnatal day P14.

Fetal and newborn *trkC*$_K^{-/-}$ or *trkC*$_E^{-/-}$ mice, as well as comparable age wild-type mice were obtained from overnight matings of adult male and female *trkC*$_K^{+/-}$ (129/c57bl/6) (Klein et al., 1994) or *trkC*$_E^{+/-}$ (129/c57bl/6) (Tessarollo et al., 1997). For each type of mutation, a minimum of five homozygous knockouts and five wild-type mice were obtained at E14, E15 and P0. At least two P0 *trkA*$_K^{-/-}$/*trkC*$_K^{-/-}$, *trkA*$_K^{+/+}$/*trkC*$_K^{-/-}$, *trkA*$_K^{-/-}$/*trkC*$_K^{+/+}$ and *trkA*$_K^{+/+}$/*trkC*$_K^{+/+}$ hybrids were obtained from matings of male and female *trkA*$_K^{+/-}$/*trkC*$_K^{+/-}$ (129/c57bl/6) (Smeyne et al., 1994; Klein et al., 1994). The fetal and postnatal offspring were genotyped by PCR. As the original observation (Fundin et al., 1997) of the impact of *Ntf3*$^{-/-}$ was based on mice with a different background from the trkC-related knockouts used in this study, P0 and P7 *Ntf3*$^{+/+}$ and *Ntf3*$^{-/-}$ mice were obtained from matings of *Ntf3*$^{+/-}$ parents with the same background (i.e. 129/c57bl/6) (L. T., unpublished). Mice with a heterozygous mutation in which the coding region of the *lacZ* gene replaced a coding exon for NT3 and bred on a c57bl/6 background were mated to obtain E14, E15 and E16 embryos that were *Ntf3*$^{+/+}$, *Ntf3*$^{+/lacZ}$ and *Ntf3*$^{lacZ/lacZ}$ (Fariñas et al., 1994). Animals were genotyped by DNA blot analysis as described (Fariñas et al., 1994).

Fetuses were obtained from dams by cesarean section after lethal anesthetic overdose (100 mg/kg sodium pentobarbital). Embryos were removed and whole embryos or heads were fixed by immersion for 4 hours in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) at pH 7.4 and 4°C. Postnatal specimens were anesthetized by hypothermia (P0-P5) or anesthetic overdose (after P5) and were perfused transcardially and postfixed for 4 hours in the above fixative. After fixation, specimens were rinsed and stored in PBS at 4°C. The relative status of mystacial pad development was used to correct for variations in the rate of development between comparable age specimens from different pregnancies and genetic backgrounds.

Fixed whole fetuses, heads or mystacial pads were cryoprotected by overnight infiltration in 30% sucrose in PBS and frozen sectioned (14 μm) in a plane parallel to the central rows of vibrissa follicles and perpendicular to the skin surface. Sections were directly thawed onto slides coated with chrome-alum gelatin and air dried overnight.

Immunofluorescence

Sections were prepared for immunofluorescence analyses (Rice et al., 1997) by incubation overnight at 4°C under primary antibodies against pan-neuronal protein gene product 9.5 (PGP) (rabbit polyclonal, 1:1000, UltraClone); calcitonin gene-related peptide (CGRP) (rabbit polyclonal, 1:1000; Peninsula); neurofilament 200 (NF200) (rabbit polyclonal, 1:400; Chemicon International), keratin 20 (mouse monoclonal, 1:50; Chemicon International), trkA (rabbit polyclonal, 1:5000) (Huang et al., 1999); trkC (goat polyclonal, 1:500) (Huang et al., 1999) and p75 (rabbit polyclonal, 1:5000) (Weskamp and Reichardt, 1991). The trkA and trkC antibodies were generated against extracellular domains and label both kinase and truncated isoforms. After rinses, sections were incubated for 1 hour at room temperature in appropriate species of secondary antibodies raised in goat or donkey and conjugated with either Cy3 or Cy2 fluorophores (1:250-500: Jackson Immuno Research Laboratories). All incubating and rinse solutions consisted of 1% bovine serum albumin and 0.3% Triton X-100 in PBS. Sections were examined with an Olympus Provis AX70 epifluorescence microscope and digital images were captured with a Sony DKC-ST5 digital photo camera interfaced with Northern Eclipse imaging and deconvolution software (Empix Imaging).

In situ hybridization

Presence of mRNAs for all isoforms of trkC or just trkC$_K$ was determined by in situ hybridization in sections of fetal mouse and rat mystacial pads (Wheeler et al., 1998). Rat trkC probes were prepared from a pBlue-script vector containing the full-length cDNA sequence [nucleotide 1 to 2003: gift of L. Parada (Tsouflas et al., 1993)]. This antisense *trkC* probes and encodes *trkC*$_K$ and recognizes mRNAs for both trkC$_K$ and trkC$_{Tr}$ isoforms. TrkC riboprobes that recognize only trkC$_K$ mRNAs were generated from the 125 bp fragment encoded within the kinase domain downstream from sequences that encode the alternatively spliced isoforms. The gene sequence runs from nucleotide 1908 to a site located in the 3′ untranslated region at nucleotide 2033. The construct was made by subcloning an *Acc*III-*Ava*I restriction fragment into the pGEM 3Z in vitro transcription vector (Promega, Madison, WI).

The RNA probes were labeled with [^{35}S]-UTP by using an in vitro transcription system and polymerases (Promega, Madison, WI). Each template (500 ng) was transcribed. Reactions were incubated at 37°C for 2 hours. Resulting RNA transcripts were hydrolyzed to an average length of 150 bp. Hybridization reactions and washes were carried out as described previously (Wheeler et al., 1998). Processed slides were dipped in photographic emulsion (NTB from Eastman Kodak, Rochester, NY), exposed at 4°C for 10 days to 3 weeks, developed, fixed and counterstained with Methyl Green. Sections were examined under both bright-field and dark-field illumination.

RESULTS

TrkA, trkC and p75 expression on normal developing Merkel innervation

Mid-follicle Merkel endings are the first innervation to develop on the nascent vibrissa follicles. Paralleling a caudal to rostral sequence of follicle development (Fig. 2), arriving axons completely engulf the deep end of the developing follicles beginning at E12.5 in mice and E13.5 in rats (not shown). As follicles elongate, these axons shift to the dorsal side and become limited to the mid-follicle level (Fig. 2). Beginning

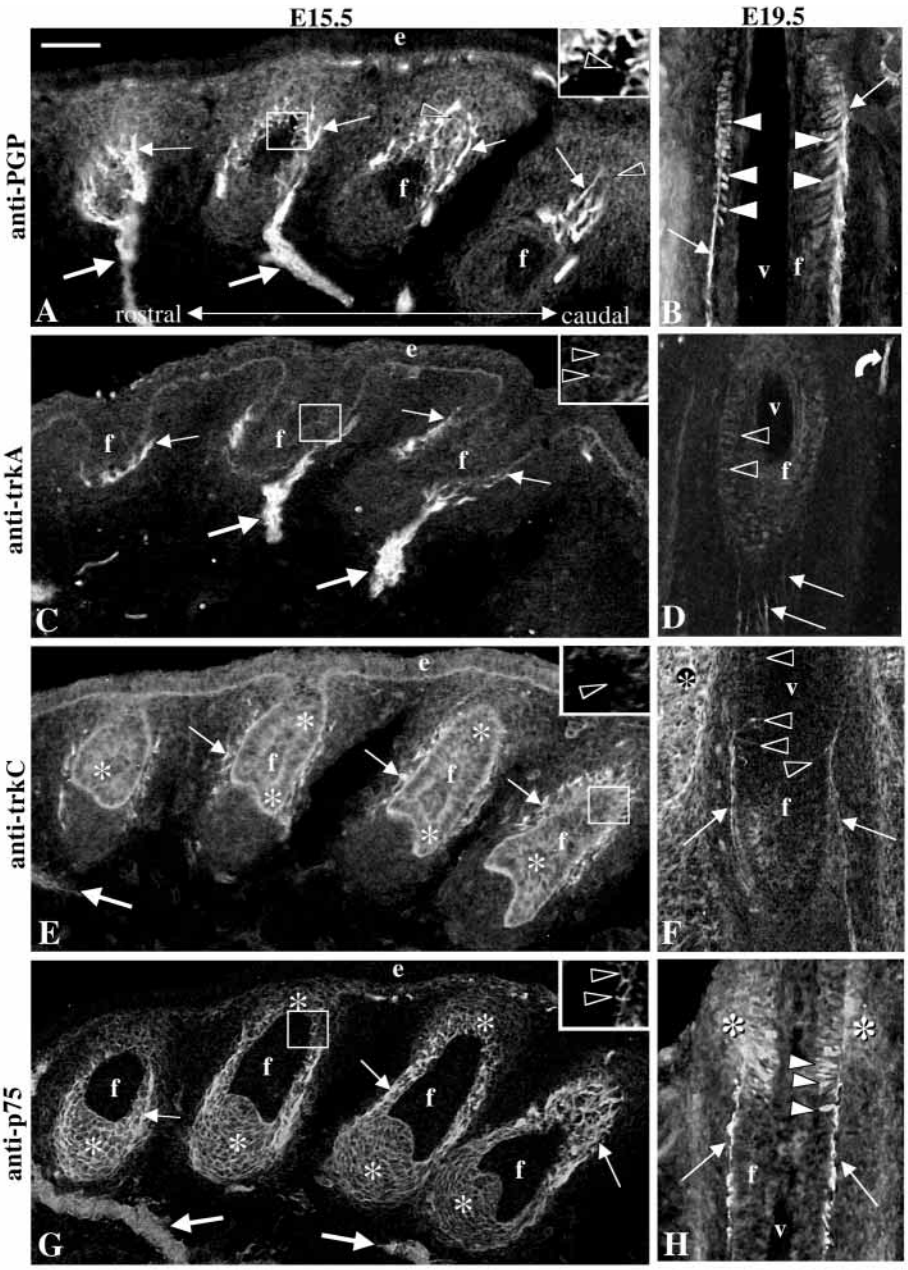

Fig. 2. Immunofluorescence images of developing vibrissae (v), vibrissa follicles (f), nerve bundles (large arrows) and individual Merkel-related axons (small arrows) in rat mystacial pads on E15.5 and the caudal region of rat mystacial pads on E19.5. White arrowheads indicate labeled Merkel endings and cells. Open arrowheads indicate just labeled Merkel endings. Inserts in A,C,E,G show Merkel endings at twice the magnification as shown in white rectangles. e, epidermis. Asterisks indicate sites of non-neuronal labeling. (A,B) Development proceeds along a caudal-to-rostral gradient (right to left). Developing axons completely engulf more immature rostral follicles but caudally have become restricted to the mid-follicle level. Merkel innervation begins to penetrate caudal follicles around E14.5 (not shown). Anti-PGP labels the Merkel endings and begins to label Merkel cells a day or two later. Labeled Merkel endings and cells are shown on E19.5. (C,D) Anti-trkA labeling is clear on axons at E15.5 and is faint on axons and endings by E19.5. By comparison, axons (curved arrow) ascending to innervate the epidermis are more intensely labeled. Labeling at E19.5 has been digitally enhanced in comparison with that at E15.5. TrkA is not clearly expressed on Merkel cells. (E,F) Anti-trkC immunoreactivity is intensely expressed on the developing follicle cells as well as on axons and Merkel endings at E15.5 and is present at low levels on dermal cells immediately surrounding the follicles. Follicle labeling rapidly diminishes over the next day and is lacking on E19.5, while trkC is still evident on axons and Merkel endings. (G,H) Anti-p75 labels axons and Schwann cells within the growing nerves and is present on individual axons at the level where they form Merkel endings. On E15.5 intense p75 immunoreactivity is expressed on dermal cells completely surrounding the follicles. On E19.5, anti-p75 labeling is present on Merkel endings and cells as well as other cells both within and adjacent to the follicles. Scale bar: 50 μm.

caudally at E13.5 in mice and E14.5 in rats, axons penetrate the basement membrane and begin to form endings among adjacent follicle cells (Fig. 2). Within a day or two, the cells that the axons terminate among become immunoreactive for anti-PGP (Fig. 2B, Fig. 3A,E) and anti-keratin 20 (not shown) which are indicative of differentiated Merkel cells (Rice et al., 1997). By E14.5 in mouse and E15.5 in rat, Merkel endings and cells are well established in the most caudal follicles, whereas axons have only begun to engulf the most rostral follicles (Fig. 2).

We previously hypothesized that this developing Merkel innervation should initially co-express $trkA_K$ and $trkC_K$ but should only express $trkC_K$ postnatally (Fundin et al., 1997). Immunolabeling revealed that axons growing towards, but not yet contacting nascent whisker follicles label with either anti-trkA or anti-trkC, not both (not shown). Detectable immunoreactivity for these two receptors remains on axons and on the earliest definitive Merkel endings for several days (Fig. 2C-F, Fig. 3B,C). Contrary to our hypothesis, double labeling revealed that trkA and trkC immunoreactivity is expressed on completely separate sets of axons and Merkel endings (Fig. 4A,B). The trkA labeling on the one set of Merkel innervation diminishes to barely detectable levels by E17.5 in the mouse (data not shown) and E19.5 in the rat (Fig. 2D) and is undetectable by birth in both species (E18.5 and E21.5 respectively). The trkC labeling on other Merkel axons and endings continues into the postnatal period and becomes barely detectable by the end of the first postnatal week.

Although our original knockout study only detected a detrimental impact of $p75^{-/-}$ on the Merkel innervation well after birth (Fundin et al., 1997), anti-p75 labeling is robust on growing nerves before they reached nascent follicles and on all

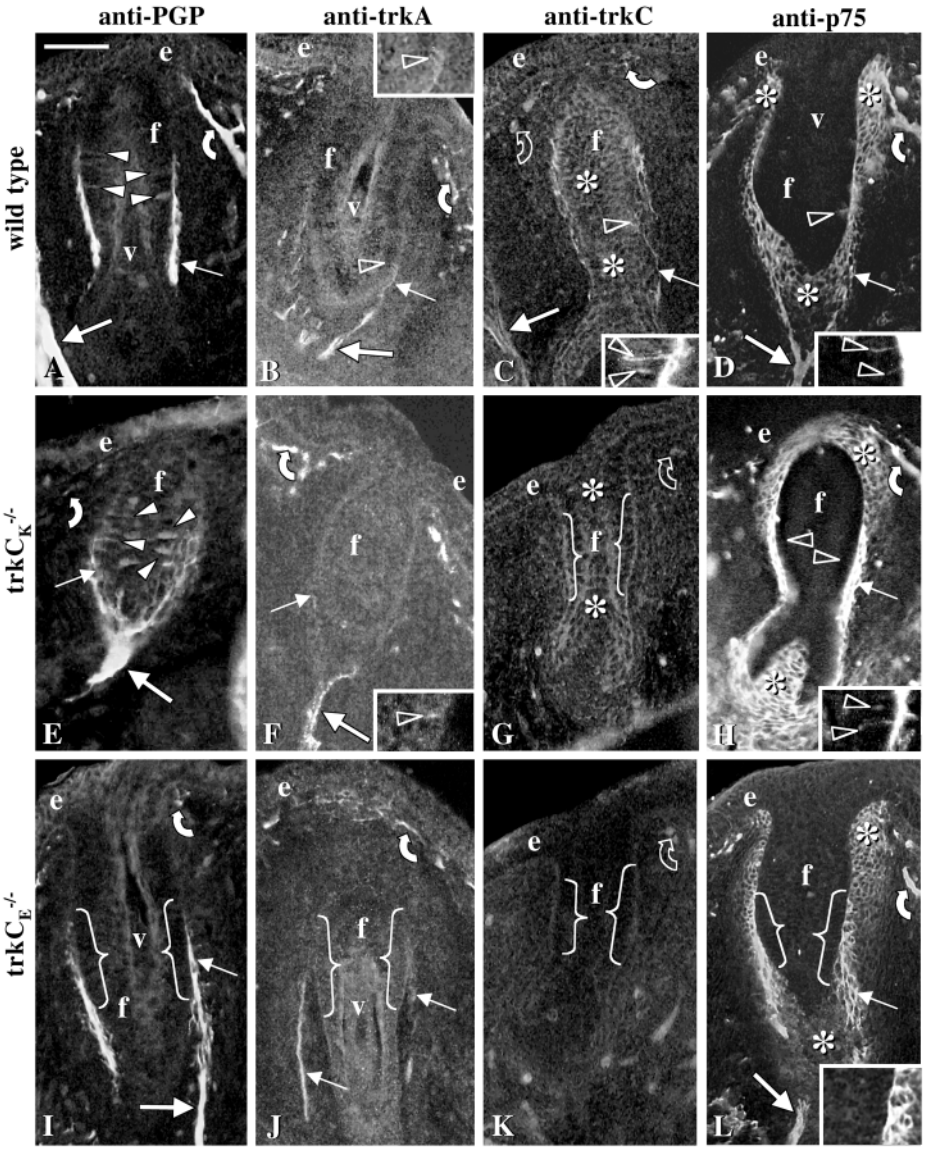

Fig. 3. Immunofluorescent images of anti-PGP, trkA, trkC and p75 labeling in and around central to caudal vibrissa follicles of E15.5 wild type, $trkC_K^{-/-}$ and $trkC_E^{-/-}$ mice 2 days after Merkel innervation has begun to develop. Small arrows, labeled axons; large arrows, larger axon bundles; white arrowheads, Merkel endings and cells; open arrowheads, Merkel endings only; curved arrows, epidermal innervation epidermis. Brackets span locations where Merkel endings are missing or are not revealed by the particular antibody. Asterisks indicate sites of non-neuronal labeling. Inserts contain higher magnification examples of Merkel endings and cells or sites where they should be located. (A-H) In wild type and $trkC_K^{-/-}$ fetuses, developing Merkel axons and endings label with anti-PGP, trkA and p75. Anti-trkA labeling is relatively faint compared with 2 days earlier (see Fig. 2C,D) and has been much more digitally enhanced than labeling in other panels. At this age only anti-PGP labels Merkel cells, which will express anti-p75 immunoreactivity within another day (see Fig. 2H). TrkC labeling is also expressed on wild-type Merkel axons and endings but is lacking on axons and endings in $trkC_K^{-/-}$. In wild type and $trkC_K^{-/-}$, non-neuronal labeling occurs with anti-p75 on dermal cells surrounding the follicles and with anti-trkC on cells within the follicles. Anti-trkC labeling within follicles is far less intense than it was 2 days earlier (see Fig. 2E,F) and has been digitally enhanced. (I-L) In $trkC_E^{-/-}$ fetuses, presumptive Merkel axons label with anti-PGP, trkA and p75; however, no endings are observed with any of these labels (see inset in L). Anti-PGP also does not reveal any Merkel cells where they are seen in wild type and $trkC_K^{-/-}$. Merkel axons do not label with anti-trkC as also occurred in $trkC_K^{-/-}$. $trkC_E^{-/-}$ fetuses have anti-p75 labeling on dermal cells surrounding the follicles comparable with that in wild-type and $trkC_K^{-/-}$ fetuses. By contrast, the follicle cells of the $trkC_E^{-/-}$ have little or no detectable trkC immunoreactivity. Scale bar: 50 μm.

of the developing and definitive Merkel-related axons and endings throughout the prenatal and postnatal period (Fig. 2G,H, Fig. 3D). Whereas, trkA and trkC labeling was limited to axons and endings, p75 labeling is robust on accompanying Schwann cells at all prenatal and postnatal ages examined, and becomes expressed on Merkel cells soon after they differentiate.

Impact of $trkA_K^{-/-}/trkC_K^{-/-}$

The expression of anti-trkA and anti-trkC labeling on separate sets of Merkel innervation is consistent with the reduction of Merkel endings at birth in $trkA_K^{-/-}$ and in $trkC_K^{-/-}$ mice (Fig. 1, Fig. 4C-E). In order to determine if all Merkel innervation is dependent on either $trkA_K$ or $trkC_K$, mystacial pads were examined from newborn offspring from crosses of $trkA_K^{+/-}/trkC_K^{+/-}$. Mystacial pads of newborn $trkA_K^{-/-}/trkC_K^{-/-}$ mice completely lacked Merkel innervation; however, some Merkel cells were still present (Fig. 4F). These results indicate that $trkA_K$ accounts for the development of one set of Merkel innervation and $trkC_K$ accounts for the remaining Merkel innervation.

$TrkC_{TR}$ and p75 expression on follicle cells and surrounding dermal cells

TrkC immunoreactivity was also present on all of the developing follicle cells on the day that the Merkel endings begin to form (E13.5 in the mouse and E14.5 in the rat) and diminishes substantially over the next 2 days (Fig. 2E, Fig. 3E, Fig. 4A,B). As seen along the caudal-to-rostral gradient within the same mystacial pads, the onset of trkC expression within the follicles slightly precedes detectable penetration of axons into the developing follicles (Fig. 2E). By the time trkC labeling disappears among the follicle cells, the Merkel endings are well established and Merkel cells are

immunodetectable with anti-PGP (Fig. 2B, Fig. 3A) and anti-keratin 20 (not shown). By E16.5 in mouse and E17.5 in rat, non-neuronal trkC labeling has shifted to the upper dermis between the vibrissa follicles (e.g. E19.5 in Fig. 2F). Interestingly, at the time trkC is expressed within the follicle, an intense complimentary expression of p75 occurs in the dermal cells immediately surrounding the developing follicles (Fig. 2D,G).

Our in situ hybridization analysis revealed that anti-trkC labeling on follicle cells in E14.5 mice was due to transient expression of $trkC_{TR}$ (Wheeler et al., 1998). Riboprobe that detects all isoforms of trkC revealed high levels of hybridization within vibrissa follicles (Fig. 5B), whereas no trkC message was detected on follicle cells with the kinase probe (Fig. 5A). Similar results were obtained on E15 rat follicles, although low levels of transcripts encoding kinase isoforms were also present (data not shown).

Impact of *trkC$_E$* deletion versus *trkC$_K$* deletion

The timing of $trkC_{Tr}$ expression in developing follicles suggested that it may be involved in the development of the Merkel innervation. To test this possibility, the impact of *trkC$_K$* deletion was compared with *trkC$_E$* deletion, as *trkC$_K$* deletion removes only the tyrosine kinase domain of $trkC_K$, whereas the *trkC$_E$* deletion should eliminate all known isoforms of trkC (Tessarollo et al., 1997). Newborn *trkC$_E^{-/-}$* lacked any Merkel endings or Merkel cells (Fig. 6E,F), whereas many endings and cells were present in newborn *trkC$_K^{-/-}$* (Fig. 6C,D) (Fundin et al., 1997). Surprisingly, the impact of *trkC$_E$* deletion was not only more severe than *trkC$_K$* deletion but was also more severe than *Ntf3* deletion (Fig. 6A,B). In *Ntf3$^{-/-}$* mice, a few Merkel cells and some innervation are present at birth. The original impact of *Ntf3* deletion (Fundin et al., 1997) was assessed in a different strain of mice (outbred from 129SV/ter and BALB/c) from those used to assess *trkC$_K$* deletion and *trkC$_E$* deletion (129/c57bl/6). Our results revealed the impact of *Ntf3* deletion was the same on the 129/c57bl/6 background. Thus, different phenotypes of *Ntf3$^{-/-}$*, *trkC$_K^{-/-}$* and *trkC$_E^{-/-}$* are not due to differences between mouse strains.

Examination of fetal E15.5 *trkC$_E^{-/-}$* (Fig. 3I,L) revealed that axons had grown to developing vibrissa follicles but, unlike in *trkC$_K^{-/-}$* or *Ntf3$^{-/-}$* fetuses (Fig. 3E, Fig. 7I), no axons penetrated the basement membrane and formed endings. Moreover, *trkC$_E^{-/-}$* fetuses lacked any evidence of Merkel cell differentiation in the follicles. Consistent with the fact that *trkC$_K^{-/-}$* is targeted at the kinase domains and may not eliminate truncated isoforms, immunolabeling was observed on the follicle cells of E14.5 and E15.5 *trkC$_K^{-/-}$* with trkC antibody (Fig. 3G), which had been generated against the extracellular domain of rat trkC. In addition, mRNA was detected in *trkC$_K^{-/-}$* with the riboprobe detecting all isoforms of trkC (Fig. 5C) but not with the kinase probe. Consistent with the fact that *trkC$_E^{-/-}$* should eliminate the extracellular domains of truncated and full-length isoforms, no obvious non-neuronal trkC immunolabeling was detected in *trkC$_E^{-/-}$* mutants (Fig. 3K) and no hybridization was obtained with the riboprobes for all isoforms or for only kinase isoforms (Fig. 5D). Both *trkC$_E^{-/-}$* and *trkC$_K^{-/-}$* fetuses had normal looking non-neuronal labeling with anti-p75 (Fig. 3H,L).

No trkC immunolabeling of any innervation was detected in *trkC$_E^{-/-}$* or *trkC$_K^{-/-}$* fetuses on E14.5-E16.5 (Fig. 3G,K) indicating that innervation at these ages lacks detectable levels of naturally occurring truncated isoforms and that the kinase mutations did not result in the formation of truncated isoforms. In these fetuses, axons projecting to the vibrissa follicles were labeled with anti-p75 and anti-trkA but Merkel endings were only seen in *trkC$_K^{-/-}$* follicles (Fig. 3F,G,J,K).

NT3 production

One possible role of transiently-expressed $trkC_{Tr}$ within the developing follicles could be to sequester and increase the local concentration of NT3, thereby promoting the development of the Merkel endings. Examination in *Ntf3$^{+/lacZ}$* and *Ntf3$^{lacZ/lacZ}$* fetuses (Fig. 7) revealed intense β-galactosidase expression in several layers of dermal cells intimately surrounding all the

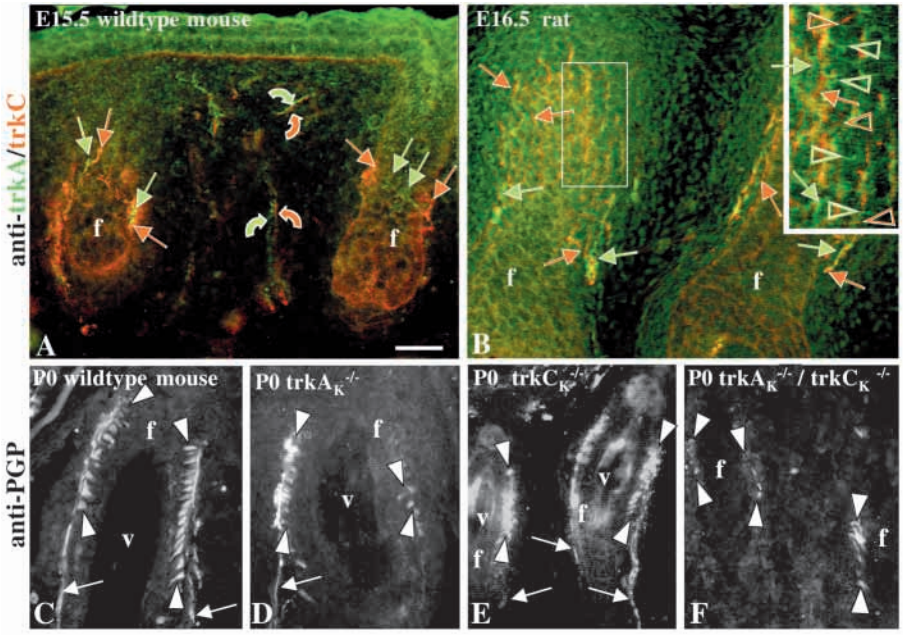

Fig. 4. Demonstration of two types of Merkel innervation: one $trkA_K$ and the other $trkC_K$ dependent. (A,B) Double labeling with anti-trkA (green) and anti-trkC (orange) of normal developing vibrissa follicles from central portions of mystacial pads in E15.5 mice and E16.5 rats. In both cases, trkA (green arrows) and trkC (orange arrows) is expressed primarily, if not entirely, on different axons. Sites of overlapping fluorophores (yellow) are due to axon intermingling. Merkel endings (open arrowheads in inset) expressed either trkA or trkC immunoreactivity, not both. Note trkC-labeling on follicle cells. (C-F) Impact of *trkA$_K$* deletion, *trkC$_K$* deletion and *trkA$_K$*/*trkC$_K$* deletion on P0 littermates. (C) Merkel axons (arrows), endings and cells (solid arrowheads) are present in wild-type mice (see Fig. 1A). (D) *TrkA$_K^{-/-}$* have reduced Merkel innervation that becomes compacted towards the upper end of the follicles (see Fig. 1B). (E) *trkC$_K^{-/-}$* have even fewer Merkel endings and cells. Many cells are uninnervated (see Fig. 1A). (F) *trkA$_K^{-/-}$*/*trkC$_K^{-/-}$* have some detectable Merkel cells but none is innervated. Scale bar: 50 μm.

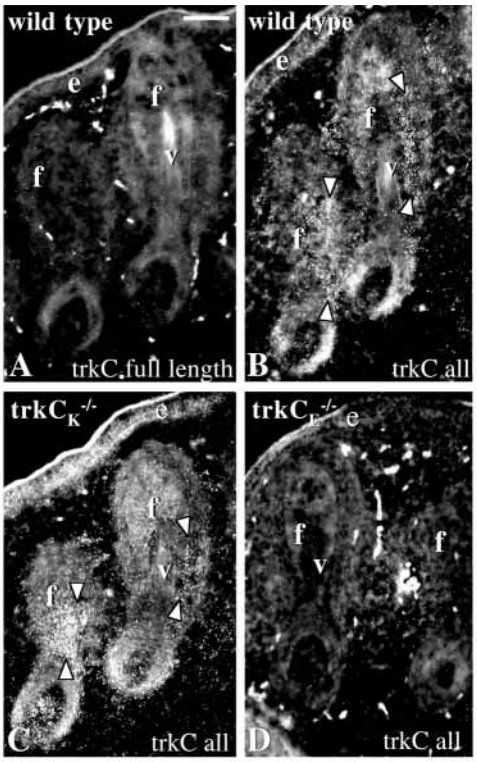

Fig. 5. Dark-field images of in situ hybridization demonstrating trkC$_{Tr}$ mRNA in developing vibrissa follicles of E14.5 mice. (A,B) In normal fetuses, extensive labeling (between arrowheads) is present in follicles with the probe recognizing all isoforms of trkC (B), but virtually no labeling is present with the probe that recognizes only full length kinase forms of trkC (A). (C) In *trkC$_K^{-/-}$* animals, labeling within the follicle is still present (between arrowheads) with the probe that recognizes all forms of trkC indicating that this mutation does not eliminate the expression of truncated trkC within the follicle. (D) Virtually no labeling occurs in the follicles of *trkC$_E^{-/-}$* mutants with the probe for all isoforms of trkC, indicating that expression of trkC$_{Tr}$ within follicles was eliminated. Scale bar: 50 μm.

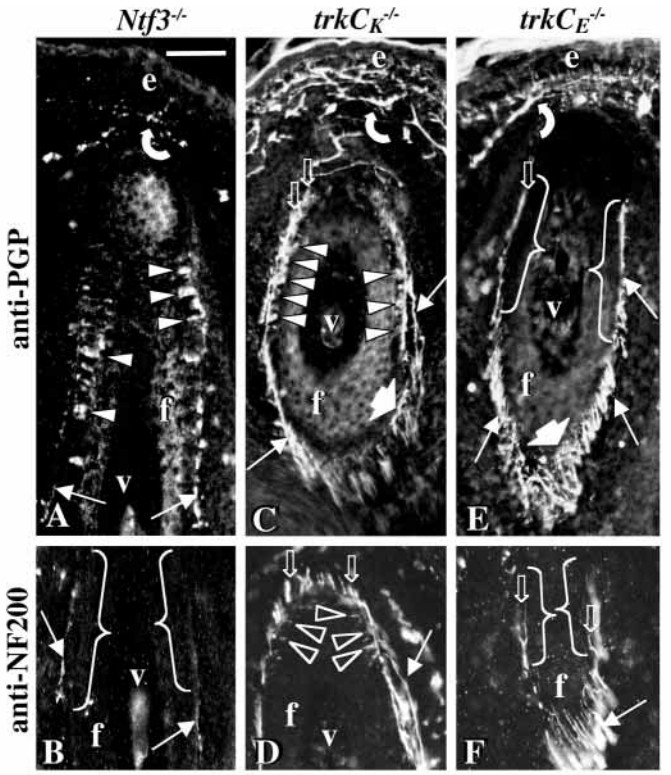

Fig. 6. Immunofluorescence images of follicle innervation labeled with anti-PGP or anti-NF200 in P0 *Ntf3$^{-/-}$*, *trkC$_K^{-/-}$* and *trkC$_E^{-/-}$* mice. In addition to axons (arrows), anti-PGP labels Merkel endings and cells (white arrowheads), and anti-NF200 labels only Merkel endings (open arrowheads). Brackets span locations where Merkel endings are missing. (A,B) Newborn *Ntf3$^{-/-}$* have only a few Merkel cells and a few fragmented axons. Anti-NF200 labels only a few axons and rarely any endings. (C,D) Newborn *trkC$_K^{-/-}$* have fewer Merkel endings and cells than normal, but much more than *Ntf3$^{-/-}$*. Most Merkel cells have endings. (E,F) Newborn *trkC$_E^{-/-}$* lack any detectable Merkel endings or cells labeled with either anti-PGP or anti-NF200. Both *trkC$_K^{-/-}$* and *trkC$_E^{-/-}$* have lanceolate endings (open arrows) and reticular endings (broad arrows) supplied by large caliber myelinated axons as well as epidermal endings (curved arrows) supplied by C-fibers that are not dependent upon intact kinase (Fundin et al., 1997) or trkC$_{Tr}$. However, these other endings are severely depleted in the absence of NT3 which, in these cases, may normally signal through trkA$_K$ or trkB$_K$ (Fundin et al., 1997; Rice et al., 1998). Scale bar: 50 μm.

nascent follicles on E13, prior to invasion of axons into caudal follicles and formation of Merkel endings (see rostral follicles at E14 in Fig. 7A,D). Concurrent with the caudal-to-rostral sequence of trkC immunolabeling throughout the follicles, a dense expression of the reporter construct also appears within the follicles along the same gradient. However, unlike trkC$_{Tr}$ which is expressed throughout the follicle (Fig. 7A-C), the *Ntf3* reporter construct is restricted to the mid-follicle level where the Merkel endings develop (Fig. 7B,C,E,F). Thus, the site of NT3 production is naturally more precisely restricted to the site of Merkel ending development than trkC$_{Tr}$. The presumptive production of NT3 in this mid-follicle location appears to be brief because the reporter gene expression is barely detectable by E15 (Fig. 7G-J), even before trkC$_{Tr}$ labeling completely disappears within the follicles (Fig. 7H).

In contrast to the complete absence of Merkel ending and Merkel cell development in *trkC$_E^{-/-}$*, analysis of the *Ntf3$^{lacZ/lacZ}$* E14 and E15 specimens revealed that at least some Merkel endings and cells were able to develop without NT3 (Fig. 7I) as had been seen in the neonates. Some of this successful innervation labeled with anti-trkA (Fig. 7G) but more was revealed with anti-p75 and anti-PGP (Fig. 7D,E,I). Anti-trkC labeling of innervation was substantially reduced in *Ntf3$^{+/lacZ}$* fetuses (Fig. 7C) and undetectable in *Ntf3$^{lacZ/lacZ}$* (Fig. 7A,B). This suggests that many axons are present that lack either trkA or trkC immunoreactivity. Therefore, many axons that would normally express anti-trkC labeling may have downregulated trkC$_K$ production, but are still present. The presence of p75 may be contributing to their development. As was also seen with *trkC$_E^{-/-}$* and *trkC$_K^{-/-}$* (Fig. 3), *Ntf3$^{lacZ/lacZ}$* did not have an obvious effect on patterns of non-neuronal labeling with anti-trkC (Fig. 7I) or anti-p75 (Fig. 7D-E). However, compared with heterozygotes, homozygous mutants have smaller follicles and the onset of vibrissa shaft differentiation was delayed (Fig. 7B,C,E,F).

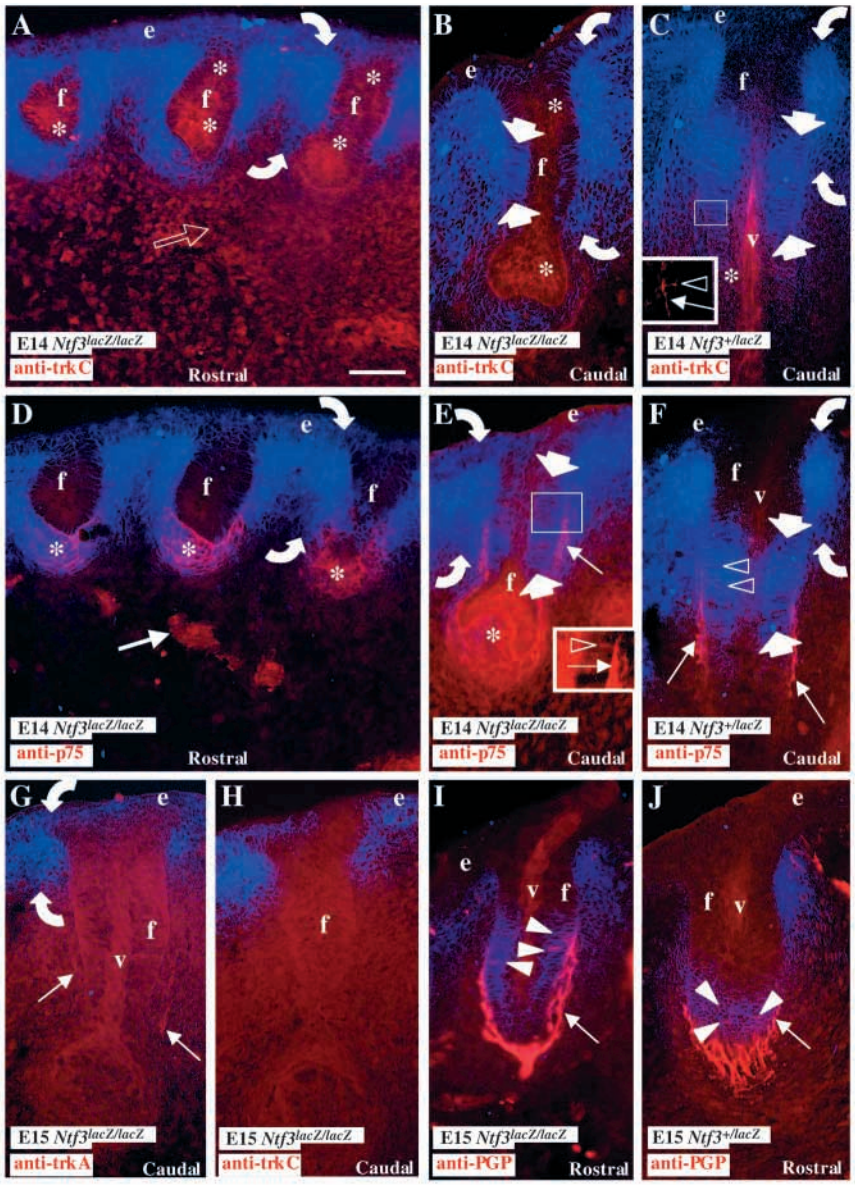

Fig. 7. Epifluorescence images of anti-PGP, p75, trkA or trkC labeling (red) combined with transmitted light images of *lacZ* reporter gene labeling (blue) in and around rostral and caudal vibrissa follicles of E14 and E15 mouse fetuses. Fetuses have either a homozygous (*Ntf3*$^{lacZ/lacZ}$) or heterozygous (*Ntf3*$^{+/lacZ}$) *lacZ* reporter gene substitution for *Ntf3*. Small arrows indicate labeled axons; large arrows, larger axon bundles; white arrowheads, Merkel endings and cells; open arrowheads, only Merkel endings. Asterisks indicate sites of non-neuronal p75 or trkC expression. (A-F) At E14, *Ntf3*/*lacZ* is expressed only in the dermis completely surrounding less mature rostral follicles (between curved arrows in A and D). Among more mature caudal follicles (B,C,E and F), *Ntf3/lacZ* is expressed in a restricted zone (between broad arrows) at mid-levels inside the follicles as well as in the dermis only surrounding the upper end of the follicles (between curved arrows). The pattern of *Ntf3/lacZ* expression is similar in homozygotes (B and E) and heterozygotes (C and F) but follicles of heterozygotes are larger and more mature. Only the upper two thirds of follicles are shown in C and F. Among the relatively immature follicles of homozygotes, anti-trkC labeling is present on cells throughout the follicles (A) whereas anti-p75 labeling is present on dermal cells surrounding the follicles (D). Non-neuronal expression of trkC has begun to decrease within more mature caudal follicles of homozygotes (B) and has nearly disappeared in relatively more mature caudal follicles of the heterozygote (C). In homozygotes and heterozygotes, Merkel innervation labels with anti-p75. Labeled endings are present only at the site where *Ntf3/lacZ* is expressed within the follicles (E,F). No innervation labels with anti-trkC in homozygotes (open arrow), but some axons (small arrow) and endings (open arrowhead) are trkC positive in heterozygotes (inset, C). The insets in C and E are enlargements of areas bounded by white rectangles but the blue *lacZ* label has been digitally suppressed and the red anti-trkC and anti-p75 label digitally enhanced. (G-J) In E15 *Ntf3*$^{lacZ/lacZ}$ or *Ntf3*$^{+/lacZ}$ fetuses, *Ntf3/lacZ* is intensely expressed primarily in the dermis surrounding upper ends of follicles and, compared to E14, is substantially reduced within the mid-level of follicles. Neuronal and non-neuronal labeling with anti-trkA (G) and anti-p75 (not shown) are comparable to normal (see Figs 2, 6). In homozygotes (H), anti-trkC labels the core of the follicle where the vibrissa is developing but does not label any innervation. Anti-PGP labels Merkel endings and cells at mid-follicle levels in homozygotes and heterozygotes (I and J). Scale bar: 50 μm.

DISCUSSION

Several important findings from this study are summarized in Fig. 8. First, some developing Merkel endings express and depend upon trkA$_K$, while others express and depend upon trkC$_K$. Thus, sensory neurons forming the same type of endings on the same target can express different receptors and depend upon a different combination of neurotrophins. Second, NT3 is briefly produced precisely at the time and place where Merkel endings develop. Thus, production of a neurotrophin can be regulated with a high degree of precision at the time and location where a set of innervation will terminate. Third, while some Merkel cells and endings develop in the absence of trkC$_K$, transient expression of trkC$_{Tr}$ within follicles is essential for development of any Merkel cells and endings. This indicates that non-neuronal expression of trkC$_{Tr}$ can play an essential developmental role for specific target cells and innervation. Fourth, the impact of *trkC*$_E^{-/-}$ on Merkel innervation and Merkel cells was more severe than absence of NT3, the only known ligand for trkC. This suggests that the essential developmental role of trkC$_{Tr}$ involves more than interactions with NT3. Finally, the same combinations of neuronal and non-neuronal receptors are expressed in mouse and rat, indicating that similar developmental mechanisms are occurring in both species for the comparable innervation.

Separate trkA and trkC sets of Merkel innervation

As has been shown previously, Merkel innervation is partially

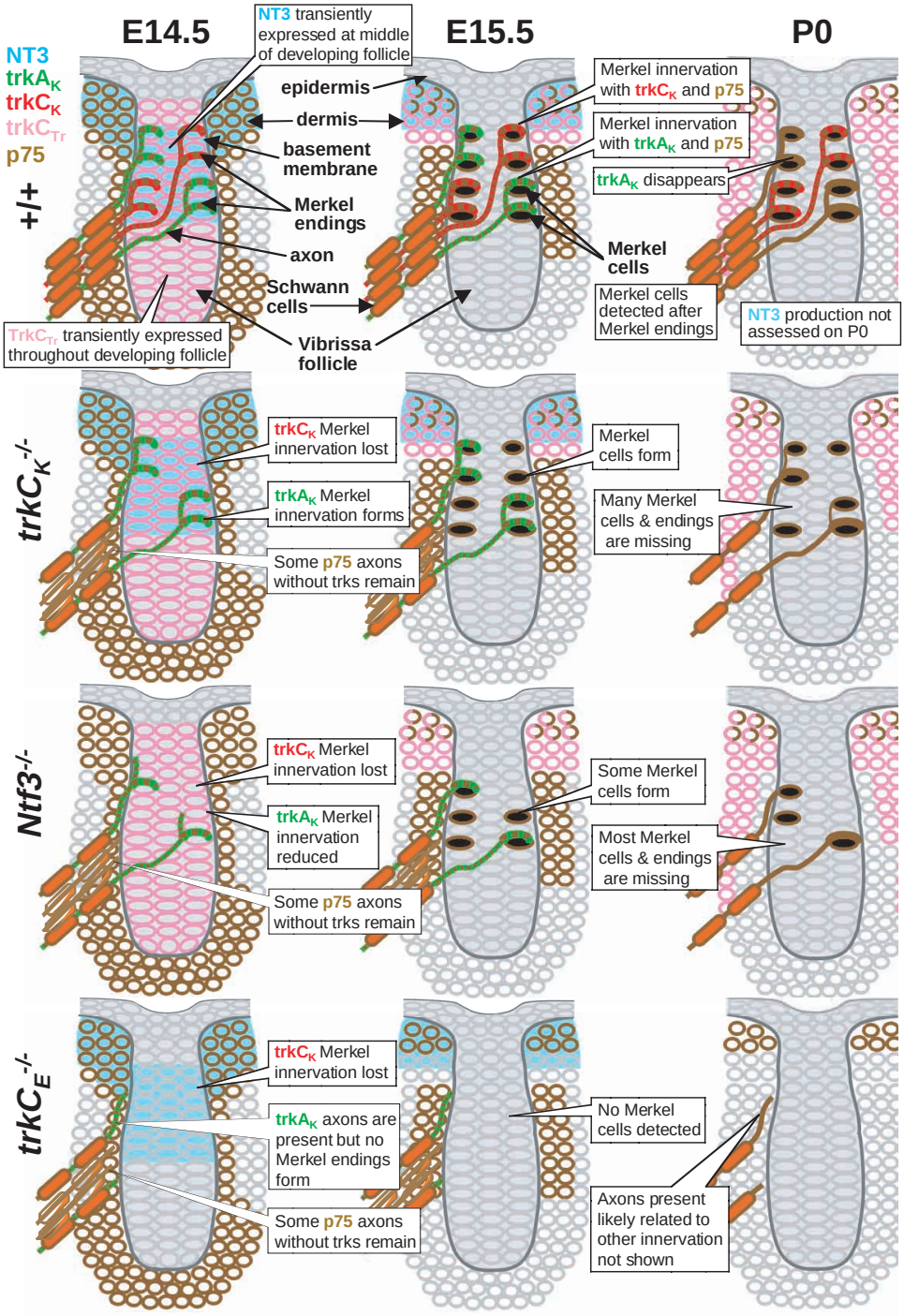

Fig. 8. Schematic compilations of immunolabeling, in situ hybridization and *lacZ* results of Merkel innervation development in wild type (+/+), *trkC$_K^{-/-}$*, *Ntf3$^{-/-}$* and *trkC$_E^{-/-}$* mouse fetuses at E14.5, E15.5 and P0. For simplification, follicles are shown as the same size instead of being larger and more mature at older ages. Locations of NT3, trkA$_K$, trkC$_K$, trkC$_{Tr}$ and p75 are color coded (upper left). Locations where NT3 should be produced is shown in +/+, *trkC$_K^{-/-}$* and *trkC$_E^{-/-}$* schematics at E14.5 and E15.5, and are based on *lacZ* expression in *Ntf3$^{+/lacZ}$* and *Ntf3$^{lacZ/lacZ}$* fetuses. *Ntf3/lacZ* was not examined on P0. Notable details are as follows. (1) Developing Merkel innervation expresses p75 along with either trkA$_K$ or trkC$_K$. (2) TrkA$_K$ disappears before birth. (3) At onset of Merkel ending development, TrkC$_{TR}$ is expressed throughout the follicle, whereas NT3 is produced only by follicle cells where the endings develop. (4) Merkel endings are detected before Merkel cells. (5) TrkA$_K$ is expressed with p75 on some axons in all three knockouts but other axons only label with p75. (6) The impact of *Ntf3$^{-/-}$* is more detrimental than *trkC$_K^{-/-}$*, although at least some Merkel endings and cells form in both. (7) No Merkel endings or Merkel cells form in the absence of trkC$_{TR}$.

reduced at mid-follicle levels in newborn *trkA$_K^{-/-}$* and *trkC$_K^{-/-}$* mice (Fundin et al., 1997). Complete absence of this Merkel innervation in newborn *trkA$_K^{-/-}$/trkC$_K^{-/-}$* hybrids indicates that each ending depends on either trkA$_K$ or trkC$_K$ for prenatal development or survival. Consistent with this dual impact, Merkel axons and endings expressed either trkA or trkC, but not both. Likewise, developing trigeminal ganglion neurons rarely if ever co-express trkA and trkC (Huang et al., 1999). Importantly, our results indicate that Merkel innervation can be supplied by neurons that have two different receptor and neurotrophin dependencies.

TrkC$_K$-dependent Merkel innervation

Presence of reduced Merkel innervation in *trkA$_K^{-/-}$* newborns and complete absence in *trkA$_K^{-/-}$/trkC$_K^{-/-}$* newborns indicates that some Merkel innervation never solely requires trkA$_K$, but does require trkC$_K$ for its development or prenatal maintenance. This set of innervation presumably is included in the increased apoptosis that occurs in *Ntf3$^{-/-}$* or *trkC$_K^{-/-}$* mice. Likewise, other sets of Merkel innervation, which develop later at the mouths of hair follicles, also require trkC$_K$, but not trkA$_K$ (Fundin et al., 1997).

The surviving mid-follicle Merkel innervation in *trkA$_K^{-/-}$*

mutants presumably is the innervation that expresses $trkC_K$. In $trkA_K^{-/-}$ mutants, surviving innervation is maintained through at least the fourth postnatal week (Fundin et al., 1997). Although trkC is immunodetectable postnatally on Merkel endings, it drops below detectable levels within a few weeks of birth. As $trkC_K$ levels decline, prolonged survival of this innervation depends upon p75 (Fundin et al., 1997), which was detected throughout development of the Merkel innervation and at least four weeks postnatally.

$TrkA_K$-dependent Merkel innervation

The presence of a reduced set of Merkel endings in newborn $trkC_K^{-/-}$ is consistent with our observation that some endings have detectable trkA immunoreactivity prenatally but not trkC. Presumably the trkA-expressing innervation is the set that is lost in $trkA_K^{-/-}$ mutants. Thus, a unique set of Merkel innervation may initially develop in response to either NGF or NT3 signaling through $trkA_K$. Consistent with such a $trkA_K$ dependency, a similar loss of Merkel innervation occurs in $Ngf^{-/-}$ mutants (Fundin et al., 1997) although not quite as severe as in $trkA_K^{-/-}$ (F. L. R., unpublished). This suggests that NT3 signaling through $trkA_K$ could partially compensate for lack of NGF (White et al., 1996). The likelihood that NT3/$trkA_K$ signaling normally does contribute to development or maintenance of some Merkel innervation is indicated by the more severe impact of $Ntf3^{-/-}$ when compared with $trkC_K^{-/-}$. Nonetheless, even though expression of *Ntf3/lacZ* construct indicates that NT3 is intensely produced at mid-follicle levels where Merkel endings develop, clearly some of the innervation requires NGF at some point, possibly early in development.

Unlike innervation that survives without NGF or $trkA_K$, surviving Merkel innervation seen at birth in $trkC_K^{-/-}$ mice deteriorates over the first 2 postnatal weeks. As trkA drops below immunodetectable levels just prior to birth, postnatal deterioration in $trkC_K^{-/-}$ suggests that the $trkA_K$-dependent innervation may switch to a dependency on $trkC_K$ for maintenance postnatally. Interestingly, neither $Ntf3^{-/-}$, $trkC_K^{-/-}$ or $trkC_E^{-/-}$ obviously increase the labeling intensity and proportion of axons expressing trkA immunoreactivity. In all three knockouts, trkA labeling remains relatively faint. Moreover, numerous other axons only label with anti-PGP and p75. Thus, $trkA_K$ is limited to a subset set of Merkel innervation and other sets do not appear to upregulate $trkA_K$ to compensate for the loss of $trkC_K$.

Surprisingly, during Merkel ending development in $Ntf3^{-/-}$ fetuses, a substantial proportion of axons that are both trkA and trkC immunonegative still labels with anti-p75. However, non-neuronal trkC labeling within and around the follicle is not obviously affected. This suggests that absence of NT3 eliminates $trkC_K$ expression on many Merkel axons but these axons are not initially lacking in the *Ntf3* knockouts (Wyatt et al., 1999). However, most of the innervation disappears before birth. Thus, NT3 or $trkC_K$ may not necessarily be essential factors for early survival of the Merkel innervation that will eventually become dependent on NT3 and $trkC_K$ (Wright et al., 1992).

Other studies have demonstrated that sensory neurons forming similar types of endings can depend upon different receptors and/or neurotrophins. For example, innervation of muscle spindles in limb musculature appears to have a different neurotrophin dependency from that of spindles in muscles of mastication (Tessarollo et al., 1994). In addition, BDNF and NT3 have both been shown to play a role in development of taste bud structure and innervation, but different components in different types of taste buds (Nosrat et al., 1997). Although, BDNF and NT3 are required for development of hair cell innervation at apical and basal ends of the cochlea, respectively (Fritzsch et al., 1999), recent evidence indicates that all of this innervation expresses trkB and trkC and that different neurotrophin survival dependencies are due to opposing gradients of BDNF and NT3 expression within the developing cochlea (Fariñas et al., 2001). By contrast, our results reveal that the same type of sensory endings (Merkel endings) terminating in the same target structure (a vibrissa follicle) can depend upon different trk receptors (trkA or trkC). Conceivably, this difference may be related to the fact that Merkel endings at the mid-follicle level of rat vibrissae can have two different types of terminal arborizations (Fundin et al., 1994; Ebara et al., 2002).

A role for $trkC_{Tr}$ in the development of Merkel endings and Merkel cells

Our results revealed that no Merkel endings or cells begin to form in $trkC_K^{-/-}$ fetuses although axons do converge at mid-follicle levels. Thus, development of Merkel endings as well as Merkel cells is completely dependent on $trkC_{Tr}$ transiently expressed in all of the developing follicle cells. Importantly, at least some Merkel cells are present at birth, even though absence of $trkA_K$ and $trkC_K$ completely eliminates their innervation. Conceivably, some Merkel endings may still develop and disappear prenatally in $trkA_K^{-/-}/trkC_K^{-/-}$.

Surprisingly, the impact of losing both the truncated and kinase isoforms of trkC was more severe than absence of $trkC_K$ alone or NT3, the only known trkC ligand. Thus, the $trkC_{Tr}$ appear to play an active developmental role beyond that of $trkC_K$. This $trkC_{Tr}$ function is at least partially independent of NT3. In this particular system, this role does not appear to be either a dominant-negative (Eide et al., 1996; Gonzalez et al., 1999; Eggert et al., 2001) or NT3 sequestering effect (Fryer et al., 1997; Das et al., 2000; Lin et al., 2000; Liebl et al., 2001). Conceivably $trkC_{Tr}$ may have constitutively active signaling that is necessary for development of Merkel ending and cells, or may have a signaling capability mediated by an unknown ligand.

NT3 and developing Merkel innervation

We initially hypothesized that non-neuronal $trkC_{Tr}$ may be concentrating NT3 within follicles to induce formation of Merkel endings (Rice et al., 1998). Our results indicate that NT3 is abruptly and transiently produced within follicles precisely at the time and place where Merkel endings develop. Merkel cells become immunodetectable shortly thereafter. Conceivably, $trkC_{Tr}$ expressed throughout follicles may be required to induce production of NT3 at this precise mid-follicle location. The loss of some Merkel innervation in $Ngf^{-/-}$ mice, coupled with formation of some Merkel endings in the absence of NT3, indicates that NGF may also be expressed in this location and that complete failure of ending formation in $trkC_E^{-/-}$ mutants may be due to a loss of both NGF and NT3 at this site. Importantly, the mid-follicle site of NT3 production is more precise than the distribution of $trkC_{Tr}$ within the follicles. Thus, if $trkC_{Tr}$ does play an essential role in the production of neurotrophins, another factor must be regulating the specific site of NT3 production.

We wish to thank Marilyn Dockum for help with tissue preparation, and Drs Sally Temple, Chris Ingraham and Ann Messer for critiques of the manuscript. This research was supported by NIH grants R01 NS34692 (F. L. R.) and P01 NS 16033 (L. F. R.), SCORE GM08194 (E. F. W.) and predoctoral training grant T32 EY 07120 (G. A. W.); by NSF IBN-9723990 (E. F. W.); and by the Howard Hughes Medical Institute (L. F. R.).

REFERENCES

Albers, K. M., Perrone, T. N., Goodness, T. P., Jones, M. E., Green, M. A. and Davis, B. M. (1996). Cutaneous overexpression of NT3 increases sensory and sympathetic neuron number and enhances touch dome and hair follicle innervation. *J. Cell Biol.* **134**, 487-497.

Buchman, V. L., Sporn, M. and Davies, A. M. (1994). Role of transforming growth factor-beta isoforms in regulating the expression of nerve growth factor and neurotrophin-3 mRNA levels in embryonic cutaneous cells at different stages of development. *Development* **120**, 1621-1629.

Das, I., Sparrow, J. R., Lin, M. I., Shih, E., Mikawa, T. and Hempstead, B. L. (2000). Trk C signaling is required for retinal progenitor cell proliferation. Trk C signaling is required for retinal progenitor cell proliferation. *J. Neurosci.* **20**, 2887-2895.

Davies, A. M. (1997). Studies of neurotrophin biology in the developing trigeminal system. *J. Anat.* **191**, 483-491.

Ebara, S., Kumamoto, K., Matsuura, T., Mazurkiewicz, J. E. and Rice, F. L. (2002). Similarities and major differences in the innervation of mystacial vibrissa follicle-sinus complexes in the rat and cat. *J. Comp. Neurol.* **449**, 103-119.

Eggert, A, Grotzer, M. A., Ikegaki, N., Zhao, H., Cnaan, A, Brodeur, G. M. and Evans, A. E. (2001). Expression of the neurotrophin receptor TrkB is associated with unfavorable outcome in Wilms' tumor. *J. Clin. Oncol.* **19**, 689-696.

Eide, F. F., Vining, E. R., Eide, B. L., Zang, K., Wang, X.-Y. and Reichardt, L. F. (1996). Naturally occurring truncated trkB receptors have dominant inhibitory effects on brain-derived neurotrophic factor signaling. *J. Neurosci.* **16**, 3123-3129.

Fariñas, I., Jones, K. R., Backus, C., Wang, X. Y. and Reichardt, L. F. (1994). Severe sensory and sympathetic deficits in mice lacking neurotrophin-3. *Nature* **369**, 658-661.

Fariñas, I., Jones, K. R., Tessarollo, L., Vigers, A. J., Huang, E., Kirstein, M., de Caprona, D. C., Coppola, V., Backus, C., Reichardt, L. F. and Fritzsch, B. (2001). Spatial shaping of cochlear innervation by temporally regulated neurotrophin expression. *J. Neurosci.* **21**, 6170-6180.

Fritzsch, B., Pirvola, U. and Ylikoski, J. (1999). Making and breaking the innervation of the ear: neurotrophic support during ear development and its clinical implications. *Cell Tissue Res.* **295**, 369-382.

Fryer, R. H., Kaplan, D. R. and Kromer, L. F. (1997). Truncated trkB receptors on nonneuronal cells inhibit BDNF-induced neurite outgrowth in vitro. *Exp. Neurol.* **148**, 616-627.

Fundin, B. T., Rice, F. L., Pfaller, K. and Arvidsson, J. (1994). The innervation of the mystacial pad in the adult rat studied by anterograde transport of HRP-conjugates. *Exp. Brain Res.* **99**, 233-246.

Fundin, B. T., Silos-Santiago, I., Ernfors, P., Fagan, A. M., Aldskogius, H., DeChiara, T. M., Phillips, H. S., Barbacid, M., Yancopoulos, G. D. and Rice, F. L. (1997). Differential dependency of cutaneous mechanoreceptors on neurotrophins, trk receptors, and P75 LNGFR. *Dev. Biol.* **190**, 94-116.

Garner, A. S. and Large, T. H. (1994). Isoforms of the avian trkC receptor: a novel kinase insertion dissociates transformation and process outgrowth from survival. *Neuron* **13**, 457-472.

Gonzalez, M., Ruggiero, F. P., Chang, Q., Shi, Y. J., Rich, M. M., Kraner, S. and Balice-Gordon, R. J. (1999). Disruption of Trkb-mediated signaling induces disassembly of postsynaptic receptor clusters at neuromuscular junctions. *Neuron* **24**, 567-583.

Huang, E. J. and Reichardt, L. F. (2001) Neurotrophins: roles in neuronal development and function. *Annu. Rev. Neurosci.* **24**, 677-736.

Huang, E. J., Wilkinson, G. A., Fariñas, I., Backus, C., Zang, K., Wong, S. L. and Reichardt, L. F. (1999). Expression of Trk receptors in the developing mouse trigeminal ganglion: in vivo evidence for NT3 activation of TrkA and TrkB in addition to TrkC. *Development* **126**, 2191-2203.

Ip, N. Y., Stitt, T. N., Tapley, P., Klein, R., Glass, D. J., Fandl, J., Greene, L. A., Barbacid, M. and Yancopoulos, G. D. (1993). Similarities and differences in the way neurotrophins interact with the Trk receptors in neuronal and nonneuronal cells. *Neuron* **10**, 137-149.

Klein, R., Conway, D., Parada, L. F. and Barbacid, M. (1990). The trkB tyrosine protein kinase gene codes for a second neurogenic receptor that lacks the catalytic kinase domain. *Cell* **61**, 647-656.

Klein, R., Silos-Santiago, I., Smeyne, R. J., Lira, S. A., Brambilla, R., Bryant, S., Zhang, L., Snider, W. D. and Barbacid, M. (1994). Disruption of the neurotrophin-3 receptor gene trkC eliminates la muscle afferents and results in abnormal movements. *Nature* **368**, 249-251.

LeMaster, A. M., Krimm, R. F., Davis, B. M., Noel, T., Forbes, M. E., Johnson, J. E. and Albers, K. M. (1999). Overexpression of brain-derived neurotrophic factor enhances sensory innervation and selectively increases neuron number. *J. Neurosci.* **19**, 5919-5931.

Liebl, D. J., Huang, W., Young, W. and Parada, L. F. (2001). Regulation of Trk receptors following contusion of the rat spinal cord. *Exp. Neurol.* **167**, 15-26.

Lin, M. I., Das, I., Schwartz, G. M., Tsoulfas, P., Mikawa, T. and Hempstead, B. L. (2000). Trk C receptor signaling regulates cardiac myocyte proliferation during early heart development *in vivo*. *Dev. Biol.* **226**, 180-191.

Middlemas, D. S., Lindberg, R. A. and Hunter, T. (1991). TrkB, a neural receptor protein-tyrosine kinase: evidence for a full-length and two truncated receptors. *Mol. Cell. Biol.* **11**, 143-153.

Nosrat, C. A., Blomlöf, J., ElShamy, W. M., Ernfors, P. and Olson, L. (1997). Lingual deficits in BDNF and NT3 mutant mice leading to gustatory and somatosensory disturbances, respectively. *Development* **124**, 1333-1342.

Pinon, L. G., Minichiello, L., Klein, R. and Davies, A. M. (1996). Timing of neuronal death in trkA, trkB and trkC mutant embryos reveals developmental changes in sensory neuron dependence on Trk signalling. *Development* **122**, 3255-3261.

Reichardt, L. F. and Fariñas, I. (1997). Neurotrophic factors and their receptors: roles in neuronal development and function. In *Molecular and Cellular Approaches to Neural Development* (ed. W. M. Cowan, T. M. Jessel, and S. L. Zipursky), pp 220-263. New York: Oxford University Press.

Rice, F. L., Fundin, B. T., Arvidsson, J., Aldskogius, H. and Johansson, O. (1997). Comprehensive immunofluorescence and lectin binding analysis of vibrissa follicle sinus complex innervation in the mystacial pad of the rat. *J. Comp. Neurol.* **385**, 149-184.

Rice, F. L., Albers, K. M., Davis, B. M., Silos-Santiago, I., Wilkinson, G. A., LeMaster, A. M., Ernfors, P., Smeyne, R. J., Aldskogius, H., Phillips, H. S. et al. (1998). Differential dependency of unmyelinated and A gamma epidermal and upper dermal innervation on neurotrophins, trk receptors, and $p75^{LNGFR}$. *Dev. Biol.* **198**, 57-81.

Smeyne, R. J., Klein, R., Schnapp, A., Long, L. K., Bryant, S., Lewin, A., Lira, S. A. and Barbacid, M. (1994). Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene. *Nature* **368**, 246-249.

Snider, W. D. and Wright, D. E. (1996). Neurotrophins cause a new sensation. *Neuron* **16**, 229-232.

Tessarollo, L., Vogel, K. S., Palko, M. E., Reid, S. W. and Parada, L. F. (1994). Targeted mutation in the neurotrophin-3 gene results in loss of muscle sensory neurons. *Proc. Natl. Acad. Sci. USA* **91**, 11844-11848.

Tessarollo, L., Tsoulfas, P., Donovan, M. J., Palko, M. E., Blair-Flynn, J., Hempstead, B. L. and Parada, L. F. (1997). Targeted deletion of all isoforms of the trkC gene suggests the use of alternate receptors by its ligand neurotrophin-3 in neuronal development and implicates trkC in normal cardiogenesis. *Proc. Natl. Acad. Sci. USA* **94**, 14776-14781.

Tsoulfas, P., Soppet, D., Escandon, E., Tessarollo, L., Mendoza-Ramirez, J. L., Rosenthal, A., Nikolics, K. and Parada, L. F. (1993). The rat trkC locus encodes multiple neurogenic receptors that exhibit differential response to neurotrophin-3 in PC12 cells. *Neuron* **10**, 975-990.

Valenzuela, D. M., Maisonpierre, P. C., Glass, D. J., Rojas, E., Nunez, L., Kong, Y., Gies, D. R., Stitt, T. N., Ip, N. Y. and Yancopoulos, G. D. (1993). Alternative forms of rat TrkC with different functional capabilities. *Neuron* **10**, 963-974.

Weskamp, G. and Reichardt, L. F. (1991). Evidence that biological activity of NGF is mediated through a novel subclass of high affinity receptors. *Neuron* **6**, 649-663.

Wheeler, E. F., Gong, H., Grimes, R., Benoit, D. and Vazquez, L. (1998). The $p75^{NTR}$ and *trk* receptors are expressed in reciprocal patterns in a wide variety of non-neural tissues during rat embryonic development, indicating independent receptor functions. *J. Comp. Neurol.* **391**, 407-428.

White, F. A., Silos-Santiago, I., Molliver, D. C., Nishimura, M., Phillips, H., Barbacid, M. and Snider, W. D. (1996). Synchronous onset of NGF and TrkA survival dependence in developing dorsal root ganglia. *J. Neurosci.* **16**, 4662-4672.

Wright, E. M., Vogel, K. S. and Davies, A. M. (1992). Neurotrophic factors promote the maturation of developing sensory neurons before they become dependent on these factors for survival. *Neuron* **9**, 139-150.

Wyatt, S., Middleton, G., Doxakis, E. and Davies, A. M. (1999). Selective regulation of trkC expression by NT3 in the developing peripheral nervous system. *J. Neurosci.* **19**, 6559-6570.