Functional interaction between p75^{NTR} and TrkA: the endocytic trafficking of p75^{NTR} is driven by TrkA and regulates TrkA-mediated signalling

Lorena PERRONE*†[1], Simona PALADINO*, Marialuisa MAZZONE*, Lucio NITSCH*, Massimo GULISANO† and Chiara ZURZOLO*

*Dipartimento di Biologia e Patologia Cellulare e Molecolare, Università degli Studi di Napoli Federico II, Centro di Endocrinologia ed Oncologia Sperimentale del Consiglio Nazionale delle Ricerche, 80131 Napoli, Italy, and †Dipartimento di Scienze Fisiologiche, Universita' degli Studi di Catania, 95125 Catania, Italy

The topology and trafficking of receptors play a key role in their signalling capability. Indeed, receptor function is related to the microenvironment inside the cell, where specific signalling molecules are compartmentalized. The response to NGF (nerve growth factor) is strongly dependent on the trafficking of its receptor, TrkA. However, information is still scarce about the role of the cellular localization of the TrkA co-receptor, p75^{NTR} (where NTR is neurotrophin receptor), following stimulation by NGF. It has been shown that these two receptors play a key role in epithelial tissue and in epithelial-derived tumours, where the microenvironment at the plasma membrane is defined by the presence of tight junctions. Indeed, in thyroid carcinomas, rearrangements of TrkA are frequently found, which produce TrkA mutants that are localized exclusively in the cytoplasm. We used a thyroid cellular model in which it was possible to dissect the trafficking of the two NGF receptors upon neurotrophin stimulation. In FRT (Fischer rat thyroid) cells, endogenous TrkA is localized exclusively on the basolateral surface, while transfected p75^{NTR} is selectively distributed on the apical membrane. This cellular system enabled us to selectively stimulate either p75^{NTR} or TrkA and to analyse the role of receptor trafficking in their signalling capability. We found that, after binding to NGF, p75^{NTR} was co-immunoprecipitated with TrkA and was transcytosed at the basolateral membrane. We showed that the TrkA–p75^{NTR} interaction is necessary for this relocation of p75^{NTR} to the basolateral side. Interestingly, TrkA-specific stimulation by basolateral NGF loading also induced the TrkA–p75^{NTR} interaction and subsequent p75^{NTR} transcytosis at the basolateral surface. Moreover, specific stimulation of p75^{NTR} by NGF activated TrkA and the MAPK (mitogen-activated protein kinase) pathway. Our data indicate that TrkA regulates the subcellular localization of p75^{NTR} upon stimulation with neurotrophins, thus affecting the topology of the signal transduction molecules, driving the activation of a specific signal transduction pathway.

Key words: compartmentalization, polarity, p75^{NTR}, trafficking, TrkA.

INTRODUCTION

The mammalian neurotrophin family, which includes NGF (nerve growth factor), brain-derived neurotrophic factor, neurotrophin 3 and neurotrophin 4/5, was initially characterized based on the ability of its members to promote the survival and differentiation of neurons in the peripheral and central nervous systems [1,2]. These effects result from the activation by the specific neurotrophin of the high-affinity tyrosine kinase receptors TrkA, TrkB and TrkC, and/or of the low-affinity neurotrophin receptor p75^{NTR}. Neurotrophin binding to the Trk receptors results in dimerization and activation of their intrinsic tyrosine kinase activity, leading in turn to activation of the p21^{Ras}/MAPK (mitogen-activated protein kinase) cascade [3,4].

The low-affinity neurotrophin receptor p75^{NTR} is a member of the tumour necrosis factor receptor superfamily, and possesses autonomous signalling capacity [5]. p75^{NTR} interacts functionally with Trk receptors to enhance responsiveness to neurotrophins, particularly at low growth factor concentrations [6]. In contrast with the Trk family receptors, the function of p75^{NTR} is still controversial, because this receptor can exert different effects depending on the cellular context [7,8]. Interestingly, the two families of NGF receptors are also expressed in epithelia, where they regulate development and morphogenesis [9–11]. An important role for the two receptors in the stimulation of carcinogenesis progression in non-neuronal cells has been demonstrated [12].

It is becoming apparent that p75^{NTR} has a fundamental role in modulating TrkA function. p75^{NTR} affects both the ligand affinity and the ligand-induced internalization of TrkA [6,13], as well as the retrograde transport of neurotrophin along the axons of nerve cells [14]. However, the molecular mechanisms involved in this regulation, as well as the site where it occurs, remain unclear. It is known that TrkA signalling can be regulated by its localization [15,16]: TrkA-dependent survival is mediated by activation of TrkA at the cell surface, while TrkA-mediated differentiation is due to the presence ot TrkA in an endocytic compartment [17]. p75^{NTR} co-operates with TrkA at the plasma membrane by modulating its endocytosis [18,19]. Nonetheless, the role of p75^{NTR} in modulating TrkA internalization is controversial. It seems that the fate of both receptors upon neurotrophin stimulation is cell type-dependent. The effect of the cellular context in the co-operation between p75^{NTR} and TrkA is very important when studying the function of these receptors in the progression of non-neuronal carcinogenesis. Since some evidence indicates that there is a strong relationship between receptor trafficking and signalling, it is important to find a model in which it is possible to dissect the trafficking of the two receptors upon neurotrophin stimulation. Oncogenic rearrangements of TrkA are frequently found in

Abbreviations used: Ag 35–40, antigen of 35–40 kDa; DPPIV, dipeptidyl peptidase IV; ERK, extracellular-signal-regulated kinase; FRT cells, Fischer rat thyroid cells; MAPK, mitogen-activated protein kinase; NFκB, nuclear factor κB; NGF, nerve growth factor; NHS, *N*-hydroxysuccinimido; NHS-SS-biotin, sulphosuccinimidyl-6-(biotinamide) hexanoate; NTR, neurotrophin receptor.

[1] To whom correspondence should be addressed (email loperron@unina.it).

thyroid carcinomas, and these chimaeric proteins show an altered subcellular localization [20]. Moreover, mutant Trks also show association with particular downstream signalling molecules in the thyroid. In fact, the fusion proteins TRK-T1 and TRK-T3 (based on the receptor for NGF encoded by *NTRK1* gene) activate FRS2 (fibroblast growth factor receptor substrate 2) and FRS3 in thyroid cells [21]. Thus the cellular localization of these receptors is a key element in the interaction and activation of downstream signalling molecules.

FRT (Fischer rat thyroid) epithelial cells have been used previously to analyse protein trafficking [22,23]. When grown in filter chambers, they form polarized monolayers in which it is possible to discriminate between the apical and basolateral surfaces, which are physically separated by the tight junctions [24,25]. Interestingly, we show here that FRT cells express endogenous and functional TrkA, which is localized exclusively on the basolateral surface, while transfected $p75^{NTR}$ is distributed selectively on the apical membrane [26]. Therefore this cellular system enables us to selectively stimulate either $p75^{NTR}$ or TrkA and to analyse their intracellular trafficking upon stimulation. We demonstrate that, after binding to NGF added from the apical surface, $p75^{NTR}$ co-immunoprecipitates with TrkA, and then the two receptors co-localize on the basolateral surface. Moreover, the TrkA–$p75^{NTR}$ interaction is necessary for relocation of $p75^{NTR}$ to the basolateral side. TrkA-specific stimulation induced by the basolateral addition of NGF also induces the TrkA–$p75^{NTR}$ interaction and $p75^{NTR}$ transcytosis at the basolateral surface. Our data indicate that TrkA drives the endocytic trafficking and subcellular localization of $p75^{NTR}$ upon stimulation with neurotrophins. Interestingly, $p75^{NTR}$-specific stimulation from the apical surface with NGF induces TrkA activation and the subsequent signalling cascade. A $p75^{NTR}$ mutant lacking the cytoplasmic tail is not transcytosed at the basolateral surface upon stimulation by NGF and does not activate TrkA. These data demonstrate that the TrkA–$p75^{NTR}$ interaction upon endocytosis induces the formation of a signalling complex.

MATERIALS AND METHODS

Reagents and antibodies

Cell culture reagents were purchased from Gibco, Protein A–Sepharose was from Pharmacia and Protein G–Sepharose was from Sigma. Sulpho-NHS (*N*-hydroxysuccinimido) derivatives and streptavidin–agarose beads were from Pierce (Rockford, IL, U.S.A.). Anti-$p75^{NTR}$ monoclonal antibody and anti-$p75^{NTR}$ rabbit polyclonal antibody were gifts from André Le Bivic (IBDM, Marseille, France). A rabbit polyclonal antibody that specifically recognizes phosphorylated TrkA and an anti-TrkA rabbit polyclonal antibody used for Western blot experiments were from Cell Signalling Technology (Beverly, MA, U.S.A.). An anti-TrkA rabbit polyclonal antibody used for immunofluorescence, anti-ERK (extracellular-signal-regulated kinase) and anti-(phosphorylated ERK) were from Santa Cruz (Santa Cruz, CA, U.S.A.). TrkA in FRT cells was also detected using anti-TrkA rabbit polyclonal antibodies from Upstate Biotechnology (Lake Placid, NY, U.S.A.).

7S NGF (a mixture of several different subunits of NGF, i.e. $\alpha_2\beta\delta_2$) was from Upstate Biotechnology. K252a was from Calbiochem–Novabiochem Corp. (La Jolla, CA, U.S.A.). Monoclonal antibodies against rat DPPIV (dipeptidyl peptidase IV) (CLB4/40), the B-subunit of rat Na/K-ATPase (IEC1/48) and Ag 35–40 (antigen of 35–40 kDa) (CLB1/41) have all been described previously [22].

Cell culture and neurotrophin treatment

FRT cells stably expressing $p75^{NTR}$ (E1A5) [26] were grown for 5 days on Transwell filters filters (Costar Corp., Cambridge, MA, U.S.A.) in F12 Coon's modified medium containing 5 % (v/v) fetal bovine serum. Cells were serum-starved in F12 Coon's modified medium containing 0.2 % (w/v) BSA for 24 h before neurotrophin treatment, and then stimulated with NGF (100 ng/ml) in either the apical or the basolateral medium. In TrkA-phosphorylation inhibition experiments, K252a was added 2 h before neurotrophin stimulation and was present throughout the stimulation time.

Immunofluorescence and confocal microscopy

FRT cells cultured on 12 mm-diameter Transwell for 5 days were fixed in 2 % (v/v) paraformaldehyde in Dulbecco's PBS solution containing 1.8 mM Ca^{2+} and 0.5 mM Mg^{2+} (CM-PBS). Cells were treated with 0.075 % (w/v) saponin in CM-PBS containing 0.2 % (w/v) gelatin and were incubated with the specific antibodies in the same buffer. After washes with CM-PBS containing saponin and gelatin, filters were incubated with fluorescein- or rhodamine-conjugated goat anti-mouse or anti-rabbit secondary antibodies, each diluted 1:50. Immunofluorescent images were collected using a confocal microscope (Zeiss LSCM 410).

Biotinylation assays

Confluent monolayers on Transwells were labelled overnight with 0.5 mCi/ml [^{35}S]Met/Cys and 1 mCi/ml [^{35}S]Cys (Amersham, Arlington Heights, IL, U.S.A.) and were biotinylated and processed for immunoprecipitation, as described previously [23]. Biotinylated antigens were then precipitated with streptavidin–agarose beads. After boiling the beads in Laemmli buffer, supernatants were analysed by SDS/PAGE and fluorography.

Immunoprecipitation of $p75^{NTR}$ and $p75^{NTR}$-associated proteins

Confluent cell monolayers on Transwells were starved for 24 h before NGF treatment, stimulated with neurotrophins from the apical or basolateral side as indicated, washed two times with ice-cold PBS containing 1 mM Na_3VO_4, and lysed in 500 μl of buffer A. Equal amounts of proteins were incubated for 2 h at 4 °C with anti-$p75^{NTR}$ monoclonal antibody at a dilution of 1:200, as described [26].

Inhibition of endocytosis

Cells were incubated in hypertonic medium [120 mM NaCl, 12 mM $MgSO_4$, 1 mM EDTA, 15 mM sodium acetate, 1 % (w/v) BSA, 100 mM Hepes, pH 7.0, 5 mM KCl, 0.4 M sucrose] for 30 min before and during stimulation, and then lysed as described previously [27].

Biotin internalization assay

Cells grown on filters were biotinylated using NHS-SS-biotin [sulphosuccinimidyl-6-(biotinamide) hexanoate] from the apical or basolateral plasma membrane domain. Sample filters were incubated at 37 °C for 15 min in the presence and or in the absence of NGF, while control filters were kept at 4 °C. After washing all filters with ice-cold PBS containing 0.1 mM $CaCl_2$ and 1 mM $MgCl_2$, reduction with glutathione (50 mM) was carried out twice from the apical or basolateral side at 4 °C. Subsequent lysis, precipitation with streptavidin beads, and SDS/PAGE were performed as described above. Western blot analysis was carried out using the appropriate antibody.

Surface immunoprecipitation and endocytosis assay

Cells grown on filters were washed with culture medium and incubated for 2 h at 4 °C with anti-p75^{NTR} antibody diluted 1:200 in the culture medium added to the apical surface. Filters were then washed five times on ice with culture medium containing 5 % (v/v) fetal bovine serum. Sample filters were incubated at 37 °C for 15 min in the presence or absence of NGF, while control filters were kept at 4 °C. Cells were lysed as described above. Protein G–Sepharose saturated with BSA was added to the lysate and rocked for 1 h at 4 °C. The immunocomplexes were subjected to SDS/PAGE, then blotted on to nitrocellulose. The supernatant of the surface immunoprecipitation was incubated overnight with anti-p75^{NTR} monoclonal antibody at a dilution of 1:200. Protein G–Sepharose was added to the lysate for 1 h. The immunocomplexes, representing the total amount of p75^{NTR} in each sample, were recovered and subjected to Western blot analysis using the appropriate antibody.

Electrophoretic mobility shift assay

Following 30 min of stimulation with NGF, cells were washed in ice-cold PBS, scraped and pelleted by centrifugation at 2300 ***g*** for 5 min at 4 °C. The cells were lysed in hypotonic buffer (20 mM Hepes, pH 7.9, 1 mM $MgCl_2$, 0.1 mM EGTA, 15 mM KCl, 1 mM PMSF). Nuclei were pelleted at 400 ***g*** for 10 min at 4 °C and then lysed in high-salt buffer (20 mM Hepes, pH 7.9, 1 mM $MgCl_2$, 0.1 mM EGTA, 15 mM KCl, 400 mM NaCl, 1 mM PMSF). The insoluble material was recovered at 16 500 ***g*** for 20 min at 4 °C. The supernatants containing the nuclear proteins were frozen at − 80 °C until used in the binding reaction. The binding of activated NFκB (nuclear factor κB) in the lysates to an oligonucleotide corresponding to the κ light chain immunoglobulin enhancer was assessed as described in [7].

RESULTS

Internalization of p75^{NTR} following stimulation by NGF from the apical membrane

To determine whether TrkA is expressed in polarized epithelial FRT cells, we used both an anti-TrkA antibody against the entire ectodomain and an anti-TrkA antibody against a C-terminal peptide. We found that FRT cells contained a protein of the same molecular mass as endogenous TrkA from PC12 cells, and that the level of expression in these two cell lines was comparable (Figure 1A, upper panel).

By confocal analysis, we found that TrkA was localized exclusively on the basolateral domain of the plasma membrane (Figure 1A, lower panels). To exclude the possibility that a mutation in the gene encoding TrkA in FRT cells was responsible for its exclusively basolateral localization, we transfected these cells with a cDNA encoding wild-type rat TrkA and analysed its distribution in stably expressing clones. The transfected receptor was also expressed exclusively on the basolateral membrane (results not shown).

It has been demonstrated that TrkA is internalized upon stimulation by NGF, and that this event plays a key role in TrkA function [15,17]. In contrast, the role of p75^{NTR} internalization is still unclear. To investigate this point, we first investigated whether p75^{NTR} is internalized following stimulation by NGF. To this end, we grew FRT cells expressing transfected p75^{NTR} and endogenous TrkA (E1A5 clone) on filters and used an endocytosis assay based on the use of a cleavable biotin analogue (NHS-SS-biotin) [28]. p75^{NTR} at the apical surface was biotinylated, and then NGF was added from the apical surface for 15 min. We found that approx. 20 % of p75^{NTR} was internalized after stimulation with NGF from the apical side (Figure 1B).

p75^{NTR} co-localizes with TrkA upon stimulation by NGF

Since p75^{NTR} was internalized upon apical loading of NGF, we decided to analyse p75^{NTR} trafficking in polarized FRT cells following neurotrophin stimulation. Surprisingly, by indirect immunofluorescence, we found that, within 15 min of apical NGF addition, a significant amount of p75^{NTR} was relocalized from the apical to the basolateral domain, where it co-localized with TrkA (Figure 1C). This behaviour was specific for p75^{NTR} in response to NGF, as localization of the apical marker DPPIV and the basolateral markers Na/K-ATPase (Figure 1D) and Ag 35–40 (not shown) [22,23] did not change following apical stimulation with NGF. To confirm these data, we quantified the membrane localization of p75^{NTR}, DPPIV and Ag 35–40 before and after NGF addition by using selective domain biotinylation (Figure 1D). Approx. 20 % of p75^{NTR} was relocalized to the basolateral membrane after 15 min of apical stimulation. In contrast, stimulation by NGF did not affect the polarity of either DPPIV or Ag 35–40 (Figure 1D), and neither did it alter the tight junctions, since the values of transepithelial resistance before and after NGF addition were unchanged (results not shown). These data demonstrated that the observed basolateral localization of p75^{NTR} was not due to a loss of polarity or to the non-specific redistribution of proteins in the plasma membrane following NGF stimulation, but rather to a specific translocation of the receptor trigged by NGF.

To rule out the possibility that the NGF-stimulated localization of p75^{NTR} to the basolateral membrane was due to mis-sorting of newly synthesized receptor, we incubated cells with cycloheximide for 2 h before and during stimulation by NGF. Under these conditions, p75^{NTR} was still present on the basolateral membrane following apical NGF treatment (results not shown), suggesting that the p75^{NTR} that was localized at the basolateral surface upon NGF addition had not been synthesized *de novo*, but was relocalized from the pre-existing plasma membrane pool via a transcytotic pathway.

Next we analysed the requirements for p75^{NTR} endocytosis. To investigate whether coated pits are involved in p75^{NTR} internalization, we inhibited receptor-mediated endocytosis by incubating the cells in hypertonic medium, which causes the disappearance of the soluble clathrin pool [27]. By confocal microscopy, we found that the endocytosis block inhibited p75^{NTR} transcytosis to the basolateral domain following 15 min of NGF apical loading (Figure 2A). Although these conditions are quite harsh for the cells, they did not affect the integrity of the monolayers on filters, or impair the signalling ability of the two receptors. Thus stimulation of p75^{NTR} by the apical addition of NGF induced the nuclear translocation of NFκB (Figure 2B) in hypertonic medium as well as in growth medium, and TrkA was also activated following 2 min of basolateral NGF loading (Figure 2C).

After its internalization, p75^{NTR} co-immunoprecipitates with TrkA

To examine whether p75^{NTR} and TrkA interact following apical stimulation with NGF in FRT cells, we performed co-immunoprecipitation experiments before and after treatment with NGF. No co-immunoprecipitation was observed in polarized E1A5 cells in the absence of stimulation (Figure 3A). However, after 15 min of p75^{NTR}-specific stimulation with NGF from the apical side, TrkA co-immunoprecipitated with p75^{NTR} (Figure 3A). To demonstrate that p75^{NTR} bound TrkA after being internalized from the apical surface following NGF stimulation, we performed

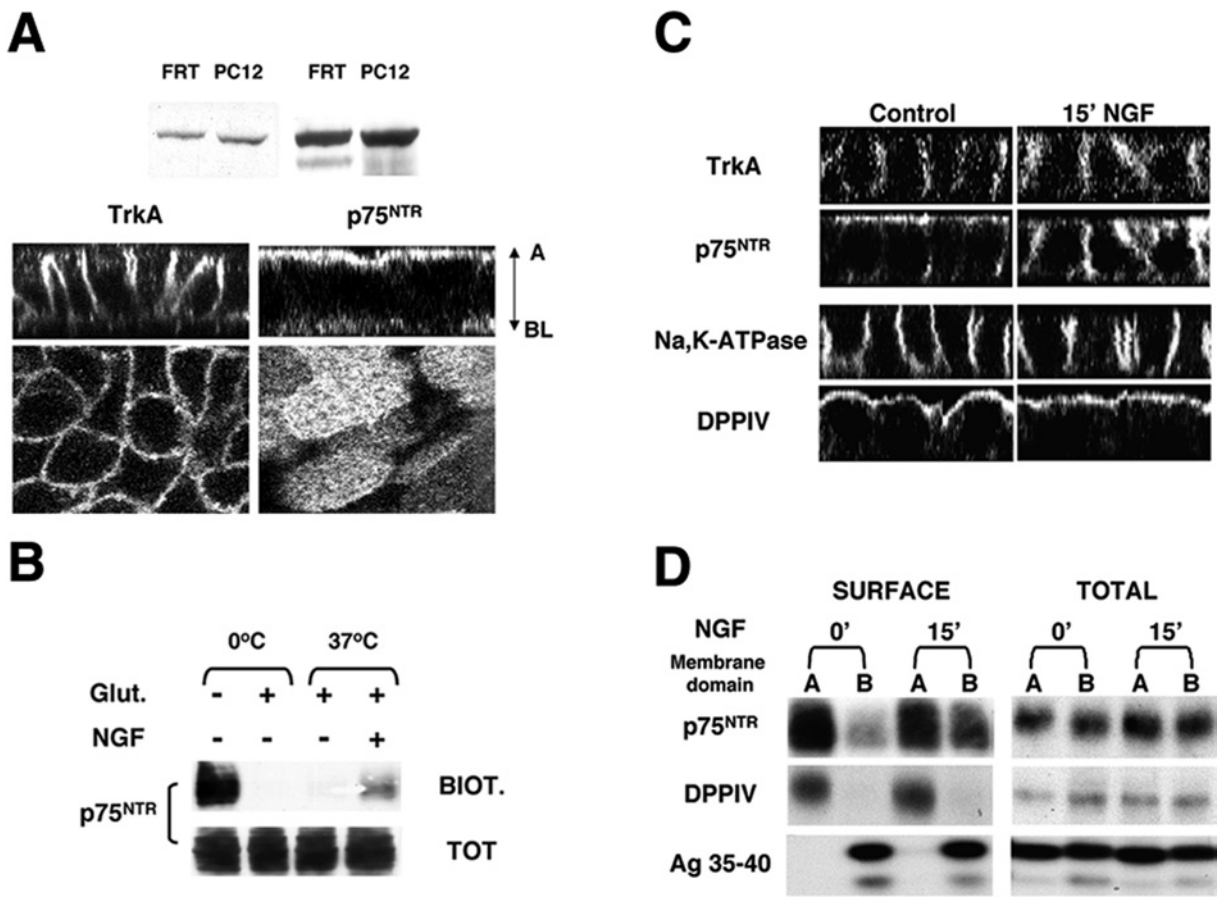

Figure 1 Stimulation of p75^{NTR} by the apical addition of NGF induces p75^{NTR} transcytosis at the basolateral surface

(**A**) Upper panel: immunoblotting with anti-TrkA antibodies against the N-terminus (left) and against a C-terminal peptide (right). Lower panel: FRT cells expressing p75^{NTR} (E1A5 clone) were grown on filters for 5 days and then subjected to indirect immunofluorescence using anti-TrkA or anti-p75^{NTR} antibodies as indicated in the Materials and methods section. A, apical membrane; BL, basolateral membrane. (**B**) E1A5 cells were biotinylated with NHS-SS-biotin (BIOT.) as described in the Material and methods section. Immunocomplexes and 1/10 of the total extracts were subjected to Western blot analysis using an anti-p75^{NTR} antibody. Glut., glutathione. (**C**) Treated and control filters were divided into four parts and subjected to indirect immunofluorescence carried out using the appropriate antibodies as indicated. (**D**) Proteins were labelled *in vivo* using [^{35}S]Met and [^{35}S]Cys for 14 h. After p75^{NTR}-specific stimulation with NGF, surface proteins were labelled from the apical or basolateral surfaces with biotin as described in the Materials and methods section. Proteins were immunoprecipitated with the appropriate antibody, boiled in 0.5 % SDS, diluted in lysis solution, and immunoprecipitated again with streptavidin to identify the biotinylated fraction. Proteins were separated by SDS/PAGE, dried and exposed to autoradiography. Aliquots containing 1/10 of the total immunoprecipitated proteins are shown as a control on the right-hand side. These experiments are representative of at least three independent experiments.

a co-immunoprecipitation assay based on the surface immunoprecipitation of p75^{NTR}. We labelled the p75^{NTR} receptor present at the apical surface with a specific antibody added to the apical medium at 0 °C, and then induced its internalization by addition of NGF to the apical surface for 15 min at 37 °C. After washing off the unbound antibody, we lysed both the stimulated and control cells and recovered p75^{NTR} bound to the antibody by precipitation with Protein G–Sepharose beads. As shown by Western blot, TrkA was present in the immunoprecipitates only following NGF stimulation (Figure 3B), whereas it was absent when the cells were not stimulated (Figure 3B). Moreover, when endocytosis was blocked, TrkA did not co-immunoprecipitate with p75^{NTR} (Figure 3B). These data demonstrate that p75^{NTR} bound to TrkA came from the apical surface receptor pool upon NGF-induced internalization.

We analysed whether TrkA is internalized at steady state in FRT cells. Using an endocytosis assay based on the use of a cleavable biotin analogue (NHS-SS-biotin), we demonstrated that, in the absence of stimulation, 25 % of TrkA was internalized, and that apical addition of NGF did not change this rate of internalization (Figure 3C), suggesting that in FRT cells TrkA recycles continuously at the basolateral surface.

p75^{NTR} is internalized following activation of TrkA by NGF stimulation from the basolateral membrane

We next assessed whether stimulation of TrkA by the basolateral addition of NGF induced p75^{NTR} transcytosis at the basolateral surface. First we analysed whether basolateral addition of NGF affected TrkA internalization. Using the endocytic assay, we demonstrated that the endocytic pool of TrkA increased following 15 or 30 min of stimulation by NGF (Figure 4A). Indirect immunofluorescence analysis showed that, after 15 min of NGF basolateral loading, p75^{NTR} was not completely apical; an intracellular and basolateral signal was also detected (Figure 4B). Indeed, following 30 min of NGF basolateral addition, p75^{NTR} was transcytosed at the basolateral surface (Figure 4B). We next investigated whether the p75^{NTR}–TrkA interaction occurred following basolateral stimulation by NGF. Immunoprecipitation experiments showed that TrkA was associated with p75^{NTR} after 15

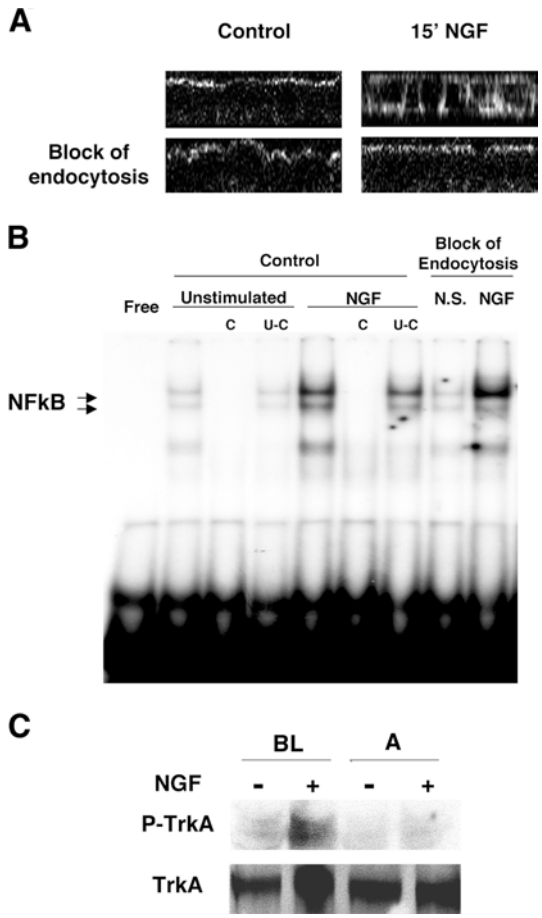

Figure 2 Blockade of endocytosis inhibits p75^{NTR} transcytosis at the basolateral surface

(**A**) Cells were incubated in hypertonic medium for 30 min before and during stimulation with NGF. Treated and control filters were subjected to indirect immunofluorescence using anti-p75^{NTR} monoclonal antibody. (**B**) Following apical addition of NGF, electrophoretic mobility shift assay analysis of nuclear extracts was performed as described in the Materials and methods section. C indicates competition of protein–probe binding with unlabelled specific double-stranded DNA oligonucleotides; U-C indicates competition carried with unspecific unlabelled oligonucleotides; N.S. indicates not stimulated. (**C**) E1A5 cells were stimulated by the apical (A) or basolateral (BL) addition of NGF. Western blot analysis was carried out using antibodies against phosphorylated TrkA (P-TrkA) and total TrkA. These experiments were repeated more than three times, with similar results.

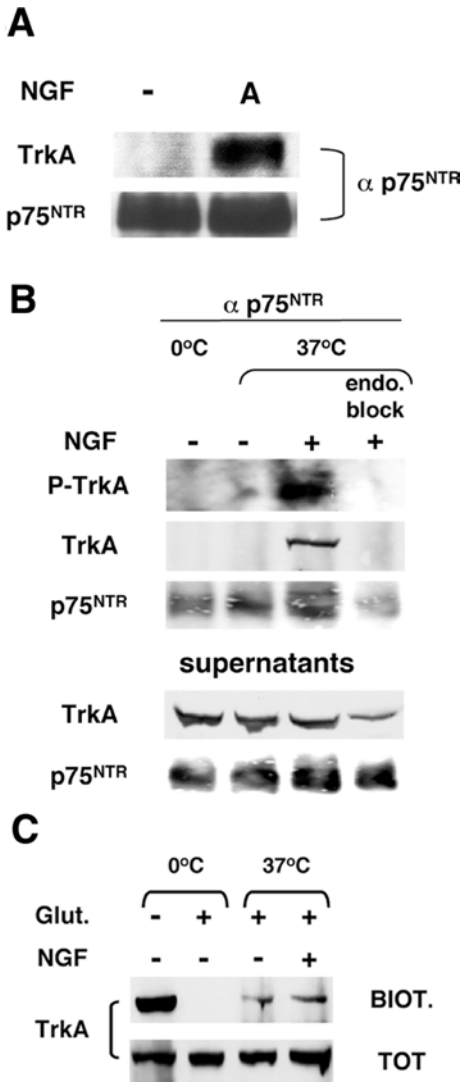

Figure 3 Stimulation of p75^{NTR} by the apical addition of NGF induces p75^{NTR}–TrkA interaction

(**A**) E1A5 cells were grown and stimulated from the apical medium, as described for Figure 1. Protein extracts from treated and control cells were immunoprecipitated with anti-p75^{NTR} antibody. Western blot analysis was carried out using an anti-TrkA (upper) or an anti-p75^{NTR} (lower) antibody. (**B**) Surface p75^{NTR} was labelled using an anti-p75^{NTR} antibody as described in the Materials and methods section. Immunocomplexes and 1/10 of the total extract were separated on SDS/8%-PAGE. Western blot analysis was carried out using anti-(phosphorylated TrkA) (P-TrkA), anti-TrkA or anti-p75^{NTR} antibodies. 'endo block' indicates that endocytosis was blocked. (**C**) E1A5 cells were grown and biotinylated with NHS-SS-biotin as described in the Materials and methods section. Protein extracts from treated and control cells were immunoprecipitated with streptavidin. Immunocomplexes (BIOT.) and 1/10 of the total extracts were separated on SDS/8%-PAGE. Western blot analysis was carried out using an anti-TrkA antibody. Glut., glutathione. These experiments are representative of at least three independent experiments.

and 30 min of NGF basolateral loading (Figure 4C). These data suggest that the TrkA–p75^{NTR} interaction occurred before p75^{NTR} transcytosis at the basolateral surface. Indeed, we detected a very low amount of TrkA associated with p75^{NTR} after 10 min of NGF basolateral loading (Figure 4C). Thus we hypothesized that when the endocytic pool of either TrkA or p75^{NTR} is increased by stimulation with NGF, the two receptors interact in an endocytic compartment.

We decided to investigate whether the p75^{NTR}–TrkA interaction that occurs upon basolateral NGF addition takes place at the plasma membrane or in an intracellular compartment. To discriminate between the surface and intracellular localization of p75^{NTR} following 15 min of NGF basolateral addition, we performed indirect immunofluorescence experiments in both the absence and the presence of saponin detergent. In the absence of saponin treatment, the antibodies could bind only the proteins localized at the cell surface, while proteins localized in intracellular compartments were undetectable. Under these conditions p75^{NTR} showed only apical localization (Figure 4D). In contrast, in the presence of the detergent we detected p75^{NTR} also on the basolateral side of the cells (Figure 4D), demonstrating that following 15 min of basolateral NGF loading, 75^{NTR} was localized in an intracellular compartment.

p75^{NTR}-specific stimulation activates TrkA and the MAPK pathway

Since we found that TrkA associated with p75^{NTR} was phosphorylated after 15 min of apical stimulation by NGF (Figure 3B),

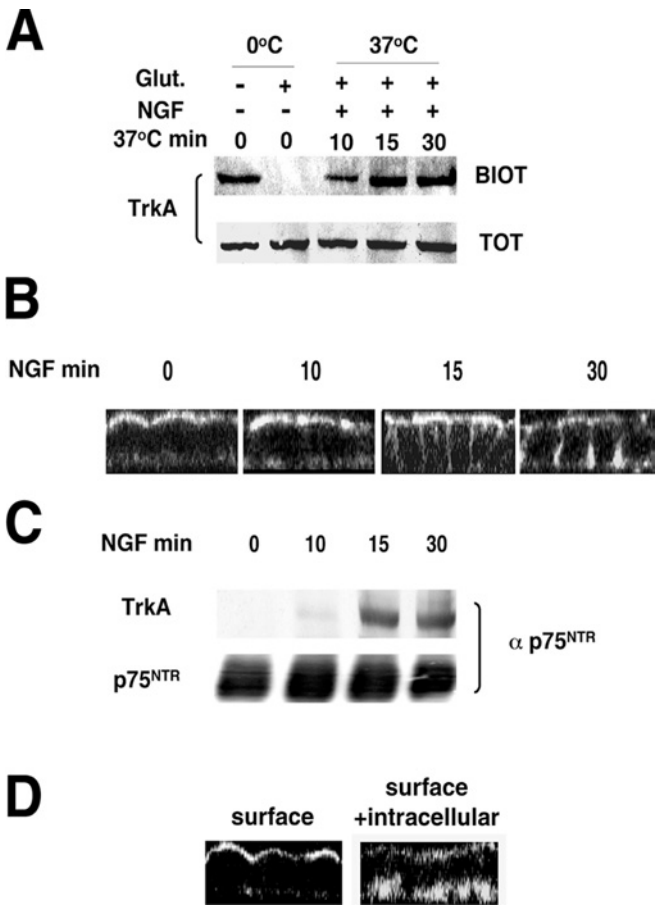

Figure 4 Stimulation of TrkA by the basolateral addition of NGF induces p75^{NTR} transcytosis at the basolateral surface

(**A**) E1A5 cells were stimulated by adding NGF from the basolateral surface. Cells were biotinylated with NHS-SS-biotin as described in the Materials and methods section. Protein extracts from treated and control cells were immunoprecipitated with streptavidin. Immunocomplexes (BIOT) and aliquots comprising 1/10 of the total extracts were separated on SDS/8 %-PAGE. Western blot analysis was carried out using an anti-TrkA antibody. Glut., glutathione. (**B**) E1A5 cells were stimulated with NGF added from the basolateral surface for the times indicated. Treated and control filters were subjected to indirect immunofluorescence using an anti-p75^{NTR} monoclonal antibody and analysed by confocal microscopy. (**C**) E1A5 cells were stimulated as described in (**B**). Protein extracts from treated and control cells were immunoprecipitated with anti-p75^{NTR} antibody. Western blot analysis was carried out using an anti-TrkA (upper) or an anti-p75^{NTR} (lower) antibody. (**D**) E1A5 cells were stimulated for 15 min by basolateral addition of NGF. The filters were divided into two parts and subjected to indirect immunofluorescence with anti-p75^{NTR} antibody in either the absence or the presence of the detergent saponin, in order to detect only cell surface (in the absence of saponin) or both cell surface and intracellular compartments. These experiments were repeated more than three times, with similar results.

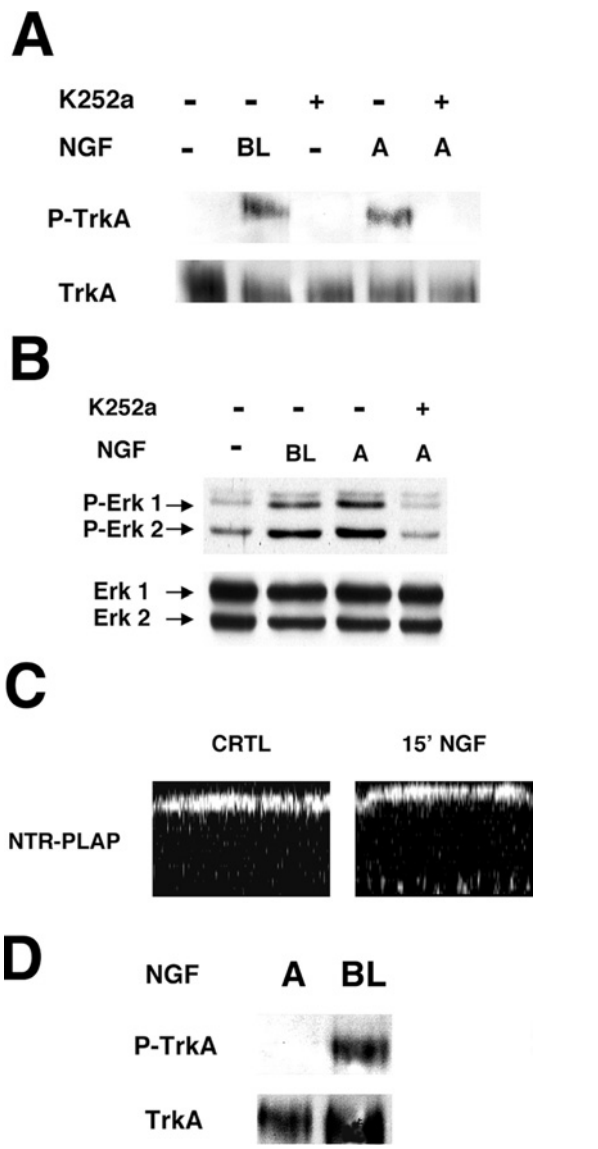

Figure 5 Stimulation of p75^{NTR} by the apical addition of NGF induces TrkA phosphorylation and MAPK activation

(**A**) E1A5 cells were stimulated by the apical (A) or basolateral (BL) addition of NGF. Inhibition of TrkA phosphorylation by 10 nM K252a was carried out 2 h before and during the stimulation time. Western blotting was carried out using an anti-(phosphorylated TrkA) (upper) or an anti-TrkA (lower) antibody. (**B**) E1A5 cells were grown and stimulated as described in (**A**). Western blotting was carried out using an anti-(phosphorylated ERK) (upper) or an anti-ERK (lower) antibody. (**C**) FRT cells expressing the p75^{NTR} mutant NTRPLAP were stimulated by apical loading of NGF for 15 min. Treated and control (CTRL) filters were subjected to indirect immunofluorescence using anti-p75^{NTR} monoclonal antibody and analysed by confocal microscopy. (**D**) FRT cells expressing NTRPLAP were stimulated from either the apical (A) or the basolateral (BL) surface with NGF. Western blotting was carried out using an anti-(phosphorylated TrkA) (upper) or an anti-TrkA (lower) antibody. These experiments were repeated more than three times, with similar results.

we investigated further whether p75^{NTR}-specific stimulation by NGF could affect TrkA signalling. We analysed TrkA activation following either apical or basolateral addition of NGF by Western blot using an anti-(phosphorylated TrkA) antibody. We found that TrkA was activated by 2 min of NGF stimulation from the basolateral side (Figure 5A). Surprisingly, we also observed TrkA phosphorylation after 15 min of apical addition of NGF (Figure 5A). The Trk-specific inhibitor K252a (10 nM) blocked TrkA phosphorylation (Figure 5A). Therefore we analysed whether the MAPK pathway was activated following apical stimulation of p75^{NTR}. Phosphorylation of ERK1/2 occurred within 2 min of direct TrkA activation by NGF addition from the basolateral side and also upon 15 min of NGF stimulation from the apical surface (Figure 5B). K252a at 10 nM completely abolished ERK1/2 activation following p75^{NTR}-specific stimulation with NGF (Figure 5B), demonstrating that ERK1/2 phosphorylation was TrkA-dependent.

To confirm further that the formation of an intracellular complex between p75^{NTR} and TrkA was responsible for TrkA activation, we transfected FRT cells with a p75^{NTR} mutant (NTRPLAP) carrying a glycosylphosphatidylinositol anchor instead of the transmembrane and cytoplasmic domains. This chimaera was also

localized exclusively on the apical surface. This mutant did not transcytose to the basolateral surface after 15 min of NGF apical loading (Figure 5C). Indeed, TrkA activation did not occur in these cells upon apical stimulation by NGF, whereas basolateral addition of NGF did activate TrkA (Figure 5D). These data clearly demonstrate that both p75^{NTR} transcytosis at the basolateral surface and TrkA activation were not a consequence of the presence of small amounts of TrkA on the apical surface that was undetectable by indirect immunofluorescence.

p75^{NTR} is transcytosed to the basolateral surface following interaction with TrkA

To further confirm that the p75^{NTR}–TrkA interaction was necessary for p75^{NTR} transcytosis at the basolateral surface, we used increasing concentrations of K252a, which has been shown to inhibit the p75^{NTR}–TrkA interaction in a dose-dependent manner [29]. In the presence of 10 nM K252a, p75^{NTR} and TrkA still co-immunoprecipitated, while at higher concentrations K252a blocked the p75^{NTR}–TrkA interaction (Figure 6A). Using confocal microscopy we found that high concentrations of K252a totally blocked p75^{NTR} transcytosis (Figure 6B). In contrast, in the presence of low K252a concentrations that did not impair formation of the complex, p75^{NTR} was transcytosed at the basolateral surface (Figure 6B). These data demonstrate that TrkA activation was not necessary for p75^{NTR} transcytosis at the basolateral surface. Indeed, TrkA activation did not occur in the presence of 10 nM K252a (Figure 6C).

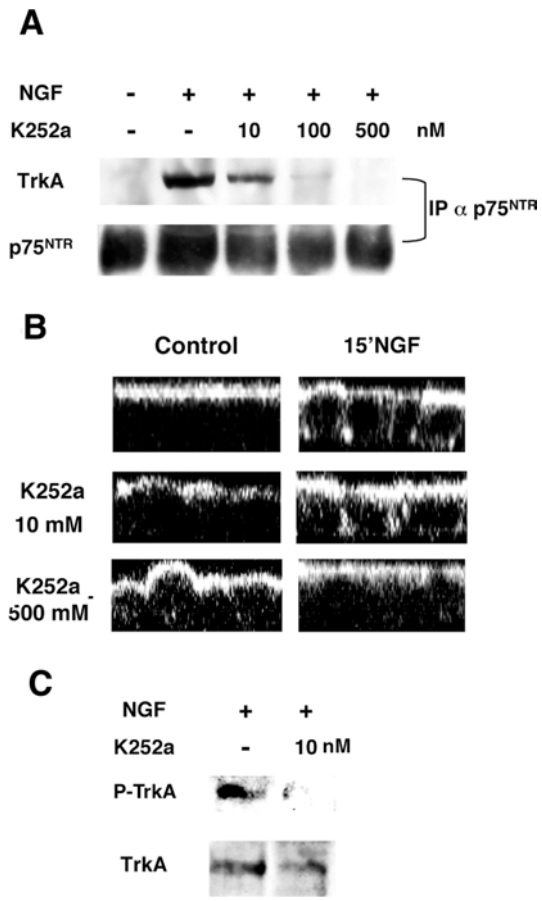

Figure 6 Phosphorylation of TrkA is not required for p75^{NTR} transcytosis at the basolateral surface

(**A**) E1A5 cells were stimulated by the apical addition of NGF for 15 min. TrkA phosphorylation was blocked using various concentrations of K252a. Protein extracts from treated and control cells were immunoprecipitated (IP) with anti-p75^{NTR} antibody. Western blot analysis was carried out using an anti-TrkA or an anti-p75^{NTR} antibody. (**B**) E1A5 cells were stimulated with NGF as described in (**A**). TrkA phosphorylation was inhibited by the addition of K252a at the concentrations indicated. Treated and control filters were subjected to indirect immunofluorescence using anti-p75^{NTR} monoclonal antibody and analysed by confocal microscopy. (**C**) E1A5 cells were stimulated for 15 min with NGF as described in (**A**). Inhibition of TrkA phosphorylation was carried out using 10 nM K252a. Western blot analysis was carried out using an anti-(phosphorylated TrkA) (upper) or an anti-TrkA (lower) antibody. These experiments were repeated more than three times, with similar results.

DISCUSSION

Two new aspects have emerged recently in the study of neurotrophin receptor signalling. First, it has been demonstrated that these receptors play a key role not only in nervous system differentiation, but also in the correct differentiation of other tissues [10,30]. Interestingly, these two receptors also play a key role in the progression of non-neuronal carcinogenesis [31–33]. Secondly, numerous analyses have demonstrated that TrkA signalling is regulated by its intracellular localization [15,17]. Although it has been shown that the low-affinity NGF receptor p75^{NTR} co-operates with TrkA and enhances its responsiveness to NGF [6,34], the role of p75^{NTR} is still debated because its function is cell-type-dependent.

The present study has demonstrated that in polarized epithelial FRT cells p75^{NTR} and TrkA are compartmentalized in the apical and basolateral domains respectively of the plasma membrane. This enabled us to selectively stimulate either p75^{NTR} or TrkA. Our new findings are summarized as follows: (1) upon p75^{NTR}-specific stimulation by NGF, this receptor binds TrkA; (2) this interaction occurs after receptor endocytosis, and leads to the transcytosis of p75^{NTR}, which then co-localizes with TrkA on the basolateral surface; (3) the p75^{NTR}–TrkA interaction is necessary for p75^{NTR} transcytosis at the basolateral surface; (4) coated pit-mediated endocytosis is involved in p75^{NTR} internalization and transcytosis at the basolateral surface; (5) TrkA stimulation by the basolateral addition of NGF also leads to p75^{NTR}–TrkA association and p75^{NTR} transcytosis; and (6) in FTR cells, the p75^{NTR}–TrkA interaction induced by p75^{NTR}-specific stimulation leads to TrkA phosphorylation and MAPK activation.

We show that p75^{NTR} and TrkA are co-immunoprecipitated following either apical or basolateral addition of NGF. Indeed, blocking the p75^{NTR}–TrkA interaction by using high concentrations of the TrkA-specific inhibitor K252a prevents p75^{NTR} transcytosis at the basolateral membrane. We demonstrate clearly for the first time that p75^{NTR}–TrkA binding induces the transcytosis of p75^{NTR} at the basolateral side, showing that TrkA drives the endocytic trafficking of p75^{NTR}, leading to a change in its topology in the cell. In fact, it is highly unlikely that p75^{NTR} would recycle by itself to the basolateral membrane after neurotrophin binding, as we and others have shown that this receptor does not contain any basolateral sorting signal [26,35]. In polarized epithelial cells, early apical and basolateral endosomes are functionally and topologically distinct [36]; however, it has been proposed that they can be interconnected via a basolateral sorting machinery [37]. Therefore, after apical internalization, p75^{NTR} could transcytose to the basolateral side only if driven by specific signals or interactions, suggesting that the same molecular mechanism could be used in other cell types. These data are in agreement with and help to explain further previous studies on the role of the p75^{NTR}–TrkA complex. It has been demonstrated that TrkA immobilizes p75^{NTR} molecules at the membrane, and that the change in p75^{NTR} mobility requires the intact cytoplasmic domains of both

p75^{NTR} and TrkA [38]. Conversely, it has also been shown that TrkA mobility at the membrane is regulated by p75^{NTR} [39].

The present study demonstrates that both p75^{NTR} and TrkA are internalized in FRT cells. We also show that endocytosis is necessary for the p75^{NTR}–TrkA interaction. Indeed, the two receptors do not co-immunoprecipitate upon the apical addition of NGF when endocytosis is inhibited. Thus we hypothesize that the two receptors interact following internalization. Our hypothesis is supported by a recent study which found that a green fluorescent protein–TrkA chimaera shows highly dynamic trafficking between the cell surface and the endocytic compartment [40].

Interestingly, our results characterize further the relationship between receptor trafficking and activity. We show for the first time that the physical interaction between TrkA and p75^{NTR} induces TrkA activation and the MAPK signalling cascade. Indeed, TrkA phosphorylation did not occur when the p75^{NTR}–TrkA interaction was abolished by blocking endocytosis. These data further demonstrate that the p75^{NTR}–TrkA interaction occurs in an endocytic compartment, because we show that blockade of endocytosis did not impair signalling starting from the plasma membrane. These results indicate that the p75^{NTR}–TrkA interaction results in the formation of a signalling complex. In agreement with the present study, it has been shown that forced dimerization of TrkA by cross-linking with anti-TrkA antibodies induces TrkA autophosphorylation and a TrkA-dependent signalling cascade [41]. Lee and Chao [42] also reported that activation of TrkA can occur in the absence of NGF. Moreover, it is known that a mutation in the cytoplasmic tail of p75^{NTR} blocks the phosphorylation of intracellular substrates of TrkA upon stimulation by NGF [43]. Indeed, it has been demonstrated recently that p75^{NTR} interacts with Shc, enhancing its TrkA-mediated activation [44]. Moreover, in sympathoadrenal cells, co-expression of p75^{NTR} induces 8-fold higher tyrosine phosphorylation of TrkA [45]. Our data clearly demonstrate that, upon activation, p75^{NTR} can recruit signalling molecules in a complex with TrkA, inducing the activation of a downstream signalling cascade.

The present study has relevance for understanding the role of p75^{NTR} and TrkA in non-neuronal carcinogenesis, especially in epithelial-derived tumours. The signalling proteins that are recruited in FRT cells by the p75^{NTR}–TrkA complex upon stimulation by NGF remain to be elucidated, and we are investigating this further. Interestingly, FRT cells expressing p75^{NTR} represent a useful cellular model for identifying signalling molecules involved in either p75^{NTR} or TrkA signalling. This cellular system enables us to dissect the endocytic trafficking of these receptors and the activation of downstream signalling molecules, shedding new light on the function of these receptors in non-neuronal carcinogenesis.

We thank A. Cattaneo for the helpful discussions. This work was supported by grant from Associazione Italiana per la Ricerca sul Cancro (AIRC) to C. Z.

REFERENCES

1 Lewin, G. and Barde, Y. (1996) Physiology of the neurotrophins. Annu. Rev. Neurosci. **19**, 289–317
2 Ip, N. and Yancopoulos, G. (1996) The neurotrophins and CNTF: two families of collaborative neurotrophic factors. Annu. Rev. Neurosci. **19**, 491–515
3 Kaplan, D. and Stephens, R. (1994) Neurotrophin signal transduction by the Trk receptor. J. Neurobiol. **25**, 1404–1417
4 Meakin, S., Gryz, E. and MacDonald, J. (1997) A kinase insert isoform of rat TrkA supports nerve growth factor-dependent cell survival but not neurite outgrowth. J. Neurochem. **69**, 954–967
5 Bracci-Laudiero, L., Aloe, L., Stenfors, C., Tirassa, P., Theodorsson, E. and Lundberg, T. (1996) Nerve growth factor stimulates production of neuropeptide Y in human lynphocytes. NeuroReport **7**, 485–488
6 Chao, M. and Hempstead, B. (1995) p75 and Trk: a two receptor system. Trends Neurosci. **18**, 321–326
7 Gentry, J., Casaccia-Bonnefil, P. and Carter, B. (2000) Nerve growth factor activation of nuclear factor kappaB through its p75 receptor is an anti-apoptotic signal in RN22 schwannoma cells. J. Biol. Chem. **275**, 7558–7565
8 Rabizadeh, S., Oh, J., Zhong, L., Yang, J., Bitler, C., Butcher, L. and Bredesen, D. (1993) Induction of apoptosis by the low-affinity NGF receptor. Science **261**, 345–348
9 Horikava, I., Miwa, T., Ishimaru, T., Furukawa, M. and Moriizumi, T. (1999) TrkA expression in olfactory epithelium and bulb during development. NeuroReport **10**, 2205–2208
10 Pincelli, C. and Marconi, A. (2000) Autocrine nerve growth factor in human keratinocytes. J. Dermatol. Sci. **22**, 71–79
11 Peeraully, M., Jenkins, J. and Trayhurn, P. (2004) NGF gene expression and secretion in white adipose tissue: Regulation in 3T3-L1 adipocytes by hormones and inflammatory cytokines. Am. J. Physiol. Endocrinol. Metab. **287**, E331–E339
12 Rabizadeh, S., Rabizadeh, S., Ye, X., Wang, J. and Bredesen, D. (1999) Neurotrophin dependence mediated by p75NTR: contrast between rescue by BDNF and NGF. Cell Death Differ. **6**, 1222–1227
13 Gargano, N., Levi, A. and Alema, S. (1997) Modulation of nerve growth factor internalization by direct interaction between p75 and TrkA receptors. J. Neurosci. Res. **50**, 1–12
14 Kramer, B., van der Zee, C. and Hagg, T. (1999) p75 nerve growth factor receptor is important for retrograde transport of neurotrophins in adult cholinergic basal forebrain neurons. Neuroscience **94**, 1163–1172
15 York, R., Molliver, D., Grewal, S., Stemberg, P., McCleskey, E. and Stork, P. (2000) Role of phosphoinositide 3-kinase and endocytosis in nerve growth factor-induced extracellular signal-regulated kinase activation via ras and rap. Mol. Cell. Biol. **20**, 8069–8083
16 Lou, X., Yano, H., Lee, F., Chao, M. and Farquhar, M. (2001) GIPC and GAIP form a complex with TrkA: a putative link between G protein and receptor tyrosine kinase pathways. Mol. Biol. Cell **12**, 615–627
17 Zhang, Z., Moheban, D., Conway, B., Bhattacharyya, A. and Segal, R. (2000) Cell surface Trk receptors mediate NGF-induced survival while internalized receptors regulate NGF-induced differentiation. J. Neurosci. **20**, 5671–5678
18 von Barteld, C., Williams, R., Lefcort, F., Clary, D., Reichardt, L. and Bothwell, M. (1996) Retrograde transport of neurotrophins from the eye to the brain in chick embryos: roles of the p75NTR and TrkB receptors. J. Neurosci. **16**, 2995–3008
19 Kohn, J., Aloyz, R., Toma, J., Haak-Frendscho, M. and Miller, F. (1999) Functionally antagonistic interactions between the TrkA and p75 neurotrophin receptors regulate sympathetic neuron growth and target innervation. J. Neurosci. **19**, 5393–5408
20 Greco, A., Mariani, C., Miranda, C., Lupas, A., Pagliardini, S., Pomati, M. and Pierotti, M. (1995) The DNA rearrangement that generates the TRK-T3 oncogene involves a novel gene on chromosome 3 whose product has a potential coiled-coil domain. Mol. Cell. Biol. **15**, 6118–6127
21 Ranzi, V., Meakin, S., Miranda, C., Mondellini, P., Pierotti, M. and Greco, A. (2003) The signaling adapters fibroblast growth factor receptor substrate 2 and 3 are activated by the thyroid TRK oncoproteins. Endocrinology **144**, 922–928
22 Zurzolo, C., Le Bivic, A., Quaroni, A., Nitsch, L. and Rodriguez-Boulan, E. (1992) Modulation of transcytotic and direct targeting pathways in a polarized thyroid cell line. EMBO J. **11**, 2337–2344
23 Zurzolo, C. and Rodriguez-Boulan, E. (1993) Delivery of Na,K-ATPase in polarized epithelial cells. Science **260**, 550–552
24 Zurzolo, C., Lisanti, M. P., Caras, I. W., Nitsch, L. and Rodriguez-Boulan, E. (1993) Glycosylphosphatidylinositol-anchored proteins are preferentially targeted to the basolateral surface in Fisher Rat Thyroid epithelial cells. J. Cell Biol. **121**, 1031–1039
25 Zurzolo, C., van't Hof, W., van Meer, G. and Rodriguez-Boulan, E. (1994) VIP21/Caveolin, glycosphingolipid clusters, and the sorting of glycosylphosphatidylinositol-anchored proteins in epithelial cells. EMBO J. **13**, 42–53
26 Lipardi, C., Nitsch, L. and Zurzolo, C. (1999) Mechanisms of apical protein sorting in polarized thyroid epithelial cells. Biochemie **81**, 531–542
27 Ilondo, M., Smal, J., de Meyts, P. and Courtoy, P. (1991) Comparison of the effects of hypertonic sucrose and intracellular potassium depletion on growth hormone receptor binding kinetics and down-regulation in IM-9 cells: evidence for a sequential block of receptor-mediated endocytosis. Endocrinology **128**, 1597–1602
28 Lipardi, C., Ruggiano, G., Perrone, L., Paladino, S., Monlauzeur, L., Nitsch, L., Le Bivic, A. and Zurzolo, C. (2002) Differential recognition of a tyrosine-dependent signal in the basolateral and endocytic pathways of thyroid epithelial cells. Endocrinology **143**, 1291–1301
29 Bibel, M., Hoppe, E. and Barde, Y.-A. (1999) Biochemical and functional interactions between the neurotrophin receptors trk and p75NTR. EMBO J. **18**, 616–622

30 Cupp, A., Kim, G. and Skinner, M. (2000) Expression and action of neurotrophin-3 and nerve growth factor in embryonic and early postnatal rat testis development. Biol. Reprod. **63**, 1617–1628
31 Descamps, S., Toillon, R., Adriaenssens, E., Pawlowski, V., Cool, S., Nurcombe, V., Le Bourhis, X., Boilly, B., Peyrat, J. and Hondermarck, H. (2001) Nerve growth factor stimulates proliferation and survival of human breast cancer cells through two distinct signaling pathways. J. Biol. Chem. **276**, 17864–17870
32 Fiorentini, C., Facchetti, M., Finardi, A., Sigala, S., Paez-Pereda, M., Sher, E., Spano, P. and Missale, C. (2002) Nerve growth factor and retinoic acid interaction in the control of small cell lung cancer proliferation. Eur. J. Endocrinol. **147**, 371–379
33 Weeraratna, A., Arnold, J., George, D., DeMarzo, A. and Isaacs, J. (2000) Rational basis for Trk inhibition for prostate cancer. Prostate **45**, 140–148
34 Maliartchouk, S. and Saragovi, H. (1997) Optimal nerve growth factor trophic signals mediated by synergy of TrkA and p75 receptor-specific ligands. J. Neurosci. **17**, 6031–6037
35 Le Bivic, A., Sambuy, Y., Patzak, A., Patil, N., Chao, M. and Rodriguez-Boulan, E. (1991) An internal deletion in the cytoplasmic tail reverses the apical localization of human NGF receptor in transfected MDCK cells. J. Cell Biol. **115**, 607–618
36 Bomsel, M., Prydz, K., Parton, R., Gruenberg, J. and Simons, K. (1989) Endocytosis in filter-grown Madin-Canine Kidney cells. J. Cell Biol. **109**, 3243–3258
37 Odorizzi, G., Pearse, A., Domingo, D., Trowbridge, I. and Hopkins, C. (1996) Apical and basolateral endosomes of MDCK cells are interconnected and contain a polarized sorting mechanism. J. Cell Biol. **135**, 139–152
38 Wolf, D., McKinnon, C., Daou, M., Stephens, R., Kaplan, D. and Ross, A. (1995) Interaction with TrkA immobilizes gp75 in the high affinity nerve growth factor receptor complex. J. Biol. Chem. **270**, 2133–2138
39 Wolf, D., McKinnon-Thompson, C., Daou, M.-C., Stephens, R., Kaplan, D. and Ross, A. (1998) Mobility of TrkA is regulated by phosphorylation and interactions with the low-affinity NGF receptor. Biochemistry **37**, 3178–3186
40 Jullien, J., Guili, V., Derrington, E., Darlix, J., Reichardt, L. and Rudkin, B. (2003) Trafficking of TrkA-green fluorescent protein chimerae during nerve growth factor differentiation. J. Biol. Chem. **278**, 8706–8716
41 Clary, D., Weskamp, G., Austin, L. and Reichardt, L. (1994) TrkA cross-linking mimics neuronal responses to nerve growth factor. Mol. Biol. Cell **5**, 549–563
42 Lee, F. and Chao, M. (2001) Activation of Trk neurotrophin receptors in the absence of neurotrophins. Proc. Natl. Acad. Sci. U.S.A. **98**, 3555–3560
43 Berg, M., Sternberg, D., Hempstead, B. and Chao, M. (1991) The low-affinity p75 nerve growth factor (NGF) receptor mediates NGF-induced tyrosine phosphorylation. Proc. Natl. Acad. Sci. U.S.A. **88**, 7106–7110
44 Epa, W., Markovska, K. and Barrett, G. (2004) The p75 neurotrophin receptor enhances TrkA signalling by binding to Shc and augmenting its phosphorylation. J. Neurochem. **89**, 344–353
45 Verdi, J., Birren, S., Ibanez, C., Persson, H., Kaplan, D., Benedetti, M., Chao, M. and Anderson, D. (1994) p75LNGFR regulates Trk signal transduction and NGF-induced neuronal differentiation in MAH cells. Neuron **12**, 733–745

Received 7 July 2004/10 August 2004; accepted 27 August 2004
Published as BJ Immediate Publication 27 August 2004, DOI 10.1042/BJ20041155