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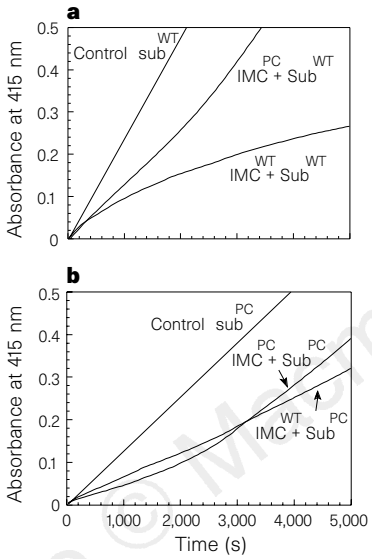
Protein memory through altered folding mediated by intramolecular chaperones

U. P. Shinde, J. J. Liu & M. Inouye

Nature **389,** 520–522 (1997)

Three of the superscripts in Fig. 3b of this Letter were incorrect. The amended figure is shown below. □



G-protein-coupled receptor of Kaposi's sarcoma-associated herpesvirus is a viral oncogene and angiogenesis activator

Carlos Bais, Bianca Santomasso, Omar Coso, Leandros Arvanitakis, Elizabeth Geras Raaka, J. Silvio Gutkind, Adam S. Asch, Ethel Cesarman, Marvin C. Gershengorn & Enrique A. Mesri

Nature **391,** 86–89 (1998)

The name of the penultimate author of this Letter was misspelled: his name is Marvin C. Gershengorn. □

correction

A GPI-linked protein that interacts with Ret to form a candidate neurturin receptor

Robert D. Klein, Daniel Sherman, Wei-Hsien Ho, Donna Stone, Gregory L. Bennett, Barbara Moffat, Richard Vandlen, Laura Simmons, Qimin Gu, Jo-Anne Hongo, Brigitte Devaux, Kris Poulsen, Mark Armanini, Chika Nozaki, Naoya Asai, Audrey Goddard, Heidi Phillips, Chris E. Henderson, Masahide Takahashi & Arnon Rosenthal

Nature **387,** 717–721 (1997)

An error in the designation of address symbols has caused confusion over the following authors' affiliations: R.D.K.'s present address is at Deltagen Inc.; L.S. Q.G. and A.G. are at Genentech Inc. □

fractions were loaded on a reverse-phase column and eluted using a linear gradient of 2–80% acetonitrile. DC-CK1 protein concentration was estimated by densitometric scanning of a coomassie blue-stained gel containing lysozyme as a standard. RANTES, MIP-1α and IL-8 were obtained from R&D Systems. T-cell migration was measured using 48-well chemotaxis chambers (Neuroprobe) as described[19]. In brief, chemokines in RPMI-1640 were added to the lower chamber and were separated from 10^5 cells in RPMI-1640 with 10% FCS by either a 8-μm or a 5 μm PVP-free polycarbonate membrane (Poretics, Livermore). After incubation for 1 h, the membrane was removed and the upper side washed with PBS, scraped to remove residual cells and washed again. After methanol fixation and staining, the number of fully migrated cells was counted microscopically in 5 high-power fields (× 400) per well. Pertussis toxin (100 ng ml^{-1}) (Calbiochem) was used for 2 h. Each experiment was performed in duplicate, and experiments with DC-CK1, RANTES, MIP-1α and IL-8 were performed in parallel in the same assay to make a direct comparison of their activities possible.

***In situ* hybridization.** Cryosections (8 μm) of tonsils and lymph nodes were fixed in 4% paraformaldehyde and pretreated with 2 μg ml^{-1} pepsin in 0.2 M HCl for 10 min and 0.1 M triethanol amine/0.25 acetic acid anhydride for 10 min. Sections were hybridized overnight with either a sense or an antisense DIG-labelled DC-CK1 RNA probe consisting of the 3′ non-coding region generated by *in vitro* transcription (Boehringer Mannheim). Before incubation with anti-DIG-alkaline phosphatase monoclonal antibody the sections were treated with 40 U ml^{-1} RNAse I (Promega) to ensure specificity. After incubation for 2–3 h with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (Boehringer Mannheim) the sections were stained with methylene green and embedded in Kaiser's. Immunostaining was performed as described[20].

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A GPI-linked protein that interacts with Ret to form a candidate neurturin receptor

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Glial-cell-line-derived neurotrophic factor (GDNF) and neurturin (NTN) are two structurally related, potent survival factors for sympathetic, sensory and central nervous system neurons[1–6]. GDNF mediates its actions through a multicomponent receptor system composed of a ligand-binding glycosyl-phosphatidylinositol (GPI)-linked protein (designated GDNFR-α) and the transmembrane protein tyrosine kinase Ret[7–12]. In contrast, the mechanism by which the NTN signal is transmitted is not well understood. Here we describe the identification and tissue distribution of a GPI-linked protein (designated NTNR-α) that is structurally related to GDNFR-α. We further demonstrate that NTNR-α binds NTN (K_d∼10 pM) but not GDNF with high affinity; that GDNFR-α binds to GDNF but not NTN with high affinity; and that cellular responses to NTN require the presence of NTNR-α. Finally, we show that NTN, in the presence of NTNR-α, induces tyrosine-phosphorylation of Ret, and that NTN, NTNR-α and Ret form a physical complex on the cell surface. These findings identify Ret and NTNR-α as signalling and ligand-binding components, respectively, of a receptor for NTN and define a novel family of receptors for neurotrophic and differentiation factors composed of a shared transmembrane protein tyrosine kinase and a ligand-specific GPI-linked protein.

In searching for a neurturin receptor, we examined sequences deposited in public databases for similarity to the GDNF receptor α component (GDNFR-α)[9,10]. Eight partial human cDNAs (Genbank accession numbers R02249, H12981, W73681, W73633, H05619, R02135, T03342 and HSC1KA111) were identified and found to encode parts of a single protein of 464 amino acids which we designated neurturin receptor α (NTNR-α). The human proteins hNTNR-α and hGDNFR-α display an overall 48% similarity, and the positions of their cysteine residues are conserved (Fig. 1). Both hNTNR-α and hGDNFR-α seem to be extracellular proteins that are attached to the outer cell membrane by means of a glycosyl-phosphatidyl inositol (GPI) modification. hNTNR-α has an amino-terminal signal peptide for secretion, three glycosylation sites, and a stretch of 17 carboxy-terminal hydrophobic amino acids preceded by a group of three small amino acids (Gly, Ser, Asn) defining a cleavage/binding site for GPI linkage (Fig. 1). Subsequently, a rat NTNR-α (rNTNR-α) was isolated and shown to be 94% identical to its human homologue (Fig. 1).

To examine whether NTNR-α could be a receptor for a neurotrophic factor such as NTN, the tissue distribution of NTNR-α mRNA was examined by *in situ* hybridization and compared with

that of GDNFR-α (Fig. 2 and data not shown). In the embryonic rat nervous system, mRNA for NTNR-α was found in the ventral midbrain where dopaminergic neurons are located, in parts of the ventral spinal cord where motor neurons are present and in the dorsal root ganglia (DRG) where sensory neurons reside. In addition, high levels of NTNR-α transcripts were found in developing sympathetic ganglia and peripheral nerves. Low levels of NTNR-α mRNA were detected in non-neuronal tissues, such as smooth and striated muscle of the embryonic gut, oesophagus, diaphragm and limb bud, which express high levels of GDNFR-α transcripts. In the adult rat brain, NTNR-α mRNA was detected in the substantia nigra, cortex, olfactory bulb and the dorsal horn of the spinal cord. Although NTNR-α mRNA and GDNFR-α mRNA were often coexpressed, the two transcripts occasionally displayed distinct tissue distribution. For instance, in the limb, GDNFR-α is expressed mainly in muscle cells but also around the brachial plexus, which innervates the muscle, whereas NTNR-α is found mainly in the brachial plexus. Similarly, in the embryonic bladder, NTNR-α is expressed in the muscle layer, whereas GDNFR-α is expressed in the underlying epithelia. Finally, in the gut, NTNR-α is expressed in the mucosal epithelium as well as in smooth muscle whereas GDNFR-α is expressed only in smooth muscle (Fig. 2). This pattern of expression is consistent with the hypothesis that NTNR-α mediates signals both inside and outside the nervous system, and suggests overlapping, yet distinct, biological roles for NTNR-α and GDNFR-α.

To determine whether NTNR-α could function as a receptor for NTN, binding experiments were performed. Equilibrium binding of human or mouse ^{125}I-NTN to recombinant soluble human (data not shown) or rat NTNR-α protein demonstrated that NTN binds specifically and reversibly to NTNR-α, and that the two proteins associate with an approximate K_d of 10 pM (Fig. 3). In contrast, neither human nor mouse NTN (at 1 nM) were able to displace ^{125}I-rGDNF from rGDNFR-α, and no binding of human or mouse ^{125}I-NTN to rGDNFR-α was detected. Taken together, these data are consistent with the idea that NTN interacts at high affinity solely with NTNR-α (Fig. 3). Although no high-affinity interaction between NTN and GDNFR-α was detected, we did observe, using higher concentrations of unlabelled NTN (10 nM), displacement of ^{125}I-rGDNF from GDNFR-α, suggesting that there is a low-affinity interaction between NTN and GDNFR-α ($K_d > 1$ nM) (data not shown).

To examine further whether NTNR-α is a specific receptor for NTN, competition binding experiments were performed using ^{125}I-rGDNF. Displaceable high-affinity binding of ^{125}I-rGDNF to recombinant soluble rGDNFR-α was readily observed ($K_d = 3$ pM) (Fig. 3), but no binding of ^{125}I-rGDNF (iodinated either on primary amino groups by using the Bolton-Hunter method, or on tyrosines by using the lactoperoxidase method) could be detected to either hNTNR-α or rNTNR-α. Similarly, no significant displacement of ^{125}I-labelled mouse NTN (^{125}I-mNTN) from NTNR-α was detected in the presence of unlabelled rGDNF (up to 1 nM). Thus GDNF seems to bind with high affinity to GDNFR-α but not to NTNR-α. As before, displacement of ^{125}I-mNTN from NTNR-α was observed when a high concentration (10 nM) of unlabelled GDNF was used, suggesting a low-affinity interaction between GDNF and NTNR-α ($K_d > 1$ nM) (data not shown). The specific high-affinity interactions between GDNF and GDNFR-α, and NTN and NTNR-α, were confirmed using competition binding to intact cells that express individual receptors (data not shown). Consistent with the prediction that NTNR-α is anchored to the cell surface by a GPI linkage, the binding of ^{125}I-NTN to cells expressing NTNR-α was significantly reduced by treatment with phosphoinositide-specific phospholipase C (PIPLC), an enzyme that specifically cleaves GPI linkages[13] (Fig. 4a). Taken together, these findings support the idea that NTNR-α is a high-affinity, GPI-linked binding protein for NTN, and that GDNFR-α is a specific high-affinity binding protein for GDNF.

Consistent with the idea that NTNR-α participates in the response to NTN, we found that NTN can prevent the death of two populations of neurons that express NTNR-α: primary embryonic dopaminergic neurons (data not shown), and motor neurons (Fig. 4b). Moreover, in agreement with the finding that NTN and GDNF use distinct receptors, we detected differences in the efficacy (but not in the potency) of the two factors. Whereas GDNF at saturating concentrations promoted the survival of 100% of BDNF-responsive motor neurons, NTN prevented the death of only 50% of these cells (Fig. 4b).

To confirm that NTNR-α is a required mediator of the NTN signal, embryonic motor neurons were treated with PIPLC, and their survival in the presence of NTN or BDNF was monitored in culture. The capacity of saturating concentrations of NTN to support the survival of embryonic rat motor neurons was completely abolished by PIPLC treatment, whereas no decrease in the

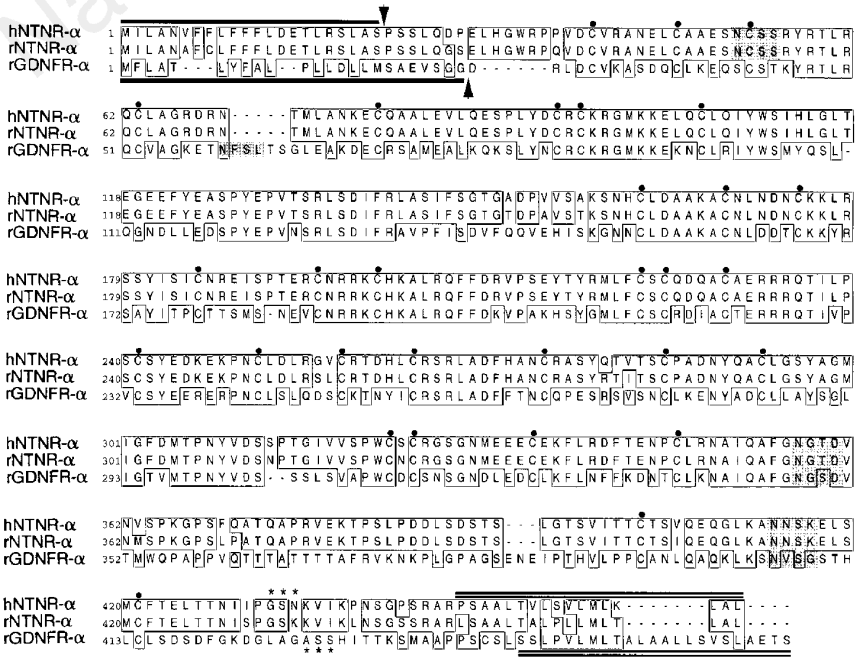

Figure 1 Primary structure of NTNR-α, and their homology to rat GDNFR-α. Signal peptides are indicated by solid lines, putative signal peptide cleavage sites are marked with arrows, potential glycosylation sites are shaded, the hydrophobic domains of the GPI attachment sites are doubly underlined, the small amino-acid residues that constitute the cleavage/attachment site for GPI-linked proteins are marked with asterisks, and the consensus cysteine residues are indicated by filled circles.

response of motor neurons to brain-derived neurotrophic factor (BDNF) was caused by PIPLC. Moreover, when NTN was added to PIPLC-treated motor neurons in combination with soluble NTNR-α, the response to NTN (Fig. 4c), but not to GDNF (data not shown), was restored. Thus NTNR-α is a necessary and specific component of the NTN signalling cascade and has the properties expected of the ligand-binding subunit of a functional NTN receptor.

NTNR-α, like GDNFR-α, lacks a cytoplasmic domain and is anchored to the outer surface of the cell by GPI. Thus, transmission of the NTN signal to the interior of the cell must involve additional proteins. As the tyrosine-kinase receptor Ret, which by itself does not bind GDNF[9,10] or NTN (data not shown), was found to be a signalling component of the GDNF receptor, we examined the possibility that it would also transduce the NTN signal following binding of NTN to NTNR-α. The human neuroblastoma TGW-I-nu cell line, which expresses endogenous c-*ret*[14,15], was exposed to NTN for 5 min, and the level of Ret tyrosine phosphorylation was determined. NTN induced phosphorylation of Ret (Fig. 4d), as well as two isoforms of the cytoplasmic kinase ERK (MAP kinase) (Fig. 4e) in this cell line. Furthermore, consistent with the idea that NTNR-α is a necessary mediator of the response to NTN, only residual tyrosine phosphorylation of Ret in response to NTN was observed in PIPLC-treated TGW-I-nu cells (Fig. 4d). The amount of tyrosine-phosphorylated Ret protein was greatly increased in PIPLC-treated TGW-I-nu cells when NTN was added together with a soluble NTNR-α (Fig. 4d).

As these findings suggested that Ret participates in the transmission of the NTN signal, we investigated whether it is part of a putative NTN receptor complex. TGW-I-nu cells were exposed to NTN and then lysed with a mild detergent. Protein complexes were immunoprecipitated with a polyclonal antibody to Ret and then analysed on a western blot using a polyclonal antibody to NTN. Consistent with the idea that NTN and Ret interact physically on the cell surface, NTN was readily co-immunoprecipitated by Ret antibodies (Fig. 4f). To confirm that NTNR-α is also present in the NTN–Ret protein complex, human embryonic kidney 293 cells were transiently transfected with c-*ret* alone, with an epitope-tagged NTNR-α alone, or with a combination of expression vectors for c-*ret* and an epitope-tagged NTNR-α, and were exposed to NTN before being lysed with a mild detergent[16]. Protein complexes were then immunoprecipitated with polyclonal antibody to Ret and analysed on a western blot using a monoclonal antibody to the epitope-tagged NTNR-α. No protein complex could be detected in cells that were transfected with Ret alone or with NTNR-α alone, either in the presence or absence of NTN (Fig. 4g, and data not shown). In contrast, in cells that expressed both proteins, residual NTNR-α could be co-immunoprecipitated by Ret antibodies in the absence of NTN. Further, addition of NTN led to a significant increase in the amount of NTNR-α that could be co-immunoprecipitated by the Ret antibodies (Fig. 4g). These findings support the hypothesis that NTN, NTNR-α and Ret form a complex on the cell surface; that Ret and NTNR-α are components of a functional NTN receptor; and that NTNR-α is an important intermediary in the interaction between NTN and Ret.

Taken together, our results define a candidate receptor for NTN and demonstrate the existence of a family of multicomponent receptors composed of a shared signalling subunit, the transmembrane tyrosine kinase receptor Ret, and receptor-specific ligand-binding subunits that are GPI-linked (Fig. 5). The findings are consistent with the observation that Ret-deficient mice display more severe deficits in the superior cervical ganglion sympathetic

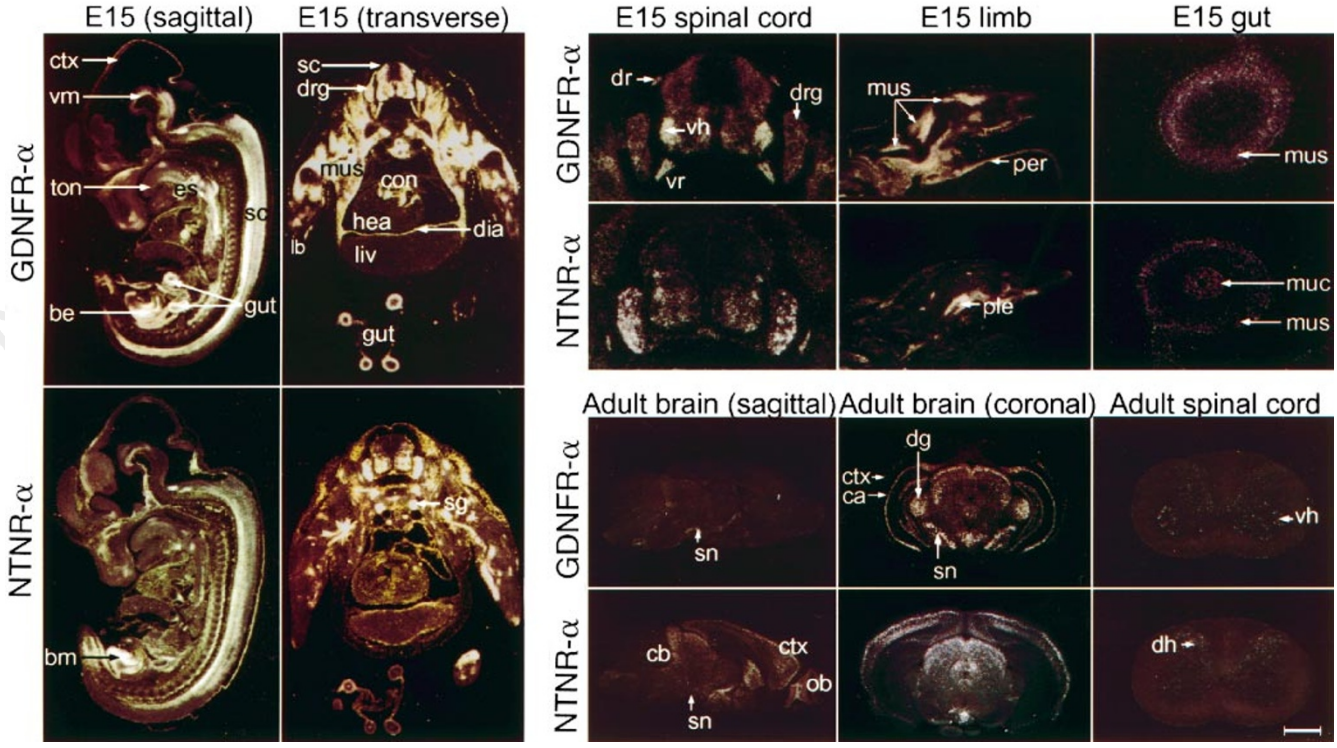

Figure 2 Tissue distribution of mRNA for NTNR-α and GDNFR-α. Abbreviations: vm, ventral midbrain; es, oesophagus; ctx, cortex; sc, spinal cord; ton, tongue; be, bladder epithelium; sg, sympathetic ganglia; bm, bladder muscles; dia, diaphragm; mus, muscle; con, cardiac conductive system; sn, substantia nigra; dg, dentaqte gyrus; ob, olfactory bulb, cb, cerebellum; ca, cornus ammonis; dr, dorsal root; drg, dorsal root ganglia; vr, ventral root; vh, ventral horn; muc, mucosal epithelial; pte, brachial plexus nerves; liv, liver; hea, heart. Scale bar, 1.1 mm in E15 sagittal section; 800 μm in E15 transverse section; 240 μm in E15 spinal cord; 590 μm in E15 limb; 93 μm in E15 gut; 3.6 mm in adult brain (sagittal); 2.4 mm in adult brain (coronal); 900 μm in adult spinal cord. The E15 transverse and spinal cord sections were exposed for different times.

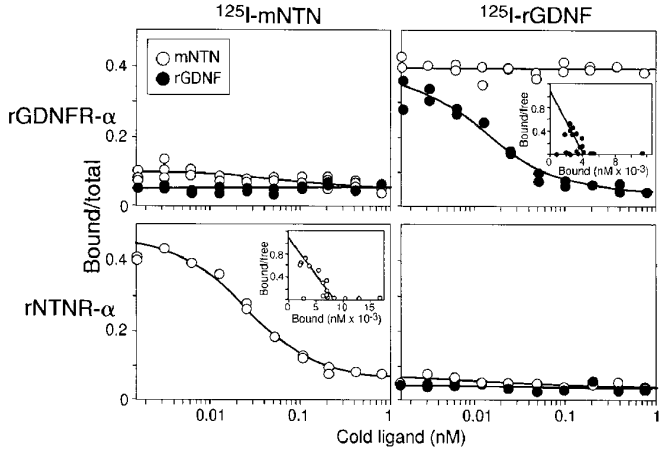

Figure 3 Binding of iodinated NTN and GDNF to NTNR-α and GDNFR-α. Binding of ^{125}I mouse NTN (^{125}I-mNTN) (5 pM) or ^{125}I rat GDNF (^{125}I-rGDNF) (5 pM) to rat NTNR-α (rNTNR-α) or rat GDNFR-α (rGDNFR-α). Human NTNR-α displayed a similar binding specificity to rat NTNR-α (data not shown). As depicted by the Scatchard analysis (insets), GDNF binds GDNFR-α with an approximate K_d value of 3 pM (similar K_d values were reported in a cell-based assay[10]), and NTN binds NTNR-α with an approximate K_d value of 10 pM.

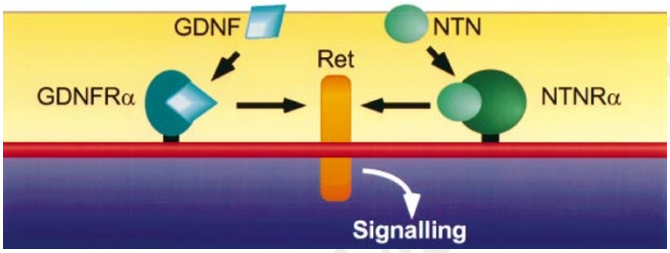

Figure 5 A schematic representation of activation of Ret by NTN or GDNF. NTN and GDNF each bind to a unique GPI-linked ligand-binding protein. This ligand–binding protein complex can bind to and activate a shared transmembrane tyrosine kinase receptor, Ret.

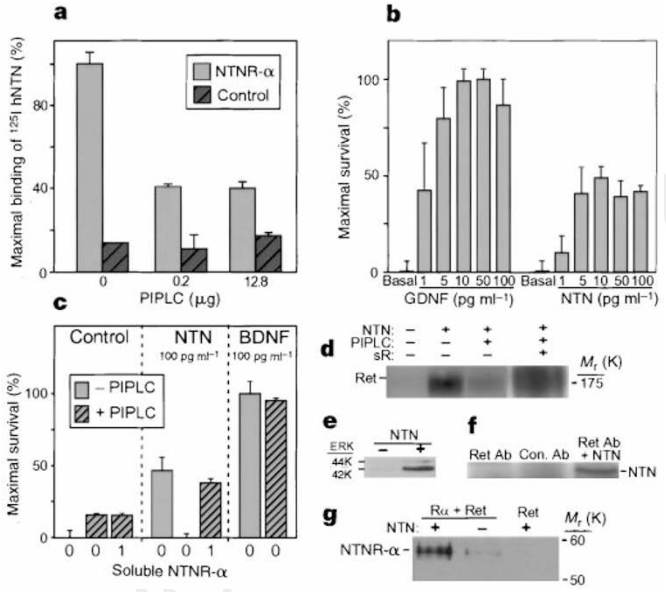

Figure 4 Interaction between NTN, NTNR-α and Ret. **a**, Binding of ^{125}I-NTN to cells expressing NTNR-α was reduced by 60% after treatment with PIPLC. **b**, Survival response of embryonic rat spinal motor neurons to GDNF or NTN, expressed as a percentage of the maximal survival in the presence of GDNF. In agreement with its receptor distribution, NTN is a potent survival factor for spinal motor neurons. **c**, Survival response of embryonic, rat spinal motor neurons to NTN or BDNF in the presence of PIPLC and soluble NTNR-α. PIPLC treatment completely abolished the survival response to NTN without changing the response to BDNF. Soluble NTNR-α restored the response of PIPLC-treated motor neurons to NTN. **d**, NTN induces tyrosine phosphorylation of Ret in TGW-I-nu cells. Phosphorylation was significantly reduced in the presence of PIPLC and is restored in the presence of soluble NTNR-α (SR). **e**, NTN induces phosphorylation of ERK in TGW-I-nu cells. **f**, NTN and Ret form a complex in TGW-I-nu cells. **g**, Recombinant NTNR-α and Ret form a protein complex in 293 cells. The amount of this protein complex is significantly increased in the presence of NTN. Ret, cells transfected with Ret alone. Rα + Ret, cells transfected with Ret and NTNR-α.

neurons[17,18] than GDNF-deficient mice[19–21] and suggest that the distinct biological activities of the GDNF and NTN may be determined by the different tissue distributions of their respective ligand-binding receptor components, rather than by their ability to activate different signalling systems.

Our findings provide a biological rationale for the evolution of what appeared to be a needlessly complex way to activate a tyrosine kinase[22]. With the discovery that Ret is shared by GDNF and NTN, it is now apparent that changing the ligand-binding molecule allows recruitment of the same signalling system by multiple growth factors. Similar 'cost-effective' strategies to make use of a single transmembrane signalling system by multiple growth factors appear to be used by cytokines[16,23], members of the transforming growth factor protein family[24], and bacterial endotoxins[25,26].

The discovery of this receptor system further defines a new model of signal transduction and highlights the diverse strategies that are used to transmit extracellular signals in the vertebrate nervous system. □

Methods

NTNR-α cloning and expression constructs. The partial cDNA sequence encoded by the expressed sequence tag (EST) cDNA clones was extended using 5′ and 3′ Marathon RACE reactions (Clontech) on human spleen mRNA, using conditions supplied by the manufacturer. Additional cDNA clones for NTNR-α were identified by screening a human fetal brain cDNA library (Stratagene) and a rat brain cDNA library (Clontech) using standard protocols. For mammalian protein expression, the complete open reading frame (ORF) was amplified using PCR and cloned into a CMV-based expression vector. The NTNR-α–IgG expression construct was made by cloning the first 432 amino acids of the receptor (which lacks a GPI linkage site) in front of the human Fc (IgG2a) sequence. For co-precipitation experiments, an epitope tag was inserted between the signal peptide and the mature coding sequence of NTNR-α. Soluble NTNR-α was produced as a C-terminal His-tagged protein in HEK 293 cells, purified by Ni-NTA chromatography[27], and tested for NTN binding.

***In situ* hybridization.** Rat embryos at embryonic day 15.5 (E15.5) were immersion-fixed overnight at 4 °C in 4% paraformaldehyde, then cryoprotected overnight in 15% sucrose. Adult rat brains and spinal cords were frozen fresh. All tissues were sectioned at 16 μm, and processed for *in situ* hybridization using ^{33}P-UTP labelled RNA probes[3]. Sense and antisense probes were derived for the N-terminal regions of NTNR-α or GDNFR-α using T7 polymerase.

Equilibrium binding analysis. For receptor-binding experiments, conditioned media from 293 cells transiently transfected with the NTNR-α–IgG or GDNFR-α–IgG constructs were incubated with approximately 5 pM ^{125}I-hNTN, ^{125}I-mNTN or ^{125}I-rGDNF, along with the appropriate cold ligand at concentrations between 0.8 nM and 1.56 pM (2-fold dilutions) in PBS containing 2 mg ml^{-1} BSA (Sigma) and 0.05% Brij 96 (Fluka) for 4 h at room temperature (in the absence of cold ligands, 15–25% of the receptors were occupied). Protein A Sepharose CL-4B beads (~50 μl; Pharmacia) were added to each reaction and the receptor–ligand complex was precipitated after

incubation for 1 h at room temperature. After washing with PBS containing 0.2 mg ml^{-1} BSA, specific counts were measured. The IGOR program was used to determine K_d. Cell-based equilibrium binding analysis[9] was used to confirm the specificity of GDNFR-α and NTNR-α for GDNF and NTN, respectively. Each binding experiment was repeated at least 4 times. For PIPLC analysis, 293 cells transiently transfected with either the full-length NTNR-α expression construct or an irrelevant plasmid were incubated with ~20,000 c.p.m. ^{125}I-hNTN in the presence of the indicated amounts of PIPLC for 90 min at room temperature. The cells were washed with ice-cold PBS containing 0.2 mg ml^{-1} BSA, and cell-associated ^{125}I was measured.

Survival assays. E14 rat motor neurons were purified, plated and grown in duplicate wells in L15 medium with the N2 supplement and 2% horse serum[28]. After 3 days in culture, only 30–40% of the motor neurons initially present survived in basal medium, whereas at saturating concentrations of GDNF or BDNF nearly all motor neurons remained alive. The increase in the number of surviving neurons in the presence of saturating concentrations of GDNF compared to basal medium was taken as 100% (maximal) survival; percentage of maximal survival under the indicated conditions is shown in Fig. 3b. PIPLC (2 μg ml^{-1}) was added to the indicated samples 2 h before, as well as 12 h and 24 h after, addition of the indicated growth factors.

Tyrosine phosphorylation. To assay for tyrosine phosphorylation, cells were incubated for 1 h at 37 °C with or without PIPLC and then exposed to NTN with or without soluble NTNR-α (10 μg ml^{-1}) for 5–10 min at 37 °C. Cells were then removed from the plates with 2 mM EDTA in PBS and lysed with ice-cold buffer (comprising, in mM 10 sodium phosphate (pH 7.0), 100 NaCl, 1% NP40, 5 EDTA, 100 sodium vanadate, 2 mM PMSF, and 0.2 units of aprotinin) and used for immunoprecipitation with antiserum raised against the 19 amino-acid C terminus of Ret, following by binding to protein A–Sepharose. The immunoprecipitated proteins were released by boiling in SDS sample buffer, separated on an 8% SDS-polyacrylamide gel, transferred to a nitrocellulose membrane, and reacted with anti-phosphotyrosine antibody (Upstate Biotechnology). Detection was with an ECL western blotting detection system (Amersham Life Science).

Co-immunoprecipitation. To examine the formation of protein complexes, TGW-I-nu cell were exposed to 500 ng ml^{-1} of NTN. Protein complexes were immunoprecipitated with Ret antibodies, transferred onto a nitrocellulose filter, and analysed with polyclonal antibody against NTN. Alternatively, HEK 293 cells were transiently transfected with expression vectors for Ret, an epitope-tagged NTNR-α, or a combination of an epitope-tagged NTNR-α and Ret. Cells were stimulated with NTN as indicated and lysed with brij 96 detergent (Fluka)[16]. Putative immune complexes were immunoprecipitated with a polyclonal antibody against Ret, transferred onto a nitrocellulose filter, and analysed with a monoclonal antibody against the epitope-tagged NTNR-α.

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Neurturin responsiveness requires a GPI-linked receptor and the Ret receptor tyrosine kinase

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Neurturin (NTN)[1] is a recently identified homologue of glial-cell-line-derived neurotrophic factor (GDNF)[2]. Both factors promote the survival of a variety of neurons[1–5], and GDNF is required for the development of the enteric nervous system and kidney[6–8]. GDNF signals through a receptor complex consisting of the receptor tyrosine kinase Ret and a glycosyl-phosphatidylinositol (GPI)-linked receptor termed GDNFR-α[9–13]. Here we report the cloning of a new GPI-linked receptor termed NTNR-α that is homologous with GDNFR-α and is widely expressed in the nervous system and other tissues. By using microinjection to introduce expression plasmids into neurons, we show that coexpression of NTNR-α with Ret confers a survival response to neurturin but not GDNF, and that coexpression of GDNFR-α with Ret confers a survival response to GDNF but not neurturin. Our findings indicate that GDNF and neurturin promote neuronal survival by signalling through similar multicomponent receptors that consist of a common receptor tyrosine kinase and a member of a GPI-linked family of receptors that determines ligand specificity.