ribonuclease-free deoxyribonuclease RQ1, extracted with phenol-chloroform, precipitated with ethanol, and dissolved in diethylpyrocarbonate-treated water to a concentration of ~1 mg/ml. For *ras* mRNA synthesis, a 0.7-kb Eco RI fragment from plasmid pJCL30 (*9*) containing the H-*ras*^{lys12} gene was subcloned into pGEM2. The resulting construct was linearized with Bam HI and transcribed in vitro with the same conditions as for *trk*.

18. Groups of two oocytes were collected before fixation with trichloroacetic acid and homogenized in buffer (20 μl per oocyte) containing 20 mM Hepes, pH 7.4, 100 mM B-glycerophosphate, 10 mM EGTA, 15 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM phenylmethylsulfonylfluoride, aprotinin (10 μg/ml), and leupeptin (10 μg/ml). Clarified extracts (10 μl) were incubated in a final volume of 15 μl with 100 μM adenosine triphosphate (ATP), 4 μCi of [γ-^{32}P]-ATP (3000 Ci/mmol) (Amersham), and 3 μg of histone H1 (Sigma) for 15 min at room temperature. The reaction was terminated by adding SDS-electrophoresis sample buffer and boiling for 3 min. Phosphorylations were visualized by autoradiography after electrophoresis on 10 to 20% polyacrylamide gradient minigels.
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Chimeric NGF-EGF Receptors Define Domains Responsible for Neuronal Differentiation

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To determine the domains of the low-affinity nerve growth factor (NGF) receptor required for appropriate signal transduction, a series of hybrid receptors were constructed that consisted of the extracellular ligand-binding domain of the human epidermal growth factor (EGF) receptor (EGFR) fused to the transmembrane and cytoplasmic domains of the human low-affinity NGF receptor (NGFR). Transfection of these chimeric receptors into rat pheochromocytoma PC12 cells resulted in appropriate cell surface expression. Biological activity mediated by the EGF-NGF chimeric receptor was assayed by the induction of neurite outgrowth in response to EGF in stably transfected cells. Furthermore, the chimeric receptor mediated nuclear signaling, as evidenced by the specific induction of transin messenger RNA, an NGF-responsive gene. Neurite outgrowth was not observed with chimeric receptors that contained the transmembrane domain from the EGFR, suggesting that the membrane-spanning region and cytoplasmic domain of the low-affinity NGFR are necessary for signal transduction.

THE BIOLOGICAL ACTIVITIES OF NGF, like those of EGF, are mediated by cell-surface receptor proteins that span the plasma membrane (*1, 2*). Receptors for NGF exist in a high- and low-affinity form. However, it is unclear whether the low-affinity form is capable of participating in signal transduction. To investigate the function of the low-affinity NGFR represented by the p75^{NGFR} protein (*2*), and the structural requirements necessary to elicit biological responses by NGF, we constructed chimeric receptors that consisted of various domains from the human EGFRs and NGFRs and tested their ability to carry out signal transduction.

The cDNA sequences (*3*) that coded for the ligand-binding domain of the human EGFR (amino acid residues −24 to 541) were fused with the sequences that encoded the transmembrane and cytoplasmic sequences (amino acids 188 to 399) of the human p75^{NGFR} (*2*), creating EN10 cDNA (Fig. 1). We also constructed two other chimeric receptor cDNAs with a similar strategy (Fig. 1, EN30 and EN31). EN30 resulted from a ligation between the extracellular domain of the EGFR (amino acids −24 to 619) with p75^{NGFR} transmembrane and cytoplasmic sequences (amino acids 204 to 399), and EN31 consisted of EGFR ligand-binding and transmembrane domains (amino acids −24 to 647) fused to only the cytoplasmic domain (amino acids 250 to 399) of the NGFR. Each hybrid cDNA was introduced into either the pMV7 (*4*) or pCMV5 (*5*) mammalian expression vectors.

Rat PC12 cells were transfected with each plasmid, and stable transfectants were selected by resistance to G418. Expression of the chimeric proteins was assessed by rosetting analysis (*2*) with the R1 monoclonal antibody, which recognizes the human EGFR (*6*). Positive clones were selected and used for immunoprecipitation and cross-linking analyses. Transfected cells were labeled with ^{35}S-labeled Cys and Met, and receptors were immunoprecipitated with the R1 antibody. In cells that expressed the EN10 and EN30 cDNAs, the chimeric receptors were compared to the native EGFR in A431 cells (Fig. 2A). The chimeric receptors migrated at an apparent molecular size of 140 kD, as would be expected for a receptor that contained the comparatively shorter NGFR cytoplasmic domain. Endogenous EGFRs were not detected, as the R1 antibody was specific for human EGFRs (*6*).

Treatment of PC12 cells with NGF led to morphological differentiation into a sympathetic cell phenotype (*7*). No changes in morphology were observed with cells treated with EGF (Fig. 3). We tested the morphological response to NGF and EGF in transfected cells that contained chimeric receptors. All clones responded to NGF at concentrations necessary to observe biological responses (1 to 10 ng/ml) (*8*). In clonal cell lines that contained the EN10 and EN30 chimeric receptors, EGF induced cell flattening within hours and extensive neurite outgrowth after 3 days of treatment with EGF. From 40 to 75% of the EN10- and EN30-containing cell lines showed a phase bright neuronal-like morphology in response to EGF, and the majority of processes were greater than one cell diameter and approached several hundred micrometers. The response of these cells to treatment with concentrations of EGF between 0.5 to 5 ng/ml was qualitatively similar to their

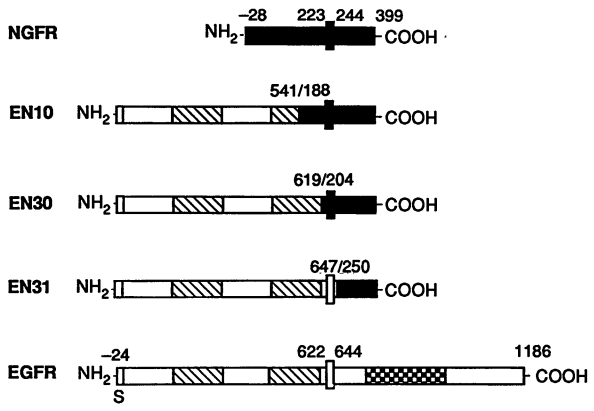

Fig. 1. Schematic representation of EGF and NGF chimeric receptors. The sequences are aligned at their transmembrane domains, shown by the vertical bar. The reading frame was verified by DNA sequencing. Filled region, NGFR; open regions, EGFR; S, signal peptide; striped regions, cysteine rich; checkerboard region, tyrosine kinase domain.

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response with NGF.

Control neomycin-resistant PC12 cells and cells transfected with full-length human EGFR cDNAs did not display any morphological response to EGF (*9*). PC12 cells that expressed the EN31 chimeric receptor did not respond to EGF, even at high concentrations (100 ng/ml). Taken together, these results imply that the response observed with EGF treatment was mediated by receptor domains from the NGFR. Our failure to observe differentiation by EGF with the EN31 chimeric receptor suggests that the transmembrane domain of the low-affinity NGFR is necessary for appropriate receptor signaling.

The lack of a response from cell lines transfected with the EN31 chimeric receptor was not due to a lack of receptor expression or its inability to bind EGF. We detected EN31 receptors by cross-linking with ^{125}I-labeled EGF, followed by immunoprecipitation with antibodies to the cytoplasmic domain of the human p75^{NGFR} (anti-NGFR). The chimeric EN31 receptor bound to ^{125}I-labeled EGF and was recognized by anti-NGFR (Fig. 2B, lane 6). Similar results were obtained with other chimeric receptor constructs. PC12 cells also expressed EGFRs that could be cross-linked to ^{125}I-labeled EGF but were not immunoprecipitated with the anti-NGFR (Fig. 2B, lanes 3 and 4). By this approach, we estimate the number of EN31 receptors to be one-fourth the number of endogenous EGFRs (Fig. 2B). In general, the numbers of chimeric receptors in transfected cells were less than those of endogenous EGFRs. Therefore, attempts to quantitate binding with ^{125}I-labeled EGF to the chimeric receptor were uninformative. Endogenous NGFRs were not cross-linked to EGF but could be detected by cross-linking with ^{125}I-labeled NGF (Fig. 2B, lane 5). These experiments indicate that the chimeric receptors were expressed on the cell surface and were capable of binding EGF.

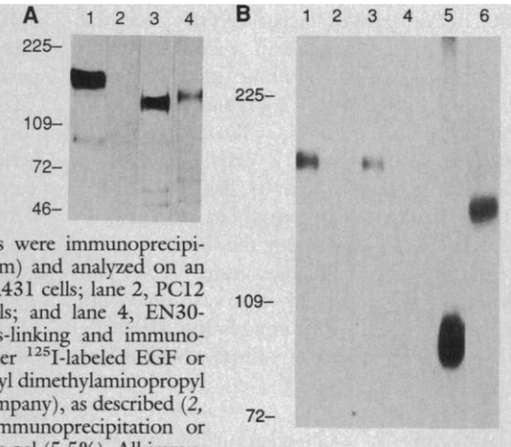

Fig. 2. Expression of chimeric receptors by immunoprecipitation. (**A**) Metabolic labeling and immunoprecipitation analysis. PC12 cells were transfected with either EN10 or EN30 cDNAs and pMV7 (neo$^+$), and stable clones were isolated by rosette analysis after growth in G418 (0.6 mg/ml). Cells were starved in Met-Cys–free media for 30 min and labeled with ExpressLabel (Du Pont Biotechnology Systems) (50 μCi/ml) overnight. Proteins were immunoprecipitated with the R1 antibody (*6*) (Amersham) and analyzed on an SDS-polyacrylamide gel (5.5%). Lane 1, A431 cells; lane 2, PC12 cells; lane 3, EN10-transfected PC12 cells; and lane 4, EN30-transfected PC12 cells. (**B**) Affinity cross-linking and immunoprecipitation. Cells were treated with either ^{125}I-labeled EGF or ^{125}I-labeled NGF and cross-linked with ethyl dimethylaminopropyl carbodiimide (EDAC, Pierce Chemical Company), as described (*2, 20*). Samples were then processed by immunoprecipitation or loaded directly onto an SDS-polyacrylamide gel (5.5%). All immunoprecipitations were carried out with polyclonal anti-NGFR. Lane 1, A431 cells cross-linked with ^{125}I-labeled EGF; lane 2, same as lane 1 plus immunoprecipitation; lane 3, PC12 cells cross-linked with ^{125}I-labeled EGF; lane 4, same as lane 3 plus immunoprecipitation; lane 5, PC12 cells cross-linked with ^{125}I-labeled NGF and immunoprecipitated; and lane 6, EN31-transfected PC12 cells cross-linked with ^{125}I-labeled EGF and immunoprecipitated. The exposure time of lane 6 was ten times the exposure time for lane 3. Molecular size markers are in kilodaltons.

To confirm that EGF was mediating a response through the chimeric receptor, we attempted to correlate receptor signaling with changes in gene expression. Many effects elicited by NGF in PC12 cells were also detected after EGF treatment (*10*). These responses include increases in 2-deoxyglucose uptake (*11*), Na^+,K^+ pump activity (*12*), Na channel density (*13*), and the rapid transcriptional induction of immediate early genes, such as c-*fos*, c-*jun*, *zif* 268/NGFI-A, and *nur77*/NGFI-B (*14, 15*).

In contrast, expression of the gene encoding the metalloprotease transin (stromelysin) was selectively induced in PC12 cells by NGF, and not by EGF treatment (*16*). Therefore, EN10-transfected cells were treated with NGF and EGF for 3 days and analyzed by RNA blot hybridization with a cDNA probe that encoded the rat transin mRNA. Uninduced cells exhibited undetectable amounts of transin mRNA; only NGF treatment of PC12 cells resulted in a significant induction of steady-state amounts of the 1.9-kb transin mRNA (Fig. 4). By contrast, in PC12 cells that expressed EN10 chimeric receptors, EGF increased transin gene expression after 3 days, approximating the response obtained with NGF in PC12 cells. The increased expression of transin mRNA with EGF was correlated with the morphological changes observed in Fig. 3 and further demonstrated that the chimeric EN10 receptor was capable of functioning in an NGF-specific manner.

Although NGF and EGF elicit a large number of common early responses, the results reported here indicate that receptor specificity is mediated by the NGFR cytoplasmic and transmembrane domains. The importance of the membrane-spanning regions is underscored by the finding that the EGFR transmembrane region cannot substitute for this domain. These results are consistent with the evolutionary conservation of transmembrane and cytoplasmic sequences of NGFR from human, rat, and chick origins (*17*) and contrasts with the EGFR, in which mutagenesis of the transmembrane region does not affect kinase activation and the oligomerization properties induced by EGF binding (*18*).

The p75^{NGFR} represents a member of a family of cell surface molecules that includes tumor necrosis factor receptors, the B cell–specific antigen CDw40, the T cell activation molecule OX40, and poxvirus receptors

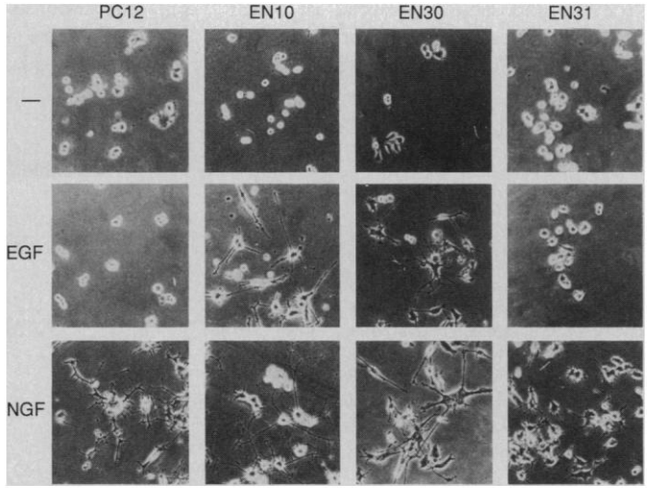

Fig. 3. Treatment of transfected PC12 cells with NGF and EGF. Phase-contrast photomicrographs of cells treated for 3 days in the presence of either NGF (5 ng/ml) or EGF (1 ng/ml) (Bioproducts for Science). Magnification, ×39.5. PC12 cells were grown in Dulbecco's minimal essential medium (DMEM) supplemented with fetal calf serum (10%) and horse serum (5%) (Gibco). Headings indicate control PC12 cells or PC12 cells transfected with the indicated cDNA. Even at high concentrations (100 ng/ml), no changes in morphology were observed with cells treated with EGF (data not shown).

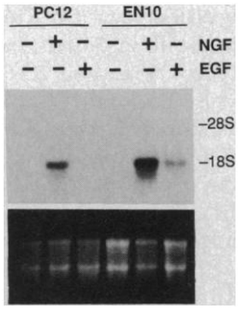

Fig. 4. Expression of transin mRNA was mediated by chimeric receptors. Cells were treated for 3 days in the presence of NGF (50 ng/ml) or EGF (5 ng/ml) in DMEM with fetal calf serum (10%) and horse serum (5%). Total RNA (20 μg) from PC12 cells and the cell line stably transfected with EN10 were isolated by guanidine thiocyanate treatment and CsCl-gradient centrifugation, separated on a formaldehyde (2.2 M)-agarose (0.8%) gel, and transferred to nitrocellulose. The filter was hybridized to a random-primed cDNA probe that encoded the rat transin gene (*16*). Ethidium bromide staining of the gel is displayed to indicate relative amounts of RNA analyzed. The markers at right indicate the sizes of ribosomal RNA.

(*19*). The common feature among these molecules is the conserved pattern of cysteine residues in the extracellular binding domains. The cytoplasmic domains, however, are unique. Deletions in the cytoplasmic domain of the $p75^{NGFR}$ result in receptors that bind ^{125}I-labeled NGF with low affinity (*20*) and lack biological responses (*21*). Affinity cross-linking and transfection studies indicate that NGF-induced biological responses require $p75^{NGFR}$ plus an associated protein (*20, 22*). The finding that the *trk* proto-oncogene binds NGF and is autophosphorylated in response to NGF (*23*) identifies $p140^{prototrk}$ as the $p75^{NGFR}$ accessory protein. The *trk* proto-onogene might be associated with the same transmembrane and cytoplasmic sequences that are found in the EN10 and EN30 chimeric receptors.

These results indicate that the low-affinity $p75^{NGFR}$ plays a crucial role in signal transduction and that the transmembrane and cytoplasmic domains are required for the initial steps of NGF-mediated neuronal differentiation. It is likely that interactions of these domains with other signal-transducing molecules are essential for NGF action. Moreover, the ability to reconstruct a growth factor response by fusion of two diverse and structurally dissimilar receptors implies that common mechanisms may function for diverse transmembrane receptor molecules in signal transduction.

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Clusters of Coupled Neuroblasts in Embryonic Neocortex

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The neocortex of the brain develops from a simple germinal layer into a complex multilayer structure. To investigate cellular interactions during early neocortical development, whole-cell patch clamp recordings were made from neuroblasts in the ventricular zone of fetal rats. During early corticogenesis, neuroblasts are physiologically coupled by gap junctions into clusters of 15 to 90 cells. The coupled cells form columns within the ventricular zone and, by virtue of their membership in clusters, have low apparent membrane resistances and generate large responses to the inhibitory neurotransmitter γ-aminobutyric acid. As neuronal migration out of the ventricular zone progresses, the number of cells within the clusters decreases. These clusters allow direct cell to cell interaction at the earliest stages of corticogenesis.

THE NEOCORTEX IS ORGANIZED INTO discrete layers of neurons that are radially subdivided into functional domains or columns (*1*). The development of this highly ordered structure is an intricate process. Neurons populate their respective layers in an inside-out pattern of development (*2, 3*). Neuroblasts synthesize DNA and undergo mitotic divisions within the deepest layer of developing neocortex, the ventricular zone (*4*). After their final division, cells migrate out of the ventricular zone along radial glia (*5*). They pass older cells that have previously migrated and end their migration in the cortical plate (*6*). Each cortical cell's position and phenotype may be determined before it migrates out of the ventricular zone (*7*). The mechanisms that operate within the ventricular zone to determine the ultimate fate of immature cortical cells are not well understood.

Cell to cell interactions could participate in determining the fates of developing neurons. Investigations into interactions between ventricular zone cells have been largely precluded by the difficulty of obtaining physiological measurements from small cells maintained in situ. The application of

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