patterning process. SAM films such as PTCS, BTCS, and APTS on Si substrates were exposed to either 193-nm or 248-nm radiation, then metallized with electroless plating techniques (*19*). Metal patterns typically several hundred angstroms thick were deposited only in the unexposed areas of the film (Fig. 4). These metal patterns can serve as plasma-hard etch barriers, conductive paths, or opaque regions for fabricating a wide variety of microelectronic devices.

Patterned two-component SAM films have also been used to produce arrays of more complex structures such as biological cells (*6, 20*). Coplanar EDA and 13F surfaces were plated with human SK-N-SH neuroblastoma cells (*21*) suspended in culture medium. On 40-μm-wide EDA regions, the cells maintained relatively spherical shapes with visible contact points. However, with 12-μm spacing, the widths of the alternating EDA/13F regions were less than the cell diameters, causing the cells to elongate to conform to the EDA lines (Fig. 5A). Subsequent neurite outgrowth after 24 hours was confined to the EDA regions (Fig. 5B).

The deep UV photochemistry of SAM films promises to be an interesting area of research and an enabling technology for manipulating molecular assemblies. SAMs are perhaps the ultimate materials for ultrahigh-resolution imaging in optical and electron-beam lithographies (*1, 2*). Also, patterned adherent cells can facilitate advances in biosensors and implant devices. This process also provides a pathway to define patterns of molecular species such as chromophores, redox reagents, catalysts, and optically active and biologically active species at the practical limits of lateral resolution. The ability to photochemically control the wettability of a SAM film over a wide range of surface energy can provide insights into adhesion and biocompatibility at the molecular level. To reach these goals, it will be necessary to understand how the surface photochemical processes in SAM films depend on the type of substrate, the nature and energy of the exposure source, the surface attachment chemistry, and the specific chemical functionalities present in the SAM molecule.

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5 December 1990; accepted 20 February 1991

The *trk* Proto-Oncogene Product: A Signal Transducing Receptor for Nerve Growth Factor

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The *trk* proto-oncogene encodes a 140-kilodalton, membrane-spanning protein tyrosine kinase ($p140^{prototrk}$) that is expressed only in neural tissues. Nerve growth factor (NGF) stimulates phosphorylation of $p140^{prototrk}$ in neural cell lines and in embryonic dorsal root ganglia. Affinity cross-linking and equilibrium binding experiments with ^{125}I-labeled NGF indicate that $p140^{prototrk}$ binds NGF specifically in cultured cells with a dissociation constant of 10^{-9} molar. The identification of $p140^{prototrk}$ as an NGF receptor indicates that this protein participates in the primary signal transduction mechanism of NGF.

THE DEVELOPMENT OF THE VERTEBRATE nervous system is characterized by a series of complex events that cause the apparently homogenous neuroepithelium of the early embryo to form the diverse, highly ordered, and interconnected neural cell types of the adult. Extensive evidence indicates that limiting diffusible factors are required for the targeting, survival, and proper synaptic arrangement of neurons (*1*). Neuronal circuits are sculpted from an initially overabundant production of neurons during development: In the midterm embryo, programmed cell death eliminates most of the neurons, leaving behind only those required for innervation of target tissues (*2*). Although neural adhesion and extracellular matrix molecules are essential for axonal migration, guidance, and growth cone targeting, most of the molecules that

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convey signals to the cell nucleus to initially induce neuronal proliferation, differentiation, and, later, survival are unknown.

The proto-oncogene *trk* is a member of the tyrosine kinase (TK) family of transmembrane receptors, which have in common a large extracellular ligand binding domain, a single transmembrane domain, and a cytoplasmic tyrosine kinase catalytic domain. Although *trk* was discovered as a rearranged oncogene from a colon carcinoma (*3*), normal *trk* expression in vivo is limited to neural crest–derived sensory neurons (*4, 5*). The low abundance of *trk* mRNA in vivo and its restricted pattern of expression in the neural crest–derived sensory nervous system indicate that the function of the *trk* receptor must be distinct from that of other tyrosine kinases because no other TK receptors display such limited expression.

The restricted expression of *trk* during development and its neuronal specificity indicated that *trk*, and related genes, might mediate morphogenic signals in the embryo during neurogenesis (*4, 6*). This notion is reinforced by experiments relating *trk*, $p140^{prototrk}$, and the neurotrophic factor NGF: *Trk* is expressed in rat pheochromocytoma PC12 cells (*7*), a transformed adrenal chromaffin cell line that differentiates in response to NGF (*8*). Furthermore, picomolar concentrations of NGF rapidly stimulate $p140^{prototrk}$ autophosphorylation (*7*), while other growth factors [insulin, basic fibroblast growth factor (bFGF), epidermal growth factor (EGF)] do not elicit $p140^{prototrk}$ tyrosine kinase activity. NGF is responsible for the maintenance and survival of sympathetic and neural crest–derived sensory neurons. In mouse embryos, NGF injected into the periphery of the trunk enhances the survival of sensory neurons that are normally targeted for cell death (*9, 10*). Exposure of embryos to antibodies to NGF reduces the survival of dorsal root ganglion (DRG) neurons, while injection of antibodies to NGF into neonatal mice inhibits the survival of sympathetic neurons (*11*).

The mechanisms by which NGF elicits its neurotrophic effects in the cell have not been fully resolved. Interaction of NGF with a cell receptor is required for the transmission of neurotrophic signals within the cell (*12*). A 75-kD receptor ($p75^{NGFR}$) that binds NGF has been identified and cloned and is present in NGF-responsive cells (*13, 14*). Nevertheless, the structural and biological properties of $p75^{NGFR}$ have provided only limited clues about the nature of the NGF signal transduction pathway inside the cell. $p75^{NGFR}$ displays the binding properties of a low affinity NGF receptor [dissociation constant (K_d) $\approx 10^{-9}$ M] when expressed in heterologous cell lines (*12*). However, the biological responsiveness to NGF depends upon its interaction with a high affinity binding site ($K_d \approx 10^{-11}$ M).

The characteristics of the signal transduction pathway activated by NGF to cause its neurotrophic effects have also been obscure. In PC12 cells the activation of tyrosine kinases in general (and $p140^{prototrk}$ tyrosine phosphorylation) is an early response to the binding of NGF (*7, 15*). These data implicate tyrosine kinases, and $p140^{prototrk}$ in particular, as candidates to transmit the neurotrophic signals mediated by the high affinity binding site for NGF. We have now examined whether $p140^{prototrk}$, a TK transmembrane receptor, can be activated by means of a direct interaction with NGF in order to investigate signal transduction mechanisms by which proliferation and differentiation in the nervous system are controlled.

The stimulation of $p140^{prototrk}$ tyrosine phosphorylation in response to addition of NGF to PC12 cells is rapid and specific, and occurs in the presence of physiological amounts of NGF (*7*). To determine whether phosphorylation of $p140^{prototrk}$ was a general response to NGF, we assayed NGF-induced $p140^{prototrk}$ phosphorylation in additional NGF-responsive neuroblastoma cell lines. NGF induced $p140^{prototrk}$ tyrosine phosphorylation in the human neuroblastoma cell lines SY5Y and LA-N-5 (*16*) (Fig. 1A). SY5Y and LA-N-6 cells express less (about fourfold) *trk* mRNA than do PC12 cells, which could account for the lower amounts of tyrosine-phosphorylated $p140^{prototrk}$ in these cell lines than in PC12 cells. NR18 cells are derived from the PC12 cell line but have lost their "high affinity" response to NGF (*17*) in part due to the absence of $p75^{NGFR}$ (*18*). These cells express much less $p140^{prototrk}$ than do normal PC12 cells. Treatment of NR18 cells with NGF resulted in no detectable phosphorylation of $p140^{prototrk}$ (Fig. 1A). Thus, in NR18 cells, the reduced tyrosine phosphorylation of $p140^{prototrk}$, as well as the absence of $p75^{NGFR}$ (*17*), correlate with the reduced ability of NGF to elicit a biological response.

The *trk* gene is expressed in embryonic sensory neural crest–derived neurons and expression is maintained in the adult (*4*). We therefore tested whether $p140^{prototrk}$ in primary embryonic neurons was phosphorylat-

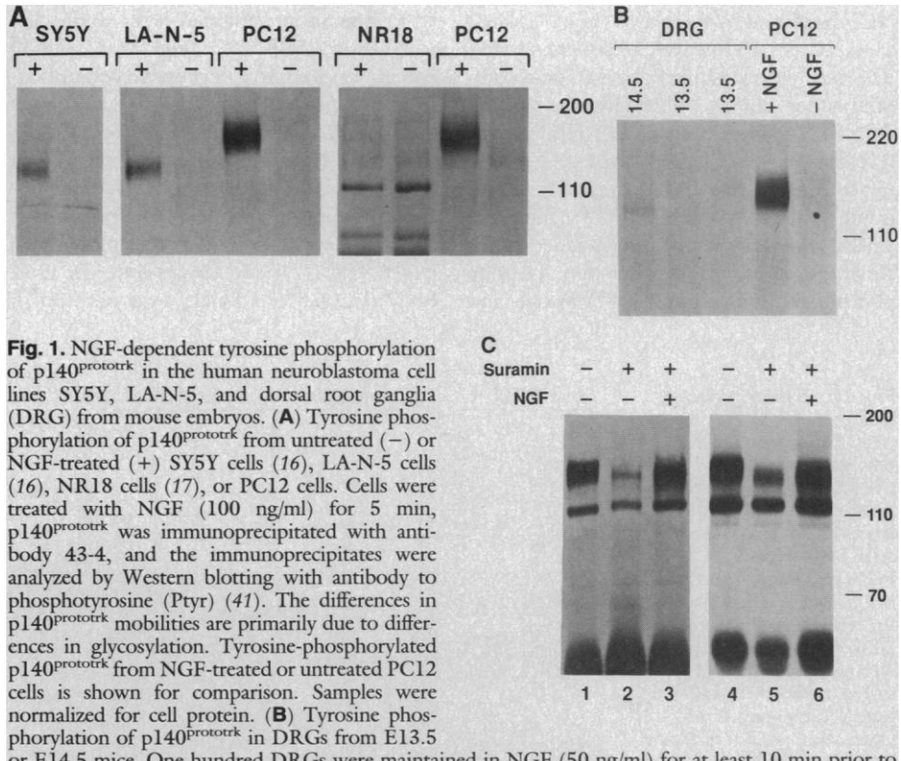

Fig. 1. NGF-dependent tyrosine phosphorylation of $p140^{prototrk}$ in the human neuroblastoma cell lines SY5Y, LA-N-5, and dorsal root ganglia (DRG) from mouse embryos. (**A**) Tyrosine phosphorylation of $p140^{prototrk}$ from untreated (−) or NGF-treated (+) SY5Y cells (*16*), LA-N-5 cells (*16*), NR18 cells (*17*), or PC12 cells. Cells were treated with NGF (100 ng/ml) for 5 min, $p140^{prototrk}$ was immunoprecipitated with antibody 43-4, and the immunoprecipitates were analyzed by Western blotting with antibody to phosphotyrosine (Ptyr) (*41*). The differences in $p140^{prototrk}$ mobilities are primarily due to differences in glycosylation. Tyrosine-phosphorylated $p140^{prototrk}$ from NGF-treated or untreated PC12 cells is shown for comparison. Samples were normalized for cell protein. (**B**) Tyrosine phosphorylation of $p140^{prototrk}$ in DRGs from E13.5 or E14.5 mice. One hundred DRGs were maintained in NGF (50 ng/ml) for at least 10 min prior to lysis, immunoprecipitated, and analyzed by immunoblotting with antibody to Ptyr (*41*). Each lane contains protein from the same number of DRGs. (**C**) Tyrosine phosphorylation of $p140^{prototrk}$ from *trk*-3T3 cells in intact cells or in vitro (*41*). (Lanes 1 to 3) $p140^{prototrk}$ immunoprecipitates from intact cells probed with antibody to Ptyr; (lanes 4 to 6) tyrosine phosphorylation of $p140^{prototrk}$ in vitro (*41*). Cells were treated with suramin (lanes 2 and 5) or with NGF (500 ng/ml) for 10 min after suramin treatment (lanes 3 and 6) (*41*). The band migrating at 110 kD is a glycosylated precursor of $p140^{prototrk}$ (*42*). The band at the bottom of the figure is immunoglobulin G. Molecular weight markers in kilodaltons are at right. Similar amounts of $p140^{prototrk}$ were present in each lane.

ed in response to NGF. Explanted DRGs from embryonic day (E) 13.5 and E14.5 mouse embryos were treated with NGF, and $p140^{Prototrk}$ phosphorylation was measured. Phosphorylation of $p140^{Prototrk}$ was detectable in E14.5 DRGs but not in two independent preparations of E13.5 DRGs (Fig. 1B). We were unable to detect tyrosine-phosphorylated $p140^{Prototrk}$ in the absence of exogenously administered NGF, although $p140^{Prototrk}$ is expressed in both E13.5 and E14.5 DRGs (*4*). The axon terminals of DRG neurons are not included in the explants, but may in fact contain more $p140^{Prototrk}$ than the cell bodies. Therefore, our data may underestimate the amount of phosphorylated $p140^{Prototrk}$. Nevertheless, our results demonstrate that embryonic DRG neurons, cells that are dependent on NGF during development, respond to NGF administration by phosphorylation of $p140^{Prototrk}$ and that this is a general response to NGF in many neuronal cells.

To assess whether NGF can activate $p140^{Prototrk}$ in heterologous, non-neuronal cells, a rat *trk* cDNA was expressed in mouse NIH-3T3 fibroblasts (*rtrk*-3T3 cells). The tyrosine phosphorylation of $p140^{Prototrk}$ expressed in *rtrk*-3T3 cells was constitutive, apparently due to autocrine stimulation by NGF produced by these cells (Fig. 1C, lanes 1 and 4) (*19*). However, treatment of *rtrk*-3T3 cells with suramin, a polyanionic compound that inhibits and reverses the binding of NGF to its receptors (*20*), reduced the tyrosine phosphorylation of $p140^{Prototrk}$ in intact *rtrk*-3T3 cells and in vitro after immunoprecipitation of $p140^{Prototrk}$ (Fig. 1C, lanes 2 and 5). When NGF was added to the suramin-treated cells for 10 min, tyrosine phosphorylation of $p140^{Prototrk}$ in cells and in immunoprecipitates was stimulated at least tenfold (Fig. 1C, lanes 3 and 6). These data indicate that $p140^{Prototrk}$ tyrosine phosphorylation is induced by NGF in a heterologous environment in the absence of other neural-specific factors.

The simplest interpretation of our results was that $p140^{Prototrk}$ directly bound NGF. To determine if NGF could bind to $p140^{Prototrk}$, several cell lines were analyzed for the ability of antisera to $p140^{Prototrk}$ to precipitate receptor-ligand complexes in affinity cross-linking experiments (Fig. 2). We tested rat PC12, human LA-N-5, human SY5Y, mouse NIH-3T3, mouse *rtrk*-3T3, and human A875 cells (*21*). NGF induced the tyrosine phosphorylation of $p140^{Prototrk}$ in PC12 and *rtrk*-3T3 cells but not in A875 melanoma or NIH-3T3 cells, which express no detectable *trk* mRNA (*19*). ^{125}I-labeled NGF was cross-linked to cells with the lipophilic photoaffinity agent *N*-hydroxylsuccinimidyl 4-azidobenzoate (HSAB). [In PC12 cells or sympathetic neurons, two NGF-containing species of 100 kD and 150 to 160 kD have been shown to be cross-linked to NGF with HSAB (*22, 23*); the 100-kD species is ^{125}I-labeled NGF bound to $p75^{NGFR}$.] After the cross-linking reaction, the cells were lysed, and the lysates were immunoprecipitated with antibodies to NGF or $p140^{Prototrk}$ (Fig. 2). A 160-kD species was immunoprecipitated by antibodies to NGF or $p140^{Prototrk}$ from PC12 (lanes 1 to 4) and *rtrk*-3T3 cells (lanes 5 to 9), but not from NIH-3T3 (lanes 10 to 12) or A875 (lanes 13 to 15) cells. The immunoprecipitation of the 160-kD species was blocked by addition of the peptide from $p140^{Prototrk}$ (*7*) used to generate the antibody (lanes 4 and 8) and was not seen if excess unlabeled NGF was added to the cells prior to cross-linking (lanes 9, 11, and 14). A cross-linked product of approximately 150 kD was also observed in LA-N-5 and SY5Y cells. The cross-linked 100-kD species was present in PC12 and A875 cells and not in the NIH-3T3 cell lines (lanes 1, 10, and 13), reflecting the absence of $p75^{NGFR}$ in NIH-3T3 cells. Thus NGF interacts with $p140^{Prototrk}$, and this interaction is found only in cell lines that show $p140^{Prototrk}$ tyrosine phosphorylation in response to NGF treatment.

To determine whether NGF directly interacts with $p140^{Prototrk}$ and whether this interaction reflects physiologically relevant conditions, equilibrium binding studies were undertaken. Crude membranes were prepared from *rtrk*-3T3 cells and assayed for binding to ^{125}I-labeled NGF. Membranes from *rtrk*-3T3 cells displayed specific, saturable binding for ^{125}I-labeled NGF, with a single binding site and a K_d of approximately 10^{-9} M. The number of receptors was approximately 500,000 per cell. Thus, $p140^{Prototrk}$, like $p75^{NGFR}$, has the binding characteristics of a low-affinity NGF receptor.

The physiological effects mediated by NGF in responsive cells have not been fully explained by the genetic and biochemical study of $p75^{NGFR}$ (*12, 14, 24, 25*). Analysis of NGF equilibrium binding with I^{125}-labeled NGF has revealed sites with two distinct affinities for NGF in PC12 cells and in primary sympathetic neurons (*12, 26*). This has led to the speculation that a high affinity form of the NGF receptor may require additional intracellular proteins to interact with NGF (*23, 25, 27, 28*) or that an additional unidentified molecule or molecules may independently mediate the high-affinity response (*9*). The 160-kD species cross-linked to NGF in PC12 cells and sympathetic neurons (*22, 23, 29*) (Fig. 2) is not recognized by antibodies to $p75^{NGFR}$ (*18, 25*). This 160-kD species comprises NGF plus a molecule of approximately 145 kD. This 145-kD protein has been referred to as the high affinity NGF receptor and contains phosphorylated tyrosine residues (*25*). Our results now indicate that the 145-kD protein is $p140^{Prototrk}$. NGF plus $p140^{Prototrk}$ corresponds in size to the high molecular weight cross-linked species in primary sympathetic ganglia and cell lines of neuronal origin, PC12, LA-N-5, and SY5Y. In these cell lines NGF also causes tyrosine phosphorylation of $p140^{Prototrk}$. Thus, $p140^{Prototrk}$ binds NGF directly, even in cells that do not contain $p75^{NGFR}$ (*trk*-expressing NIH-3T3 cells): $p140^{Prototrk}$ can bind NGF independently of the low affinity $p75^{NGFR}$ and of neural cell–specific factors.

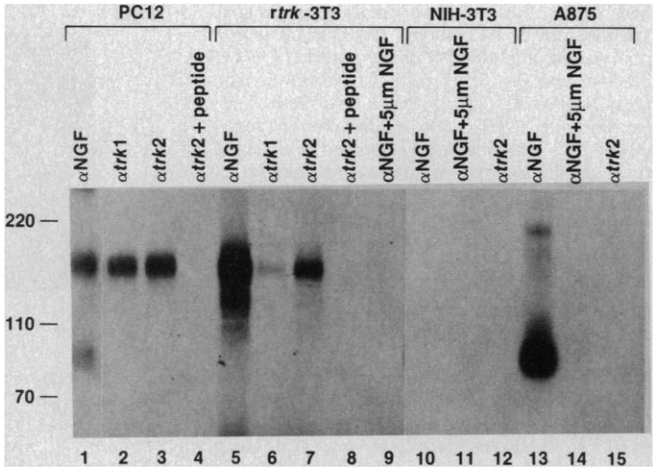

Fig. 2. Affinity cross-linking of NGF to $p140^{Prototrk}$ on PC12 cells and *rtrk*-3T3 cells. $p140^{Prototrk}$ was labeled by cross-linking of ^{125}I-labeled NGF to cultured cells with HSAB (*43*). PC12 cells (lanes 1 to 4), *rtrk*-3T3 cells (lanes 5 to 9), NIH-3T3 cells (lanes 10 to 12), A875 human melanoma cells (lanes 13 to 15). Lysates from cells were immunoprecipitated with antibody to NGF (αNGF) (lanes 1, 5, 9 to 11, and 13 to 14), $p140^{Prototrk}$ antibody 7-4 (α*trk*1) (lanes 2 and 6), or $p140^{Prototrk}$ antibody 43-4 (α*trk*2) in the absence (lanes 3, 7, 12, and 15) or presence (lanes 4 and 8) of competing peptide (10 μg/ml) (*7*). Cross-linking was performed in the presence of excess unlabeled NGF (5 μM) in lanes 9, 11, and 14. The antibody 7-4 immunoprecipitates three- to fivefold less $p140^{Prototrk}$ than does antibody 43-4 (*44*). Molecular weight markers in kilodaltons are indicated on the left.

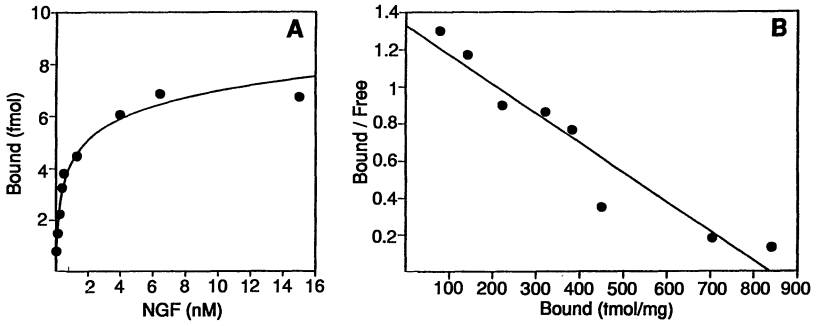

Fig. 3. Equilibrium binding analysis of NGF to cell membranes prepared from *trk*-3T3 cells. Binding of ^{125}I-labeled NGF was analyzed in membrane preparations by filter binding as described (*29*). Reactions were carried out in triplicate in the presence or absence of excess unlabeled NGF (5 μM) (to assess nonspecific binding) with 10 μg of membrane protein for 1 hour at 30°C and filtered under vacuum through Millipore HVLP filters. Specific binding was over 80% of total binding. (**A**) Saturation binding curve. (**B**) Data in (A) plotted according to the method of Scatchard. Only values above 50% specific binding were used. The LIGAND program was used to determine K_d.

Although the 160-kD species containing cross-linked NGF has been previously identified, its precise role in NGF-mediated events has remained controversial. This species has been referred to as the high affinity NGF receptor. Other investigators have argued (*29*), on the basis of biochemical data, that one NGF receptor ($p75^{NGFR}$) can account for both binding affinities in PC12 cells. In addition, the 160-kD species has been suggested to include $p75^{NGFR}$ plus additional intracellular factors (*30*).

Our results demonstrate that the 140-kD *trk* gene product ($p140^{prototrk}$) can function as an NGF receptor in PC12 cells and in *trk*-expressing NIH-3T3 cells. However, under our experimental conditions $p140^{prototrk}$ alone does not have the binding characteristics of a high affinity NGF receptor. Indeed, the K_d observed for $p140^{prototrk}$ is similar to that observed for $p75^{NGFR}$. Thus, the 160-kD cross-linked species ($p140^{prototrk}$ plus NGF) does not by itself seem to constitute the high affinity receptor. However, because $p140^{prototrk}$ alone can be activated by NGF, albeit with a low binding affinity, conditions may exist where $p140^{prototrk}$ acts independently of $p75^{NGFR}$ (*18*) and a reinterpretation of previous experiments predicated on the expression of $p75^{NGFR}$ may be necessary. Therefore, PC12 cells contain two different receptors each of which can bind NGF. It remains to be demonstrated whether these molecules interact or act independently to mediate NGF responses (*18*).

During postimplantation embryonic development *trk* mRNA is expressed only in neural crest–derived sensory neurons (DRG, trigeminal, superior, and jugular ganglia) (*4*). These sensory neurons all display trophic responses to NGF, a result consistent with mediation of NGF responses by $p140^{prototrk}$. However, *trk* transcripts cannot be detected in other sites where NGF-responsive neurons have also been identified, such as sympathetic ganglia and brain. This may be because *trk* is expressed in amounts that are below our threshold of detection in embryonic brain and sympathetic ganglia or that *trk* expression does not appear in these cells until very late prenatal development (or after birth). Sensory neurons require NGF during early development but are less dependent on NGF in the adult, whereas sympathetic neurons are most NGF-dependent in late embryogenesis and in the adult (*10, 31*). In fact, in situ experiments with *trk* cDNA probes in adult mouse brain show *trk* transcripts in a small number of neurons in the basal forebrain region where we had not previously detected expression in the embryo (*32*).

In vertebrates, TK receptors first came under scrutiny on the basis of their participation in neoplastic transformation (*33, 34*). Analysis of nononcogenic forms of these receptors led to a general view of TK receptors (such as platelet-derived growth factor receptor and fibroblast growth factor receptor) as mediators of mitogenic signals (*34, 35*). Indeed, ligands and normal substrates of TK receptors can also be proto-oncogenes or modulators of proto-oncogene activity (for example, Sis, Raf, and GAP) (*36, 37*). However, in *Drosophila melanogaster*, TK receptors function in the transduction of morphogenic signals. Thus, *sevenless* encodes a TK receptor required for the formation of retinal photoreceptor number seven, while *torso* encodes a TK receptor that mediates signals leading to the anteroposterior orientation of embryonic terminal structures (*38*). No associated mitogenic activity has been reported for the *sevenless* or *torso* gene products.

Our present observations indicate that nonmitogenic signal transduction is not unique to invertebrates and that $p140^{prototrk}$ mediates cell differentiation and survival signals rather than mitogenic signals (*4, 5*). It is unlikely that $p140^{prototrk}$ will be an isolated example of a TK receptor providing a mechanism for neurotrophic signal transduction. The products of *trk*b and other members of the *trk* gene family are likely to mediate related neurotrophic signals as receptors to NGF-related neurotrophic factors (such as NT-3, and BDNF). TK receptors that are not members of the *trk* subfamily may also have neurotrophic functions. Thus, the product of the proto-oncogene c-*kit*, a TK receptor, may provide nonmitogenic developmental signals in certain tissues, including the neural tube and brain (*39*).

Intracellular substrates of TK receptors include phospholipase C–γ, Raf-1, RasGAP, and PI-3 kinase (*34*). Indeed, tyrosine phosphorylation of PLC-γ in PC12 cells by $p140^{prototrk}$-associated tyrosine kinase activity has been observed and P1-3 kinase activity is induced by NGF (*40*). However, it is unlikely that known TK substrates, such as PLC-γ, which are activated by various TK receptors, articulate the signals that distinguish a $p140^{prototrk}$-mediated neurotrophic response from TK-mediated mitogenic responses (*34*). The wealth of existing biochemical information and expertise that has been generated through identification of tyrosine kinase substrates can now be directed toward identifying new substrates of $p140^{prototrk}$ and should allow advances in understanding how neurotrophic signals are transmitted.

The *trk* gene product may only be the first of a host of as yet unidentified TK receptors that are utilized in vertebrate embryonic cells of neural and other lineages to mediate the subsequent molecular steps toward terminal cell differentiation.

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41. Cells (2×10^7) or DRGs were treated with NGF, washed, and lysed in buffer containing 1% NP40; the lysates were then immunoprecipitated with antibody 43-4 to $p140^{prototrk}$. Immunoprecipitates were either electrophoresed on 7.5% SDS-polyacrylamide gels and subjected to immunoblot analysis with the phosphotyrosine (Ptyr) monoclonal antibody 4G10 as described (7) or were analyzed in kinase assays (*7, 34*). DRGs were subjected to Dounce homogenization during lysis in buffer containing 1% NP40. *rtrk*-3T3 cells were generated by $CaPO_4$-mediated transfection of a mouse-rat *trk* cDNA hybrid into NIH-3T3 cells. Rat *trk* cDNAs were obtained from an embryonic rat DRG cDNA library. The longest *trk* cDNA obtained (2.4 kb) was missing approximately 150 bp of the coding region as compared to available mouse and human *trk* sequence. The missing bases plus minimal (~50 bp) 5′ flanking noncoding sequences were replaced from mouse first coding exon sequences, and the reconstructed gene was placed downstream of an MSV-LTR (D. Martin-Zanca, E. Sanz, L. F. Parada, manuscript in preparation). *rtrk* 3T3 cells (2×10^7) were treated with 1 mM suramin in Dulbecco's modified Eagle medium (DMEM) for 2 hours or mock-treated. After extensive washing of the cells with DMEM, NGF was added.
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43. ^{125}I-labeled NGF was prepared by lactoperoxidase treatment to specific activities of 2500 to 3500 cpm/fmol. Cross-linking of $p140^{prototrk}$ to ^{125}I-labeled NGF was performed as described (*18*). Cells (2×10^6/ml) were incubated with 0.5 nM ^{125}I-labeled NGF for 2 hours at 4°C. HSAB (50 μM) was added, and the reaction was exposed to long ultraviolet wavelength light (365 nm) for 10 min. After washing in 50 mM lysine in phosphate buffered saline, the cells were lysed in buffer containing 1% NP40, and the lysates were immunoprecipitated and analyzed by 7.5% SDS–polyacrylamide gel electrophoresis as described (7).
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45. We thank M. C. Fishman for the rat DRG cDNA library, J. Maragos, J. Blair, and S. Rabin for technical help, and R. Handley for preparation of the manuscript. Supported in part by NCI contract NO1-CO-74101 with Advanced Biosciences Laboratory (D.R.K., D.M.-Z., and L.F.P.) and by grants from the March of Dimes, Andrew W. Mellon Foundation, and the National Cancer Institute (B.L.H.) and from the National Institutes of Health, the American Cancer Society, Hirschl-Caulier Trust Fund, Parke Davis, and the special group of donors to the Dorothy Rodbell Foundation (M.V.C.).

18 March 1991; accepted 30 March 1991

Induction by NGF of Meiotic Maturation of *Xenopus* Oocytes Expressing the *trk* Proto-Oncogene Product

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The effect of nerve growth factor (NGF) was assessed in *Xenopus* oocytes expressing the human *trk* proto-oncogene product, $p140^{prototrk}$. Oocytes injected with *trk* messenger RNA expressed polypeptides recognized by antibodies to the *trk* gene product. Exposure of these oocytes to nanomolar amounts of NGF resulted in specific surface binding of ^{125}I-labeled NGF, tyrosine phosphorylation of $p140^{prototrk}$, and meiotic maturation, as determined by germinal vesicle breakdown and maturation promoting factor ($p34^{cdc2}$) kinase activation. Thus the *trk* proto-oncogene product can act as a receptor for NGF in a functionally productive manner.

THE MEMBERS OF THE *TRK* GENE family (*1*) code for receptor-like, tyrosine kinase molecules that are transcribed in a pattern that suggests that they have a specialized role in neural tissue development (*2*). NGF (*3*) induces tyrosine phosphorylation and tyrosine kinase activity of the *trk* proto-oncogene product ($p140^{prototrk}$) in PC12 cells (*4*), suggesting that this protein may transduce NGF signals. In *Xenopus* oocytes, factors that control cell proliferation and regulate the cell cycle (including hormones, growth factors and their receptors, and oncogenes) can easily be studied (*5–7*). We have now expressed the product of the human *trk* proto-oncogene in *Xenopus* oocytes by injecting them with *trk* mRNA synthesized in vitro and analyzing the biochemical and biological effects produced by NGF in these oocytes.

A cDNA fragment coding for the complete human *trk* proto-oncogene product (*8*) was inserted into vector pGEM2 to generate plasmid pGEMTRK-R1 from which full-length sense *trk* mRNA can be transcribed. An analogous H-ras^{lys12} construct, used as a control in these studies, was generated with a 670-bp EcoR I fragment from plasmid pJCL30 (*9*). These constructs were used to synthesize in vitro the full-length *trk* mRNA (2.7 kb) and *ras* mRNA (0.7 kb) (Fig. 1A), which were then injected into the cytoplasm of full-grown *Xenopus* oocytes. Expression of the protein products, after incubation to allow for translation of the injected mRNA, was assessed by immunoprecipitation of lysates of radiolabeled, injected oocytes with specific antibodies (Fig. 1B). Immunoprecipitation with two different antibodies to either the whole $p70^{trk}$ oncogene protein (*10*) (lanes 1 and 2) or the COOH-terminal region of $p140^{prototrk}$ (*8*) (lanes 3 and 4) revealed significant de novo accumulation of two polypeptide bands of 110 and 140 kD (Fig. 1B, lanes 2 and 4) that corresponded in size to the glycosylated protein products of human *trk* (*8*). The antibodies did not react with these molecules in control oocytes injected with water (lanes 1 and 3).

Expression of $p21^{ras}$ in oocytes was carried out in a similar manner by injecting the

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