Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 11133–11137, November 1994
Neurobiology

An alternatively spliced form of the nerve growth factor receptor TrkA confers an enhanced response to neurotrophin 3

(PC12 cells/neurite outgrowth)

DOUGLAS O. CLARY* AND LOUIS F. REICHARDT

The Neuroscience Program, Department of Physiology, and Howard Hughes Medical Institute, University of California, San Francisco, CA 94143

Communicated by Ann M. Graybiel, July 1, 1994 (received for review April 20, 1994)

ABSTRACT **TrkA, a member of the receptor tyrosine kinase family, binds nerve growth factor (NGF) and subsequently activates intracellular signaling pathways. Previous studies have found variable and weak interaction of the TrkA receptor with neurotrophin 3 (NT-3), another member of the NGF family. TrkA is expressed in two splice forms, differing in the presence of an 18-bp exon in the extracellular domain. The biological responses of each isoform of the TrkA receptor were tested after transfection into the cell line PC12^{nnr5}, a variant of PC12 cells lacking functional TrkA protein. NGF was found to activate each form of the receptor comparably. However, the TrkA isoform containing the variable exon showed significantly higher activation by NT-3, which was detected by stimulation of TrkA autophosphorylation, induction of ZIF268 transcription, and cellular differentiation. Function-perturbing antibodies to the p75 low-affinity NGF receptor potentiated the NT-3 responses of both forms of TrkA in the transfected PC12^{nnr5} cell lines, suggesting that the low-affinity NGF receptor suppresses the ability of TrkA to respond to NT-3.**

The mechanism of signal transduction for nerve growth factor (NGF) and other members of the neurotrophin family has been clarified by the finding that members of the Trk family of receptor tyrosine kinases are essential components of the neurotrophin signaling pathway (reviewed in refs. 1–3). Earlier studies of neurotrophin receptors had focused on the p75 low-affinity NGF receptor (LNGFR), which binds each of the neurotrophins with a similar, low affinity. The discovery of a family of Trk receptors (known as TrkA, TrkB, and TrkC) suggested that each neurotrophin would bind specifically to one Trk receptor, leaving the role of the LNGFR in doubt. In fact, the data regarding Trk receptor specificity remain somewhat perplexing. For example, while it is clear that TrkA binds to and is efficiently activated by NGF (4, 5), there has been considerable disagreement over its interactions with another member of the neurotrophin family, neurotrophin 3 (NT-3). In fibroblast cell lines expressing ectopic TrkA, NT-3 was found to activate TrkA substantially (6) or negligibly (7). The pheochromocytoma cell line PC12 responds to activation of TrkA by differentiating into a neuronal phenotype (8), but aside from one early report (9), the only observed biological effect of NT-3 on PC12 cells is a small increase in serum-free survival at very high concentrations of NT-3 (7).

The reasons for these inconsistencies are not known; however, at least some of the differences in Trk receptor activity arise from their cellular context (7). In addition, there may also be differences in the TrkA cDNA constructs themselves. TrkA cDNAs have been isolated from rat and human (10, 11). Comparison of these two cDNAs revealed a high degree of homology with the exception of an additional 18 bp of sequence found in the extracellular domain of the rat *trkA* clone. Further characterization of the TrkA transcripts from both rat and human has revealed that this difference is a result of alternative splicing of the pre-mRNA encoding TrkA, with tissue-specific localization of the two TrkA isoforms (12). When the neurotrophin responses of the two TrkA isoforms were tested in fibroblast cell lines, no obvious differences were detected (12).

To test possible differences in the two isoforms of TrkA in a more neuronal environment, we have expressed them in the TrkA-deficient cell line PC12^{nnr5}. We find that in this context one of the variants of the TrkA receptor shows a marked enhancement of activation by NT-3.

MATERIALS AND METHODS

Cell Culture. PC12 cells and derivatives were grown on collagen-coated plastic (13). For outgrowth experiments, cells were cultured in Dulbecco's modified Eagle's medium H21 containing 1% horse serum and neurotrophins as indicated. At least 200 cells per condition were quantitated.

Construction of TrkA-expressing Cell Lines. Isolation of rat TrkA cDNAs has been described (13). The DNA sequences of the two splice forms agreed with those presented previously (11, 12). cDNAs encoding the two TrkA isoforms were inserted into the retroviral expression vector LNCX-XHO', a derivative of the LNCX vector (14). These plasmids were transfected into the ψ-2 packaging cell line, leading to the production of retroviruses encoding the two forms of rat TrkA. PC12^{nnr5} cells were infected with either the $TrkA_I$ or $TrkA_{II}$ virus and selected with G418. The effects of neurotrophins on NTA cells were confirmed with populations derived from two independent infections of each virus.

Immunoblot, Cell Surface Labeling, and Immunoprecipitations. The production of antibodies recognizing the extracellular domains of rat TrkA (RTA) and the LNGFR (REX) and conditions of cell lysis, immunoprecipitation, and immunoblotting have been described (13, 15). For cell surface labeling, 10^7 cells were incubated in phosphate-buffered saline (PBS) containing 1 mCi (37 MBq) of $Na^{125}I$, 60 μg of lactoperoxidase, and 0.001% hydrogen peroxide for 30 min. TrkA was precipitated from 10^7 cpm of each resulting lysate. For histochemical analysis of TrkA expression, the cultures were fixed with 4% paraformaldehyde in PBS and quenched in 10% methanol/3% hydrogen peroxide/PBS. TrkA was detected with the peroxidase method using the ABC protocol (Vector Laboratories). Background staining was determined by omitting the primary antibody.

RNA Analysis. RNA from PC12, NTA_{II}, and NTA_I cells treated with NGF or NT-3 was prepared as described (16), separated in a 1.3% agarose/formaldehyde gel, and trans-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: NGF, nerve growth factor; NT-3, neurotrophin 3; LNGFR, low-affinity NGF receptor.

*Present address: SUGEN, Inc., 515 Galveston Drive, Redwood City, CA 94063.

ferred to Hybond-N (Amersham). The top and bottom of the membrane were hybridized with ZIF268 and cyclophilin probes, respectively (17–19). Quantitation was performed with a PhosphorImager (Molecular Dynamics).

RESULTS

During isolation of cDNAs encoding the TrkA receptor from the PC12 cell line (13), we found that two isoforms of the receptor were encoded in our cDNA library, differing in 18 bp in the extracellular domain (Fig. 1). A recent study confirms that these two forms of TrkA arise from the addition of a variable exon during pre-mRNA splicing (12); they have been denoted $TrkA_I$ (lacking the exon) and $TrkA_{II}$ (containing the exon). However, no differences were detected in the neurotrophin responses of the two splice forms of TrkA after expression of each in fibroblast cell lines (13).

As splicing differences can alter the biochemical properties of a receptor, we examined the two forms of TrkA by using a cell line which can respond to TrkA activation by expressing a neuron-like differentiation pathway. A derivative of the PC12 cell line, $PC12^{nnr5}$ (20) can no longer respond to NGF because it lacks TrkA expression (21); introduction of a TrkA receptor restores NGF responsiveness which is essentially indistinguishable from the parental cell line (22). The $PC12^{nnr5}$ cell line was therefore used for transfections to test the biological activities of the two TrkA isoforms. To obtain constructs which would express the two forms of TrkA equivalently, two *Bgl* II sites (Fig. 1) were used to exchange the central section of $TrkA_I$ (cDNA isolate 2) into $TrkA_{II}$ (cDNA isolate 6), producing two cDNA constructs which had identical 5′ and 3′ untranslated regions and differed only in the presence of the variable exon. The two cDNA constructs were cloned into a retroviral vector, and the resulting retroviruses were used to infect $PC12^{nnr5}$ cells, yielding hundreds of G418-resistant colonies. The $PC12^{nnr5}$ cell line exhibits a high degree of variability in its morphology; therefore we cultured the resulting G418-resistant cells from each infection as a population. The infection with the $TrkA_I$ virus resulted in a cell line named NTA_I (nnr5 $TrkA_I$), and the population deriving from the $TrkA_{II}$ virus was named NTA_{II}.

We tested the levels of TrkA expression in each of the NTA cell lines. By immunoblot using an antibody against the extracellular domain of TrkA, or by immunoprecipitation of surface-iodinated cells, the two forms of the receptor were judged to be synthesized in comparable amounts and transported to the cell surface equivalently (Fig. 2). The NTA lines expressed 140-kDa and 110-kDa *trkA* products (Fig. 2*A*), of which only the 140-kDa form was expressed on the surface of the cell, as judged by surface iodination (Fig. 2*B*). The populations taken as a whole expressed 4-fold higher levels of TrkA than PC12 cells, as determined by quantitation of the 140-kDa band from an immunoblot probed with ^{125}I-labeled protein A. The apparent molecular masses of the two receptor isoforms varied slightly, probably due to glycosylation differences; note that there is a potential N-linked glycosylation site encoded 5 bp downstream of the variable exon. In addition, the levels of the LNGFR were tested in all three cell lines; the NTA_{II} and NTA_I cell lines expressed equivalent amounts of the LNGFR, 60% less than was expressed by PC12 cells (Fig. 2).

The first detectable event following binding of neurotrophin to the TrkA receptor is receptor autophosphorylation on tyrosine. We screened the two cell lines for neurotrophin responsiveness by examining increases in receptor phosphotyrosine levels. Both the NTA_{II} and NTA_I cell lines were able to respond strongly to NGF, as was the PC12 cell line. In contrast, NT-3 had no detectable effect on PC12 cells and a minimal effect on NTA_I cells but, surprisingly, had a marked effect on NTA_{II} cells expressing the TrkA isoform containing the variable exon. To test whether the observed increase in $TrkA_{II}$ phosphorylation in response to NT-3 was accompanied by activation of signaling pathways, we sought to quantitate a downstream signaling event. Several of the intermediate early class of transcriptional regulators are induced in PC12 cells 30–60 min after NGF treatment (23). We tested for induction of ZIF268, also known as NGFI-A (17, 24), 30 min after treatment of PC12 and NTA cultures with NGF or NT-3. Table 1 shows quantitation of six RNA blot experiments in which the induction of ZIF268 gene expression is normalized to the expression of cyclophilin, a

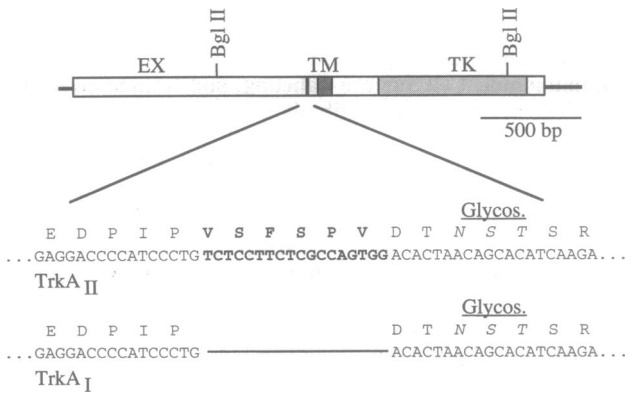

FIG. 1. Alternative splicing of rat TrkA. The TrkA cDNA is shown diagrammatically above with the extracellular (EX), transmembrane (TM), and intracellular tyrosine kinase (TK) domains and noncoding sequence (thin lines) indicated. Also shown are the *Bgl* II sites used in the construction of the cDNA expression constructs. The variable exon is depicted as a thin black line. Below, the region of the alternative splice is expanded with the nucleotide and amino acid sequences of the two splice forms shown. The region included only in the $TrkA_{II}$ splice form is depicted in boldface, and a nearby potential N-linked glycosylation site (Glycos., NST sequence) is also indicated.

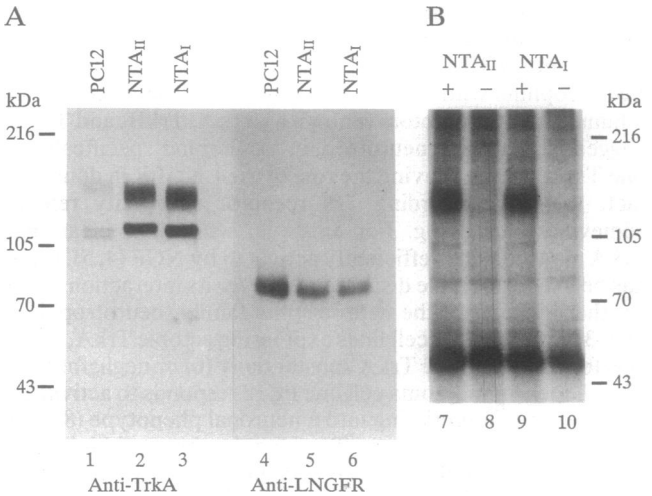

FIG. 2. Expression of LNGFR and TrkA in PC12 cells and in transfected $PC12^{nnr5}$ cells. (*A*) Immunoblot of TrkA and LNGFR in PC12 (lanes 1 and 4), NTA_{II} cells (expressing $TrkA_{II}$; lanes 2 and 5), and NTA_I cells (expressing $TrkA_I$; lanes 3 and 6). Each lane contains glycoproteins from 25 μg of cell lysate concentrated by adsorption to wheat germ agglutinin-agarose. Lanes 1–3 and lanes 4–6 were incubated with the RTA anti-TrkA antibody and the REX anti-LNGFR antibody, respectively. (*B*) Cell surface expression of $TrkA_{II}$ and $TrkA_I$ in $PC12^{nnr5}$ cells. NTA_{II} or NTA_I cells were surface iodinated and TrkA immunoprecipitated from each lysate is shown in + lanes. The − lanes show the results of identical precipitations performed without the primary antibody.

Table 1. Ligand-dependent induction of the ZIF268 (NGFI-A) gene

No.	Cell line	Treatment	ZIF268 expression
1	PC12	No addition	0.07 ± 0.013
2	PC12	NGF (50 ng/ml)	1.65 ± 0.400
3	PC12	NT-3 (50 ng/ml)	0.03 ± 0.006
4	NTA_{II}	No addition	0.03 ± 0.010
5	NTA_{II}	NGF (50 ng/ml)	0.99 ± 0.257
6	NTA_{II}	NT-3 (50 ng/ml)	0.37 ± 0.108
7	NTA_I	No addition	0.04 ± 0.011
8	NTA_I	NGF (50 ng/ml)	0.85 ± 0.295
9	NTA_I	NT-3 (50 ng/ml)	0.07 ± 0.018

The levels of ZIF268 and cyclophilin mRNA in each condition were determined as described in *Materials and Methods*. The results were normalized by calculating the ZIF268/cyclophilin signal ratio, and the means and their standard errors derived from six experiments are shown. The relatively high experimental error derives from variation in the specific activity of the two probes between experiments. Analysis with the two-tailed *t* test for paired samples showed the following means to differ significantly ($P < 0.05$): 2 vs. 3; 5 vs. 6; 8 vs. 9; 6 vs. 9. The following were tested and found not to differ significantly: 2 vs. 5; 2 vs. 8; 5 vs. 8.

transcript whose expression is unchanged by NGF (23). NGF efficiently induced the ZIF268 gene in all three cell lines. NT-3 showed no significant effect on PC12 or NTA_I cells but was able to promote a considerable induction of ZIF268 (37% of NGF) in the NTA_{II} cell line.

To test whether the entire NGF signaling pathway had been activated, we determined whether cellular differentiation occurred, which is most easily ascertained by the elaboration of neuronal processes. PC12, NTA_{II}, and NTA_I cells were cultured in the presence of NGF or NT-3 for 6 days. Within 3 days, NTA_{II} and NTA_I cells were extending long processes and developing somatic hypertrophy in response to NGF. This rapid response to NGF by PC12 cells overexpressing TrkA has been noted previously (25). Shorter processes were detected at this time in the PC12/NGF wells and the NTA_{II}/NT-3 wells. After 6 days (Fig. 3), PC12, NTA_{II}, and NTA_I cells each had produced a large network of processes in response to NGF. PC12 or NTA_I cells cultured in the presence of NT-3 showed no significant outgrowth,

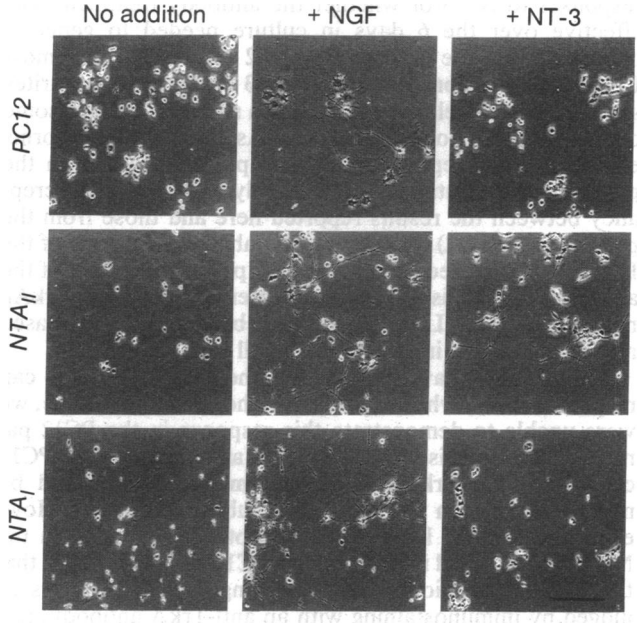

FIG. 3. NT-3-dependent differentiation of NTA_{II} cells. PC12, NTA_{II}, and NTA_I cells were treated with NGF at 50 ng/ml, NT-3 at 50 ng/ml, or no neurotrophin for 6 days. (Bar = 100 μm.)

while the NTA_{II} cells, cultured with NT-3, exhibited considerable process outgrowth at this time. The average lengths of the processes generated by the NTA_{II} line in the presence of NT-3 was shorter than that promoted by NGF on any of the three cell lines, however.

Process outgrowth was quantitated by determining the percentage of cells in each condition bearing a process over two cell diameters in length. NGF promoted a very similar level of differentiation in both NTA_{II} and NTA_I cells, reaching a plateau of 70% differentiated cells (Fig. 4). The percentage of NTA_{II} or NTA_I cells which differentiate in the presence of high concentrations of NGF defines the potential neurotrophin-responsive population; the 30% of NTA_{II} or NTA_I cells which did not respond to NGF expressed insufficient amounts of TrkA to differentiate. This was determined by a histochemical analysis of the NGF-responsive and -unresponsive populations. NTA cultures were fixed and subjected to TrkA histochemistry following 2 days of treatment with NGF at 50 ng/ml. The percentage of cells in the NTA_{II} and NTA_I cultures which expressed detectable levels of TrkA was 73% and 70%, respectively, consistent with the numbers of NGF-responsive cells. Furthermore, all of the cells in the culture which had developed processes were immunopositive for TrkA, indicating that TrkA expression was linked to NGF responsiveness.

About 35% of the NTA_{II} cells extended processes in the presence of the higher concentrations of NT-3 (half as many cells as responded to the same concentrations of NGF); this dropped to 12% of the population for NT-3 at 10 ng/ml. Immunolocalization of TrkA in these cultures revealed that no unstained cells responded to NT-3 at 50 ng/ml after 2 days of culture, while 41% of the TrkA-positive cells had extended processes. Only a very few cells in the NTA_I cell line extended processes in response to NT-3, and only at the highest concentrations. However, NT-3 had a morphological effect on NTA_I cells; a high proportion of them became spindle shaped (a morphology seen in all three cell lines in the presence of NGF).

Benedetti *et al.* (26) have characterized a transfected variant of the PC12 cell line which expresses an extracellular truncation of the LNGFR and also has a greatly reduced expression of full-length LNGFR. This line exhibits a substantial response to NT-3, including neurite outgrowth. As it

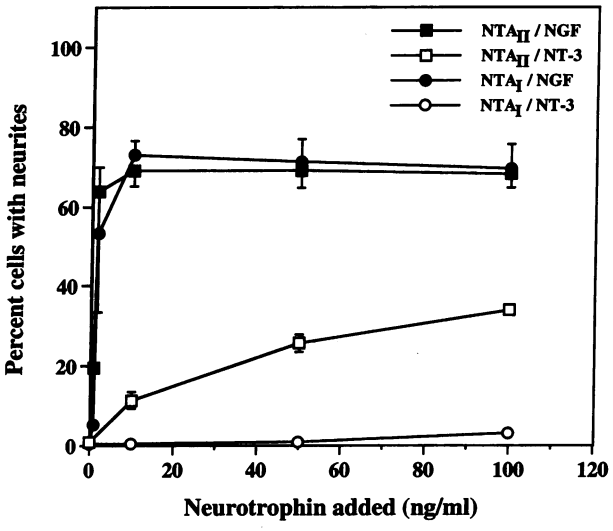

FIG. 4. Quantitation of neurite outgrowth. PC12, NTA_{II}, and NTA_I cells were cultured in the presence of varying concentrations of NGF or NT-3. These results are the means of four similar experiments. The difference between NTA_{II}/NT-3 and NTA_I/NT-3 was found to be significant ($P < 0.01$) at each concentration tested (two-tailed *t* test for paired samples).

has previously been postulated that PC12 cells have a more restrictive environment for neurotrophin responsiveness than fibroblast cell lines (7), it seems plausible that at least one of the mechanisms that restricts NT-3 response by TrkA in the PC12 line and its derivatives is provided by the LNGFR. We tested this potential role of the LNGFR by using the polyclonal anti-LNGFR antibody REX (15) to bind the LNGFR for 1 hr prior to neurotrophin addition. To minimize the culture volumes in these experiments, we chose to measure the activity of the neurotrophins by following the burst of neurotrophin-dependent protein tyrosine phosphorylation in whole cell lysates (13, 27). A marked upregulation of NT-3 responsiveness was detected in both the NTA_{II} and NTA_{I} cell lines after preincubation with the REX antibody (Fig. 5). A strong response to NT-3 in the NTA_{II} line became more robust (lanes 5 and 6), and the response in the NTA_{I} line became easily detectable (lanes 7 and 8). The response of the NTA lines to NGF was partially inhibited under the same conditions (lanes 9–12). The decrease in the strength of the NGF response was also detected in the PC12 cell line after preincubation with the anti-LNGFR antibody, similar to an earlier report where the anti-LNGFR monoclonal antibody 192 was able to partially inhibit the NGF-dependent induction of Fos mRNA in PC12 cells (28).

DISCUSSION

It has been postulated that the cellular context of a Trk receptor modulates the specificity of receptor activation (7). For example, TrkA has been demonstrated to become phosphorylated in response to NT-3 when expressed ectopically in fibroblasts (6), yet the TrkA expressed endogenously in PC12 cells is not activated by NT-3 as shown by several criteria. A cell could use mechanisms which are intrinsic to the receptor for modulating the response to neurotrophins; for example, control of receptor splicing, glycosylation, and receptor copy number. In addition, extrinsic mechanisms, such as expression of other receptor subunits and signaling molecules, may also be employed to modulate receptor activity.

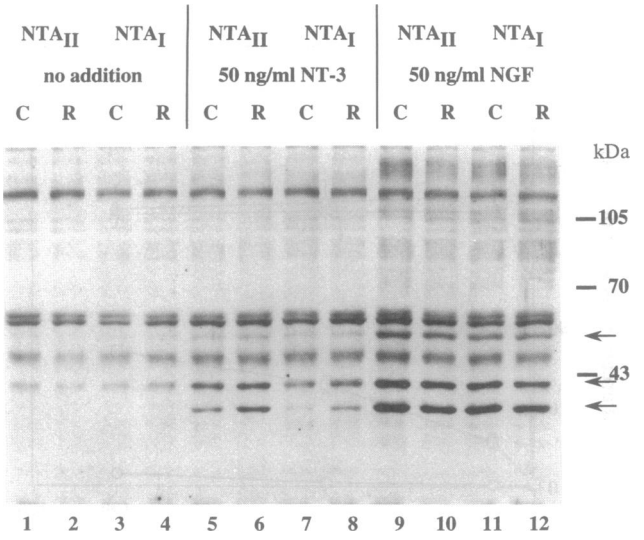

FIG. 5. The REX antibody increases NT-3 response in the NTA cell lines. NTA_{II} and NTA_{I} lines were preincubated with the anti-LNGFR IgG REX (R) or control nonimmune IgG (C) prior to addition of NGF or NT-3 at 50 ng/ml for 5 min as indicated. Changes in phosphotyrosine were detected by antigen blot. The arrows at right denote several proteins which became hyperphosphorylated during the assay.

We have found that two isoforms of TrkA which vary in a small exon in the extracellular domain do show significant differences in their ability to be activated by NT-3 when expressed in a variant of the PC12 cell line. While it is also possible that they also differ somewhat in their response to NGF, we have not detected this. This finding is in contrast to a previous report in which similar receptor constructs were expressed in fibroblast cell lines and each of the receptor isoforms was found to be phosphorylated modestly by NT-3 (12). Although those authors did not examine downstream consequences of receptor activation, it is quite possible that the differences between this study and the previous one come not from methodology but from the cell type used for expression. As noted above, fibroblast cell lines have been found to be in general more permissive for Trk receptor activation than the PC12-derived cell lines.

If a difference exists between the fibroblast lines and the $PC12^{nnr5}$ cell lines, what is it likely to be? A leading candidate for one of the control mechanisms is the p75 LNGFR, which may affect both NGF and NT-3 responsiveness by TrkA and which is not expressed in fibroblast cell lines. A previous report (26) examined the NT-3 responsiveness of a PC12 variant which expressed very low levels of the full-length LNGFR. NT-3 was found to promote substantial neurite outgrowth from this cell line. We used a polyclonal antibody to the LNGFR which has been shown to block NGF binding to the LNGFR (15) to perturb its interactions with TrkA, NGF, and NT-3. We found only a modest decrease in the response to NGF in the presence of the anti-LNGFR antibody, consistent with the inhibition seen with an anti-LNGFR monoclonal antibody with NGF at 50 ng/ml (28). At lower concentrations of NGF, the inhibition by the anti-LNGFR monoclonal (28) or polyclonal (unpublished results) antibody is more striking, but not complete. This is most likely the reason that the polyclonal antibody was unable to affect neuronal survival under fairly high NGF concentrations (15). However, using a similar experimental paradigm we were able to detect an increase in TrkA-dependent protein tyrosine phosphorylation in response to NT-3 from both of the TrkA receptor isoforms. We also attempted to use this approach to affect NT-3-dependent neurite outgrowth but were unable to detect any clear increases. It is unknown whether blocking the LNGFR influences only short-term responses to NT-3 or whether the antibody treatment was effective over the 6 days in culture needed to generate outgrowth, but the finding that PC12 cells which lack most LNGFR expression respond to NT-3 by extending neurites supports the model that LNGFR can suppress at least some of the NT-3 responses of TrkA. As both TrkA isoforms exhibited NT-3-dependent tyrosine phosphorylation in the presence of the anti-LNGFR antibody, some of the discrepancy between the results reported here and those from the previous study (12) could be attributable to the effects of the LNGFR. It also seems possible that part of the effect of the alternative exon is to decrease interaction of the $TrkA_{II}$ receptor with the LNGFR and thereby permit an increased response to NT-3 in the $PC12^{nnr5}$ cell line.

Although we have shown that the $TrkA_{II}$ isoform can respond to NT-3 when expressed in the $PC12^{nnr5}$ cell line, we were unable to demonstrate this response in the PC12 parental line. Both isoforms of TrkA are expressed in PC12 cells, with the $TrkA_{II}$ form predominating as judged by mRNA expression (ref. 12 and unpublished results). However, the average level of TrkA protein expression in the NTA lines is 4-fold increased over PC12 cells. We found that the NTA populations express a range of TrkA levels as judged by immunostaining with an anti-TrkA antibody, and therefore some cells in each population express >4-fold higher TrkA levels. Increasing TrkA expression in PC12 cells does have profound effects on NGF signaling, including

higher and more prolonged activation of signaling molecules and a much more rapid differentiation response (25). While the effects of NT-3 on PC12 cells overexpressing TrkA have not been characterized previously in great detail, at least some responses to NT-3 have been reported, including the NT-3-dependent phosphorylation of the SNT protein (29). Furthermore, the level of endogenous LNGFR in the NTA lines is reduced to 40% of the level in PC12 cells (Fig. 2 and ref. 21). In light of the proposed suppressant role of the LNGFR on TrkA responsiveness to NT-3, it seems likely that the discrepancy between the actions of NT-3 on the NTA_{II} cell line and PC12 cells arises from differences in receptor concentration as well as the ratio of TrkA to the LNGFR. However, the inability of the $TrkA_I$ receptor relative to the $TrkA_{II}$ receptor to respond to NT-3 must be a consequence of its splice form, as the levels of TrkA and the LNGFR expressed in the two NTA lines are the same.

If the potential mechanisms discussed above function in neurons *in vivo* to control TrkA receptor specificity, Trk receptors may exhibit ligand-activation specificities which are distinct from one type of neuron to another. The two isoforms of TrkA were found to be distributed in a tissue-specific manner, with the $TrkA_{II}$ isoform predominating in neural tissues (12). In sensory and sympathetic neurons, the $TrkA_{II}$ receptor is coexpressed with the LNGFR (30); however, the ratio of the two receptors is unknown. On the other hand, TrkA has been found to be expressed in striatal neurons without LNGFR coexpression (31, 32), although the TrkA isoform has not been defined. Therefore, in each of these examples, NT-3 could potentially play a role in receptor activation and neuronal function. Differential mRNA splicing is likely to be one of a number of mechanisms that is used to control the specificity of Trk receptors throughout the life of the animal.

We thank Dr. Lloyd Greene for the gift of $PC12^{nnr5}$ cells, Dr. Patricia A. Olson at Chiron for the gift of NT-3, Dr. W. Mobley for the gift of NGF, and Dr. J. Feder for the ZIF268 and p1B15 plasmids. We thank Drs. Isabel Fariñas, Frances Lefcort, and Uli Muller for helpful comments on the manuscript. Marcia Stubblebine aided in the sequence analysis. D.O.C. gratefully acknowledges the support of the Helen Hay Whitney Foundation. This research was supported by National Institute of Mental Health Center Grant 48200. L.F.R. is an Investigator of the Howard Hughes Medical Institute.

1. Bothwell, M. (1991) **65**, 915–918.
2. Chao, M. V. (1992) *Neuron* **9**, 583–593.
3. Meakin, S. O. & Shooter, E. M. (1992) *Trends Neurosci.* **15**, 323–331.
4. Klein, R., Jing, S. Q., Nanduri, V., O'Rourke, E. & Barbacid, M. (1991) *Cell* **65**, 189–197.
5. Kaplan, D. R., Hempstead, B. L., Martin-Zanca, D., Chao, M. V. & Parada, L. F. (1991) *Science* **252**, 554–558.
6. Cordon-Cardo, C., Tapley, P., Jing, S. Q., Nanduri, V., O'Rourke, E., Lamballe, F., Kovary, K., Klein, R., Jones, K. R., Reichardt, L. F. & Barbacid, M. (1991) *Cell* **66**, 173–183.
7. Ip, N. Y., Stitt, T. N., Tapley, P., Klein, R., Glass, D. J., Fandl, J., Greene, L. A., Barbacid, M. & Yancopoulos, G. D. (1993) *Neuron* **10**, 137–149.
8. Greene, L. A. & Tischler, A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2424–2428.
9. Rosenthal, A., Goeddel, D. V., Nguyen, T., Lewis, M., Shih, A., Laramee, G. R., Nikolics, K. & Winslow, J. W. (1990) *Neuron* **4**, 767–773.
10. Martin-Zanca, D., Oskam, R., Mitra, G., Copeland, T. & Barbacid, M. (1989) *Mol. Cell. Biol.* **9**, 24–33.
11. Meakin, S. O., Suter, U., Drinkwater, C. C., Welcher, A. A. & Shooter, E. M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 2374–2378.
12. Barker, P. A., Lomen-Hoerth, C., Gensch, E. M., Meakin, S. O., Glass, D. J. & Shooter, E. M. (1993) *J. Biol. Chem.* **268**, 15150–15157.
13. Clary, D. O., Weskamp, G., Austin, L. R. & Reichardt, L. F. (1994) *Mol. Biol. Cell* **5**, 549–563.
14. Miller, A. D. & Rosman, G. J. (1989) *BioTechniques* **7**, 980–990.
15. Weskamp, G. & Reichardt, L. F. (1991) *Neuron* **6**, 649–663.
16. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
17. Christy, B. A., Lau, L. F. & Nathans, D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7857–7861.
18. Danielson, P. E., Forss-Petter, S., Brow, M. A., Calavetta, L., Douglass, J., Milner, R. J. & Sutcliffe, J. G. (1988) *DNA* **7**, 261–267.
19. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
20. Green, S. H., Rydel, R. E., Connolly, J. L. & Greene, L. A. (1986) *J. Cell Biol.* **102**, 830–843.
21. Loeb, D. M., Maragos, J., Martin, Z. D., Chao, M. V., Parada, L. F. & Greene, L. A. (1991) *Cell* **66**, 961–966.
22. Loeb, D. M. & Greene, L. A. (1993) *J. Neurosci.* **13**, 2919–2929.
23. Feder, J. N., Jan, L. Y. & Jan, Y.-N. (1993) *Mol. Cell. Biol.* **13**, 105–113.
24. Milbrandt, J. (1987) *Science* **238**, 797–799.
25. Hempstead, B. L., Rabin, S. J., Kaplan, L., Reid, S., Parada, L. F. & Kaplan, D. R. (1992) *Neuron* **9**, 883–896.
26. Benedetti, M., Levi, A. & Chao, M. V. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 7859–7863.
27. Maher, P. A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6788–6791.
28. Milbrandt, J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4789–4793.
29. Rabin, S. J., Cleghon, V. & Kaplan, D. R. (1993) *Mol. Cell. Biol.* **13**, 2203–2213.
30. Schecterson, L. C. & Bothwell, M. (1992) *Neuron* **9**, 449–463.
31. Holtzman, D. M., Li, Y., Parada, L. F., Kinsman, S., Chen, C. K., Valletta, J. S., Zhou, J., Long, J. B. & Mobley, W. C. (1992) *Neuron* **9**, 465–478.
32. Steininger, T. L., Wainer, B. H., Klein, R., Barbacid, M. & Palfrey, H. C. (1993) *Brain Res.* **612**, 330–335.