**Nerve Growth Factor Binding Site on TrkA Mapped to a Single 24-Amino Acid Leucine-rich Motif**

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The extracellular domains of the TrkA nerve growth factor (NGF) receptor and its homologs harbor a modular mosaic of potential ligand binding motifs, namely two immunoglobulin (Ig)-like modules and an LRM3 cassette consisting of a tandem array of three leucine-rich motifs (LRMs) flanked by cysteine-rich clusters (Schneider, R., and Schweiger, M. (1991) Oncogene 6, 1807-1811). Identification of a structural motif capable of specifically recognizing the various neurotrophins was achieved by assessing their affinities to isolated recombinant modules of TrkA and TrkB. In both receptors the LRM3 cassette alone could mediate the respective neurotrophin selectivities and affinities. Further tracking down of this NGF-binding site in TrkA strikingly revealed that a single LRM of 24 amino acids could bind NGF selectively with nanomolar affinity. Since this is the first example of a single LRM with a highly specific, well defined function, it might serve as a valuable tool to elucidate the general structural requirements of substrate recognition and high affinity binding in the large superfAMILY of LRM-containing proteins.

Nerve growth factor (NGF) was first discovered over 40 years ago (2). It belongs to the growing family of neurotrophins that promote the survival and differentiation of distinct neuronal populations (3–6). Neurotrophins bind to two discrete receptor types, which can be distinguished pharmacologically. p75NGFR binds all known neurotrophins with similar nanomolar affinities (7, 8), whereas cells expressing TrkA, a tyrosine kinase receptor originally identified as a human oncogene (9), bind solely NGF and exhibit significantly slower dissociation kinetics (10, 11).

There are two receptors homologous to TrkA, namely TrkB and TrkC, which are correlated with the binding of other neurotrophins. TrkB is a receptor for three distinct ligands, brain-derived neurotrophic factor (BDNF) (12), neurophin-3 (NT-3) (5), and neurophin-4 (NT-4) (13). TrkC exclusively binds NT-3 (14). Although only cells expressing Trk-type receptors show functional responses upon neurotrophin binding (15, 16), it is still a matter of debate whether the binding phenomena observed in different cellular systems (17–20) are solely due to the action of these receptors (21, 22) or rather to larger protein complexes involving the concerted action of additional components such as p75NGFR. Other imponderabilities of cell culture-based systems arise from internalization events, temperature dependence, lysosomal degradation, and cell type-specific differences. This prompted us to conduct a systematic biochemical characterization of isolated receptor modules to achieve a clear assignment of affinities and kinetic properties to defined molecular structures.

The extracellular ligand-binding domains of receptor tyrosine kinases harbor various protein-protein interaction motifs that potentially function in ligand binding and/or receptor dimerization (1, 23, 24). In many cases mutations in the extra-cellular domains lead to a ligand-independent constitutive activation of the receptor (25–27). In the case of Trk-type receptors the modular structure of the extracellular domain was elucidated by the application of sequence comparison algorithms (1) capable of detecting even highly degenerate motifs (28). Several distinct protein modules were identified that could serve as docking sites for either neurotrophins or other components involved in a functional receptor complex (1). Among these are immunoglobulin-like domains and leucine-rich motifs (LRMs) containing regions.

LRMs are short amino acid sequences (22–30 residues) that are repeated in tandem in individual protein and contain hydrophobic residues at conserved positions (29). In the case of Trk-type receptors, there are three tandem LRM repeats of, on the average, 24 residues in the N-terminal region of the molecule that are flanked by two cysteine-rich regions, so-called cysteine clusters (1). LRM repeats are potent mediators of strong and specific homo- and heterophilic protein-protein interactions. They have been found in proteins as diverse as human platelet glycoprotein IX (30), Drosophila Toll (31), and Drosophila Chaoptin (32), where they mediate cell-cell interactions and communication, and yeast adenylate cyclase, where they form the interaction site with the Ras protein (33). This clear involvement of LRM repeats in protein-protein interactions made them highly interesting candidates for the NGF binding site within the TrkA receptor.

On the other hand, immunoglobulin-like domains are firmly established as potent ligand binding domains. The keratinocyte growth factor receptor (34), the macrophage colony-stimulating factor receptor (35), and the cellular adhesion molecule 1 (ICAM-1) (36) are prominent examples of receptors that utilize Ig-like domains for ligand binding. Recently two groups have shown independently that the immunoglobulin-like domains of TrkA, TrkB, and TrkC play quantitatively important roles in the binding of the neurotrophin ligands and in the activation of the receptors (37, 38).

**EXPERIMENTAL PROCEDURES**

Cloning and Expression of Receptor Modules—The regions coding for the TrkA/B domains were amplified from rat/mouse brain mRNA by reverse transcription-polymerase chain reaction and cloned into the PMal™-p expression vector. The sequences of the fragments were identical to the ones published in Refs. 19 and 39. The recombinant maltose binding protein (MBP)-TrkA/B fusion proteins were expressed in...
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Escherichia coli and purified essentially as described in the manufacturer’s protocol (New England Biolabs, 1990). For the negative controls a fusion protein composed of MBP and β-galactosidase (MBP-βGal) was expressed. The purified proteins were extensively dialyzed against 20 mM Tris-Cl, pH 7.4, 200 mM NaCl, 1 mM EDTA (column buffer) before they were used in the experiments.

Mouse submaxillary gland NGF-β was purchased from Sigma. Recombinant E. coli and vaccinia virus-expressed mouse BDNF and mouse NT-3 (40) were kind gifts of R. Kolbeck and Y.-A. Barde. 125I-NGF-β and 125I-BDNF were prepared using the lactoperoxidase method (see “Acknowledgements”) or purchased from Amersham Corp.

Binding Assays Using Receptor Affinity Columns—100 µg (~1.5 nmol) of fusion protein were loaded onto a 100-µl amylase resin column equilibrated with column buffer. After washing with 10 volume volumes of the same buffer containing 5 mg/ml bovine serum albumin and 0.1 mg/ml cytochrome (bovine heart) to prevent nonspecific binding to the column material and with another 20 column volumes of the above buffer without protein, 2 µg of the respective neurotrophin in 100 µl of column buffer were loaded onto the column. To determine whether the applied neurotrophin had bound to the respective immobilized Trk domain, 15 µl of the flow-through were loaded onto a 10–20% SDS-polyacrylamide gradient gel. After electrophoresis the gels were silver-stained according to Heukeshoven and Dernick (41). A reduction in the strength of the neurotrophin band as compared with the flow-through of the binding control column (with immobilized βGal-MBP) represents specific binding of the neurotrophin to the respective Trk domain.

The application of column buffer containing 10 mM maltose led to the co-elution of the bound neurotrophins with the recombinant receptor components as determined by SDS-polyacrylamide gel electrophoresis and silver staining. These gels are not shown in this paper because, in addition to the neurotrophins, the eluted fractions contained a greater than 50-fold excess of the respective receptor protein, making it impossible to produce a perspicuous figure.

Equilibrium Binding Assays—100 ng (1.25 pmol) of recombinant protein/assay were batch loaded onto 5 µl of amylase resin in 25 µl of column buffer. After centrifuging, the supernatants were removed, and the pellets were washed three times with column buffer in order to minimize nonspecific binding, the column material was resuspended in 90 µl of column buffer, 5 mg/ml bovine serum albumin, 0.1 mg/ml cytochrome c (bovine heart), 2 mg/ml heat-denatured bovine serum albumin, 0.1 mg/ml heat-denatured cytochrome c and incubated with gentle shaking for 30 min. The denatured proteins were included to give a more solid amylase resin pellet after centrifugation. 5 µl of 125I-NGF at 0.32 pM (equivalent to 1.25 pmol) were added and incubated with gentle shaking at 20 °C for 90 min to reach equilibrium binding. Final concentrations of 125I-NGF ranged from 7.8125 × 10^{-12} to 4 × 10^{-9} M. Each binding reaction was carried out in duplicate, and for each concentration of 125I-NGF a MBP-βGal control was made to detect nonspecific binding to MBP. Differences between duplicate values were generally very small. After centrifuging for 3 min, the supernatants were transferred to fresh tubes (SN1). The pellets were washed three times in column buffer containing 20 mM maltose to elute the receptor-ligand complexes. The supernatants of all three centrifugation steps were combined in a new tube (SN2). SN1, SN2, and the amylase resin pellet were measured on a “Cobra Auto Gamma” counter (Packard). SN1, therefore, represented free 125I-NGF, SN2 corresponded to specifically bound 125I-NGF. The small amounts of radioactivity trapped in the pellet were added to the free 125I-NGF. Nonspecific binding to the MBP in the control experiments was low and subtracted from the specific binding in each case.

Essentially the same experiment was conducted with the other receptors of TrkA and TrkB (using 125I-BDFN). For the binding reactions 1.25 pmol or 2.5 pmol of receptor protein were used.

Kinetics of Association—Binding reactions were prepared as described above and contained 1.25 pmol of receptor/100 µl. The start volume of all reactions was 700 µl. The reactions were brought to final concentrations of 125I-NGF ranging from 5 × 10^{-12} to 2 × 10^{-9} M. Aliquots of 100 µl were taken at the different time points (0, 2, 5, 15, 30, 60, and 90 min) and processed as described above. All data points are means of duplicates.

Calculations of the Kd and the kinetic values were performed according to Rodriguez-Tebar and Barde (42) using the GraFit program (Erithacus).

RESULTS AND DISCUSSION

The goal of this study was to investigate the distinct roles these individual structural modules of Trk-type receptors play in the binding of neurotrophin and how strong of a contribution these receptors make in generating high affinity binding. For an initial experiment, the entire extracellular domains of TrkA and TrkB were expressed in recombinant soluble form and purified as described under “Experimental Procedures.” Immobilized on a column, these recombinant domains were found to exhibit the same ligand binding specificities observed in the cellular system (5, 12, 18), i.e. NGF bound to TrkA, and BDNF and NT-3 bound to TrkB. This is direct evidence that Trk proteins can specifically bind neurotrophins in the absence of their intracellular kinase domain and without membrane attachment or contribution of other receptor components.

Quantification of the affinities of neurotrophins to the recombinant extracellular domains using binding assays with 125I-NGF and 125I-BDNF (see “Experimental Procedures”) led to Kd values of ~10^{-9} M for the respective ligand-receptor pairs (NGF-TrkA and BDNF-TrkB) (Table I). No affinity of 125I-NGF to TrkB and of 125I-BDNF to TrkA was detected (Table I), which verifies the finding of high binding specificity in Trk receptors described above. These results directly show that the NGF binding sites with nanomolar Kd values observed in NIH 3T3, COS, and NR18 cells ectopically expressing TrkA (16, 18) can be attributed entirely to the affinity of the TrkA receptor. The same is true for the BDNF-TrkB interaction. The nanomolar
lar affinities observed by us for this ligand-receptor pair are in good agreement with the data obtained by Dechant et al. (43) for A293 cells ectopically expressing chick TrkB and by Soppet et al. (12) for NIH 3T3 cells ectopically expressing rat TrkB, the receptor used in this work.

Scatchard plot analyses of the interactions of 125I-NGF with the TrkA extracellular domain (Fig. 1) and of 125I-BDNF with the respective region of TrkB (data not shown) revealed no binding sites with affinities in the range of 10^-12 M as observed on NIH 3T3-derived cell lines overexpressing TrkA (44) or even in the picomolar range as exhibited by chick embryonic sensory neurons (17) and other neuronal cells. No affinities of 125I-NGF to TrkB and of 125I-BDNF to TrkA could be detected (Table I). In vivo more than 50% of the extracellular domains of Trk receptors are made up of carbohydrates. Our studies with nonglycosylated recombinant proteins suggest that glycosylation does not enhance the affinity and specificity of the Trk-neurotrophin interactions but they do not exclude the possibility that glycosylation may reduce ligand-binding affinity, possibly even in a regulatory mechanism.

The first step in the identification of the ligand binding sites of TrkA and TrkB was the expression of the two major structural components of their extracellular domains, the LRM3 cassette and the Ig2 domain. As mentioned above, both types of structures have been shown to be capable of exerting strong and specific protein-protein interactions (29, 34–36, 45, 46) and thus were good candidates for the ligand binding site. The recombinant domains were immobilized on column matrices, and their ability to bind NGF, BDNF, and NT-3 was tested (Fig. 2).

The binding experiments with the recombinant Ig2-domains revealed no detectable affinity of NGF, BDNF, or NT-3 to this segment of any of the two Trk receptors investigated (Fig. 2A). Additional binding assays using 125I-NGF and 125I-BDNF (Table I) confirmed these results. Therefore, our experiments did not support a role of the Ig2-domain in the binding of NGF to TrkA and the binding of BDNF and NT-3 to TrkB, but rather suggested the LRM3 cassette as the major neurotrophin binding site. Since the immunoglobulin-like domains have recently been shown to significantly contribute to ligand binding in Trk receptors (37, 38) we assume the lack of glycosylation or some other system-inherent problem prevented the binding of NGF to the immunoglobulin-like domains in our assays. We therefore concentrated on investigating the role of the N-terminal LRM3 cassette in Trk receptor function.

This structural entity of TrkA and TrkB could still discriminate between NGF, BDNF, and NT-3 in that the LRM3 cassette of TrkA specifically bound NGF (but not BDNF and NT-3) and the LRM3 cassette of TrkB specifically bound BDNF and NT-3 (but not NGF) (Fig. 2B). These results demonstrate that the LRM3 cassettes of TrkA and TrkB contain neurotrophin binding sites displaying the same ligand binding specificities as the entire receptors. Quantitative binding assays revealed that the LRM3 cassettes of TrkA and TrkB bind 125I-NGF and 125I-BDNF, respectively, with the same nanomolar affinities as the complete recombinant extracellular domains (Table I).

Since the expression of trkB-derived proteins is significantly hampered by their high toxicity to E. coli, we chose TrkA to systematically trace down the exact location of a neurotrophin binding site. Creating appropriate expression vector constructs, the two cysteine clusters flanking the LRM3 cassette were removed first (L1–3) followed by the elimination of the first (C1L1–1.5) and the second (C1L1–2) LRM repeat in two separate approaches. All these soluble recombinant receptor proteins showed the same nanomolar affinities and specificities for 125I-NGF (Table I), suggesting that the 24 amino acids of the middle LRM are sufficient to constitute a ligand binding site.

This could be demonstrated by the expression of three further fusion proteins, the isolated second LRM (L2) and two proteins disrupting the second LRM (L2 and L2-L2) in the very center from opposite sides (C1L1-L1.5 and L1.5-L2C2). The L2 region exhibited full binding affinity (Kd for 125I-NGF ~ 10^-9 M) and specificity, being able to discriminate between NGF, BDNF, and NT-3 in that no affinity of L2 for the latter two neurotrophins was detected (Table I). Scatchard plot analysis revealed the existence of a single type of binding site (data not shown). In contrast, C1L1-L1.5 and L1.5-L2C2 showed no detectable affinity for 125I-NGF and 125I-BDNF (Table I). These data unambiguously
The L2 fusion protein is capable of forming oligomers in solution, plexes. Preliminary results from our laboratory show that the correlation to the formation of di- or oligomeric receptor complexes is unclear. The observed negative cooperativity may somehow be related to the kinetics of association and dissociation. For this reason, as for the association rate, the calculation of a second LRM is the only repeat in Trk-type receptors that matches perfectly with the general LRM consensus sequence making it a classic structural and functional unit.

To investigate in more detail what part of the binding characteristics of the NGF receptor complex observed in the cellular system can be attributed to TrkA or, more precisely, to an unprecedentedly small ligand binding site of TrkA, the binding kinetics of the recombinant TrkA extracellular domain as well as of L2 were examined and compared with the data that have been determined for different in vivo systems.

The kinetics of association of L2 with 125I-NGF were found to be complex (Fig. 4A). The relationship between the observed association rate (k_{obs}) and the ligand concentration is linear at low concentrations of NGF (5 × 10^{-10} to 4 × 10^{-11} M), allowing the calculation of a k_{1} close to 1 × 10^{7} M^{-1} s^{-1} (Fig. 4A). At higher concentrations of ligand (around the K_{d}), a mechanism of negative cooperativity seems to take effect again, leading to a linear relationship between k_{obs} and NGF concentration, allowing the calculation of a second k_{1} value of about 3 × 10^{5} M^{-1} s^{-1} (Fig. 4A). These data correlate well with the ones obtained with the recombinant extracellular domain. The off rate that was observed at low concentrations of ligand is akin to that observed on PC12 cells (48). Experiments by Mahadeo et al. (49) using mutants of PC12 cells lacking p75NGFR indicated that in vivo p75NGFR may in some way assist TrkA in recruiting ligand since only in the presence of this second neurotrophin receptor were k_{1} values in the range of 10^{7} M^{-1} s^{-1} observed. Our results indicate that TrkA may be sufficient to generate on rates in this range even though such a mechanism seems to be only effective at low concentrations of ligand.

The dissociation kinetics revealed a similarly complex scenario apparently displaying a curve composed of two separable components (Fig. 4B). For this reason, as for the association rates, two different half-lives (-16 and -110 min) for the ligand-receptor complex and two different k_{-1} values (-5 × 10^{-4} s^{-1} and -1 × 10^{-4} s^{-1}) could be calculated, again with L2 exhibiting the same behavior as the entire extracellular domain of TrkA (Fig. 4B). Remarkably, not only this biphasic behavior but also the measured values are in good agreement with the ones observed by Meakin et al. (19) in COS cells expressing rat TrkA (half-lives of 10 and 90 min, respectively). All of these values clearly represent slow dissociation kinetics, indicating that the second leucine-rich motif may be involved in determining the complex kinetics of dissociation that define TrkA as the "slow NGF receptor" (50).

The molecular mechanism of this complex behavior with respect to the kinetics of association and dissociation is as yet unclear. The observed negative cooperativity may somehow be correlated to the formation of di- or oligomeric receptor complexes. Preliminary results from our laboratory show that the L2 fusion protein is capable of forming oligomers in solution, albeit probably with lower efficiency than the entire extracellular domain. Taking into account the concentration of the recombinant proteins in the assays it seems quite possible that also in this system most of the L2 proteins are present in di-/oligomeric form. These dimers could bind two NGF dimers and plot according to the total concentrations of NGF. The k_{1} values were obtained from the slopes of the regression lines. Binding reactions were prepared as described under "Experimental Procedures." The reactions were brought to final concentrations of 125I-NGF ranging from 5 × 10^{-12} M to 2 × 10^{-9} M. Aliquots were taken at the different time points and processed as described above. All data points are means of duplicates. The data are corrected for nonspecific binding. The k_{1} values for the second LRM of TrkA (L2, O) and the TrkA extracellular domain ( ) were (8.54 ± 1.37 × 10^{-6})(2.87 ± 0.68 × 10^{-6}) M^{-1} s^{-1} and (7.03 ± 1.03 × 10^{-6})(2.53 ± 0.72 × 10^{-6}) M^{-1} s^{-1}, respectively. B, kinetics of dissociation. Binding reactions were prepared and brought to equilibrium binding as described under "Experimental Procedures." Concentrations of 125I-NGF ranged from 5 × 10^{-10} to 4 × 10^{-9} M. Dissociation of 125I-NGF was induced by the addition of a 100-fold excess of unlabeled NGF. Aliquots were taken at the different time points and processed as described above. All data points are means of duplicates. Two separate curves can be drawn through the above data leading to two different t_{1/2} values for each data set. The data for the second LRM of TrkA (L2, ●) and the TrkA extracellular domain (Ex, ○) are almost identical, with t_{1/2} values of -16/110 min and (-5 × 10^{-4} s^{-1})(1 × 10^{-4} s^{-1}), respectively.

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Fig. 3. Leucine-rich motifs and the NGF binding site of TrkA. The first two lines show an alignment of the second LRM from TrkA and TrkB. The third line is the consensus of both sequences. The last line shows the general consensus sequence of a leucine-rich motif.

FIG. 4. The binding kinetics of 125I-NGF to the 24-amino acid second LRM of TrkA are equivalent to the ones observed for the entire recombinant extracellular domain. A, kinetics of association. The observed association rates (k_{obs}) were calculated from the times at which half of the equilibrium binding was reached (t_{1/2} = ln 2/t) and plotted against the total concentrations of NGF. The k_{1} values were obtained from the slopes of the regression lines. Binding reactions were prepared as described under "Experimental Procedures." The reactions were brought to final concentrations of 125I-NGF ranging from 5 × 10^{-12} M to 2 × 10^{-9} M. Aliquots were taken at the different time points and processed as described above. All data points are means of duplicates. The data are corrected for nonspecific binding. The k_{1} values for the second LRM of TrkA (L2, O) and the TrkA extracellular domain ( ) were (8.54 ± 1.37 × 10^{-6})(2.87 ± 0.68 × 10^{-6}) M^{-1} s^{-1} and (7.03 ± 1.03 × 10^{-6})(2.53 ± 0.72 × 10^{-6}) M^{-1} s^{-1}, respectively. B, kinetics of dissociation. Binding reactions were prepared and brought to equilibrium binding as described under "Experimental Procedures." Concentrations of 125I-NGF ranged from 5 × 10^{-10} to 4 × 10^{-9} M. Dissociation of 125I-NGF was induced by the addition of a 100-fold excess of unlabeled NGF. Aliquots were taken at the different time points and processed as described above. All data points are means of duplicates. Two separate curves can be drawn through the above data leading to two different t_{1/2} values for each data set. The data for the second LRM of TrkA (L2, ●) and the TrkA extracellular domain (Ex, ○) are almost identical, with t_{1/2} values of -16/110 min and (-5 × 10^{-4} s^{-1})(1 × 10^{-4} s^{-1}), respectively.

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istics of the TrkA receptor are apparently not dependent on glycosylation as far as the LRM₃ cassette is concerned. The E. coli expressed modules show functionality, and the middle LRM is active even though it is normally located between two potential glycosylation sites (1). The carbohydrate chains attached to these sites may be a reason why other groups (37, 38) using different in vitro and heterologous in vivo systems were unable to detect the interaction observed by us. On neurons there may be a mechanism during receptor activation that exposes the second LRM so it is accessible for NGF and can fulfill its biological role.

Our studies, however, did not demonstrate binding affinities in the range of 5–10 pM as have been described for some cell lines overexpressing TrkA receptors (10, 44). It has been suggested that this high affinity binding may be a result of the formation of di-/oligomeric complexes that represent the activated state of most receptor tyrosine kinases (10). Even though the recombinant protein corresponding to the Trk extracellular domains is capable of forming homodimer/oligomers in vitro, we cannot, with respect to the fairly high ratio of available MBP docking sites on the immobilizing matrix to the amount of Trk-MBP protein present, exclude the possibility that the number of active Trk di-/oligomers in this experimental system is too low for high affinity in the picomolar range to be detected. The observation of negative cooperativity in binding, however, is a good indication that di-/oligomers are indeed formed in our system.

An interesting possibility arising from our results is that the promiscuity observed in Trk-type receptors might be based on the allocation of the binding sites for the different neurotrophins to distinct LRMs within the LRM₃ cassette. This would, for example, allow different neurotrophins to simultaneously bind to one and the same Trk-type receptor.

Finally, the recently determined three-dimensional structure of an LRM protein (51) offers ideal opportunities for molecular modelling studies on the detailed interaction mechanism not only in the case of Trk-type receptors and neurotrophins but in general for the whole superfamily of LRM containing proteins and their substrates. Hence, the precise mapping of this binding site for NGF in TrkA receptors could facilitate the design of new neurotrophic drugs with enhanced performance and a wider range of supported neurons for the efficient treatment of damaged nerves after injuries or in the course of neurodegenerative diseases.

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REFERENCES
1. Schneider, R., and Schweiger, M. (1991) Oncogene 6, 1807–1811
2. Levi-Montalcini, R. (1987) Science 237, 1154–1162
3. Thoenen, H. (1991) Trends Neurosci. 14, 165–170
4. Raffioni, S., Bradshaw, R. A., and Buxser, S. E. (1993) Annu. Rev. Biochem. 62, 823–850
5. Barbacid, M. (1993) Oncogene 8, 2033–2042
6. Götz, R., Köster, R., Winkler, C., Rauff, F., Lottspeich, F., Schard, M., and Thoenen, H. (1994) Nature 372, 266–269
7. Rodriguez-Tebar, A., Dechant, G., and Barde, Y.-A. (1990) Neuron 4, 487–492
8. Barker, P., A., and Murphy, R. A. (1992) Mol. Cell. Biochem. 110, 1–16
9. Martín-Zanca, D., Hughes, S. H., and Barbacid, M. (1986) Nature 321, 743–748
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