

NERVE GROWTH FACTOR IN NEURONAL DEVELOPMENT AND MAINTENANCE

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SUMMARY

One of the major roles of nerve growth factor (NGF) is to mediate the selective survival of a proportion of the developing sympathetic and sensory neurones as they innervate their particular target tissues. The underlying basis of this phenomenon is the synthesis of limited amounts of NGF in the target, its secretion around, and uptake by, the nerve terminal and its retrograde transport along axons to the neuronal cell bodies. The cascades of reactions which lead to neuronal survival and maintenance are initiated by signal transduction somewhere in this pathway. Retrograde transport and the initial signal transduction step begin when NGF binds to NGF receptors on the nerve terminal. Receptor-mediated internalization and the survival and maintenance function of NGF are mediated by the higher affinity receptors. These receptors have relative molecular masses of approx. 145 000 and are trypsin-resistant when occupied. In contrast, the larger population of lower affinity receptors have relative molecular masses of 85 000 and are rapidly degraded by trypsin. Clustering of the lower affinity receptors by a variety of agents gives them many of the characteristics of the higher affinity receptors, suggesting receptor interconversion may play a role in NGF actions. The structure of the lower affinity NGF receptor, determined by gene transfer and cloning, shows it to be a unique, heavily glycosylated protein. The extracellular domain is rich in cysteine-containing repeat units while the intracellular domain lacks the consensus sequence for an endogenous kinase activity. It is likely that the higher affinity receptor contains this protein as the NGF binding subunit together with a second protein which determines both the nature of the signal transduction mechanism and the process of internalization.

INTRODUCTION

The discovery of the flow of nerve growth factor (NGF) from the target tissue of an appropriate neurone to the neuronal cell body provides the framework for an understanding of how the action of NGF leads to the selective survival of a fraction of a group of nerve cells at a critical time in development. The influence of the target on this process of naturally occurring neuronal cell death has been known for a long time, stemming from the findings that neuronal cell death is enhanced by removal of target tissue but reduced by provision of additional target tissue (reviewed in Oppenheim, 1981). That this influence is mediated in targets of sympathetic and

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some sensory neurones by a trophic substance became apparent in the experiments which led to the discovery of NGF (Levi-Montalcini & Hamburger, 1951, 1953). A particularly striking demonstration of the role of NGF in controlling neuronal survival came from the experiments of Levi-Montalcini & Booker (1960a), who found that injections of NGF antiserum in neonatal mice produced massive neuronal death in sympathetic ganglia. Different methods of delivering anti-NGF antibodies extended these observations to dorsal root ganglion sensory neurones (Gorin & Johnson, 1979, 1980; Johnson, Gorin, Brandeis & Pearson, 1980), while all the antibody experiments emphasized that the antibody-induced neuronal cell death occurred at a specific time in the development of the particular neurones. Furthermore, considerable evidence exists to show that neuronal cell death is a result of depriving the animals of endogenous NGF rather than of a complement-mediated cytotoxicity (Goedert *et al.* 1980). The *in vitro* experiments of Campenot (1977) using multi-compartment chambers also demonstrated that NGF interacting with the nerve terminals of sympathetic neurones is sufficient to support neuronal survival. All these data suggest that sensory and sympathetic neurones compete for limiting quantities of NGF when their axons reach their peripheral targets and that the supply of NGF determines the extent of neuronal cell death. A consequence of this hypothesis is that neuronal cell death should be reduced by providing NGF over and above that derived from the target tissue, and this has been found to be true. Again, some of the earlier work showed that NGF administration to neonatal animals increased the size of sympathetic and sensory ganglia as a result of both increased neuronal cell numbers and size (Levi-Montalcini & Angeletti, 1968; Levi-Montalcini & Booker, 1960b; Hendry, 1976; Hendry & Campbell, 1976), processes which correlated well with the timing of neuronal cell death (Cowan, 1975; Carr & Simpson, 1978). More recently, the timing and extent of neuronal cell death of VL and DM sensory neurones has been determined and exogenous NGF has been shown to rescue a significant fraction of VL and essentially all DM neurones (Hamburger, Brunso-Bechtold & Yip, 1981). Clearly NGF acts as an endogenous trophic factor for perhaps all sensory neurones, although it is present in their targets in amounts insufficient to sustain all the neurones during development. It is reasonable to assume that the same conclusion applies to sympathetic neurones.

Besides this key role in development, NGF continues to play an important role in the maintenance of the differentiated state of mature sympathetic and sensory neurones. This is demonstrated by the reduction in protein content, norepinephrine content and tyrosine hydroxylase activity in sympathetic ganglia after administration of anti-NGF antibodies to adult rodents (Goedert, Otten & Thoenen, 1978; Otten, Goedert, Schwab & Thibault, 1979; Ebendal, Olsen, Seiger & Hedlund, 1980) as well as the reduction of substance P levels in the sensory neurones of these animals (Otten, Goedert, Mayer & Lembeck, 1980; Ross *et al.* 1981; Mayer, Lembeck, Goedert & Otten, 1982; Schwartz, Pearson & Johnson, 1982; Kessler, Bell & Black, 1983). Correspondingly, increases in neuronal cell volume and tyrosine hydroxylase activity in the superior cervical ganglion are observed when NGF is injected into the targets (eye or submaxillary gland) of these sympathetic neurones (Paravicini,

Stoeckel & Thoenen, 1975; Hendry, 1977). NGF also partially obviates the decrease in substance P and extralysosomal acid phosphatase seen in dorsal root ganglia after peripheral axotomy (Fitzgerald, Wall, Goedert & Emson, 1985). Since NGF is found in adult rat sciatic nerve in culture (Richardson & Ebendal, 1982) and is produced by non-neuronal cells at the site of and distal to an injury of the same nerve *in vivo* (Heumann, Korsching, Bandtlow & Thoenen, 1987), it is possible that NGF acts to promote sympathetic and sensory axon regrowth *in vivo* as it does *in vitro* (Levi-Montalcini, Meyer & Hamburger, 1954).

THE RETROGRADE FLOW OF NERVE GROWTH FACTOR

Underlying the actions of NGF is its ability to convey information from the peripheral targets to the neuronal cell body by retrograde flow (Fig. 1). The phenomenon was first demonstrated by injecting [125 I]NGF unilaterally into the anterior chamber of the eye of a mouse and observing accumulation of radioactivity, subsequently shown to be in the form of intact, biologically active [125 I]NGF, in the superior cervical ganglion on the side of the injection (Dumas, Schwab & Thoenen, 1979; Johnson, Andres & Bradshaw, 1978). Transection of postganglionic nerves abolishes the preferential labelling, showing that transport occurs in axons. This has also been confirmed by following retrograde transport of [125 I]NGF in sympathetic neurites in culture in compartmentalized chambers (Claude, Hawrot, Dunis & Campenot, 1982). More recently, the retrograde flow of endogenous NGF in the rat sciatic nerve has been demonstrated by its accumulation on the distal, but not the proximal, side of a crush (Korsching & Thoenen, 1983*b*). This experiment and measurement of both NGF and NGF mRNA in a typical sympathetic target (iris) provide the most convincing proof that endogenous NGF is produced in the target, sequestered by nerve terminals and carried retrogradely to the neuronal cell bodies

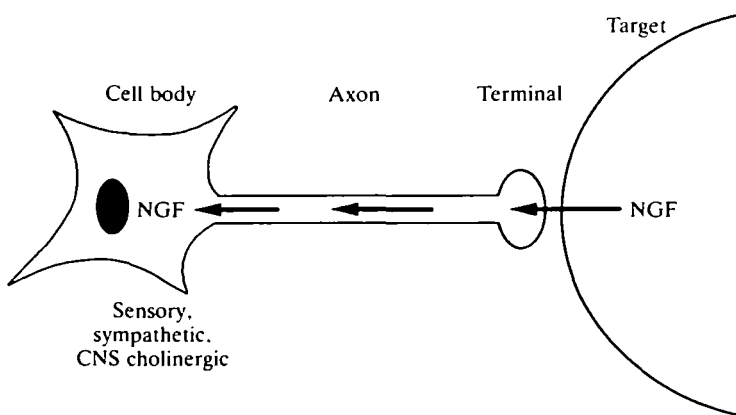


Fig. 1. NGF in development, maintenance and regeneration. The retrograde flow of NGF from the target to the neuronal cell body has been directly demonstrated in sensory and sympathetic fibres and indirectly in the fibres of the CNS cholinergic neurones in the basal prebrain. Any manipulation which reduces or stops the flow of NGF is detrimental to the maintenance of the differentiated state of the neurone.

(Korsching & Thoenen, 1983a; Heumann, Korsching, Scott & Thoenen, 1984; Shelton & Reichardt, 1984). The actual flow of NGF occurs in membrane-bound vesicles along microtubules (Schnapp, Vale, Sheetz & Reese, 1985), at a rate similar to the fast axonal flow of other molecules (Grafstein & Forman, 1980). Interruption of the retrograde flow of NGF leads to degeneration of developing – and chromatolysis and loss of differentiated functions in mature – sympathetic and sensory neurones. This may be achieved by severing the axon, disintegrating the microtubules with chemical agents such as vinblastine or colchicine (Purves, 1976; Chen, Chen, Calissano & Levi-Montalcini, 1977; Johnson, 1978) or by destroying the nerve terminals with 6-hydroxydopamine (Levi-Montalcini *et al.* 1975). The effects of such treatments are similar to the changes seen after administration of anti-NGF antibodies has been used to deplete supplies of NGF. The retrograde transport of NGF is independent of neuronal activity but depends instead on a specific interaction with NGF receptors at the nerve terminal (Dumas *et al.* 1979; Schwab, Heumann & Thoenen, 1982) (Fig. 2). The NGF is then internalized by receptor-mediated endocytosis, a process characterized in some detail in the NGF-responsive pheochromocytoma cell line, PC12 (Layer & Shooter, 1983). After transport of the vesicles (endosomes) along microtubules to the cell body, the endosomes are acidified by insertion of proton pumps into their membranes, causing the NGF to dissociate from the NGF receptor at the low pH (Vale & Shooter, 1984). The NGF, now in the fluid phase of the endosome, is translocated finally to lysosomal structures and degraded (Schwab, 1977; Schwab & Thoenen, 1977; Claude *et al.* 1982). The fate of the NGF receptor is unknown, although if it behaves in an analogous manner to other receptors which efficiently internalize ligands, it will be recycled to the nerve terminal for further use.

As NGF proceeds from target tissue to neuronal cell body along the retrograde pathway it generates intracellular signals which mediate its various functions at the molecular level. However, the exact location at which this signal or signals are produced is not yet known. The possibilities are (1) at the receptor, (2) after internalization in the nerve terminal, (3) during retrograde flow or (4) in the cell body, or various combinations of these. Certain possibilities have been eliminated. The degradation of NGF in the lysosomes appears not to play a role in nerve growth factor action since inhibition of lysosomal function by chloroquine has no effect on NGF-induced neurite outgrowth (Shooter, Yankner, Landreth & Sutter, 1981) or on choline acetyltransferase activity in PC12 cells (Heumann, Schwab, Merkl & Thoenen, 1984). Similarly anti-NGF antibodies introduced into the cytoplasm of PC12 cells have no effect on the ability of these cells to respond to NGF, indicating that immune-precipitable fragments of NGF produced by lysosomal degradation are not involved (Heumann, Schwab & Thoenen, 1981; Seeley, Keith, Shelanski & Greene, 1983). Furthermore, since neurite outgrowth from PC12 cells is not induced by introducing NGF into the cells' cytoplasm, any signals generated by NGF with the cell must come from NGF within vesicles. NGF enters these vesicles by interacting with the specific NGF receptors on the nerve terminal. Considerable

effort has gone into characterizing these interactions and the receptors themselves since it is at this level that intracellular signals may first be generated.

THE NERVE GROWTH FACTOR RECEPTORS

All the NGF-responsive cells display two classes of NGF receptors, one of high affinity and low capacity and the other of low affinity and high capacity (Vale & Shooter, 1984) (Table 1). Both types of receptor are observed when NGF binding experiments are carried out at low, as well as at higher, temperatures, indicating the cell surface localization of both the receptors (Sutter, Riopelle, Harris-Warrick & Shooter, 1979a). The high-affinity receptors show a relatively low rate of dissociation, and are referred to as slow receptors or SNGFRs, while the dissociation rate of the low-affinity receptors is approx. 100-fold faster, leading to their

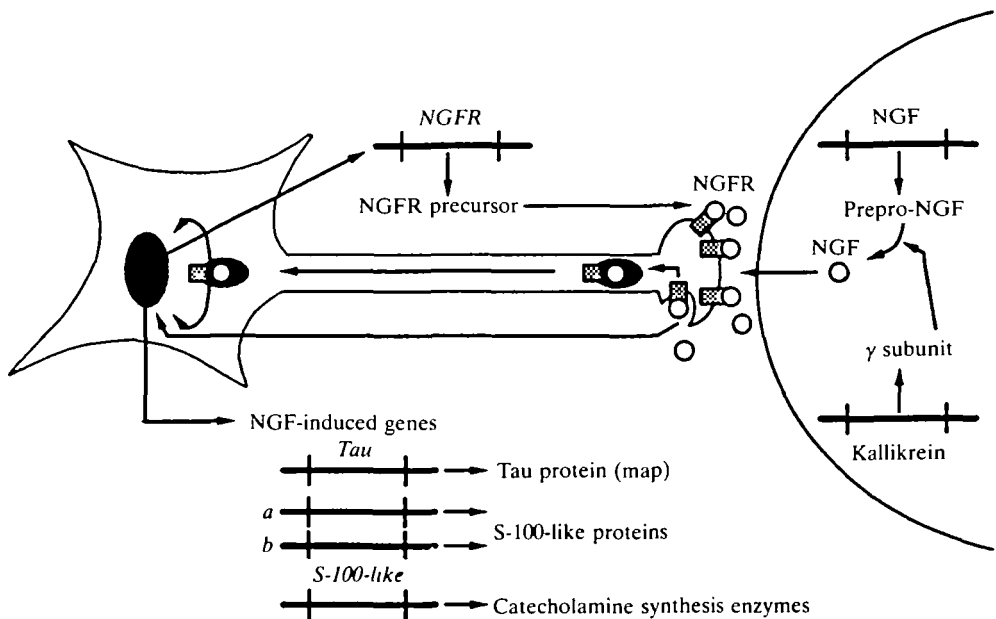


Fig. 2. The NGF pathway. NGF, synthesized under the control of a single gene and processed from two precursor proteins, is secreted by the several cell types in the target tissue into the vicinity of the nerve terminal. Binding to the NGF receptors on the nerve terminal initiates signal transduction and internalization. The internalized vesicles carrying NGF bound to the NGF receptor are transported along microtubules to the neuronal cell body. After the pH of the vesicles (endosomes) has been lowered by the action of proton pumps, their fluid phase, including free NGF, is transferred to lysosomes and the proteins in it are degraded. The receptors are free to recycle to the nerve terminal after anterograde transport along the microtubules, although there is no evidence, as yet, for this process. The arrows emanating from the NGF-NGF-receptor complex indicate locations at which intracellular signals might arise. These are (i) at the nerve terminal membrane, (ii) after internalization and/or retrograde flow up the axon and (iii) in the neuronal cell body. The cascade of reactions started by one or more of these signals leads finally to modulation of gene expression and several of the genes whose transcription is increased by the action of NGF are listed in the diagram.

Table 1. *Properties of the two nerve growth factor receptors*

Property	SNGFR (HNGFR)	FNGFR (LNGFR)
K_d for [125 I]NGF binding	10^{-11} mol l $^{-1}$	10^{-9} mol l $^{-1}$
Dissociation ($t_{1/2}$) for [125 I]NGF release	≈ 10 min slow	≈ 3 s fast
Trypsin (occupied receptor)	stable	labile
Triton X-100 (occupied receptor)	insoluble	soluble
Relative molecular mass: complex receptor	158 000 140 000	100 000 80 000

identification as fast receptors or FNGFRs. Pharmacological dose-response curves indicate that the SNGFRs mediate long-term NGF responses such as neurite outgrowth (Sutter, Riopelle, Harris-Warrick & Shooter, 1979b). High-affinity SNGFR occupancy also correlates with survival and neurite outgrowth of sensory neurones (Zimmermann, Sutter, Samuelson & Shooter, 1978). In contrast, the low-affinity FNGFRs mediate at least some of the rapid responses to NGF, such as stimulation of amino acid uptake (McGuire & Greene, 1979; Kedes, Gunning & Shooter, 1982). This receptor heterogeneity can be explained by one or more models. For example, the two receptor types could be independent receptor molecules derived from different genes or by differential splicing of mRNA or protein precursors. Alternatively, the SNGFRs could be generated from the FNGFRs by association with another protein, by crosslinking with the divalent ligand NGF or by a change in conformation. These last three hypotheses imply that NGF binds first to the low-affinity FNGFRs thereby converting them to high-affinity SNGFRs. Current evidence favours the idea that the FNGFR is the common NGF binding subunit of the two receptor types with a second protein associating with the FNGFR to create the SNGFR.

The key pieces of evidence for this conclusion are the relative molecular masses (M_r) of the two receptor species (determined by crosslinking experiments), the structure of the FNGFR (determined by gene transfer and cloning) and the demonstration of possible receptor conversion by agents which cluster the receptors.

RECEPTOR CONVERSION

Several experiments suggest that rapidly (FNGFR) and slowly (SNGFR) dissociating receptors are interconvertible. Addition of the plant lectin wheat germ agglutinin (WGA) alters the ratio of the two receptor types on PC12 cells without affecting total binding (Vale & Shooter, 1982; Grob & Bothwell, 1983). Specifically, WGA converts a major proportion of the FNGFRs which are trypsin-sensitive to trypsin-resistant, slowly dissociating receptors. Whether these receptors are exactly the same as the naturally occurring trypsin-resistant SNGFRs is unclear. However, the WGA-induced change in the dissociation characteristics of the receptor also

correlates with the conversion of the receptor to a form insoluble in Triton X-100: another characteristic of the SNGFRs (Vale & Shooter, 1982). It seems likely that WGA acts by crossbridging the FNGFR to one or more proteins in the cell membrane which themselves are anchored to the cytoskeleton (Vale, Ignatius & Shooter, 1985). That receptor clustering may be involved in the conversion is also shown by the finding that anti-NGF antibodies, but not monovalent Fc fragments, convert occupied FNGFRs to a slowly dissociating, trypsin-resistant form insoluble in Triton X-100 (Vale & Shooter, 1983). Since NGF is potentially divalent with respect to receptor binding, receptor clustering may occur simply through the action of NGF. However, the differences in the relative molecular masses of the two receptors and the finding that PC12 plasma membranes display only FNGFRs (Block & Bothwell, 1983) suggest that another component in the intact cell, possibly a cytoskeletal-linked protein, may be responsible for receptor conversion.

RECEPTOR SIZE

Following earlier work (Massague *et al.* 1981), which showed that rabbit superior cervical ganglion membranes had two differently sized NGF receptor species, Hosang & Shooter (1985) used the same hetero-bifunctional, photoactivated crosslinking agent to demonstrate that on PC12 cells [125 I]NGF also crosslinked into two major complexes with relative molecular masses of 158 000 and 100 000, respectively. Assuming that only one chain of NGF is covalently crosslinked in these complexes, it follows that the actual values for the receptors are approx. 85 000 and 145 000, respectively. Moreover, on the basis of its low rate of dissociation and resistance to trypsin (binding of [125 I]NGF is unaffected but the receptor loses a peptide with an M_r of 10 000), the larger receptor was identified as the SNGFR. Similarly, since the species with the lower M_r dissociated rapidly and was degraded by trypsin it has the characteristics of the FNGFR. Treatment of both receptors with neuraminidase removes terminal sialic acid residues and lowers the apparent relative molecular masses of both receptors without significantly affecting their capacity to bind NGF (Vale, Hosang & Shooter, 1985).

The relative molecular mass of the purified low-affinity human NGF receptor from the A875 melanoma cell is 85 000 (Puma *et al.* 1983) and a similar figure was obtained for the biosynthetically labelled receptor in these cells (Ross *et al.* 1984). Taken together with the results obtained from the cloning of the rat low-affinity FNGFR (see later), it is clear that the low-affinity NGF receptor from both species is a single, glycosylated peptide chain with a relative molecular mass of approx. 85 000.

The crosslinking technique can be used to follow the internalization and intracellular fate of the [125 I]NGF-receptor complexes in PC12 cells. When the latter are incubated for relatively long periods with [125 I]NGF prior to crosslinking, a progressive decrease in the labelling of the M_r 158 000, but not the M_r 100 000, complex is observed, suggesting internalization only of the former (Hosang & Shooter, 1987). In keeping with this idea is the further finding that the lower relative molecular mass receptor remains trypsin-sensitive during the prolonged incubation,

indicating a cell surface localization, while the larger receptor is protected from the characteristic tryptic release of the small peptide, indicating that it is internalized. It is of considerable interest that the receptor which is internalized in PC12 cells, the SNGFR, is also the one implicated in mediating NGF-induced neurite outgrowth. The internalized, crosslinked SNGFR remains in the endosomes of the PC12 cell where, apparently, the inability of the low pH environment to bring about the usual dissociation of the (crosslinked) [125 I]NGF from its receptor (Vale & Shooter, 1984) inhibits both transfer of the [125 I]NGF to the lysosomes and the potential recycling of the receptor.

GENE TRANSFER AND CLONING OF THE LOW-AFFINITY NERVE GROWTH FACTOR RECEPTOR

The identification of the initial intracellular signal generated by the binding of NGF to its receptors requires information on the molecular structures of the receptors. Since no information was available for the amino acid sequences of either receptor, the technique of gene transfer was used (i.e. the transfer of the gene for the

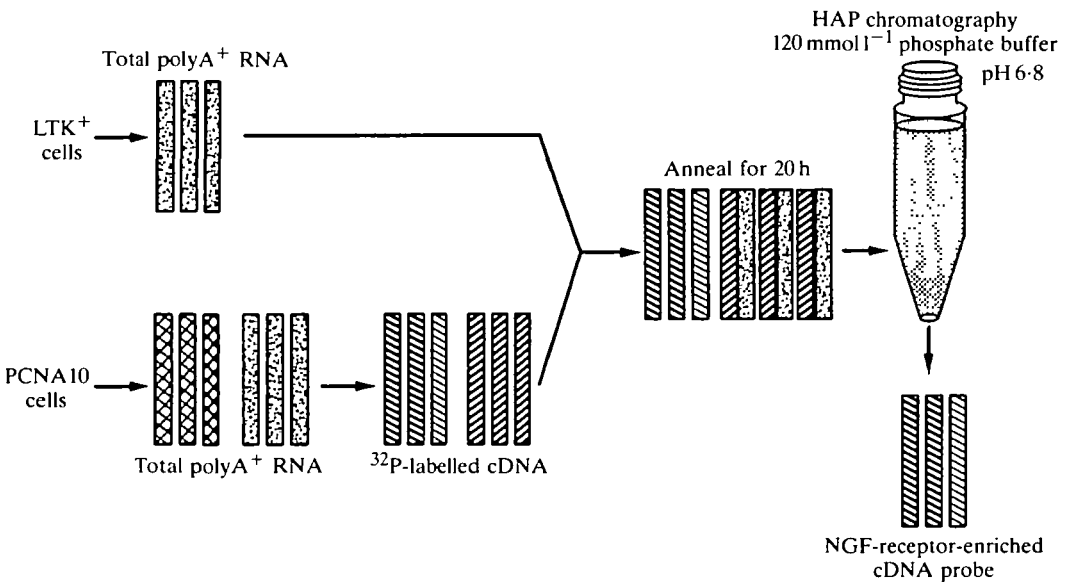


Fig. 3. Preparation of the NGF-receptor-enriched probe. The rat FNGFR polyA⁺ RNA is identified by light cross-hatching and the corresponding single-stranded cDNA by heavy downward-sloping lines. The other rat polyA⁺ RNAs, stemming from rat genes on either side of the rat FNGFR gene, are indicated by heavy cross-hatching and their single-stranded cDNAs by light downward-sloping lines. Common mouse polyA⁺ RNAs are shown by stippled blocks and it is these species which form RNA:DNA hybrids when the polyA⁺ RNA from the LTK⁺ cells is hybridized to the single-stranded cDNA from the transfected PCNA10 cells. The double-stranded hybrids are removed by hydroxyapatite (HAP) chromatography leaving a single-stranded cDNA pool enriched in sequences for the FNGFR cDNA. The latter is then used to screen a double-stranded cDNA library from the PCNA10 cells.

rat NGF receptor to a cell line from another species, mouse). The success of this approach depends on the availability of a monoclonal antibody reacting with one or other of the rat, but not the mouse, NGF receptors. One such antibody exists that enhances NGF binding on rat PC12 cells by specifically increasing the affinity of the low-affinity FNGFR (Chandler, Parsons, Hosang & Shooter, 1984). This antibody was used in conjunction with a second fluorescein-conjugated goat anti-mouse antibody to detect, and isolate in a cell sorter, cells expressing the rat FNGFR. Transfection of mouse *LTK⁻* cells with 100 to 200-kb pieces of PC12 genomic DNA and a plasmid containing the chicken thymidine kinase gene was followed by selection of cells in a medium which allows only for the survival of cells integrating and expressing the thymidine mouse genes (Radeke *et al.* 1987). These cells also integrate and express one of the PC12 genomic fragments and it is from this population that the few cells expressing the rat FNGFR gene were selected with the anti-receptor antibody as described above. In one of the transfectants obtained in this manner the phenotype was unstable, resulting in a continuous amplification of the expression of the FNGFR during growth in the selective medium after cell sorting. The phenotype finally stabilized after 10 rounds of division, giving a transfected cell line (PCNA10) expressing approx. 2.5×10^6 FNGFRs per cell. All the receptors expressed were rapidly dissociating with an equilibrium dissociation constant the same as that of the low-affinity NGF receptors. Their relative molecular mass was 83 000, again consistent with the size of the FNGFR. It is of interest that the

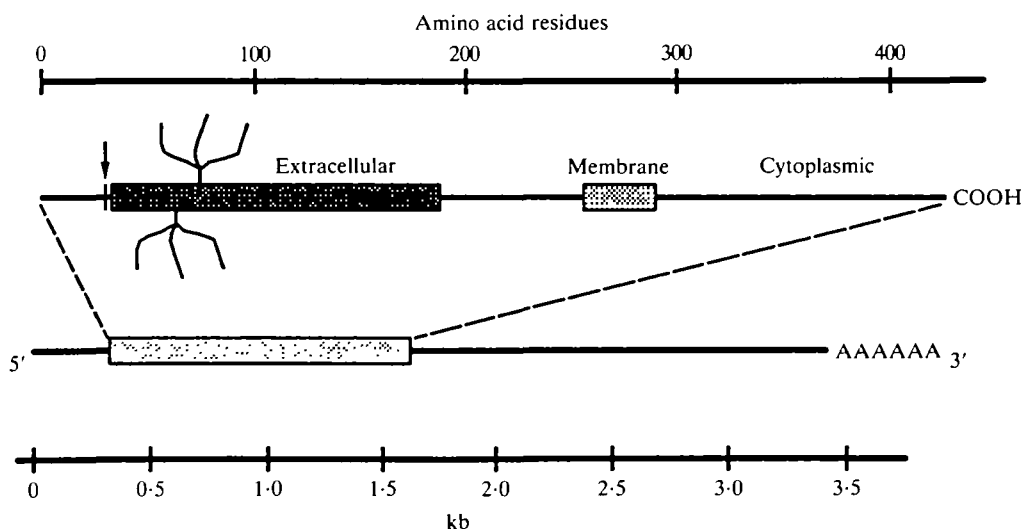


Fig. 4. The NGF receptor mRNA and protein. The open reading frame in the FNGFR mRNA is shown by the box in the 5'-half of the line immediately above the scale for the numbers of bases (kb). The protein corresponding to the open reading frame is shown in the line above the mRNA. The arrow near the left-hand end indicates the site of cleavage for removal of the signal peptide, the larger box shows the position of the four cysteine-rich repeating units, the trees show the position of N-linked glycosylation sites and the smaller box shows the membrane-spanning domain.

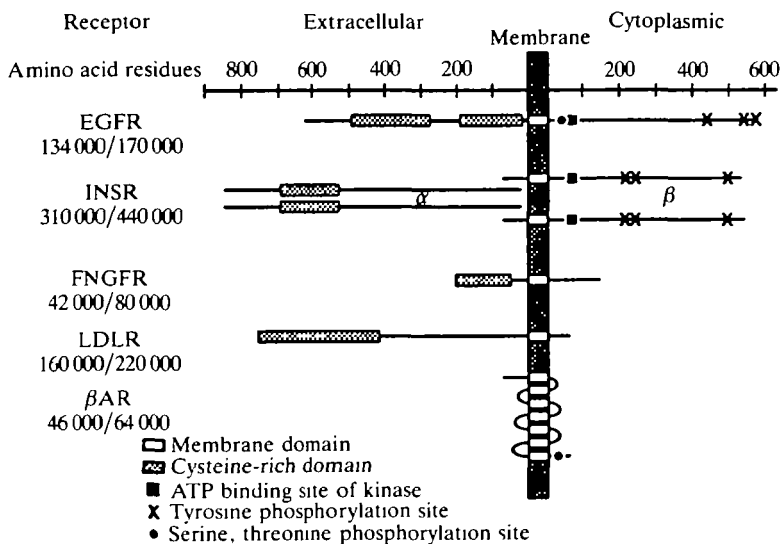


Fig. 5. The structure of receptors. A comparison of the known sequences of a number of receptors. The epidermal growth factor receptor (EGFR) is typical of a number of mitogen receptors which have continuous or split tyrosine kinase cytoplasmic domains. The insulin receptor (INSR) also has a tyrosine kinase in each of its β subunits. The LDL receptor (LDLR) is one of the receptors which efficiently internalize ligand and are recycled whereas the β -adrenergic receptor (β AR) is typical of the receptors with multimembrane spanning domains which interact with G proteins within the cell. The figures beneath each receptor name give the relative molecular masses of the protein part of the receptor (left) and of the mature glycosylated receptor (right). The comparisons of receptor structure at this level do not reveal common features, other than the cysteine repeating units, which are responsible for the various functions of the receptors, including internalization.

expression of the rat FNGFR gene in the mouse L cell gives rise only to the FNGFR and not to SNGFRs.

The rat FNGFR cDNA was recovered by subtractive hybridization of the transfectant cell single-stranded cDNA with polyA⁺ RNA from the recipient L cell (Fig. 3), and the use of this FNGFR-enriched cDNA pool to screen a double-stranded transfectant cell cDNA library. Of the 19 clones which hybridized very strongly with this probe, six cross-hybridized and the longest insert was approx. 3.4 kb. The latter hybridized with a common message from all cell types expressing NGF receptors but not from cells lacking the receptors and, when transfected in an appropriate expression vector into mouse L cells, gave rise to the expression of FNGFR. The nucleotide sequence of this FNGFR cDNA showed the start of an open reading frame close to the 5'-end and continuing for 1275 bases, resulting in a receptor precursor containing 425 amino acids (Fig. 4). The FNGFR receptor itself, with 396 residues, has a relative molecular mass of 42 478, indicating that it is heavily glycosylated to bring its size up to that of the mature membrane-bound receptor (M_r 83 000) indicated by electrophoretic analysis. It has one membrane-spanning segment separating an extracellular domain containing four cysteine-rich repeating units, characteristic of protein- or peptide-binding receptors (Fig. 5), from an

intracellular domain rich in serine and threonine, but lacking an ATP-binding site characteristic of an endogenous kinase. The FNGFR shows no significant homologies with any of the other known receptors (or indeed any other protein), in spite of the extracellular repeating units, and by itself offers no clue to the receptor-mediated transduction signal. The structure of the human FNGFR on melanoma cells is, in contrast, highly homologous to that of the rat FNGFR (Johnson *et al.* 1986).

The fact that the FNGFR cDNA hybridizes to a single mRNA species in cells which express both types of NGF receptors suggests that the two receptors either have no relationship to one another, except for the property of binding NGF, or that they share the FNGFR protein as a common subunit. It follows, if the latter explanation is correct, that the high-affinity SNGFR will contain a second cellular protein, with a relative molecular mass of approx. 60 000, which is the key to the signal transduction mechanism. As anticipated from the demonstration that only the high-affinity SNGFR is internalized after NGF binding to PC12 cells, the low-affinity FNGFR expressed in mouse L cells does not internalize NGF, suggesting that the ability to internalize is also dependent on the interaction of the FNGFR with another protein. One of the possible candidates for this second protein is the product of the proto-oncogene *c-src*, a 60 000 M_r tyrosine-specific kinase. One of the advantages of the gene transfer technique is that it can be used to identify the gene coding for this putative second protein in the SNGFR by its ability to convert some or all of the FNGFR in the mouse L cell transfectant to the high-affinity SNGFR. These and similar studies using, for example, the antisense mRNA for the FNGFR to suppress FNGFR expression in PC12 cells, should finally permit the relationship of the two receptor types to be firmly established.

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