Neurotrophin-7: a novel member of the neurotrophin family from the zebrafish

Ann-Sofie Nilsson, Michael Fainzilber[1], Pierre Falck[2], Carlos F. Ibáñez*

Division of Molecular Neurobiology, Department of Neuroscience, Karolinska Institute, 171 77 Stockholm, Sweden

Abstract A novel member of the neurotrophin family, zebrafish neurotrophin-7 (zNT-7), was isolated from the zebrafish *Danio rerio*. The amino acid sequence of zNT-7 is more closely related to that of fish nerve growth factor (NGF) and neurotrophin-6 (NT-6) than to that of any other neurotrophin. zNT-7 is, however, equally related to fish NGF and NT-6 (65% and 63% amino acid sequence identity, respectively) indicating that it represents a distinct neurotrophin sequence. zNT-7 contains a 15 amino acid residue insertion in a β-turn region in the middle of the mature protein. Recombinant zNT-7 was able to bind to the human p75 neurotrophin receptor and to induce tyrosine phosphorylation of the rat TrkA receptor tyrosine kinase, albeit less efficiently than rat NGF. zNT-7 did not interact with rat TrkB or TrkC, indicating a similar receptor specificity as NGF. We propose that a diversification of the NGF subfamily in the neurotrophin evolutionary tree occurred during the evolution of teleost fishes which resulted in the appearance of several additional members, such as zNT-7 and NT-6, structurally and functionally related to NGF.

© 1998 Federation of European Biochemical Societies.

Key words: Brain-derived neurotrophic factor; Evolution; Neurotrophic factor; Nerve growth factor; p75; Receptor tyrosine kinase

1. Introduction

The neurotrophins are a family of structurally and functionally related polypeptide hormones that control the survival, differentiation, plasticity and regeneration of vertebrate neurons [1]. Four different neurotrophins are known in mammals and amphibians: nerve growth factor (NGF) [2,3], brain-derived neurotrophic factor (BDNF) [4], neurotrophin-3 (NT-3) [5–9] and neurotrophin-4 (NT-4) [10,11], also known as neurotrophin-4/5 [12]. Neurotrophins bind to two classes of cell surface receptors. All members of the family interact with a transmembrane protein without catalytic function, p75 [13,14], as well as with distinct members of a subfamily of receptor tyrosine kinases known as Trk [15]. NGF interacts with TrkA, BDNF and NT-4 with TrkB and NT-3 with TrkC. Mammalian NGFs show higher sequence similarity to NT-3 than to either BDNF or NT-4. The latter are more closely related to each other, which is also reflected in the fact that

both these neurotrophins utilize the same receptor tyrosine kinase, TrkB. Mammalian NT-3 is equally distant from NGF and the BDNF/NT-4 subfamily [10]. The crystal structure of neurotrophins contains three pairs of anti-parallel β-strands connected by turns and β-hairpin loops containing most of the variable residues among the four mammalian neurotrophin proteins [16–18].

Homologous sequences to NGF, BDNF and NT-3 have also been isolated from teleost fishes (salmon, zebrafish and platty fish among others), while no sequences resembling NT-4 have so far been found in any fish species [10,19]. However, a distinct neurotrophin gene, known as neurotrophin-6 (NT-6), has been identified in two species of platty fish [20]. NT-6 is more closely related to NGF than to any other neurotrophin, and like NGF, recombinant NT-6 has trophic activities on embryonic sympathetic neurons. NT-6 has an unusually long extension in a highly variable β-turn region, rich in positively-charged residues, which has been shown to mediate the interaction of this neurotrophin with heparin in vitro [21]. Despite big efforts by several laboratories, sequences related to NT-6 have so far not been found in mammals, suggesting that NT-6 may be a diversification of the NGF subfamily of neurotrophins unique to fishes.

Using the polymerase chain reaction (PCR), we have isolated a novel neurotrophin from the zebrafish *Danio rerio* which we have named zebrafish neurotrophin-7 (zNT-7). zNT-7 is both structurally and functionally related to fish NGF and NT-6. The apparent absence of sequences homologous to either zNT-7 or NT-6 in mammals suggests diverse evolutionary histories of the neurotrophin family tree in different groups of vertebrates.

2. Materials and methods

2.1. PCR, molecular cloning and sequence analyses

Six separate mixtures of 28-mer oligonucleotides (including restriction sites) representing all possible codons corresponding to the amino acid sequences KQYFYET (upstream oligonucleotide) and WRFIR-ID (downstream oligonucleotide) were used to prime the amplification of about 1 μg of zebrafish genomic DNA. PCR products were subcloned into pBS KS$^+$ (Stratagen) and sequenced. Several independent clones were sequenced for each gene isolated. Approximately 1 000 000 clones from a zebrafish genomic library were screened using conventional procedures with a PCR fragment of zNT-7 labeled with [α-^{32}P]dCTP. Hybridization was carried out in 4× SSC (1× SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.0), 40% formamide, 1× Denhardt's solution and 10% dextrane sulfate at 42°C. The filters were washed at 60°C in 0.1× SSC, 0.1% SDS. DNA was prepared from a hybridizing phage clone and directly sequenced using oligonucleotide primers 5′-ACC TGA CCC ATG CCA CAG ACT TAG-3′, 5′-TCG CTT GGA GGT TCA TCC GCA TCG-3′, 5′-CAA GCG ATC TGG TTT TTG TAG GAC-3′, 5′-CCA ACC CAG TGC TCT TCC GTG TCG-3′ and 5′-TCC GTC GGA TGC GTG GAC GCC TGG-3′. A full-length zNT-7 DNA fragment was obtained by PCR

*Corresponding author. Fax: (46) (8) 341960.
E-mail: carlos@cajal.mbb.ki.se

[1] *Present address*: Department of Biological Chemistry, The Weizmann Institute, 76100 Rehovot, Israel.

[2] *Present address*: Department of Zoology, Uppsala University, Uppsala, Sweden.

A

```
AAAAAATGGCCAGGTGTGTGTTTTTTTTAATTAAGAGGTTTAAATGACAAATTAGATTAAA        60

AGTTTATTTAAAAAAAATAAAATAAAACAAATAAATGTTATTAAATGCCTATGACAAAGA       120

AAAATAGACAATACATTGTGAAATTTCCACAGATGACATACATTCCATGCATGACGTGAC       180

TTCAATATGAACAGTTCTGTATTTCTGCTGAAATGTTAAGCAGTCAGGGATACTGTGCAG       240

GCCACTTCACTAATAGAAAGCAAGCTGTTTCTTAACAAACTGGAGAATTAATTTTCCATG       300

TTGCTGATTATCTGAGGTGTGTCTGTTTCTGCAGGACGAGTGCCCATGAGGTCGTTGACG       360
                                                  M   R   S   L   T          5

CTGGTCCTGCTCATCCTGATCAGCATTCAGGCTGCACTCATTATGGGAGGCCACACCCAG       420
L   V   L   L   I   L   I   S   I   Q   A   A   L   I   M   G   G   H   T   Q         25

GCACAGGCGTCAGCCAATCAGCACGCGGCAACACAAAACTCACCCAGGCTTTACAGCCAA       480
A   Q   A   S   A   N   Q   H   A   A   T   Q   N   S   P   R   L   Y   S   Q         45

TCAAAAGAGGACTTGCACTATGACGAACTAATTCCCACCGTGGACCCCAAACTCTTCAAC       540
S   K   E   D   L   H   Y   D   E   L   I   P   T   V   D   P   K   L   F   N         65

AAGCGCCGCTACCGCTCACCTCGCGTAGTGTTTAGTGATGTGATCACTGCTGAAAAAGAA       600
K   R   R   Y   R   S   P   R   V   V   F   S   D   V   I   T   A   E   K   E         85

ATGGGAACCCCCAGGCGTCCACGCATCCGACGGAAGGCAAACGACTTCCTGCATCGTGGT       660
M   G   T   P   R   R   P   R   I   R   R   K   A   N   D   F   L   H   R   G        105

GAGTACTCGGTGTGTGACAGCGAAGAGCACTGGGTTGGCAACCTGACCCATGCCACAGAC       720
E   Y   S   V   C   D   S   E   E   H   W   V   G   N   L   T   H   A   T   D        125

TTAGGGGGCAATGAAGTCATGGTGCTACCACATTTTCGCATCAACAACGTTGTAAAGAAG       780
L   G   G   N   E   V   M   V   L   P   H   F   R   I   N   N   V   V   K   K        145

CAGCTCTTCTACGAGACCACATGTCGTGTGAAGAAACCTATAGGGGCCCCTAAGCCAGGT       840
Q   L   F   Y   E   T   T   C   R   V   K   K   P   I   G   A   P   K   P   G        165

CAAGGAGCCAGTGGCGTTAAAGCGGGAACCTCTAGCTGTCGTGGCATTGACAGCAAGCAC       900
Q   G   A   S   G   V   K   A   G   T   S   S   C   R   G   I   D   S   K   H        185

TGGAACTCTTACTGCACCAACACACACACCTATGTGCGGGCGCTAACGTCCTACAAAAAC       960
W   N   S   Y   C   T   N   T   H   T   Y   V   R   A   L   T   S   Y   K   N        205

CAGATCGCCTGGAGGTTCATCCGAATCAACGCCGCATGTGTCTGTGTGCTCAGCCGTAAC      1020
Q   I   A   W   R   F   I   R   I   N   A   A   C   V   C   V   L   S   R   N        225

TCCTGGAGGCATGGACTGAAGTATTGACTGTTATCTCAGCAATCCAATGCAGCCTCCTGT      1080
S   W   R   H   G   L   K   Y   *                                                   233

TGAAAGTCCCTTTCACCCATCATTATTATCATCTGTACTGCCAACGCCACTGAGTCCCAT      1140

CCTTTACCTGTATTCTTTATGACATCGCATCCTAGGACCTTCTACCCACCTCACTCTGTA      1200

AATTATGGTCAATTTA                                                                  1216
```

Fig. 1. Primary structure of zNT-7. A: DNA and deduced amino acid sequence of zNT-7. Putative signal sequence (single underline) and proteolytic cleavage site (double underline) are indicated. B: Alignment of amino acid sequences of zNT-7 and other neurotrophins. Variable region III is indicated. chi, chicken; hum, human; mou, mouse; xen, *Xenopus*; xip, platty fish (*Xiphophorus maculatus*); zeb, zebrafish.

amplification of phage clone DNA with primers 5′-GAT GGA TCC TGC CCA TGA GGT CGT TGA CGC T-3′ and 5′-GAT GGA TCC GGG ACT CAG TGG CGT TGG CAG T-3′, subcloned into pCDNA3 (Invitrogen), and confirmed by further DNA sequencing reactions. Alignments of amino acid sequences were performed with programs GAP, PILEUP and LINEUP; phylogenetic trees were con-

structed using DISTANCES, GROWTREE, PAUPSEARCH and PAUPDISPLAY. All programs were from the Genetics Computer Groups package version 9.1 [22].

2.2. Production of recombinant zNT-7

COS cells grown to about 70% confluency were transfected with

B

```
                                                                 --------region III-------
zebnt7     ANDFLHRGEYSVCDSEEHWG..NLTHATDLGGNEVMVLPHFRI.NNVKKQLFYETTCRVKK.....PIGAPKPGQGA.SGVKAGTSSCRGILDSKHVNSYCTNTHTYVRALTS.YKNQIAWRFIRINAACVCVLSRNSWRHGLKY
xipnt6     KAVSHTMHRGEYSVCDSINIVW...NKTRAITMSGNEVTVLSHVTV.NNKVKKQLFYETTCRSPTHRSSGIVIGGFSGERGGKQGSKTGNSGCRGIDSRYWNSHCTNTDIYVSALTV.FKEQTAWRFIRINAACVCVLSRNSWSRRPGH
zebngf     AGPPQHRGVYSVCERLSHWEG..NKTKA--------QYFFETTCSSG........................ATGGSSCLGIDARHWNSYCTHSHTFVRALTS.FKNLVAWRLIRINVACVCVLSRKSWRA
xipngf     QPQ.HRGVYSVCESVSVWVG.NKTKAITDISGKEVTVLPYVNI.NNVKKKQYFFETTCHSP...........PSGGSRCLGIDARHWNSHCTNSHTFVRALTSSENQ.VAWRLIRINVACVCVLSRKSMQH
chingf     TAHFVLHRGEFSVCDSVSMWVG..DKTTATDIKGKEVTVLGEVNI.NNNVFKQYFFETKCRDP..........RPVSSGCRGIDAKHWNSYCTTTHTFVKALTMEGKQ.AAWRFIRIDTACVCVLSRKSGRP
xenngf     TVHFVLHKGEYSVCDSVSMWVG..EKTKATDIKGKEVTVLGEVNI.NNSVFKQYFFETKCRDP.........KPFVSSGCRGIDAKHWNSYCTTTHTFVKALTMEGKQ.AAWRFIRIDTACVCVLSRK.GRT
humngf     SSSHPIFHRGEFSVCDSVSVWVG..DKTTATDIKGKEVMVLGEVNI.NNSVFKQYFFETKCRDP........NFVDSGCRGIDSKHWNSYCTTTHTFVKALTMDGKQ.AAWRFIRIDTACVCVLSRKAVR
ratngf     SSTHVFHMGEFSVCDSVSVWVG..DKTTATDIKGKEVTVLGEVNI.NNSVFKQYFFETKCRAP.........NFVESGCRGIDSKHWNSYCTTTHTFVKALTTEDKQ.AAWRFIRIDTACVCVLSRKAAR
humbdnf    HSDPARRGELSVCDSISEWTRADKKTAVDMSGGTVTVLEKVPVSKGQ.LKQFYFETKCNPM...........GYTKEBGCRGIDKRHWNSQCRTTQSYVRALTMDSKKRIGWRFIRIDTSCVCTLTIKRGR
mousebdnf  HSDPARRGELSVCDSISEWTRADKKTAVDMSGGTVTVLEKVPVSKGQ.LKQFYFETKCNPM...........GYTKEBGCRGIDKRHWNSQCRTTQSYVRALTMDSKKRIGWRFIRIDTSCVCTLTIKRGR
chibdnf    HSDPARRGELSVCDSTSEWTAAEKKTAVDMSGATVTVLEKVPVPKGQ.LKQFYFETKCNPK..........GYTKEBGCRGIDKRHWNSQCRTTQSYVRALTMINKRVGWRFIRIDTSCVCTLTIKRGR
xenbdnf    HSDPARRGELSVCDSISEWTRANKKTAVDMSGATVTVLEKVPVSKGQ.LKQFYFETKCNPM..........GYMKEBGCRGIEKRYWNSQCRTTQSYVRAFTMDSKKKVGWRFIRIDTSCVCTL
xipbdnf    HSDPSRRGELSVCDSLSQWTRAVDKKTALDMSSQTVTVMEKVPVPNGQ.LKQFYEHKCNPM..........GYTKDGCRGIDKRHYTSQCRTTQSYVRALTMDSKKIGWRFIRIDTSCVCTLTIKRGR
humnt3     YAEHKSHRGEYSVCDSESLWVT.DKSSAIDIRGHQVTVLGEIKT.GNSPVKQYFYETRCKEA..........RPVKNGCRGIDDKHWNSQCKTSQTVRALTSENNKLVGWRWIRIDTSCVCALSRKIGRT
mmnt3      YAEHKSHRGEYSVCDSESLWVT.DKSSAIDIRGHQVTVLGEIKT.GNSPVKQYFYETRCKEA..........RPVKNGCRGIDDKHWNSQCKTSQTVRALTSENNKLVGWRWIRIDTSCVCALSRKIGRT
ratnt3     YAEHKSHRGEYSVCDSESLWVT.DKSSAIDIRGHQVTVLGEIKT.GNSPVKQYFYETRCKEA..........RPVKNGCRGIDDKHWNSQCKTSQTVRALTSENNKLVGWRWIRIDTSCVCALSRKIGRT
chint3     YAEHKSHRGEYSVCDSESLWVT.DKSSAIDIRGHQVTVLGEIKT.GNSPVKQYFYETRCKEA..........KPVKNGCRGIDDKHWNSQCKTSQTVRALTSENNKLVGWRWIRIDTSCVCALSRKIGRT
humnt4     GVSETAPASRRGELAVCDAVSGWVT.DRRTAVDLRGREVEVLGEVPAAGGSPLRQYFFETRCKAD.......NAEBGGPGAGGGGGCRGVDRRHWVSECKAKQSYVRALTADAQGRVGWRWIRIDTACVCTLLSRTGRA
ratnt4     GVSETAPASRRGELAVCDAVSGWVT.DRRTAVDLRGREVEVLGEVPAAGGSPLRQYFFETRCKAE.......SAGEGGPGVGGGGCRGVDRRHWVSECKAKQSYVRALTADAQGRVGWRWIRIDTACVCTLLSRTGRA
xemnt4     ASGSDSVSLSRRGELSVCDSVNVWVT.DKRTAVDDRGKIVTVMSEIQTLTG.PLKQYFFETKCNPS.......GSTTRGCRGVDKKQMLSECKAKQSYVRALTIDANKLVGWRWIRIDTACVCTLLSRTGRT
```
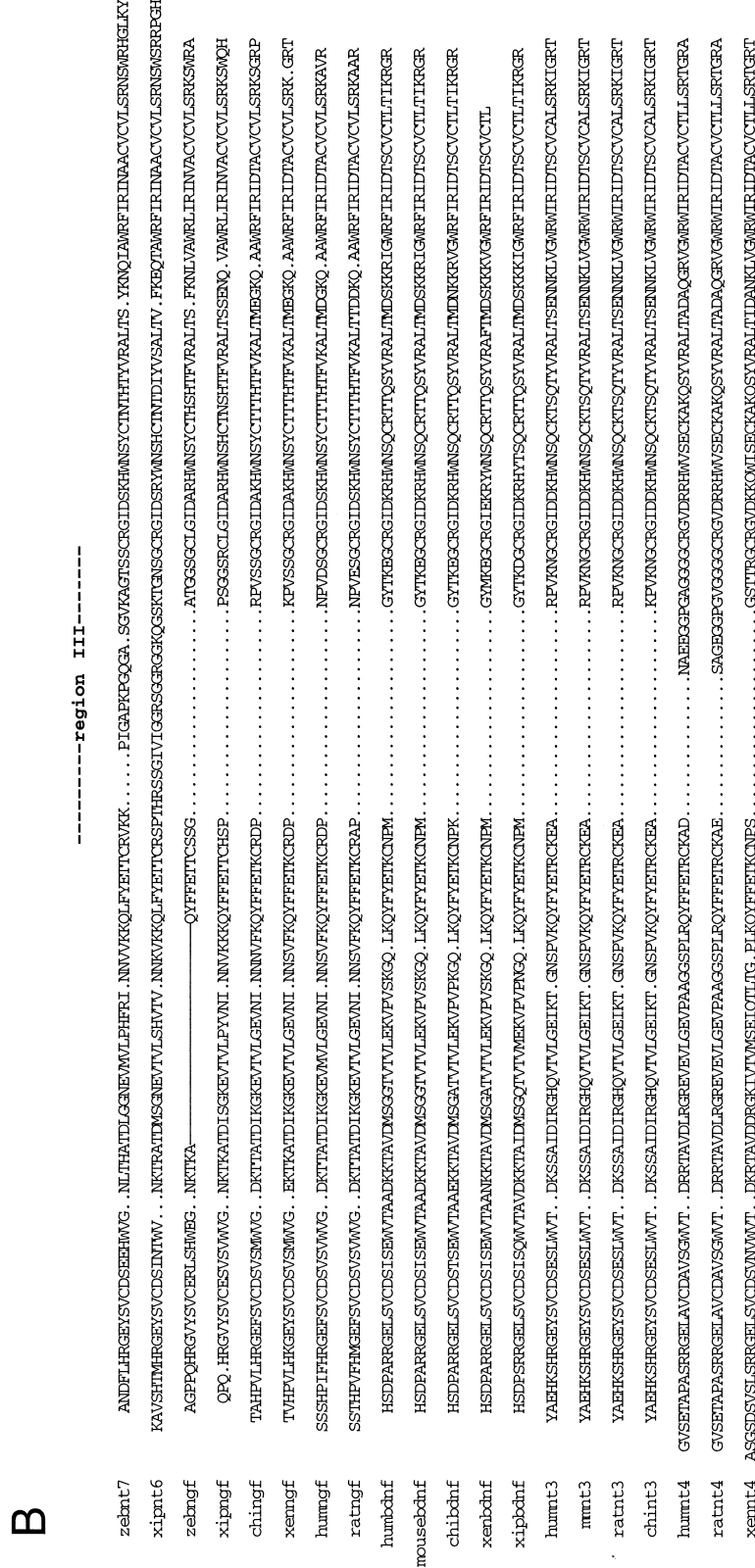
Fig. 1. (*continued*)

25 μg plasmid DNA per 100-mm dish using the DEAE dextran-chloroquine protocol. After transfection, cells were grown in complete medium (DMEM plus 10% FCS) for 24 h, after which they were changed to serum-free medium supplemented with transferrin and insulin (1 μg/ml each) and left for three days. Conditioned medium was collected, cleared by centrifugation, and concentrated by ultrafiltration (Amicon). For metabolic labeling, 35-mm dishes transfected in parallel were grown over the third night after transfection in the
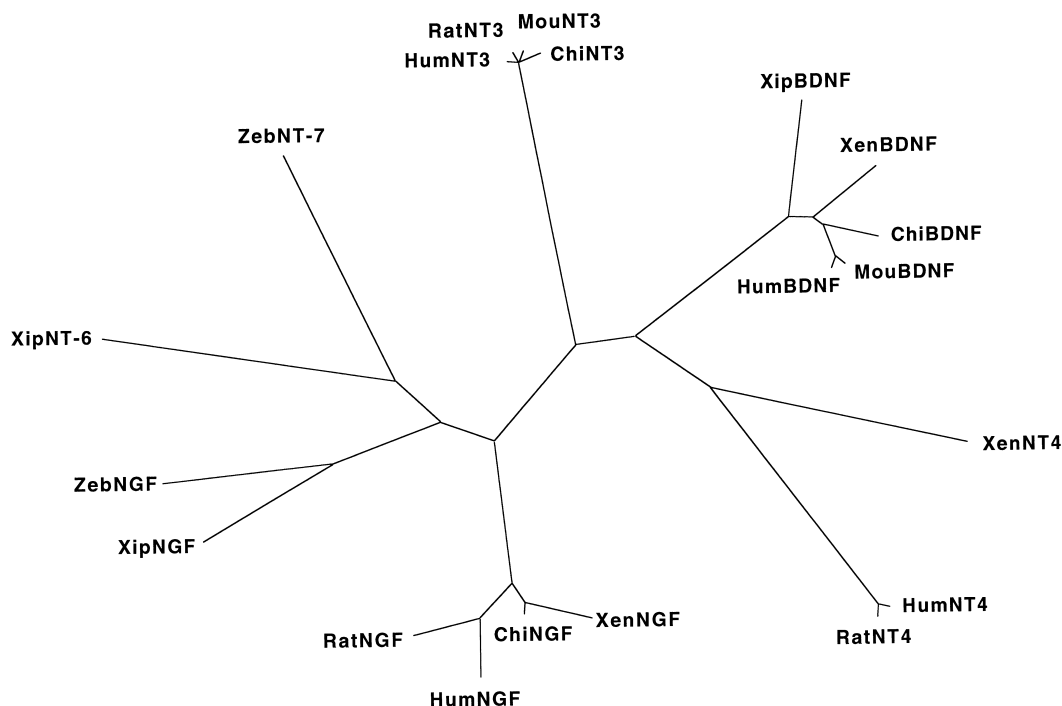
Fig. 2. Unrooted phylogenetic tree of neurotrophins. The tree shown was obtained using a neighbor-joining algorithm. Trees of similar topology were also obtained when a heuristic algorithm of parsimony analysis was applied to the same alignment.

presence of 200 μCi/ml [^{35}S]cysteine (Amersham). Aliquots of 100 to 200 μl of conditioned media were analyzed by SDS-polyacrylamide electrophoresis (SDS-PAGE) in 16% polyacrylamide gels. After fixation, the gels were exposed to phosphorimaging screens and analyzed in a Storm 840 phosphorimager (Molecular Dynamics).

2.3. Receptor binding and Trk phosphorylation assays

Purified murine NGF (Promega) was labelled by the lactoperoxidase method with ^{125}I-Na to an average specific activity of 1×10^8 cpm/μg. Steady-state binding to human A875 melanoma cells was measured in competition assays performed in 96-well filter plates (Millipore) at 4°C with 1.5×10^{-9} M ^{125}I-NGF, 10×10^5 cells/well, and serial dilutions of COS cell conditioned medium containing zNT-7. After two washings with ice-cold binding buffer, dried filters were counted in a gamma counter (LKB). Non-specific binding was measured in a parallel incubation to which at least 100-fold molar excess of unlabelled NGF was added. All results were corrected for this non-specific binding. Control experiments using medium from mock-transfected COS cells showed that other proteins present in the medium had no effect on ^{125}I-NGF binding. The generation of MG87 fibroblasts expressing Trk receptors has been described previously [23]. After exposure to serial dilutions of COS cell conditioned medium or purified factors, Trk-expressing cells were lysed and cell lysates were immunoprecipitated with specific anti-Trk antibodies (kindly provided by David Kaplan), fractionated by SDS-PAGE, blotted onto nitrocellulose filters and probed with anti-phosphotyrosine antibodies (UBI). Western blots were analyzed by enhanced chemifluorescence (ECF, Amersham) and scanned in a Storm 840 fluorimager (Molecular Dynamics).

3. Results and discussion

To enable a reconstruction of the evolutionary history of the neurotrophin family, we have previously used PCR to isolate neurotrophin gene sequences from several organisms [10]. Using a collection of degenerate oligonucleotide primers complementary to conserved regions in the neurotrophins, we have now isolated partial neurotrophin-like sequences from genomic DNA of the zebrafish *Danio rerio*. One of these se-

quences, herein called zebrafish NT-7 (zNT-7), was significantly different from previously isolated neurotrophins and was selected for further analysis.

A zebrafish genomic library was screened with the PCR fragment of zNT-7, and a single hybridizing clone was isolated. Phage DNA from this clone was used as template in automated DNA sequencing reactions using primers based in the sequence of the zNT-7 PCR fragment. The complete open reading frame of zNT-7 was deduced in this way (Fig. 1A). Oligonucleotide primers complementary to the beginning and end of this open reading frame were used in subsequent PCR reactions to amplify a DNA fragment containing the full length coding sequence of zNT-7. The zNT-7 sequence begins with a hydrophobic stretch of amino acids – a putative secretion signal sequence – and has a typical dibasic motif in the middle – a putative proteolytic cleavage site for release of the mature polypeptide form (Fig. 1A). The amino acid sequence corresponding to mature zNT-7 was aligned to those of other neurotrophins. zNT-7 showed a similar arrangement of variable and conserved regions as seen in other members of the neurotrophin family (Fig. 1B).

A characteristic feature of the neurotrophin structure is a β-turn of variable length flanked by two cysteine residues known as variable region III [16]. This region bulges out of the elongated neurotrophin backbone and is highly variable, even among species orthologs of the same neurotrophin. A model of NGF-TrkA association has been proposed in which each receptor molecule makes extensive contact with the two NGF promoters along the side of the dimer interface [24]. In this model, the amino acid residues in variable region III are predicted to accommodate outside the receptor-ligand binding surface in between the two receptor molecules. Perhaps because of this, the β-turn of region III can tolerate considerable expansions in the number of residues between the two cys-

teines. Mammalian NT-4s have up to 16 amino acid residues in this region [7,11], while fish NT-6 has 31 [20]. Variable region III in zNT-7 contains 24 amino acid residues in between the two conserved cysteines (Fig. 1B). Apart from being rich in glycine and proline residues, the sequence in this region is unlike that of any other member of the neurotrophin family.

An unrooted phylogenetic tree including representative full-length neurotrophin amino acid sequences from different vertebrates was constructed using a neighbor-joining algorithm (Fig. 2). Trees of similar topology were also obtained using heuristic algorithms of parsimony analysis (not shown). zNT-7 appeared more closely related to members of the NGF subfamily than to other neurotrophins. A subgroup of teleost fish NGF-like sequences, including platty fish and zebrafish NGF, platty fish NT-6 and zebrafish zNT-7 was distinguished by this analysis (Fig. 2). Direct comparisons of amino acid sequences established that zNT-7 is equally related to fish NGF (65% sequence identity to either platty fish or zebrafish NGF) and platty fish NT-6 (63% sequence identity), indicating that it represents a distinct branch of the NGF subfamily tree. This analysis also showed that paralogous genes in the fish NGF subfamily share 63–65% sequence identity (platty fish NT-6 and NGF, for example), while orthologous neurotrophins (platty fish and zebrafish NGF, for example) share above 80% sequence identity.

The structural similarity of zNT-7 to members of the NGF subfamily was supported by its receptor binding specificity. Recombinant zNT-7 was produced in the conditioned medium of transiently transfected COS cells. Metabolic labeling of transfected COS cell monolayers followed by SDS-PAGE analysis indicated that zNT-7 and rat NGF were produced at comparable levels in conditioned media (not shown). COS cell conditioned medium containing zNT-7 displaced ^{125}I-NGF binding from the p75 neurotrophin receptor expressed on A875 human melanoma cells in a dose-dependent manner (Fig. 3). Thus, like all other neurotrophins, zNT-7 also interacts with the p75 neurotrophin receptor. Conditioned medium containing zNT-7 was also tested on MG87 fibroblasts expressing different members of the Trk subfamily of receptor

Fig. 4. zNT-7 induces tyrosine phosphorylation of rat TrkA (A) but not TrkB (B) or TrkC (C). Purified BDNF and NT-3 were used at 100 ng/ml. Concentrations of conditioned medium of COS cells transfected with empty vector (vector), NGF or zNT-7 are indicated. Unstimulated cells (blank) were also included as negative controls. The migration of tyrosine phosphorylated Trk receptors is indicated (arrowhead).
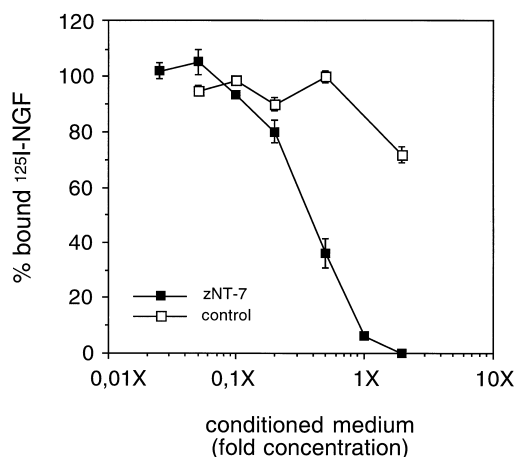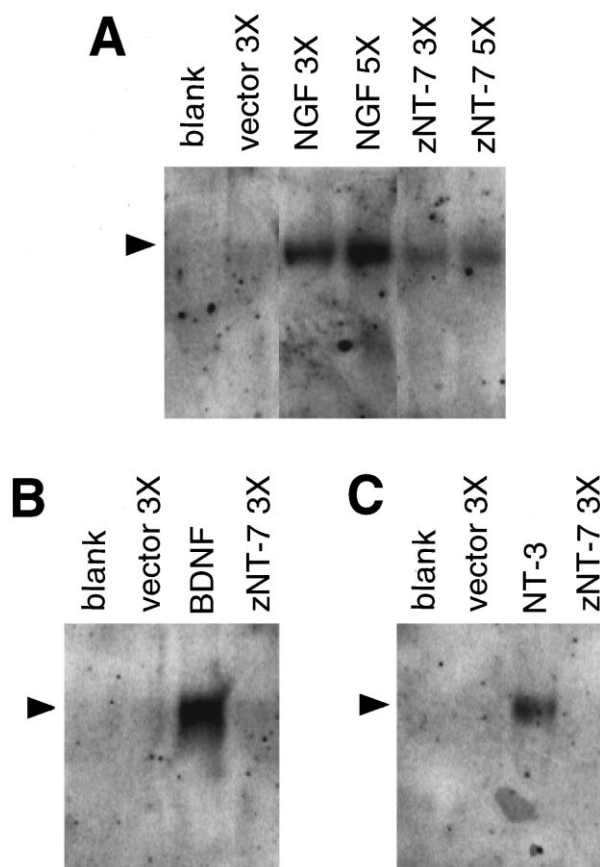

Fig. 3. Binding of zNT-7 to the p75 neurotrophin receptor. Serial dilutions of COS conditioned medium containing zNT-7 (solid squares) were used to displace the binding of ^{125}I-NGF from p75 receptors on the human melanoma cell line A875. Little or no displacement was seen with conditioned medium from COS cells transfected with a control plasmid (open squares).

tyrosine kinases. After exposure to conditioned medium, cells were lysed and analysed for tyrosine phosphorylation of Trk receptors by immunoprecipitation and Western blotting. This analysis demonstrated that zNT-7 was able to induce tyrosine phosphorylation of rat TrkA (Fig. 4). However, conditioned medium containing zNT-7 was much less potent than that containing NGF at stimulating TrkA activation, despite the fact that both media contained comparable amounts of neurotrophin protein, indicating a suboptimal interaction of zNT-7 with mammalian TrkA receptors. zNT-7 was unable to induce tyrosine phosphorylation of rat TrkB or rat TrkC receptors (Fig. 4). Thus, although less potent than murine NGF, the receptor specificity of zNT-7 supports the higher relatedness of this neurotrophin to members of the NGF subfamily.

Orthologs of NGF, BDNF and NT-3 have been found in diverse groups of vertebrates including mammals, amphibians, reptiles, birds and fishes [10], suggesting that these are ancestral neurotrophins common to all major vertebrate groups. In contrast, no orthologs of NT-4 have so far been found in fish, nor of NT-6 in mammals, despite big efforts by several laboratories. We have so far been equally unsuccessful in identifying orthologs of zNT-7 in mammals. If the absence of detectable NT-6 and zNT-7 sequences in mammals is taken as evidence that the corresponding neurotrophin genes are not

present in these organisms, the NT-6 and zNT-7 genes might have first appeared in a common ancestor of teleost fishes after this lineage split from the ancestor of amphibians and mammals. Likewise, if orthologs of NT-4 are not present in fish, the NT-4 gene might have first appeared in a common ancestor of the amphibian/mammalian lineage after this diverged from fishes [25]. In this scenario of neurotrophin evolution, NT-4 and NT-6/zNT-7 could be seen as diversifications of the BDNF and NGF subfamilies in amphibians/mammals and teleost fishes, respectively, that occurred after these two groups split from a common ancestor more than 400 million years ago. In this respect, it is also of interest to note that NT-4, NT-6 and zNT-7 are the only neurotrophins known to have amino acid insertions in the β-turn variable region III; the fact that these insertions are all of different size (i.e. 7, 22 and 15 residues, respectively) suggests that they originated independently. We propose that, after the divergence of fishes from other vertebrate lineages leading to amphibians and mammals, diversifications of the neurotrophin gene family occurred in the NGF subfamily in fishes and in the BDNF subfamily in amphibians and mammals which resulted in the acquisition of additional members characterized by the presence of amino acid insertions in variable region III and the shared use of a common Trk receptor.

The radiation of teleost fishes was a remarkable event during vertebrate evolution both because of its magnitude and speed. The possibility that teleost fishes have a number of neurotrophin genes not present in other vertebrate lineages should therefore not be so surprising. In fact, up to five different Trk gene paralogs have been described in the zebrafish [26], compared to only three in all other vertebrate groups investigated so far, suggesting that teleost fishes may indeed have more neurotrophin/Trk pairs than mammals. The identification of the cognate Trk receptor for zNT-7 among the five zebrafish Trk proteins previously identified is clearly of great interest.

References

[1] Lewin, G. and Barde, Y.-A. (1996) Ann. Rev. Neurosci. 19, 289.
[2] Angeletti, R.H. and Bradshaw, R.A. (1971) Proc. Natl. Acad. Sci. USA 68, 2417–2420.
[3] Levi-Montalcini, R. (1987) 237, 1154–62.
[4] Leibrock, J., Lottspeich, A.H., Hofer, M., Hengerer, B., Masiakowski, P., Thoenen, H. and Barde, Y.-A. (1989) Nature 341, 149–152.
[5] Hohn, A., Leibrock, J., Bailey, K. and Barde, Y.-A. (1990) Nature 344, 339–341.
[6] Ernfors, P., Ibáñez, C.F., Ebendal, T., Olson, L. and Persson, H. (1990) Proc. Natl. Acad. Sci. USA 87, 5454–5458.
[7] Rosenthal, A., Goeddel, D.V., Nguyen, T., Lewis, M., Shih, A., Laramee, G.R., Nikolics, K. and Winslow, J.W. (1990) Neuron 4, 767–773.
[8] Kaisho, Y., Yoshimura, K. and Nakahama, K. (1990) FEBS Lett. 266, 187–191.
[9] Jones, K.R. and Reichardt, L.F. (1990) Proc. Natl. Acad. Sci. USA 87, 8060–8064.
[10] Hallböök, F., Ibáñez, C.F. and Persson, H. (1991) Neuron 6, 845–858.
[11] Ip, N.Y. et al. (1992) Proc. Natl. Acad. Sci. USA 89, 3060–3064.
[12] Berkemeier, L., Winslow, J., Kaplan, D., Nicolics, K., Goeddel, D. and Rosenthal, A. (1991) Neuron 7, 857–866.
[13] Chao, M.V. and Hempstead, B.L. (1995) Trends Neurosci. 18, 321–326.
[14] Dechant, G. and Barde, Y.-A. (1997) Curr. Op. Neurobiol. 7, 413–418.
[15] Barbacid, M. (1994) J. Neurobiol. 25, 1386–1403.
[16] McDonald, N., Lapatto, R., Murray-Rust, J., Gunning, J., Wlodawer, A. and Blundell, T. (1991) Nature 354, 411–414.
[17] Holland, D.R., Cousens, L.S., Meng, W. and Matthews, B.W. (1994) J. Mol. Biol. 239, 385–400.
[18] Robinson, R.C., Radziejewski, C., Stuart, D.I. and Jones, E.Y. (1995) Biochemistry 34, 4139–4146.
[19] Götz, R., Raulf, F. and Schartl, M. (1992) J. Neurochem. 59, 432–442.
[20] Götz, R., Koster, R., Winkler, C., Raulf, F., Lottspeich, F., Schartl, M. and Thoenen, H. (1994) Nature 372, 266–269.
[21] Li, Y.L., Franz, J., Lottspeich, F. and Gotz, R. (1997) Biochemical Journal.
[22] Genetics Computer Group (1994), Madison, Wisconsin, USA 53711.
[23] Ip, N.Y. et al. (1993) Neuron 10, 137–149.
[24] Ibáñez, C.F. (1995) Trends Biotechnol. 13, 217–227.
[25] Ibáñez, C.F. (1996) Neurochem. Res. 21, 787–793.
[26] Martin, S.C., Marazzi, G., Sandell, J.H. and Heinrich, G. (1995) Dev. Biol. 169, 745–758.