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# Dynamic expression of *neurexophilin1* during zebrafish embryonic development ☆

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ABSTRACT

Neurexophilin 1 (Nxph1) is a specific endoligand of α-neurexins that is essential for trans-synaptic activation. Here, we report its dynamic expression during development in zebrafish. Our study revealed an early onset of expression of *nxph1*. RT-PCR on a series of embryonic stages showed that it is maternally deposited, although only readily detectable by whole mount *in situ* hybridization by 22 hpf. During embryogenesis and larval stages, the zygotic transcript is expressed dynamically in various clusters of post-mitotic neurons and in glia in the central nervous system.



Neurexophilin is a highly conserved secreted cysteine-rich glycoprotein in vertebrates. It was discovered as a 29-kDa protein that is co-purified with neurexin 1α on immobilized α-latrotoxin (Petrenko et al., 1993). The binding of the protein to neurexin is very tight, hence the name neurexophilin (Petrenko et al., 1996).

The protein neurexophilin comprises of four domains: a signal peptide, a non-conserved N-terminal region, a highly conserved central *N*-glycosylated domain, and an equally conserved C-terminal cysteine-rich domain. Expression and protein purification experiments revealed that neurexophilins are secreted glycoproteins that are proteolytically processed from the primary translation product, first by signal sequence cleavage and then by cleavage at the boundary between the N-terminal non-conserved and the central conserved domain. The proteolytic processing observed in neurexophilin, reminiscent of maturation processes in neuropeptides, together with their tight binding to neurexins indicates that they are endogenous ligands for α-neurexins (Missler and Sudhof, 1998). Neurexins (NRXNs) are neuronal cell surface proteins that are required for trans-synaptic activation of synaptic transmission but not synapse formation (Missler et al., 2003). They are polymorphic synaptic receptors encoded by at least three genes (NRXN1, NRXN2 and NRXN3) in mammals, each of which has two independent promoters directing transcription of long α-neurexins and short β-neurexins.

Molecular cloning revealed that there are at least four neurexophilins genes (*nxph1*,*nxph2*,*nxph3* and*nxph4*) in mammals (Missler and Sudhof, 1998; Petrenko et al., 1996). Comparisons of neurexophilins show that they are closely related to each other in the C-terminus regions but diverge considerably in their N-terminal regions (Missler and Sudhof, 1998). Rodents have all four genes but only neurexophilins 1, 3, and 4 are expressed at detectable levels. Nxph2 is however expressed in bovine, strongly suggesting evolutionary changes in nxph gene expression. Nxph4 has a different linker region and doesn't bind to α-neurexins, unlike other neurexophilins (Missler et al., 1998).

Although neurexophilin gene family has been studied in mammals, there is lack of data for these genes in lower vertebrates. Very little is known about the expression of neurexophilins *in vivo*. Zebrafish (*Danio rerio*) has a quite simple and well-characterized nervous system with broad developmental and physiological similarities to humans. Recent studies in zebrafish showed that *nxph1* is expressed in discrete clusters in the habenula, pallium, and ventral thalamus at 72 hpf and is involved in Notch signaling (Hortopan and Baraban, 2011). We provide here a comprehensive description of *nxph1* expression during zebrafish development.



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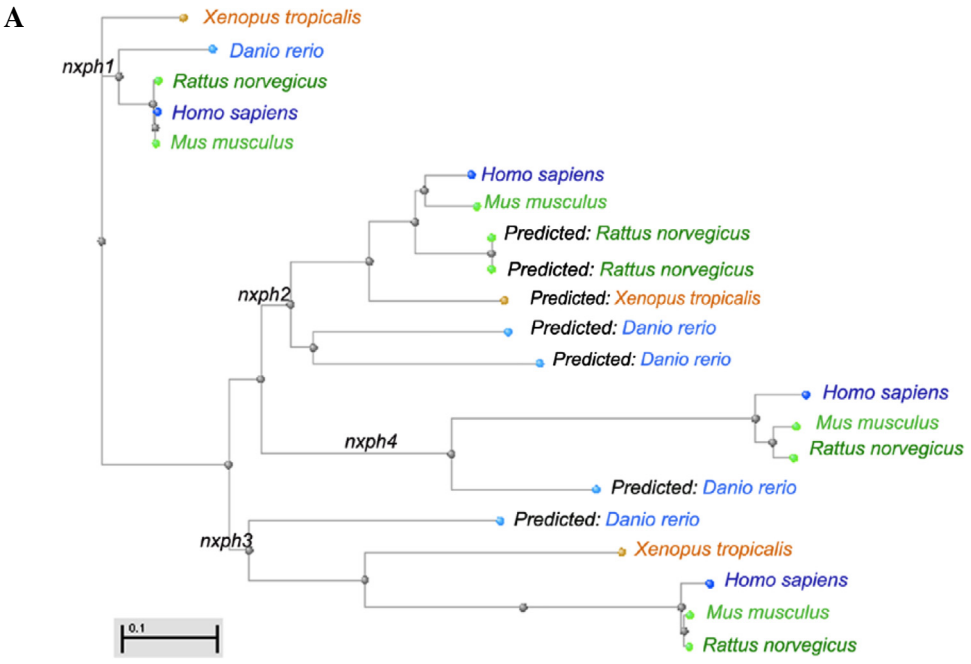


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Danio rerio          MRATCWCAVFLITPAVYLVSSAHGSVPGKSELLKSGSPKSTLKHIWTESSKDLSISRLLS
Homo sapiens         MQAACWYVLFLLQPTVYLVTCANLTNGGKSELLKSGSSKSTLKHIWTESSKDLSISRLLS
Mus musculus         MQAACWYVLLLLQPTVYLVTCANLTNGGKSELLKSGSSKSTLKHIWTESSKDLSISRLLS
Rattus norvegicus    MQAACWYVLLLLQPTVYLVTCANLTNGGKSELLKSGNSKSTLKHIWTESSKDLSISRLLS
Xenopus tropicalis   MQPVYWLSVLLLQATLQLMICTNSVGAGKIGNQKSGNSISTLKHIWTESNKELSISRLIS
                     *    *    *    *          **    ***   ********** * ****** *

Danio rerio          QTLHGKENATALDLHYDTPEPYSEQDLWDWLRNTTDPQDSRPRAKRRPMVKTGKFKKMFG
Homo sapiens         QTFRGKENDTDLDLRYDTPEPYSEQDLWDWLRNSTDLQEPRPRAKRRPIVKTGKFKKMFG
Mus musculus         QTFRGKENDTDLDLRYDTPEPYSEQDLWDWLRNSTDLQEPRPRAKRRPIVKTGKFKKMFG
Rattus norvegicus    QTFRGKENGTDLDLRYDTPEPYSEQDLWDWLRNSTDLQEPRPRAKRRPIVKTGKFKKMFG
Xenopus tropicalis   QTFRGKENQTALNMRYDTPEPYTEQELWEWLRNSTDLQEPRPRAKRRPIVKTGKFKKMFG
                     **  **** * *   ******* ** ** **** ** *  ******** ***********

Danio rerio          WGDFHSNIKTVKLNLLITGKIVDHGNGTFSVYFRHNSTGQGNVSVSLVPPTKIVEFDLTA
Homo sapiens         WGDFHSNIKTVKLNLLITGKIVDHGNGTFSVYFRHNSTGQGNVSVSLVPPTKIVEFDL-A
Mus musculus         WGDFHSNIKTVKLNLLITGKIVDHGNGTFSVYFRHNSTGQGNVSVSLVPPTKIVEFDL-A
Rattus norvegicus    WGDFHSNIKTVKLNLLITGKIVDHGNGTFSVYFRHNSTGQGNVSVSLVPPTKIVEFDL-A
Xenopus tropicalis   WGDFHSNIKTVKLNLLITGKIVDHGNGTFSVYFRHNSTGQGNVSVSLVPPTKIVEFDL-A
                     ************************************************************ *

Danio rerio          QQSVIDAKDSKSFNCRIEYEKVERGSKNTLCNFDPSKTCFQEQTQSHVSWLCSKPFKVIC
Homo sapiens         QQTVIDAKDSKSFNCRIEYEKVDKATKNTLCNYDPSKTCYQEQTQSHVSWLCSKPFKVIC
Mus musculus         QQTVIDAKDSKSFNCRIEYEKVDKATKNTLCNYDPSKTCYQEQTQSHVSWLCSKPFKVIC
Rattus norvegicus    QQTVIDAKDSKSFNCRIEYEKVDKATKNTLCNYDPSKTCYQEQTQSHVSWLCSKPFKVIC
Xenopus tropicalis   QQSVIDAKDSKSFNCRIEYEKVDKATKNTLCNYDPSKTCYQEQTQSHVSWLCSKPFKVIC
                     ** ******************    ****** ****** ********************

Danio rerio          IYISFYSTDYKLVQKVCPDYNYHSDTSYFPSG
Homo sapiens         IYISFYSTDYKLVQKVCPDYNYHSDTPYFPSG
Mus musculus         IYISFYSTDYKLVQKVCPDYNYHSDTPYFPSG
Rattus norvegicus    IYISFYSTDYKLVQKVCPDYNYHSDTPYFPSG
Xenopus tropicalis   IYISFYSTDYKLVQKVCPDYNYHSDTPYFPSG
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**Fig. 1.** Phylogenetic tree and amino acid alignment of neurexophilins. (A) Phylogenetic tree of the neurexophilin homologues of human (NP_689958.1, NP_009157.1, NP_009156.2, NP_009155.1), NP_067712.2), mouse (NP_032777.3, NP_032778.1, NP_570928.1, NP_899120.2), rat (NP_037126.1, XP_001055001.1, XP_002726131.1, NP_067711.1, frog (NP_001120374.1, XP_002933980.1, NP_001096477.1) and zebrafish (NP_001002733.1, XP_698522.2, XP_001337769.1, XP_691473.2, XP_002667270.2) was generated based on the full-length amino acid sequence available at NCBI. (B) Multiple sequence alignment of nxph1 in human, rat, mouse, frog and zebrafish. The *nxph1* aminoacid sequences of *Danio rerio*, *Homo sapiens*, *Mus musculus*, *Rattus norvegicus* and *Xenopus tropicalis* were aligned based on Clustal W(2.1) algorithm. Conserved amino acids are indicated by an asterisk (identical in all cases), colon (conserved substitutions observed) or dot (semi-conserved substitutions observed).