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GDNF expression during *Xenopus* development

Jun-ichi Kyuno, Elizabeth A. Jones *

Molecular Physiology, Department of Biological Sciences, Warwick University, Gibbet Hill Road, Coventry CV4 7AL, UK

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Abstract

Glial cell line-derived neurotrophic factor (GDNF) has multiple roles in kidney morphogenesis, spermatogenesis, and neurogenesis during development. In this study, we report the cloning and expression pattern of *Xenopus laevis* GDNF. The *X. laevis* GDNF cDNA sequence has a complete open reading frame of 684 bases, predicting 227 amino acid residues at the protein level. Comparison of the *X. laevis* GDNF amino acid sequence with those of chick, human, mouse, rat and zebrafish indicates that *X. laevis* GDNF has 60%–52% and 75%–62% identity over the whole amino acid sequence and for the putative mature forms, respectively. All known functional motifs of GDNF were conserved in the *X. laevis* sequence. Temporal expression analysis by RT-PCR indicated that *GDNF transcripts* were first detectable at stage 12 at a low level, and gradually increased up to stage 22. From stage 24, the expression sharply increased and continued at a similar level as development progressed. Spatial expression analysis by whole-mount *in situ* hybridization showed that the *GDNF mRNA* was predominantly detected in somites, pronephros, pharyngeal arches, epibranchial placodes, digestive tract and some of the lateral line structure. These results suggest that this *X. laevis* gene is the orthologue for GDNF.

Keywords: Glial cell line-derived neurotrophic factor; GDNF; *Xenopus laevis*; Pronephros

1. Results and discussion

Glialcell line-derived neurotrophic factor (GDNF) has multiple roles during development. GDNF was originally identified as a potent survival factor for midbrain dopaminergic neurons (Lin et al., 1993). GDNF regulates the survival and the differentiation of several neuronal populations in the central and peripheral nervous system (reviewed in Enomoto, 2005). In addition, GDNF regulates ureteric branching in kidney morphogenesis and differentiation in spermatogenesis (reviewed in Sariola and Saarma, 2003). GDNF belongs to the GDNF family ligands (GFLs) that form a distinct subgroup of the transforming growth factor-β (TGF-β) superfamily, because they contain seven cysteine residues in the same relative spacing as other members of the superfamily (Lin et al., 1993). In this study, we report the cloning and expression pattern of *Xenopus laevis* GDNF.

1.1. Sequence analysis of X. laevis GDNF

The partial coding sequence of *X. laevis* GDNF was obtained by polymerase chain reaction (PCR) amplification, using primers designed from the *X. tropicalis* genomic sequence identified as homologous to mouse GDNF at the amino acid sequence level. Extension of this sequence with rapid amplification of cDNA ends (RACE)-PCR identified an expressed sequence tag (EST) clone for *X. laevis* GDNF (GenBank BQ735429, IMAGE 5571059). Complete mRNA sequence was determined and registered with the GenBank (Accession No. DQ779994).

Xenopus laevis GDNF was obtained as a clone containing a complete open reading frame of 684 bases. Conceptual translation of GDNF yielded a predicted protein containing 227 amino acid residues with a putative molecular weight of 25.8 kDa (Fig. 1A). A BLAST search with

* Corresponding author. Tel.: +44 2476 523061; fax: +44 2476 523701.
E-mail address: Elizabeth.jones@warwick.ac.uk (E.A. Jones).

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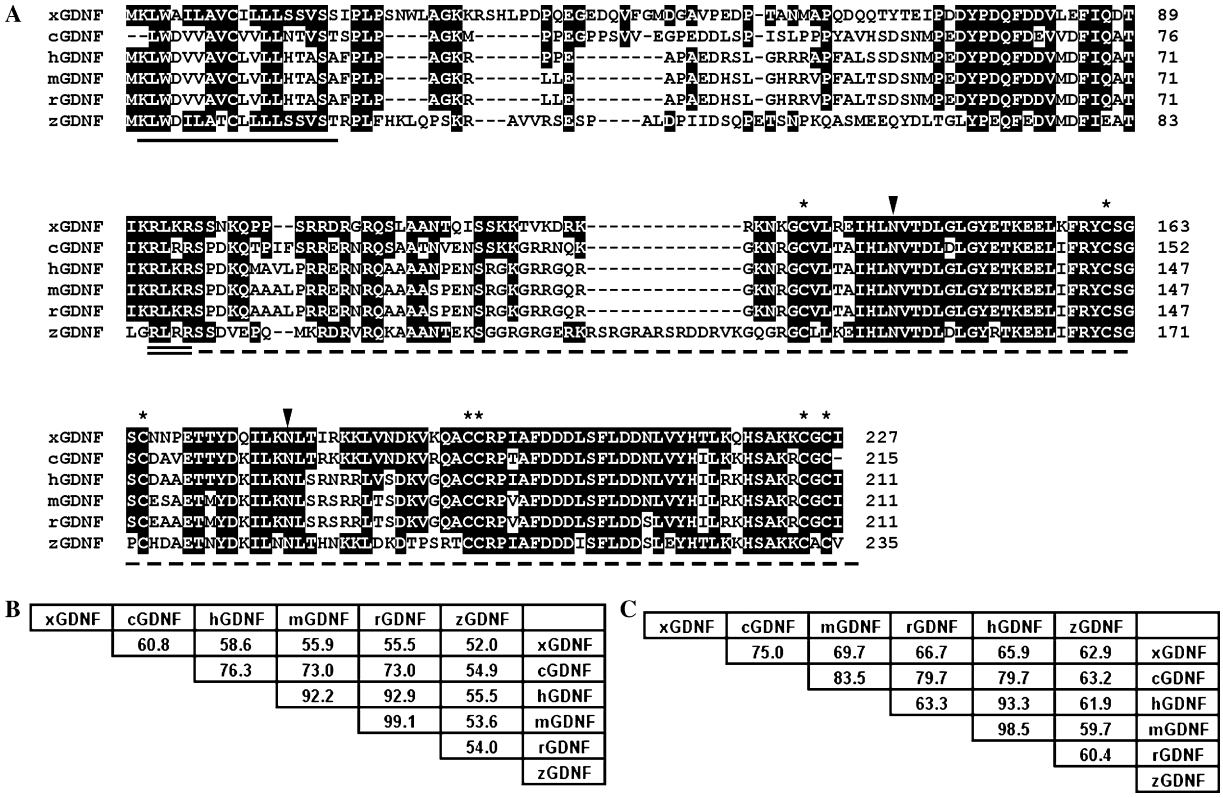

Fig. 1. Comparison of GDNF amino acid sequence between vertebrates. Predicted GDNF amino acid sequences of *X. laevis* (xGDNF, GenBank Accession No. DQ779994), chick (cGDNF, GenBank Accession No. AF176017), human (hGDNF, GenBank Accession No. BC069369), mouse (mGDNF, GenBank Accession No. D88352), rat (rGDNF, GenBank Accession No. NM_019139), and zebrafish (zGDNF, GenBank Accession No. AF329853) are compared. (A) Multiple alignment of GDNFs. Black boxes indicate the amino acid residues identical to *X. laevis* GDNF. The number on the right side refers to the position in the amino acid sequence. The solid underline indicates potential secretion signals. The double underline indicates predicted proteolytic processing sites for production of mature GDNF. The predicted amino acid sequence of mature GDNF is marked with a broken underline. Asterisks indicate the seven Cys residues conserved in the TGF-β superfamily. Two putative N-linked glycosylation sites are marked by arrowhead. (B and C) Homology between whole amino acid sequences (B) and predicted mature forms (C) of GDNFs. The figures indicate percent identities at amino acid level.

the amino acid sequence of *X. laevis* GDNF revealed that the *X. laevis* gene is most similar to other GDNF orthologues.

GDNF is synthesized as a precursor protein and processed as a mature protein after secretion. As with other vertebrate GDNFs, *X. laevis* GDNF has a potential secretion signal after the initial Met that is predicted to be cleaved after Ser^{19}. *X. laevis* GDNF also has a predicted proteolytic processing site that is predicted to be cleaved after Arg^{95} to produce mature GDNF. The GDNF family is a distantly related member of TGF-β superfamily. *X. laevis* GDNF contains the seven Cys residues in predicted mature regions, which are conserved in other members of the TGF-β superfamily. Two putative N-linked glycosylation sites were also conserved as in other vertebrate GDNFs.

Alternative splice forms of GDNF transcript have been reported for human (Grimm et al., 1998.), rat (Springer et al., 1995.), and chick (Homma et al., 2000).

In the short forms of these GDNF transcripts, deletions are located in the prodomain of the precursor protein sequence. *X. laevis* GDNF cloned in this study indicated higher homology with long form of GDNF orthologues rather than short form of these. *X. laevis* GDNF protein sequence was compared with long forms of GDNFs because the short form of *X. laevis* GDNF was not detectable during this study. At the whole amino acid sequence level, *X. laevis* GDNF displays 60.8%, 58.6%, 55.9%, 55.5%, and 52.0% identity with chick, human, mouse, rat, and zebrafish GDNF, respectively (Fig. 1B). Among the predicted mature forms of GDNFs, *X. laevis* protein has 75.0%, 69.7%, 66.7%, 65.9%, and 62.9% identity with chick, human, mouse, rat, and zebrafish GDNF, respectively (Fig. 1C). Therefore conserved motifs and high similarity among vertebrate GDNFs suggest that this *X. laevis* gene is an orthologue of GDNF.

1.2. Temporal and spatial expression pattern of X. laevis GDNF

The temporal expression profile of *GDNF* transcripts was analyzed by reverse transcription (RT)-PCR (Fig. 2). Maternal expression of *GDNF* was not detected. Zygotic expression was first detectable at a low level at stage 12,