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# Evidence for the coevolution of axon guidance molecule Netrin and its receptor Frazzled

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ABSTRACT

Coevolution of a ligand and its receptor is critical for maintaining their function in different species, but how ligand and its receptor coevolve is poorly understood. The axon guidance molecule Netrin and its receptor Frazzled (Fra) are useful to study the mechanisms of ligand–receptor coevolution. Here, we have applied codon substitution models to identify positive selection of the *netrin* and *fra* genes. The sites under positive selection in *netrin* and *fra* were detected in same lineage, such as nematode, dipteran, hymenopteran, hemichordate, and teleost. Several amino acid residues that are under positive selection were identified in the interaction domains. Here we provide evidence that positive selection is essential for the coevolution of Netrin and Fra during central nervous system evolution.

## 1. Introduction

The coevolutionary mechanism underlying the conserved interaction between paired-interaction proteins is essential for understanding the evolutionary process of protein interaction networks. Several examples of molecular coevolution of ligands and their receptors have been identified by testing their correlated changes using phylogenetic and interaction methods (de Juan et al., 2013; Pazos and Valencia, 2008; Reitzel and Tarrant, 2010). However, the evolutionary role of interaction domains in paired protein coevolution is largely unknown. As the interaction domains of receptors and their ligands play key functional roles, the correlated changes of amino acid sites in the interaction domains might be critical to their coevolution. The axon guidance molecule Netrin and its receptor Frazzled (Fra) are good models for investigating the role of interaction domains in ligand–receptor coevolution. Netrin and Fra bind to each other and direct the proper wiring of the central nervous system (CNS) (Cebria and Newmark, 2005; Kolodziej et al., 1996). To achieve this interaction, the VI and V domains of Netrin-1 bind to the FN4 and FN5 domains of Deleted in Colorectal Carcinoma (DCC), an ortholog of Fra in vertebrates (Geisbrecht et al., 2003; Kruger et al., 2004). The interaction between Netrin and Fra appears to have been conserved during the evolutionary process from planarians to humans (Cebria and Newmark, 2005; Chan et al., 1996; Kolodziej et al., 1996; Lai Wing Sun et al., 2011; Rajasekharan and Kennedy, 2009). However, the evolutionary mechanism underlying the conserved interaction between Netrin and Fra remains unclear.

## 2. Materials and methods

### 2.1. Sequence collection and alignment

123 *netrin* homologous sequences from 58 species and 82 *fra* homologous sequences from 57 species were identified by BLAST searches against National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov) and Ensembl Genome Browser (http://asia.ensembl.org/index.html). Accession numbers were compiled in Tables S1 and S2. Sequence alignments (Files S1–S7) were performed using MUSCLE in MEGA 5 with default parameters (Edgar, 2004; Tamura et al., 2011).

### 2.2. Phylogenetic tree constructions

The neighbor-joining (NJ) trees (Saitou and Nei, 1987) for Netrin and Fra were constructed by MEGA 5 (Tamura et al., 2011). Bootstrap analysis with 1000 replicates was used to assess the support for tree nodes (Felsenstein, 1985).

### 2.3. Positive selection tests

Selective pressure was measured by the ratio of nonsynonymous to synonymous nucleotide substitutions rates ($\omega = dN / dS$). This ratio

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*Abbreviations:* Fra, Frazzled; CNS, central nervous system; DCC, deleted in colorectal carcinoma; NCBI, National Center for Biotechnology Information; NJ, neighbor-joining; LRT, likelihood ratio test; FN, fibronectin; Ig, immunoglobulin.

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A
Vertebrate Netrin-4
Ggor Netrin-4
Ppan Netrin-4
Hsap Netrin-4
Pabe Netrin-4
Mmul Netrin-4
Cjac Netrin-4
Mdom Netrin-4
Lafr Netrin-4
Ogar Netrin-4
Cfam Netrin-4
Btau Netrin-4
Cgri Netrin-4
Rnor Netrin-4
Mmus Netrin-4
Ecab Netrin-4
Acar Netrin-4a
Mgal Netrin-4a
Ggal Netrin-4a
Xtro Netrin-4
Acar Netrin-4b
Ggal Netrin-4b
Mgal Netrin-4b
Trub Netrin-4a2
Tnig Netrin-4a2
Onil Netrin-4a2
Olat Netrin-4a2
Olat Netrin-4a1
Onil Netrin-4a1
Trub Netrin-4a1
Tnig Netrin-4a1
Trub Netrin-4b
Drer Netrin-4b
Drer Netrin-4a
Skow Netrin-4
Planarian Netrin
Smed Netrin-2
Djap Netrin-2
Smed Netrin-1
Djap Netrin-1
Nvec Netrin
Nematode UNC-6
Crem UNC-6
Cele UNC-6
Cbri UNC-6
Asuu UNC-6
Mocc Netrin-1B
Mocc Netrin-1A
Mocc Netrin-1C
Dsec Netrin-A
Dmel Netrin-A
Dere Netrin-A
Dyak Netrin-A
Dana Netrin-A
Dvir Netrin-A
Dmoj Netrin-A
Dper Netrin-A
Dpse Netrin-A
Dwil Netrin-A
Dvir Netrin-B
Dmel Netrin-B
Dwil Netrin-B
Dpse Netrin-B
Dper Netrin-B
Agam Netrin-A
Agam Netrin-B
Tcas Netrin
Nvit Netrin
Mrot Netrin
Aflo Netrin
Bimp Netrin
Bter Netrin
Phum Netrin
Apis Netrin
Arthropod Netrin
Skow Netrin
Trub Netrin-1b
Onil Netrin-1b
Olat Netrin-1b
Drer Netrin-1b
Drer Netrin-1a
Olat Netrin-1a
Onil Netrin-1a
Trub Netrin-1a
Tnig Netrin-1a
Xtro Netrin-1
Ggal Netrin-1
Acar Netrin-1
Mdom Netrin-1
Lafr Netrin-1
Ptro Netrin-1
Mmul Netrin-1
Ogar Netrin-1
Btau Netrin-1
Hsap Netrin-1
Cfam Netrin-1
Rnor Netrin-1
Mmus Netrin-1
Oana Netrin-1
Vertebrate Netrin-1
Olat Netrin-2
Onil Netrin-2
Trub Netrin-2
Tnig Netrin-2
Drer Netrin-2
Acar Netrin-2
Ggal Netrin-2
Xtro Netrin-2
Mgal Netrin-2
Shar Netrin-3
Mdom Netrin-3
Ppan Netrin-3
Cjac Netrin-3
Amel Netrin-3
Ecab Netrin-3
Cpor Netrin-3
Lafr Netrin-3
Ggor Netrin-3
Hsap Netrin-3
Mmul Netrin-3
Pabe Netrin-3
Cfam Netrin-3
Mput Netrin-3
Btau Netrin-3
Ogar Netrin-3
Cgri Netrin-3
Rnor Netrin-3
Mmus Netrin-3
Vertebrate Netrin-2/3
B
Nematode UNC-40
Planarian Fra
Smed Fra
Djap Fra
Cbri UNC-40
Crem UNC-40
Cele UNC-40
Asuu UNC-40
Mocc Fra
Apis Fra
Phum Fra
Bter Fra
Bimp Fra
Aflo Fra
Mrot Fra
Nvit Fra
Tcas Fra
Agam Fra
Dwil Fra
Dmoj Fra
Dvir Fra
Dpse Fra
Dper Fra
Dana Fra
Dere Fra
Dsec Fra
Dmel Fra
Dyak Fra
Arthropod Fra
Skow Fra
Ppan DCC
Pabe DCC
Ptro DCC
Hsap DCC
Ggor DCC
Cjac DCC
Mmul DCC
Ogar DCC
Lafr DCC
Ecab DCC
Cfam DCC
Cgri DCC
Rnor DCC
Mmus DCC
Btau DCC
Mdom DCC
Oana DCC
Acar DCC
Xtro DCC
Drer DCC
Olat DCC
Onil DCC
Trub DCC
Tnig DCC
Vertebrate DCC
Olat Neogenin
Onil Neogenin
Trub Neogenin
Tnig Neogenin
Drer Neogenin1
Drer Neogenin2
Xtro Neogenin
Acar Neogenin
Ggal Neogenin
Mgal Neogenin
Oana Neogenin
Shar Neogenin
Mdom Neogenin
Rnor Neogenin
Mmus Neogenin
Cgri Neogenin
Ogar Neogenin
Cpor Neogenin
Lafr Neogenin
Amel Neogenin
Cfam Neogenin
Mput Neogenin
Ecab Neogenin
Btau Neogenin
Cjac Neogenin
Mmul Neogenin
Pabe Neogenin
Ppan Neogenin
Ptro Neogenin
Hsap Neogenin
Ggor Neogenin
Vertebrate Neogenin