Developmental Biology 374 (2013) 142–152

Contents lists available at SciVerse ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

From the Society for Developmental Biology

Discoidin domain receptors guide axons along longitudinal tracts in *C. elegans*

Thomas Unsoeld, Ja-On Park, Harald Hutter*

Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada

ARTICLE INFO

Article history:
Received 20 September 2012
Received in revised form
1 November 2012
Accepted 3 November 2012
Available online 9 November 2012

Keywords:
Neuronal development
Nervous system
Neuron
PVP
Pioneer
Axon guidance
Axon navigation
Discoidin domain receptors
Receptor tyrosine kinase
DDR-1
DDR-2
Ventral nerve cord
Dorsal nerve cord
C. elegans

ABSTRACT

Discoidin domain receptors are a family of receptor tyrosine kinases activated by collagens. Here we characterize the role of the two discoidin domain receptors, *ddr-1* and *ddr-2*, of the nematode *C. elegans* during nervous system development. *ddr-2* mutant animals exhibit axon guidance defects in major longitudinal tracts most prominently in the ventral nerve cord. *ddr-1* mutants show no significant phenotype on their own but significantly enhance guidance defects of *ddr-2* in double mutants. *ddr-1* and *ddr-2* GFP-reporter constructs are expressed in neurons with axons in all affected nerve tracts. DDR-1 and DDR-2 GFP fusion proteins localize to axons. DDR-2 is required cell-autonomously in the PVPR neuron for the guidance of the PVPR pioneer axon, which establishes the left ventral nerve cord tract and serves as substrate for later outgrowing follower axons. Our results provide the first insight on discoidin domain receptor function in invertebrates and establish a novel role for discoidin domain receptors in axon navigation and axon tract formation.

Introduction

During development neurons send out axons, which integrate a vast number of extracellular cues in order to reach their target cells. These guidance cues are detected by receptors on the growth cone at the tip of an extending axon. Receptor–ligand binding triggers intracellular signaling pathways, which affect actin and microtubule assembly, leading to growth cone stabilization or collapse resulting in directed axon outgrowth (Vitriol and Zheng, 2012). Several receptor tyrosine kinases (RTKs) are involved in axon guidance including Ryk/Derailed, which has been implicated in Wnt-mediated axon repulsion (Callahan et al., 1995; Keeble and Cooper, 2006) and Eph receptors which are of particular importance in topographic mapping (Reber et al., 2007). RTKs are type I transmembrane proteins consisting of an intracellular tyrosine kinase domain, a single-pass transmembrane domain and an extra-cellular ligand-binding domain. Human RTKs are classified into 20 families (Hanks and Hunter, 1995; Manning et al., 2002; Robinson et al., 2000). The small family of discoidin domain receptors consists of two members in mammals, DDR1 and DDR2. Likewise, *C. elegans* has two discoidin domain receptor genes, *ddr-1* and *ddr-2*, while only one gene has been identified in *Drosophila* (Vogel et al., 2006). Phylogenetic analysis (Fig. 1A) suggests that the two discoidin domain receptor genes in mammals and *C. elegans* evolved independently from a single ancestral gene (Vogel et al., 2006). In contrast to other RTKs, which typically bind small soluble proteins, mammalian discoidin domain receptors are activated by collagens. Both receptors interact with fibrillar collagens and require a native triple-helical structure for receptor activation (Shrivastava et al., 1997; Vogel et al., 1997). Discoidin domain receptors also interact with members of the network-forming collagens. Collagen type IV and VIII act as ligands for DDR1 while type X collagen interacts with DDR2 (Hou et al., 2001; Leitinger and Kwan, 2006; Shrivastava et al., 1997; Vogel et al., 1997). Unlike most RTKs, which usually dimerize upon ligand binding, discoidin domain receptors form stable, disulfide-linked dimers in the absence of collagen stimulation (Abdulhussein et al., 2008; Mihai et al., 2009; Noordeen et al., 2006). Dimerization of the extracellular domains is a prerequisite for ligand binding (Leitinger, 2003).

* Corresponding author.
E-mail address: hutter@sfu.ca (H. Hutter).

0012-1606/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ydbio.2012.11.001

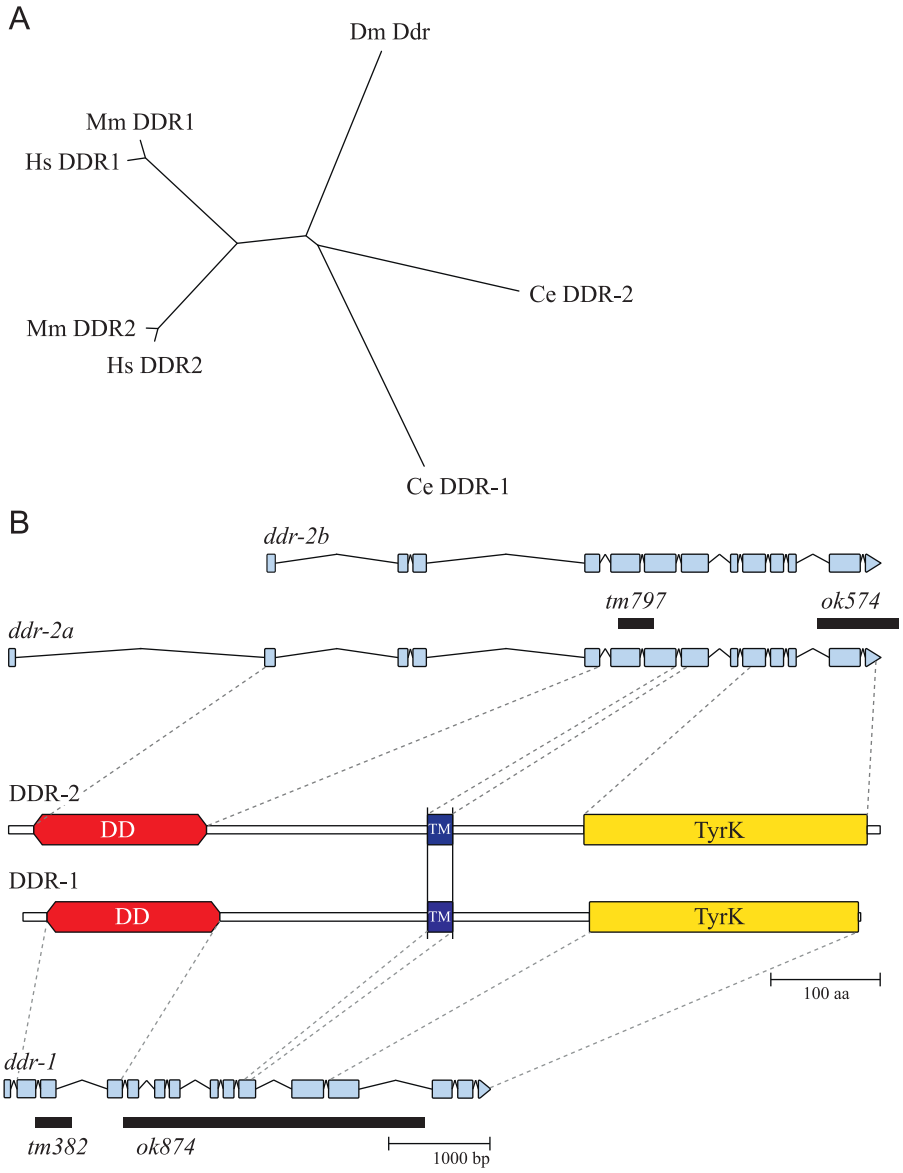

Fig. 1. (A) Relationship between discoidin domain receptors. Unrooted phylogenetic tree of human (Hs DDR), mouse (Mm DDR), *Drosophila* (Dm Ddr) and *C. elegans* (Ce DDR) discoidin domain receptors. (B) Gene models and protein domain organization of discoidin domain receptors in *C. elegans*. The locations of the deletions used in this study are indicated by black bars. DD: discoidin domain; TM: transmembrane domain; TyrK: tyrosine kinase.

Compared to the rapid autophosphorylation of other RTKs, discoidin domain receptor activation is slow and is sustained for a prolonged period of time (Vogel et al., 1997).

Discoidin domain receptors are involved in numerous processes during development such as regulating cell proliferation, adhesion, migration and remodeling of the extracellular matrix in part through the activation of metalloproteinases (Curat and Vogel, 2002; Hou et al., 2001,2002; Labrador et al., 2001; Olaso et al., 2002). Both discoidin domain receptors are expressed in the nervous system. DDR2 expression was found in the developing and mature brain of rats (Lai and Lemke, 1994). DDR1 is expressed in proliferating areas in the central nervous system of rats and mice (Sanchez et al., 1994; Zerlin et al., 1993). Postnatally, DDR1 expression follows the progress of myelination and was detected in both myelin and oligodendrocytes (Franco-Pons et al., 2006; Roig et al., 2010). In humans DDR1 has been suggested as a susceptibility gene for schizophrenia (Roig et al., 2007).

In this study we characterize discoidin domain receptors in the nematode *C. elegans*. We found that *ddr-1* and *ddr-2* are expressed in the nervous system *ddr-2* mutants show a variety of axon navigation defects in major longitudinal tracts, most notably the left ventral nerve cord. DDR-2 is required cell-autonomously in the left ventral nerve cord pioneer neuron PVPR for the proper guidance of both the pioneer and follower axons. Mutants in *ddr-1* show no phenotype alone but act synergistically with *ddr-2* highlighting a novel role for the two discoidin domain receptors in axon guidance during nervous system development of *C. elegans*.

Results

Discoidin domain receptors in C. elegans

Discoidin domain receptors are single-pass transmembrane proteins. Their extracellular region contains the ligand-binding discoidin domain and a stalk region. The intracellular region consists of a juxtamembrane region and the catalytic tyrosine kinase domain (Fig. 1B). The *ddr-1* gene of *C. elegans* encodes a