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Zebrafish orthologue of mental retardation protein IL1RAPL1 regulates presynaptic differentiation☆

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ABSTRACT

IL1-receptor accessory protein-like 1 (IL1RAPL1), a member of interleukin-1/toll receptor (TIR) family, is responsible for a nonsyndromic form of mental retardation (MR). The zebrafish orthologue of mammalian IL1RAPL1, designated as Il1rapl1b, was expressed widely in the brain and in the olfactory placode. We employed an olfactory sensory neuron-specific gene manipulation system in combination with *in vivo* imaging of transparent zebrafish embryos to examine the functional role of Il1rapl1b in synaptic vesicle accumulation and subsequent morphological remodeling of axon terminals, the characteristic features of presynaptic differentiation of zebrafish olfactory sensory neurons during synapse formation. Antisense morpholino oligonucleotide against *il1rapl1b* suppressed both the synaptic vesicle accumulation and axon terminal remodeling. Consistently, the overexpression of Il1rapl1b stimulated synaptic vesicle accumulation. Swapping the carboxyl-terminal domain of Il1rapl1b with that of mouse IL-1 receptor accessory protein abolished the stimulatory effect. On the other hand, a substitution mutation in the TIR domain suppressed the morphological remodeling of axon terminals. Thus, the regulation of synaptic vesicle accumulation and subsequent morphological remodeling by Il1rapl1b appeared to be mediated by distinct domains. These results suggest that Il1rapl1b plays an important role in presynaptic differentiation during synapse formation.

Introduction

Mental retardation (MR), defined as a failure to develop cognitive abilities, is the most frequent cause of serious handicap in children and young adults (Chiurazzi and Oostra, 2000; Chelly and Mandel, 2001). In syndromic form of MR, cognitive impairments are usually associated with other physical and neurological deficits. On the other hand, nonsyndromic MR is characterized by reduced cognitive function without any other clinical features, thus providing the most direct approach to specifically study the neurobiology of cognition and pathogenesis of MR. Recently, several genes associated with X-linked nonsyndromic MR have been identified by positional-cloning strategies, providing clues to investigate the molecular and cellular dysfunctions underlying the disorder (Chelly and Mandel, 2001; Chelly et al., 2006). IL1-receptor accessory protein-like 1 (IL1RAPL1) responsible for a nonsyndromic form of X-linked MR belongs to a novel class of the interleukin-1/Toll receptor (TIR) family (Carrié et al., 1999; Born et al., 2000). IL1RAPL1 protein consists of three immunoglobulin (Ig)-like domains, a single transmembrane segment, the TIR domain and the carboxyl-terminal domain. Recent studies suggest that IL1RAPL1 interacts with neuronal calcium sensor-1 (NCS-1) through the carboxyl-terminal domain (Bahi et al., 2003). When expressed in PC12 cells, IL1RAPL1 suppressed N-type Ca^{2+} currents and neurite elongation (Gambino et al., 2007).

Formation and refinement of synaptic connections are key steps of neural development to establish elaborate brain networks. The initial interaction between presynaptic and postsynaptic cells triggers intracellular signals that induce assembly of synaptic machineries and structural alterations to form a stable synapse (Friedman et al., 2000). During presynaptic differentiation of zebrafish olfactory sensory neurons, synaptic vesicles visualized with vesicle-associated membrane protein 2 (VAMP2)-enhanced green fluorescent protein (EGFP) fusion protein markedly accumulate in axon terminals between 36 h postfertilization (hpf) and 60 hpf, while the morphological remodeling of axon terminals from complex shapes with filopodia to simple shapes without filopodia proceeds in next 24 h (Yoshida and Mishina, 2005). Correspondingly, in the olfactory bulb, the stereotyped pattern of glomerular arrangement appears within 3 days postfertilization (Wilson et al., 1990; Dynes and Ngai, 1998) and odor responses become detectable at 60–72 hpf (Li et al., 2005). To investigate the functional role of IL1RAPL1 in neural development, we employed an olfactory sensory neuron-specific gene manipulation system with olfactory marker protein gene (*omp*) promoter-driven effector-reporter double-cassette vectors in transparent zebrafish embryos (Yoshida and Mishina, 2003). The injection of antisense morpholino oligonucleotide (MO) against *il1rapl1b* into embryos

☆ IL1RAPL1 1 and presynaptic differentiation.

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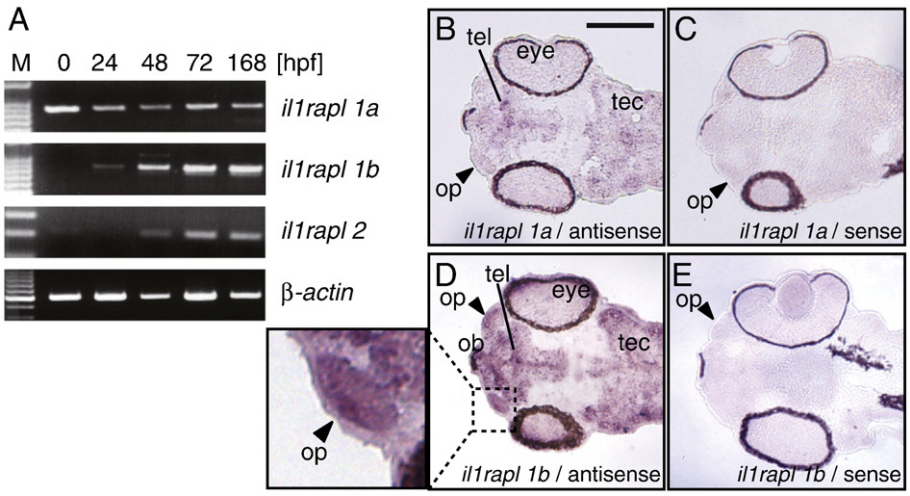

Fig. 1. Expression of zebrafish *il1rapl* genes. (A) Expression of zebrafish *il1rapl1a*, *il1rapl1b* and *il1rapl2* mRNA in the 0 hpf, 24 hpf, 48 hpf and 72 hpf zebrafish embryos and 168 hpf larva estimated by RT-PCR. The sizes of *il1rapl1a*, *il1rapl1b* and *il1rapl2* fragments were 825 bp, 828 bp and 211 bp, respectively. β-*actin* fragment of 490 bp served as a positive control. (B, C) Sections of 60 hpf embryos stained by in situ hybidization with DIG-labeled antisense (B) and sense (C) probes of the zebrafish *il1rapl1a* mRNA. (D, E) Sections of 60 hpf embryos stained by in situ hybidization with DIG-labeled antisense (D) and sense (E) probes of the zebrafish *il1rapl1b* mRNA. The dashed region in (D) including the olfactory placode is magnified on the left. eye, eye; ob, olfactory bulb; op, olfactory placode; tec, tectum; tel, telencephalon. Arrowheads point olfactory placodes. Scale bar, 100 μm.

suppressed both VAMP2-EGFP punctum formation and axon terminal remodeling. Consistently, the overexpression of zebrafish Il1rapl1b in the olfactory sensory neurons stimulated VAMP2-EGFP punctum formation in the axon terminals during synapse formation. Swapping the carboxyl-terminal domain of Il1rapl1b with that of mouse IL-1 receptor accessory protein (IL-1RAcP) abolished the stimulatory effect

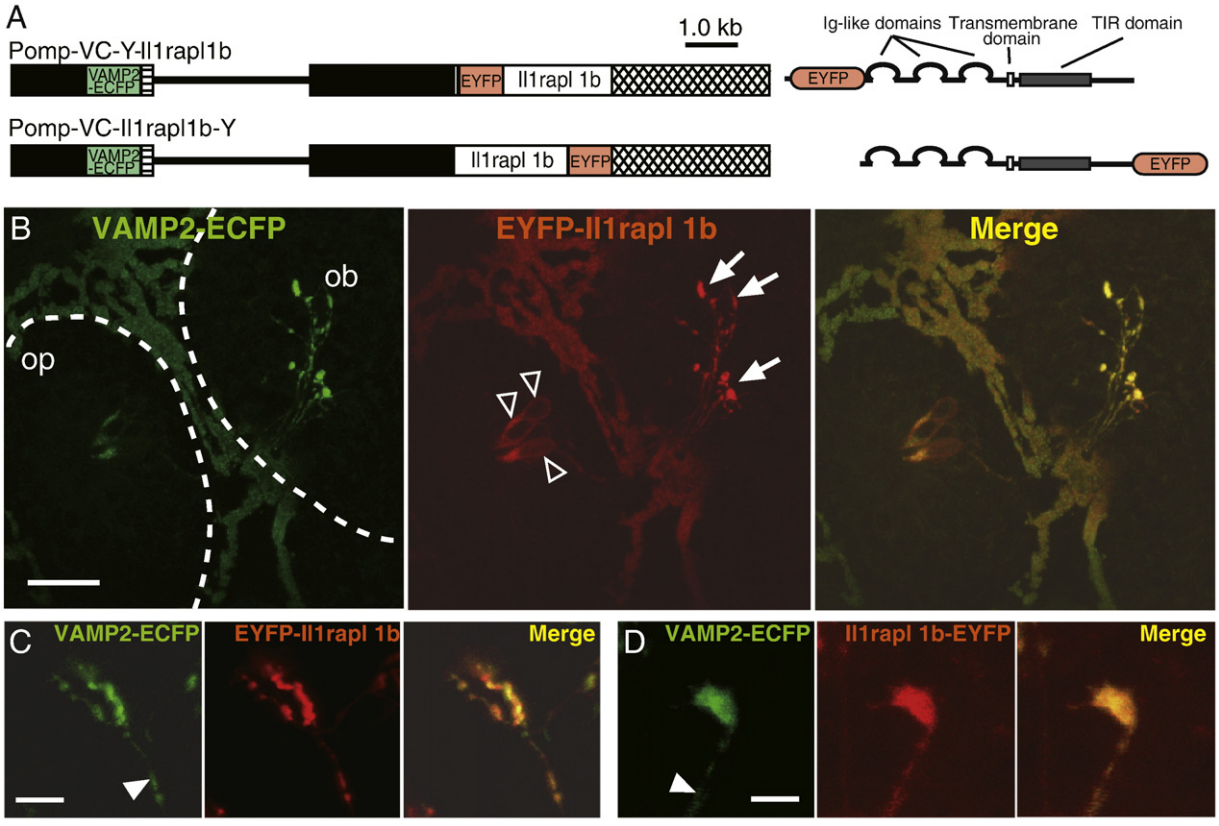

Fig. 2. Localization of zebrafish Il1rapl1b tagged with EYFP in developing zebrafish olfactory sensory neurons. (A) *Omp* promoter-driven expression vectors for EYFP-Il1rapl1b fusion protein and VAMP2-ECFP (Pomp-VC-Y-Il1rapl1b, top) and for Il1rapl1b-EYFP-fusion protein and VAMP2-ECFP (Pomp-VC-Il1rapl1b-Y, bottom) in olfactory sensory neurons. Black boxes, the *omp* promoter; crosshatched boxes, the 3′ downstream sequence of the *omp* gene; hatched boxes, SV40 polyadenylation signal sequence; lines, pBluescript II SK+. Schematized structures of EYFP-tagged Il1rapl1b proteins are on the right. (B) Representative VAMP2-ECFP signals (left, green) and EYFP-Il1rapl1b signals (middle, red) in olfactory sensory neurons of Pomp-VC-Y-Il1rapl1b-injected embryos at 60 hpf. These signals are merged on the right. op, olfactory placode; ob, olfactory bulb. Arrows and arrowheads point cell bodies and axon terminals, respectively. Bar = 20 μm. (C, D) Representative VAMP2-ECFP signals (left, green) and EYFP-fusion protein signals (middle, red) in the axon terminal of olfactory sensory neuron of Pomp-VC-Y-Il1rapl1b (C)-or Pomp-VC-Il1rapl1b-Y (D)-injected embryos at 60 hpf. These signals are merged on the right. Arrowheads point axonal shafts. Bar = 5 μm.