Neuroscience Research 69 (2011) 87–92

Contents lists available at ScienceDirect

Neuroscience Research

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

Review article

Modulation of neurotransmitter receptors and synaptic differentiation by proteins containing complement-related domains

Minoru Nakayama, Chihiro Hama*

Department of Molecular Biosciences, Faculty of Life Sciences, Kyoto Sangyo University, Kita-ku, Kyoto 603-8555, Japan

ARTICLE INFO

Article history:
Received 9 September 2010
Received in revised form 25 October 2010
Accepted 9 November 2010
Available online 17 November 2010

Keywords:
CUB
CPP
Auxiliary protein
TARPs
LTP
Epilepsy
Glutamate receptor
Acetylcholine receptor

ABSTRACT

Neurotransmitter receptors play central roles in basic neurotransmission and synaptic plasticity. Recent studies have revealed that some transmembrane and extracellular proteins bind to neurotransmitter receptors, forming protein complexes that are required for proper synaptic localization or gating of core receptor molecules. Consequently, the components of these complexes contribute to long-term potentiation, a process that is critical for learning and memory. Here, we review factors that regulate neurotransmitter receptors, with a focus on proteins containing CUB (complement C1r/C1s, Uegf, Bmp1) or CCP (complement control protein) domains, which are frequently found in complement system proteins. Proteins that contain these domains are structurally distinct from TARPs (transmembrane AMPA receptor regulatory proteins), and may constitute new protein families that modulate either the localization or function of neurotransmitter receptors. In addition, other CCP domain-containing proteins participate in dendritic patterning and/or synaptic differentiation, although current evidence has not identified any direct activities on neurotransmitter receptors. Some of these proteins are involved in pathologic conditions such as epileptic seizure and mental retardation. Together, these lines of information have shown that CUB and CCP domain-containing proteins contribute to a wide variety of neuronal events that ultimately establish neural circuits.

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1. Introduction

Synaptic function largely depends on neurotransmitter receptors in postsynaptic membranes that extend to presynaptic active zones. Neurotransmitter receptors directly or indirectly affect synaptic ionic currents and either mediate or inhibit the transmission of neural signals. Therefore, both the local densities and activities of neurotransmitter receptors are critically important for synaptic functioning.

Molecular mechanisms that underlie synaptic differentiation have been most elucidated in a study of Agrin (McMahan, 1990), a large proteoglycan that mediates acetylcholine receptor clustering with receptor complexes and cytoplasmic factors at neuromuscular junctions (Wu et al., 2010). Recently, several other proteins that are secreted or inserted in the plasma membrane have been found to regulate neurotransmitter receptor clustering or functioning. For example, the TARPs family of transmembrane proteins recruits AMPA receptors at excitatory synapses via vesicle trafficking and

* Corresponding author. Tel.: +81 75 705 3120; fax: +81 75 705 1914.
E-mail address: hama@cc.kyoto-su.ac.jp (C. Hama).

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doi:10.1016/j.neures.2010.11.006

also modulates the gating of the receptors (Milstein and Nicoll, 2008; Tigaret and Choquet, 2009). In this review, we focus on proteins that contain a CUB (complement C1r/C1s, Uegf, Bmp1) or CCP (complement control protein) domain, which frequently appears in components of the complement system, and discuss how these proteins regulate neurotransmitter receptors.

In the second half of this review, we describe three CCP domain-containing proteins that are involved in synaptic differentiation, dendritic patterning, or neural circuit formation. Defects in these proteins cause a number of phenotypes, notably seizures and epilepsy. Thus, proteins with CCP or CUB domains play crucial roles in the development and function of neural circuits.

2. CUB and CCP domains

We will focus on two types of proteins that contain CUB or CCP domains, both of which are present in a wide variety of secreted or membrane-bound proteins where they are thought to mediate protein–protein interactions. The CUB domain – originally identified in the complement subunits C1r/C1s, sea urchin epidermal growth factor, and bone morphogenetic protein 1 (BMP1) – comprises approximately 110 amino-acid residues that form a β-barrel with a jellyroll fold. Most CUB domains contain four conserved cysteine residues that probably form two disulphide bridges (C1 – C2 and C3 – C4; see InterPro and SMART: Simple Modular Architecture Research Tool).

The CCP domain, also referred to as the SCR (short consensus repeat) or Sushi domain (Ichinose et al., 1990), is frequently found in mammalian regulators of complement activation. This domain contains approximately 60 amino-acid residues in a β-sandwich arrangement, and includes four cysteine residues that form two disulfide bonds (Norman et al., 1991).

3. LEV-10 and LEV-9 mediate acetylcholine receptor (AchR) clustering in *Caenorhabditis elegans*

Genetic screening for *C. elegans* mutants that exhibited weak resistance to the drug levamisole identified the *lev-10* gene (Gally et al., 2004). *lev-10* encodes a transmembrane protein with five CUB domains and one low-density lipoprotein receptor domain class A (LDLa) in the extracellular region (Fig. 1). Levamisole is a nematode-specific cholinergic agonist that causes muscle

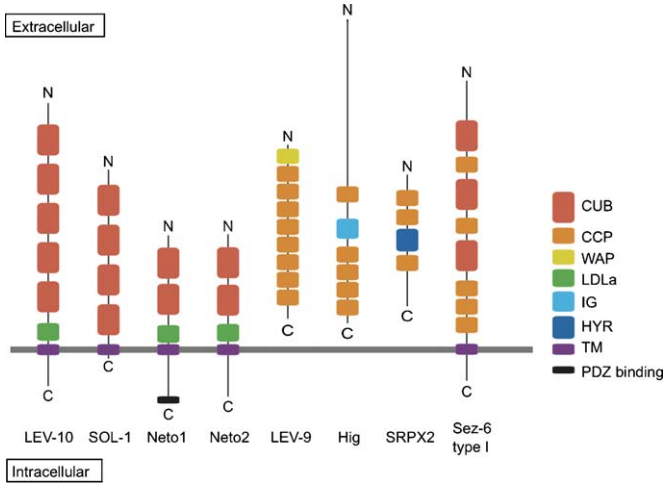

Fig. 1. Domain organization of CUB- and CCP-containing proteins described in this review. CUB, complement C1r/C1s; Uegf, Bmp1 domain; CCP, complement control protein domain; WAP, whey acidic protein domain; LDLa, low-density lipoprotein receptor domain class A; IG, immunoglobulin domain; HYR, hyaline repeat; TM, transmembrane domain.

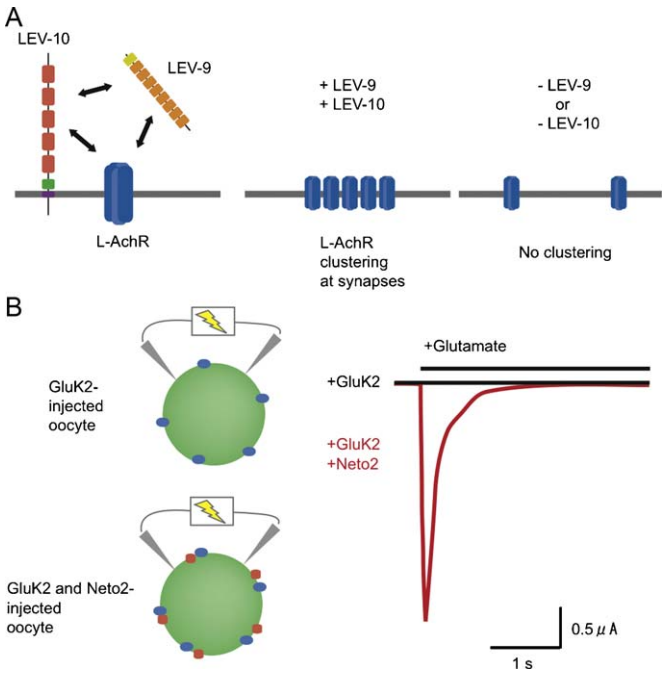

Fig. 2. CUB- and CCP-containing proteins regulate clustering or functional properties of neurotransmitter receptors. (A) LEV-10 (CUB protein) and LEV-9 (CCP protein) are required for L-AchR clustering. LEV-10, LEV-9, and L-AchR form a protein complex (left). In the absence of LEV-9 or LEV-10, L-AchR clustering was not detected at neuromuscular junction synapses (right). (B) Neto2 modulates GluK2 function. Glutamate-evoked currents were recorded using a two-electrode voltage-clamp method in GluK2-injected *Xenopus laevis* oocytes (left). Large currents were observed in oocytes coinjected with GluK2 and Neto2 cRNA, whereas currents in oocytes injected with only GluK2 were small (right).

hypercontractions and death, suggesting that the phenotype in *lev-10* mutants reflected a reduction in cholinergic transmission. Immunostaining for UNC-29 – a non-α-subunit of levamisole-sensitive acetylcholine receptors (L-AchRs) – revealed markedly reduced levels in 'en passant' synapses along cholinergic motor nerves innervating muscle cells, although total UNC-29 levels were similar to those observed in wild-type worms. This altered distribution of the receptor subunit was consistent with a decrease in motor neuron-stimulated evoked currents in muscle cells. In addition, LEV-10 did not affect the distribution of muscle $GABA_A$ receptor or evoked responses by levamisole-insensitive AchRs. Thus, LEV-10 is specifically required for the localization of levamisole-sensitive AchRs on muscle cell surfaces at neuromuscular junctions (Fig. 2A).

LEV-10 is expressed in muscle cells and concentrated at cholinergic neuromuscular junctions together with L-AchRs (Gally et al., 2004). When UNC-29 was absent, no LEV-10 was detected at neuromuscular junctions, although the total LEV-10 expression level was not altered. The interaction between these proteins is mediated by an extracellular region of LEV-10 because fusion proteins containing the extracellular domain and either GFP or a heterologous transmembrane domain allowed the receptor to accumulate at synapses. These data suggest that LEV-10 interacts directly or indirectly with L-AchR in a multiprotein complex that is recruited to or stabilized at synapses.

One of the components of this complex is encoded by the *lev-9* gene, which was identified based on a mutant phenotype that was similar to that of the *lev-10* mutant (Gendrel et al., 2009). The predicted LEV-9 protein contains 622 amino-acid residues that form a WAP (whey acidic protein) domain and eight CCP modules (Fig. 1). LEV-9 is expressed in muscle cells, secreted into extracellular spaces, and localized near L-AchR clusters and LEV-10, probably in synaptic clefts. In *lev-9* mutants, immunofluorescence