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2
3 **Cadherins: potential regulators in the faculty of language**

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25 **ABSTRACT**

26 The cadherin superfamily is a large (now more than 100 proteins) protein family
27 originally identified as cell adhesion molecules. Each cadherin shows distinct
28 expression patterns in the nervous system, and their expressions are both
29 spatially and temporally regulated and diverse among different species.
30 Mounting evidence has suggested that cadherins play multiple roles in neural
31 development and functions. Recently, using songbirds and mice, the potential
32 role of cadherins in vocal behavior has been demonstrated. Here, we will briefly
33 introduce general function of cadherins, and analyze the potential involvement of
34 cadherins in vocal behaviors and their evolution.

35

36 **Introduction**

37 Human beings are the only animals that use language. However, human studies
38 are basically limited to non-invasive ones, therefore, animal models are needed
39 to explore human language properties on the molecular level. Although animals
40 other than human beings do not use language, they share part of the faculty of
41 language [1,2]. Therefore, several animals have been successfully used as
42 models for studying human language properties. For example, songbirds are
43 good animal models to study the neural mechanisms of vocal learning [3–5].
44 Mammals such as mice or marmosets are good anatomical and behavioral
45 models to study human vocalization [6–8]. These animals are powerful tools for
46 exploring the molecular mechanisms of human vocal learning.

47
48 Recently, using songbirds and mice, the involvement of cadherins in vocal
49 behavior was demonstrated. Cadherins are transmembrane proteins originally
50 identified as cell adhesion molecules, and play multiple roles in the nervous
51 system, from neuronal wiring to synaptic plasticity [9]. Here, we briefly introduce
52 the general roles of cadherins in the vertebrate nervous system, and review the
53 information regarding the possible involvement of cadherins in vocal behaviors
54 and their evolution.

55

56 **Cadherin: a large protein superfamily**

57 Cadherins (Cdhs) are transmembrane proteins originally identified as cell
58 adhesion molecules [9–13], whose superfamily now includes more than 100
59 distinct proteins. According to their sequence similarities, family members are

further classified into subfamilies, including classical cadherins, desmosomal cadherins, protocadherins, Flamingo/CELSRs, Fat cadherins, and others. All family members have conserved repeated sequences called "cadherin repeats" (ECs) in their extracellular domains, but otherwise their structures are highly diverse. In this review, we limit discussion mainly to classical cadherins.

Classical cadherins contain five ECs in the extracellular portion that are necessary for homotypic binding [9–13]. In their cytoplasmic portion, there are binding sites for beta-catenin and p120-catenins that are necessary for intracellular signal transduction. Classical cadherins are further subdivided into 5 type-I (Cdh1-4, Cdh15) and 13 type-II (others) according to their sequence similarities in the cytoplasmic region. These cadherins function as cell adhesion molecules via homotypic or heterotypic binding. In general, homotypic binding mediates stronger interaction than heterotypic binding, although binding specificities have not fully been established and are highly complex. For example, a cell aggregation assay revealed that type-I cadherins such as Cdh1 (E-cadherin) and Cdh2 (N-cadherin) form strong homotypic bonds and weak heterotypic bonds, whereas these cadherins cannot bind with Cdh6, a type-II cadherin [14]. Some type-II cadherins, such as Cdh6, Cdh9, and Cdh10, share similar amino acid sequences in the extracellular domains to allow for heterotypic interactions amongst themselves [15], whereas Cdh8 and Cdh11 are able to bind each other. However, the former group (Cdh6, 9, 10) is not able to bind to the latter group (Cdh8, 11) because of the low sequence similarity between the two. Altogether, it can be said that classical cadherins have both

some similarity and diversity in their structure and function.

In fact, protein-protein interactions of cadherins are more complicated than previously thought. Classical cadherins form heterotypic bonds with protocadherins [16], and the interactions change the adhesive properties of the protocadherins [17]. Cadherins also bind directly to other proteins besides the cadherin superfamily. For example, Cdh2 can bind directly to α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA), GluR2, via its extracellular domain [18].

General function of cadherins in the nervous system

Cadherins are broadly expressed in the nervous system. Each cadherin shows distinct but overlapping expression [19–21], thus demonstrating both diversity and redundancy at the expression level. Some cadherins show strong expression in a number of brain areas connected with each other, suggesting their functional roles in certain neural circuit formations [19]. It has been proposed that combinatorial expressions of multiple cadherins make adhesive diversity so that each neuron is able to bind to a specific target [20]. In mature neurons, cadherin proteins are accumulated in synapses [22]. Cadherin expression is spatially and temporally regulated, a mechanism through which expression changes can control morphological events during neural development [23,24]. Though some attempts have been made to understand this mechanism [25], regulation of cadherin expression remains largely unknown and seems to be complicated. Regulation by alternatively splicing has been also

demonstrated [26]. In the case of protocadherins, it has been shown that their expression is neuronal-activity dependent and modified via epigenetic regulation [16,27].

Cadherins play multiple roles in various developmental steps, including nuclear formation, axon guidance, synapse formation, synapse function, and plasticity [16,24,28–38]. In studying cadherin functions in the synapse, the type-I classical cadherin Cdh2 and its intracellular components such as alpha-N-catenin and beta-catenin have been most extensively analyzed. Cadherins are localized to and accumulated in the synapse to make a large, complex synaptic structure with other synaptic molecules and receptors, through direct or indirect binding (Figure 1A) [12,34]. Overexpression or functional blocking studies in cultured neurons revealed that the Cdh2/catenin complex stabilizes synapse structure [30,33,34]. Cdh2 is broadly expressed in the nervous system, and its loss in constitutive knockout mice is embryonic lethal. The conditional knockout of *Cdh2* in the cerebral cortex and hippocampus disturbs the neuroepithelial structure, disrupting cortical architecture [35].

In contrast to type-I classical cadherins, type-II classical cadherins show more localized expression related to neural circuits [19], and their loss-of-function mutations cause no gross morphological abnormalities and show defects limited to some specific neural circuit formations or synapse functions. For example, *Cdh6* mutant mice showed abnormal innervation of retinal axons in *Cdh6*-positive target midbrain areas [37]. Knockdown of *Cdh9* showed pre- and

post-synaptic defects in the hippocampal dentate gyrus (DG)-CA3 connections, suggesting that the DG-CA3 connection is formed via homotypic binding of Cdh9 [38]. *Cdh11* mutant mice demonstrated enhancement of long-term potentiation (LTP) in the hippocampal CA1 region, increasing the level of LTP saturation and reducing anxiety-related behaviors, while showing no clear histological defect in the brain [28]. *Cdh8* mutant mice showed a reduced cold-sensitive response of neurons in the dorsal horn (DH) of the spinal cord, although the synaptic structure is intact between sensory neurons in the dorsal root ganglia (DRG) and DH neurons [10,36]. Thus, each cadherin works in a different manner, depending on its situation, where sometimes it controls synapse formation and other times it modulates neuronal activity.

Role of cadherins in vocal behaviors

Many studies have examined the molecular mechanisms controlling vocal learning processes (summarized in review articles [39,40]). *In situ* hybridization screening was performed in the Bengalese finch, and classical cadherins and protocadherin were identified as candidate molecules involved in avian vocal learning [41,42]. During the process of vocal learning, birds first listen to and memorize their fathers' songs as template songs (sensory learning stage). Then, they practice singing by themselves, trying to imitate their fathers' songs via trial and error (sensorimotor learning stage) [3–5]. Interestingly, cadherin expression in the robust nucleus of the archistriatum (RA) is changed from *Cdh7*-positive to *Cdh6B* (the avian homologue of *Cdh6*)-positive during the transition from sensory learning to the sensorimotor learning stage [41]. Using lentiviral

vector-mediated gene transduction, overexpression of *Cdh7* prevented vocal development in the Bengalese finch, particularly in the harmonic sounds typically seen in adult songs [43]. It has been shown that *Cdh6* enhances, and *Cdh7* suppresses, spine or branch formation and neuronal activity [44,45] via different intracellular signaling pathways [44]. Thus, it seems that the change of cadherin expression modulates neural morphology and electrophysiological properties in RA neurons, to control vocal development of the songbird via enhancement of synapse formation (Figure 1B).

The involvement of cadherins in vocal behaviors has also been shown in the rodent. Mice produce ultrasonic vocalizations (USVs) as an isolation call or a courtship song. Although USV seems to be innate, unlike songbirds' songs [46], mice can produce a complex courtship song [6]. As songbirds, *Cdh6* expression is broadly seen in vocal control areas of the mouse brain (Figure 2; [19] and our unpublished data). *Cdh6* knockout mice showed abnormal USVs, with high pitch for an isolation call and a courtship song [47]. Since other vocalizations such as aggressive calls are intact, vocalization defects in *Cdh6* mutant mice seem to be specific for USVs.

Possible cadherin function in the evolution of faculty of language

Expression patterns of cadherins have been extensively analyzed, particularly in classical cadherins and delta protocadherins in avian and mammalian species [10,12,13,19–21], and have also been shown to be diverse among species. Comparative gene expression analysis among several vocal learners

(Bengalese finch, budgerigar) and non-leaners (dove and quail) revealed that cadherin expressions are particularly diverse in the vocal system versus other systems like the visual system (highly constrained among these avian species), suggesting that diverse cadherin expressions are related to differential vocal behaviors among different species [48]. Comparative analysis between the rodent (mouse) and the non-human primate (common marmoset) showed that more differential and less redundant cadherin expressions are seen in the primate cerebral cortex [42,49], whereas overlapping and more redundant cadherin expressions are seen in the mouse cortex [21]. These data suggest there are more specialized functions of cadherins to distinguish between the rodents and primates [49]. Although the pallial architecture is different between avian species and mammalian species, and it is therefore not easy to compare cadherin expressions between these species, in both cases expression patterns of cadherins are diverse between different species, even when belonging to the same class. It appears that differential expressions of cadherins give rise to species diversity in neuronal connections and activity for example, allowing for novel functions or speciation among neural functions.

It has been recently shown that genetic variations in each cadherin gene are associated with various human psychiatric disorders such as autism spectrum disorder (*Cdh9*, *Cdh10*, *Cdh13*, *Pcdh10*), schizophrenia (*Cdh8*, *Cdh12*, *Cdh18*, *Pcdh11Y*), bipolar disorder (*Cdh7*) [12,13], and language developmental defect (*Pcdh11X/Y*) [50], although no reports have been published thus far regarding mental disease-like phenotypes in cadherin knockout mice. Taken together with

differential cadherin expressions between rodents and primates, these recent findings also support the notion that novel brain functions emerge by differential cadherin expression.

Interestingly, the cadherin superfamily contains many family members with moderate levels of redundancy and diversity. Both properties are needed for evolution and diversification. In some cases, genetic changes negatively affect the survival of the animals. Since cadherins share some redundancy with other family members, they can compensate for these genetic mutations in other cadherin genes. Thus, cadherins can easily accumulate genetic mutations that are necessary for preparing the way for evolution and diversification in the future. Eventually, the accumulation of genetic mutations might have diversified cadherin expressions at key evolutionary steps, causing different connection patterns or electrophysiological properties to emerge in neurons and consequently generating vocal learning ability (Figure 3).

It has been shown that other synaptic transmembrane proteins such as Eph-Ephrin, Semaphorin-Plexin and Neurexin-Neurologin regulate synapse formation, function and its plasticity [51–53]. Neurexin-Neurologin have been identified as responsible factors for human psychiatric disorders such as schizophrenia or autism [52] and language disability [54]. As cadherins, these families also contain redundancy and diversity at gene expression and function level. Thus, these molecules may also be good candidates to cause evolutionary changes in neural circuits subserving human language.

228

229 On the other hand, despite species differences, some similarity has been
230 demonstrated in molecular basis of the faculty of language. For example, *FoxP2*,
231 a responsible gene for human language disorder, is shown to be involved in
232 avian vocal learning [55], suggesting that some mutual molecular mechanisms
233 exist between birdsong and human language properties. Considering cadherin
234 function in vocal behaviors of a songbird and a mouse and similar expressions in
235 vocal pathways, cadherins may be also a potential component of 'deep
236 homology' in the faculty of language of animal kingdom.

237

238 **Conclusion**

239 Cadherins play multiple roles in the nervous system, from neuronal wiring to
240 regulating synaptic function. Cadherin expression patterns are highly diverse
241 among different species, even among members of the same classes, either
242 Aves or Mammalia. Functional analyses in songbirds and mice suggested the
243 involvement of cadherins in vocal behaviors. Moreover, recent evidence
244 indicates that genetic mutations in cadherin genes are associated with human
245 psychiatric disorders that include deficits in language ability. Thus, it appears
246 that cadherins are potential target genes for development of human language
247 properties, as well as the evolution of language itself.

248

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Figure legends

Figure 1. (A) Cadherins in the synapse. Classical cadherins bind directly or indirectly to neurotransmitter receptors (e.g. NMDAR or AMPAR), or to transmembrane proteins such as nectin, Pcdh, RTKs, and to various intracellular signaling molecules, to form a large synaptic complex. (B) Temporal change of cadherin expressions during the vocal learning process and model of cadherin function in vocal learning of the songbird. Combinatorial expressions of classical cadherins (Cdh6, Cdh7, Cdh9, Cdh10, Cdh12) [41] and protocadherins (e.g. Pcdh11X) [42] seem to modulate spine morphology and neuronal activity in the vocal nuclei to control vocal development. Abbreviations: AMPAR, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; Cdh, cadherin; NMDAR, N-methyl-D-aspartate receptor; Pcdh, protocadherin; PSD, postsynaptic density; RTK, receptor tyrosine kinase

Figure 2. Brief summary of *Cdh6* expressions in Bengalese finches and mice. Mutual cadherin expressions are seen in neural pathways underlining vocalization of songbirds and mice. *Pcdh11X* also shows similar expressions in the vocal system between the two [42]. Am, Nucleus ambiguus; CPu, Caudate-Putamen; DLM, nucleus dorsolateralis anterior, pars medialis; DM, dorsal medial nucleus of the midbrain; GP, Globus pallidus; M1, primary motor area; LMAN, Lateral magnocellular nucleus of anterior nidopallium; nXIIIts, tracheosyringeal part of the hypoglossal nucleus; VL/VA, ventral lateral / ventral anterior nuclei of the thalamus; PAG, periaqueductal gray; Ram, nucleus retroambiguus; RF, reticular formation.

475

476 Figure 3. Hypothetical scenario of the emergence of novel brain function by
477 cadherin gene expressions. Divergent cadherin expressions might have induced
478 novel functional circuits to generate new cognitive faculties.

479

Figure 1

A

B

Figure 2

Songbird

Mouse

● Strong expression ● Weak or partial expression ○ No clear expression

Figure 3