

# Supplementary Material

corresponding to:

## **Expression of protocadherin 18 in the CNS and pharyngeal arches of zebrafish embryos**

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## PODCAST TEXT

Hello researchers,

I am Fumitaka Kubota, a graduate student at Gunma University Graduate School of Medicine in Maebashi, Japan.

I am going to make a brief introduction to our paper entitled "Expression of protocadherin 18 in the CNS and pharyngeal arches of zebrafish embryos" which appeared in "International Journal of Developmental Biology", 2008.

Differential adhesion is a major mechanism of morphogenesis in embryonic development. Cadherins are thought to provide a molecular basis for such cell adhesions. We are interested in protocadherins, a subset of cadherins, as a machinery of delicate morphogenesis as those occur in the CNS development.

Many of protocadherin genes form several clusters on the genome, analogous to gamma globulin clusters. Recent findings, though, indicate allelic and combinational gene regulations for the clustered protocadherins. That makes non-clustered protocadherins attractive as a diversely controlled adhesion machinery for CNS development. In the course of our research of non-clustered protocadherins, we cloned a zebrafish protocadherin 18, and made its expression analyses in embryonic development.

Zebrafish protocadherin 18 protein has a typical structure of protocadherins, including a signal sequence, 6 cadherin repeats, a single transmembrane domain, and a cytoplasmic domain. It shows 65 or 66% identity, and 78 or 79% homology with known counterparts of other vertebrates.

It has "Disabled-1" binding motif in its cytoplasmic domain, which is characteristic of known counterparts. It also has a "CM-2" motif, which is shared by other non-clustered protocadherins such as protocadherin 8, 10, and 19.

Basically, protocadherin 18 was expressed in the rostral regions and the CNS in the zebrafish embryos.

The expression started by the early gastrula stage, 6 hours post-fertilization, in their animal cap but not in the germ ring or the shield. Protocadherin 18 was expressed in the neural tube and the CNS from 12 hours. Some populations of cells in the lateral neural tube and spinal cord of 12 through 18-hour embryos expressed protocadherin 18, but expression in these cells disappeared by 24 hours.

The expression pattern in the hindbrain was particularly interesting. The hindbrain of embryos at 24 through 56 hours expressed protocadherin 18 in cells closely adjacent to the rostral and caudal rhombomeric boundaries in a thread-like pattern running in the dorsoventral direction. The protocadherin 18-positive cells were localized in the ventral part of the hindbrain at 24 hours and in the dorsal part from 36 hours.

Some signaling molecules and transcription factors, including deltaA, rasgef1b, and beta3.1, have been documented to show similar expression patterns in the hindbrain. We enjoy imagining that protocadherin 18 is involved in the rhombomeric boundary formation driven by Delta-Notch signaling.

Protocadherin 18 was also expressed in the telencephalon, diencephalon, tectum, upper rhombic lip, retina, and otic vesicle. Expression in the CNS decreased markedly before hatching. Pharyngeal arches, jaws, and gills expressed protocadherin 18, and the molecule was also expressed in some endodermal cells in late embryos.

We are currently investigating functional significances of protocadherin 18 in the CNS and pharyngeal arch development.

Thank you for listening. This podcast was produced for the International Journal of Developmental Biology by Fumitaka Kubota, Tohru Murakami, Yuki Tajika, and Hiroshi Yorifuji at Gunma University Graduate School of Medicine, Maebashi, Japan, January 2008.