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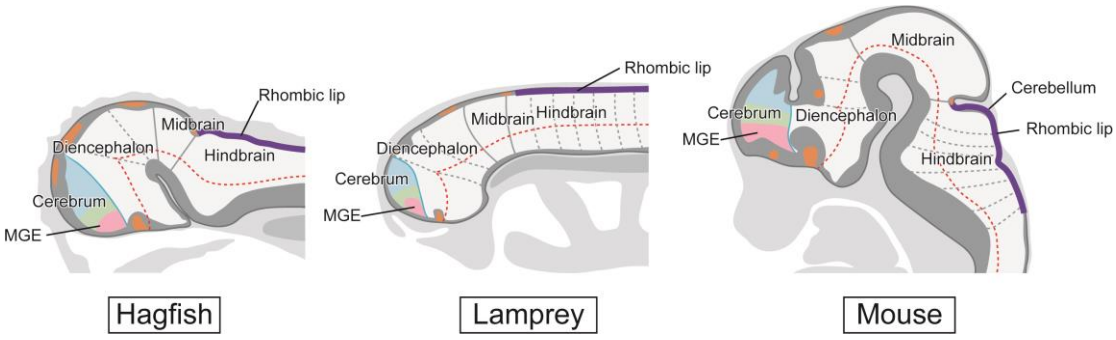
Origins of complex vertebrate brain older than previously thought

March 10, 2016– Cyclostomes are jawless vertebrate fish that lack paired fins, and have only one nostril. They are regarded as being more primitive forms of vertebrates, and there are presently only two surviving groups of cyclostomes—hagfish and lampreys. The latest common ancestor they shared with gnathostomes, the jawed vertebrate species including humans, was thought to be 500 million years ago. The acquisition of a highly regionalized complex brain is considered the hallmark of vertebrates, and the brain of cyclostomes have been characterized as being ancestral forms of the vertebrate brain due its more simple regionalization. The actual evolutionary origins of the vertebrate, however, remain unclear.

Now RIKEN visiting scientist Fumiaki Sugahara and research scientist Juan Pascual-Anaya of the Evolutionary Morphology Laboratory (Shigeru Kuratani, Chief Scientist) and others have carried out detailed analyses of the embryonic brain of cyclostomes and discovered the existence of two key brain domains—the medial ganglionic eminence (MGE) and the rhombic lip—common across all gnathostomes but previously thought to be absent in cyclostomes. Their discovery provides strong evidence that the fundamental plan for vertebrate brain development was already established before the divergence of cyclostomes and gnathostomes over 500 million years ago. This work, published in *Nature*, was carried out when the laboratory was part of the CDB. Sugahara has since moved to and continues his research at the Hyogo College of Medicine, Japan.



Embryonic brain regionalization in cyclostomes (hagfish, lamprey) and gnathostomes (mouse)

The blueprint for brain development is remarkably well conserved across gnathostome species. In the embryonic brain, the MGE emerges from the ventral region of the subpallium (future cerebrum), from which GABAergic interneurons migrate to the cerebral cortex. The rhombic lip, which gives rise to the cerebellum important for motor control, appears along the dorsal hindbrain. The apparent absence of the MGE and rhombic lip in developing lamprey brains suggested that these domains emerged in gnathostomes after divergence of cyclostomes and gnathostomes. However, GABAergic interneurons, presumed to be of MGE origin, have been found in adult lampreys, which alludes to the existence of an MGE region in embryonic lamprey brains, conflicting with conventional evolutionary models.

Sugahara and his collaborators began by making a detailed examination of brain development in hagfish, the only other extant cyclostome groups. They made serial sections of the embryonic head region at several different developmental stages, and used immunostaining and in situ hybridization methods to stain for specific gene-expressing regions and tissues, and then reconstructed the brain in 3D using computer software. These results were then compared to those obtained from brains of lamprey and cloudy cat sharks, an example of a gnathostome offshoot to cyclostomes, at similar developmental stages. Hagfish had many common developmental brain features such as the positioning of key structures and nerve fibers as lamprey and shark. However, unlike in lamprey, the team identified expression patterns of MGE marker genes, *Nkx2.1* and *Hedgehog*, in the subpallium region, and of rhombic lip marker genes, *Pax6* and *Atoh1*, along the dorsal hindbrain region, confirming that hagfish form an MGE and rhombic lip domain during brain development.

Then how can the absence of the MGE and rhombic lip in lamprey, which exist in the hagfish and gnathostomes, be explained? One possibility is that common brain features between cyclostomes and

gnathostomes were lost secondarily in lamprey, and another is that the gnathostomes and hagfish evolved similar features independently through convergent evolution. The team revisited the embryonic lamprey brain armed with newly published genomic data for lamprey, and uncovered additional orthologues of MGE and rhombic lip marker genes that were previously undetected. They confirmed that orthologous genes for *Nkx2.1*, *Pax6*, and *Atoh1* in the lamprey were expressed in patterns comparable to those seen in hagfish and gnathostomes, indicating that an MGE and rhombic lip-like domain exist in the embryonic lamprey brain.

“The brain architecture of cyclostomes were presumed to be less complex than those of gnathostomes because two key brain domains common across all vertebrate brains could not be found. But our current study shows that these brain domains are indeed present in cyclostomes, indicating that the fundamental vertebrate brain plan was established well over 500 million years ago,” says Kuratani. “Most gnathostomes subsequently developed more elaborate brain regions leading to the forms seen today, so our next step is to understand how each brain region was acquired and evolved. Also of interest is the origins and evolution of cerebral neocortex and cerebellum.”