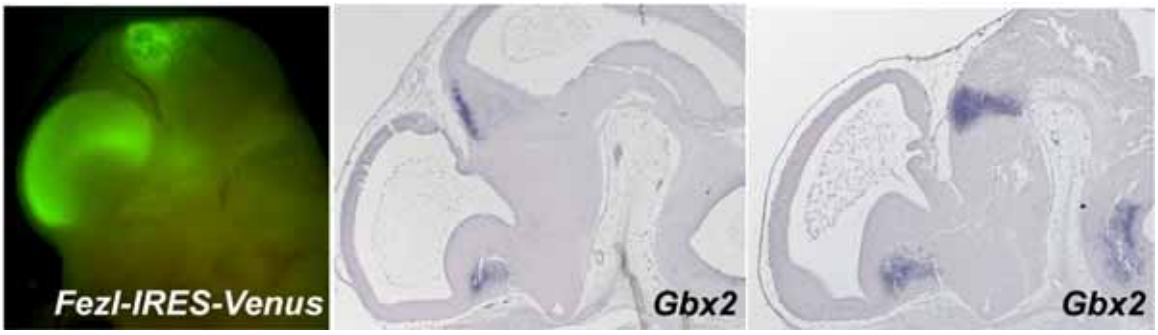
RIKEN Center for Developmental Biology (CDB)
2-2-3 Minatojima minamimachi, Chuo-ku, Kobe 650-0047, Japan

Fez and _Fezl_ share the burden in rostral brain regionalization

October 4, 2006 – The mammalian brain emerges progressively during development, unfolding as a linear series of sections from spinal cord to forebrain. The embryonic forebrain can be subdivided into rostral and the caudal regions, and the caudal forebrain (or diencephalon) itself comprises three main subdivisions: prethalamus, thalamus and pretectum. Similar to other forms of organogenesis, brain development relies on tightly regulated and highly specific patterns of gene expression to ensure that each part integrates seamlessly with the whole. A number of genetic markers have been identified for each of the specified caudal forebrain regions, but the means by which the boundaries between these subdivisions are established earlier in development has remained elusive.

Now, in a study published in the journal *Development*, Tsutomu Hirata and Masato Nakazawa and colleagues in the Laboratory for Vertebrate Axis Formation (Masahiko Hibi; Team Leader) identify a pair of genes that work together to set up the initial subdivisions of the mouse diencephalon. Hirata and Nakazawa, working with researchers from a number of other CDB labs, describe how the zinc-finger genes *Fez* and *Fezl* cooperate to delineate and maintain forebrain patterning by repressing caudal specification in the rostral forebrain.



Misexpression of *Fezl* in the caudal diencephalon (marked by Venus expression, left panel) represses the formation of thalamus (middle panel), which normally expresses *Gbx*2 (right panel).

In the vertebrate diencephalon, the border region between thalamus and prethalamus is known as the zona limitans intrathalamica (ZLI). It has been suggested that the ZLI lies at the intersection of mutually inhibiting rostral-caudal position determinant genes, but direct genetic evidence for this model has been scant. Hirata and Nakazawa noticed that although the transcriptional repressors *Fez* and *Fezl* had been shown to have striking loss-of-function phenotypes in a number of aspects of neurodevelopment, but minimal effect on the forebrain when disrupted individually, which prompted them to create mice deficient for both genes as a means of identifying possible complementary function.

The strategy paid off, as *Fez*/*Fezl* double homozygous mice showed severe caudal forebrain defects, including the loss of the prethalamus and ZLI, and a significantly reduced thalamus (the pretectum, however, was intact). The Hibi team's analyses of genes normally expressed in diencephalic regions showed that many genes directly or indirectly involved in the regionalization patterning of the forebrain were affected by the double homozygous mutation, suggesting that *Fez* and *Fezl* work together to regulate rostro-caudal patterning.

Contact: Douglas Sipp sipp@cdb.riken.jp TEL: +81-78-306-3043
RIKEN CDB, Office for Science Communications and International Affairs

Interestingly, in contrast to the deleterious effects on prethalamus and ZLI, the double homozygous phenotype showed a rostral expansion of the caudal regions of the diencephalon. Where the expression of genes normally associated with the more rostral prethalamus was lost, that of typically caudal markers was found to have shifted to the anterior, even from the earliest stages of neural patterning. For example, the expression of *Irx1*, a gene whose area of expression is ordinarily delimited at its rostral edge by the ZLI, was found to be shifted rostrally in the double mutant, bringing up the possibility that *Fez* and *Fezl* exert a repressive effect that normally prevents *Irx1* (and other caudal diencephalic genes) from encroaching into the prethalamus. Tests using misexpressed *Fez* and *Fezl* tended to support the interpretation that these two genes in combination work to repress caudal diencephalon fate.

Although morphologically abnormal, the diencephalon is not significantly reduced in size in the *Fez*/*Fezl* mutants, which led Hirata and Nakazawa to propose that these two genes function early in neural patterning to repress caudal gene expression in the rostral diencephalon, while at the same time promoting the establishment of the prethalamus. The reductive effects on the thalamus (which initially expands in these mutants) appear to be secondary to the loss of the ZLI, as this region serves as an inductive center for the thalamus, which it borders rostrally. It remains to be seen whether the repressive effects of *Fez* and *Fezl* on caudal fate determinants are direct or indirect, and how these genes themselves are regulated. Similarities to the genetic regulation of rostro-caudal regionalizaton of the avian brain, which has been extensively studied in chicken, may provide a basis for further study and, ultimately, a better understanding of how this complex pattern is laid down early in the development of the mammalian brain.

“Until now, the establishment of rostral-caudal polarity in the diencephalon has been something a black box, as we knew essentially nothing about the molecular mechanisms behind this process,” says Hibi. “I think that this study of *Fez* and *Fezl* provides the first genetic evidence for factors that regulate that patterning, and it’s going to be interesting to see what these two transcriptional repressors are targeting, which will certainly deepen our understanding of forebrain development.”

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