

Headshrinker's secrets revealed: A role for Ssdp1 in head development

June 1, 2005 – Early vertebrate embryogenesis is guided by the activity of signaling centers, patches of tissue capable of inducing the differentiation of surrounding regions and establishing many of the body plan's most basic features, such as the anterior-posterior and dorsal-ventral axes, the neural tube and the digestive tract. One such signaling center, known as the head organizer, plays a fundamental role in the ontogeny of the head by instructing the anteriorization of and formation of head structures from the neural tube. A number of genes have already been identified as involved in the formation of the head organizer, but their specific functions and the interactions and spatiotemporal relationships that characterize these genes' expression patterns have yet to be worked out in their entirety.

Now, in a research article published in the June issue of the journal *Development*, members of the Laboratory for Embryonic Induction (Hiroshi Sasaki; Team Leader) report new insights into the function of the transcriptional co-activator protein Ssdp1 in the later stages of head organizer development. The study, led by Noriyuki Nishioka, a research scientist in the Sasaki lab, in collaboration with labs in the Universities of Osaka, Hokkaido, Minnesota and Texas and the NIH casts the genetic regulation of head induction in a fresh light by revealing one more component of the complex interplay between transcription factors needed to establish the structures of the head at the appropriate sites and developmental stages.



Failure of head development in mouse carrying the *hsk* mutation of the *Ssdp1* gene (right)

The story began with a single mouse mutant, in which the embryo failed to develop any head structures whatsoever anterior to the ear, a hallmark of disturbed head organizer function. The mutation was discovered fortuitously during a transgenesis experiment involving an entirely different gene, which led the Sasaki team to surmise that they'd serendipitously interfered with a gene involved in head organizer formation or its activity and, dubbing this striking phenotype *headshrinker* (*hsk*), they set off working at once to identify the gene in question. The results of fluorescence in situ hybridization (FISH), a method for tagging and mapping genetic genes to their chromosomal locations, showed that their target was a gene on chromosome 4 named *Ssdp1* (for single-stranded DNA binding protein). The *headshrinker* phenotype results when Ssdp1 expression is downregulated and they found that they were able to rescue this phenotype by creating mice transgenic for Ssdp1, in which head organizer was apparently restored as pups were born with heads intact.

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

Nishioka et al next looked at head organizer development in the *hsk* mutants in closer detail and found that although early head organizer tissues such as the anterior visceral endoderm and anterior definitive endoderm formed appropriately, at day E7.5 the prechordal plate failed to take shape. When they next studied the effects of this prechordal plate defect on the neural ectoderm, they observed that all ectoderm-derived head structures anterior to the midbrain-hindbrain boundary were absent, suggesting that the prechordal plate works to suppress posteriorization of the neural ectoderm, a function important to the induction and maintenance of normal head development.

Previous studies had shown that another transcription factor, *Lim1*, is also essential for head organizer function, as indicated by the headless phenotype of *Lim1* mutant mice. The *Lim1* protein is a member of the LIM homeodomain family of transcription factors, and relies on its co-factor, *Ldb1*, which, interestingly, is also known to interact with *Ssdp1*. Intrigued by this connection, Nishioka hypothesized that *Ssdp1* forms a ternary complex with *Lim1-Ldb1*, enhancing its transcriptional activator function. The team showed that all three of these factors are expressed in prechordal plate mesenchyme, and that simultaneous co-expression of *Ssdp1*, *Lim1* and *Ldb1* in vitro caused the marked upregulation of a prechordal plate-specific reporter gene. Indeed, even *Ssdp1* alone caused dose-dependent transcriptional activation when expressed in the presence of *Ldb1*.

This series of experiments strongly points to a model in which a tripartite *Ssdp1-Lim1-Ldb1* complex regulates head morphogenesis by driving the expression of genes in the late-stage head organizer, the prechordal plate. But the story doesn't end there as, in addition to its headlessness, the *hsk* mutant also exhibits a range of general defects in cell survival and proliferation and thoracic development, leaving questions of *Ssdp1* function in those phenomena open to further study.