

Tead Start: Activation of the *Foxa2* enhancer in the node

October 26, 2005 –The great diversity of vertebrate forms is the result of developmental processes that shape the body as it grows, an intricately orchestrated activity that relies on signals from centers within the embryo capable of directing tissue movement and differentiation. Perhaps the best known of these signaling centers is the “organizer,” first identified by Hans Spemann and Hilde Mangold in the newt as a region capable of directing neighboring tissues to adopt specific fates. Studies in other organisms including frog, chick and mouse have shown that organizing centers in these species all share certain properties and characteristics as they guide the embryo through gastrulation, in which it sorts itself into three germ layers. Perhaps even more interestingly, many organizers themselves comprise a diverse and dynamic assembly of cells, marked by specific patterns of gene expression and playing a continuously changing series of roles as the embryo matures.



Node-specific expression of *Foxa2* is achieved by a core-element sequence of the enhancer.

In the mouse, the organizer forms at the anterior end of a structure known as the primitive streak about six and a half days after conception. Throughout embryogenesis, the organizer plays home to a number of cell populations, first coordinating the emergence of the head before assuming a new identity as the “node,” which regulates trunk development beginning at about embryonic day 7. The node remains a morphologically distinct region thereafter, and contains cells that serve as the progenitors of the notochord, a rod-like stretch of mesoderm that underlies the nascent central nervous system along the midline of the body and itself serves as a signaling center regulating the dorsoventral patterning of the trunk. It is characterized by the expression of a number of node-specific molecules, one of the most significant of which is *Foxa2*, a transcription factor known to regulate the development of other midline signaling centers as well. Given its prevalence and importance in organizing the embryonic midline in all taxa studied, the question of the regulation of *Foxa2* expression is one of widespread interest to developmental biologists. Now, in an article published in the November issue of *Development*, the Laboratory for Embryonic Induction (Team Leader, Hiroshi Sasaki) and colleagues in the CDB and Osaka University report an advance in the understanding of how *Foxa2* gene expression is driven in the node.

Building on previous knowledge of a *Foxa2* enhancer specific to the node and notochord, the team made transgenic mice in which a core-element sequence containing a motif critical to the enhancer's activity was used to drive the expression of β -galactosidase, enabling them indirectly to track *Foxa2* activation over space and time. Equipped with a monitor of enhancer activity, they studied patterns of expression in the embryo and noticed an intriguing similarity to the footprint of Wnt/ β -catenin, a molecular pathway involved in many aspects of cell signaling and differentiation. Co-transfection of canonical Wnt factors with the core element (CE) into embryonic carcinoma cells revealed that Wnt does act upstream of the *Foxa2* enhancer, albeit in an indirect manner.

Looking at the CE more closely, Sasaki et al found that its function relies on a pair of three-nucleotide regions in its 27-base sequence, suggesting that the cooperation of two transcription factors is essential for the enhancer's ability to activate *Foxa2* expression in the node. Now curious, they used the CE sequence as bait in a yeast one-hybrid screening against a mouse cDNA library, intended to isolate factors that interact with the core element. A pair of members of the TEAD family of transcription factors turned up in many of the positive clones. *Tead* genes and their cofactor Yap65 are expressed widely in the embryo during the E6.5 to E8.5 stages, including in regions of *Foxa2* activity, marking them as promising candidates for further study, which was borne out by transfection of members of the *Tead* family and the Yap65 cofactor into carcinoma cells. All four *Tead* factors appear to be involved in the regulation of the *Foxa2* enhancer core element, although at varying intensities, with *Tead1* and *Tead4* showing the strongest activity. Importantly, this *Tead* effect was found to be independent of the Wnt/ β -catenin pathway, whose precise molecular nature remains unknown but is thought to be linked to the CE 5' region.

Looking next *in vivo*, they electroporated a repressor form of *Tead* into transgenic mouse embryos and found that interference with *Tead* function disturbed both *Foxa2* expression and the normal development of the notochord. Parallel studies in zebrafish showed that *Tead* proteins regulate *foxa2* in the embryonic shield, an organizer with similar function to the mouse node, in fish as well.

The pervasive expression of *Foxa2* and *Tead* in multiple embryonic regions and stages suggests that both play fundamental and varied roles in development, but their convergence at the relatively narrow spatiotemporal window of the node/notochord indicates that their functional interaction there is highly context-specific. The evidence from the Sasaki study provides a compelling case for an induction of the *Foxa2* enhancer by *Tead* in cooperation with a transcription factor downstream of Wnt that is localized exclusively to the node, which may serve to explain the unique activity of this particular incarnation of the organizer. The identity of *Tead*'s co-agent remains to be found, but these latest steps forward by the Sasaki team have brought the picture of vertebrate embryonic induction that much clearer into focus.