duplication after the radiation of the protochordate lineages. In developmental terms, the data presented here suggest that developmental gene loss was highly significant as well.

doi:10.1016/j.mod.2009.06.443

11-P007

Mechanisms of left–right asymmetric signal generation around the node

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Despite recent progress in understanding of how left–right (L–R) asymmetry is generated during vertebrate development, many important questions still unanswered. One such question concerns the mechanism by which the signal responsible for the generation of L–R asymmetry is transferred from the node to the lateral plate. This signal, whose identity remains unknown, is generated in the node, and its arrival in the left lateral plate induces the asymmetric expression of Nodal. Although it is known that L–R asymmetry-breaking event in the mouse embryo is the leftward fluid flow (nodal flow) on the node, it is unclear what kinds of molecules and signals become asymmetric around the node due to nodal flow.

We found that the transcriptional regulatory element, Asymmetric Node Enhancer (ANE), which is located within the 7.5-kb upstream region of human LEFTY-1, could direct left side specific enhancement of reporter gene expression in the node at 1-somite stage, shortly after the beginning of nodal flow. To identify the molecules and signals responsible for the asymmetric activity of ANE, we explored ANE activities in several types of L–R mutant embryos, in which asymmetric Nodal expression in LPM is abnormal. There was a good correlation between the laterality of ANE activity and that of Nodal expression in the LPM. Our results suggest that ANE responds to the L–R asymmetric signals responsible for L–R axis determination. In addition, we want to show how these players interact with each other leading to generate L–R asymmetric signal in the node.

doi:10.1016/j.mod.2009.06.444

11-P008

Identification of genes with asymmetric expression in the zebrafish brain

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Asymmetry is a conserved and fundamental feature of the brain, however, little is known of the genetic mechanisms that establish asymmetry during development. Here we used zebrafish to identify novel genes involved in asymmetric brain morphogenesis using a reverse genetic approach. We isolated differential expressed sequences enriched in either left (L) or right (R) adult brain tissue using subtractive hybridisation technology. These sequences were employed as probes to hybridise commercial zebrafish genetic libraries from which 132 differential clones were identified and amplified by PCR to construct a differential and non-redundant custom microarray. Of these clones, 41% corresponded to genes of well-known function and 15% were unknown. Asymmetric expression is currently being tested by in situ hybridisation in embryos and adult brain tissue. We have already found clone P5A3 as a novel gene with asymmetric expression in the epithalamic habenular region from larval to adult stages. P5A3 loss and gain of function analyses indicates a role in asymmetric habenular morphogenesis.

doi:10.1016/j.mod.2009.06.445

11-P009

The roles of Tbx5 and Tbx4 in limb bud initiation and symmetry

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Tbx5 and Tbx4 are two closely related transcription factors that are expressed in the forelimb and hindlimb, respectively. Both genes are thought to play equivalent roles in the initiation of the forelimb bud and the hindlimb bud. Deletion of Tbx5 from the presumptive forelimb area leads to a failure of the limb bud to form. However, following deletion of Tbx4 in the presumptive hindlimb area, a hindlimb bud still forms, although it fails to grow. I am studying this phenotype in order to elucidate what factors may be compensating for the absence of Tbx4, and am consequently exploring this. Haploinsufficiency of TBX5 in humans is associated with a congenital disorder known as Holt Oram Syndrome. Clinical characteristics of HOS include heart defects and upper limb abnormalities. A striking feature of the limb defects is left sided asymmetry. Using mouse models in which we have disrupted activity of Tbx5, we have recapitulated the left sided bias of limb asymmetry. We are currently using this mouse strain and others to uncover the origins of asymmetry seen in this limb phenotype.

doi:10.1016/j.mod.2009.06.446

11-P010

Zebrafish and medaka: Model organisms for a comparative developmental approach of brain asymmetry

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