論文の内容の要旨

論文題目：Evolutionary developmental study of transcriptional regulatory networks in the gastrula organizer

（原腸胚オーガナイザーにおける転写制御ネットワークの進化発生学的研究）

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In the field of Evolutionary Developmental Biology, it remains unsolved as to how the gastrula organizer evolved from its ancient prototype in eumetazoans. To address this issue, I investigated gene regulatory mechanisms in the organizer of various organisms by focusing on organizer-specific transcription factors.

The LIM homeobox gene *lim1* (*lhx1*) is specifically expressed in the vertebrate gastrula organizer, and Lim1 acts as a transcriptional activator to exert ‘organizer’ activity in *Xenopus* embryos. Its ancient paralog, *lim3* (*lhx3*), is expressed around the blastopore in amphioxus and ascidian but not vertebrate gastrulae. These two genes are thus implicated in organizer evolution. In Chapter I, I addressed the evolutionary origins of their blastoporal expression and organizer activity. Gene expression

analysis of organisms ranging from cnidarians to chordates suggests that blastoporal expression has its evolutionary root in or before the ancestral eumetazoan for *lim1*, but possibly in the ancestral chordate for *lim3*. Analysis of organizer activity of Lim1 and Lim3 from various organisms using secondary axis forming assays in *Xenopus* embryos suggests that activation of Lim1 by its co-factors, Ldb and Ssbp, is evolutionarily conserved in eumetazoans, and that Lim1 acquired organizer activity in the bilaterian lineage, whereas Lim3 acquired organizer activity in the deuterostome lineage. In addition, ascidian Lim3 acquired a specific transactivation domain to confer organizer activity on this molecule. Gain and loss of function analyses using cnidarian embryos suggest that Lim1 has organizer activity in cnidarian embryos by regulating expression of blastoporal genes such as *chordin* and *foxa*, depicting the evolutionary origin of the organizer.

In vertebrates, homeodomain proteins Otx2 and Lim1 are required for head formation, but the regulatory principles underlying their functions in the head organizer remain unsolved. In Chapter II, I show using ChIP-seq analysis that Otx2, Lim1, the coactivator p300 and the corepressor TLE/Groucho colocalize on cis-regulatory modules (CRMs) of thousands of genes including almost all ‘head-organizer’ genes in the *Xenopus tropicalis* gastrula. Comprehensive analysis of CRMs with RNA-seq data

revealed that Lim1/Otx2-bound CRMs co-localizing with TLE rather than p300 are strongly associated with region/tissue-specific genes. Together with reporter analyses, my data suggest that Otx2 activates head-organizer genes with Lim1 and represses non-head-organizer genes with transcriptional repressors such as the head-organizer specific homeodomain protein Goosecoid (Gsc). Furthermore, ChIP-seq analysis for Gsc and histone modifications supported this regulatory model. Thus, it is likely that each of thousands of genes interprets Otx2 as a 'positional tag' to determine its expression in the head organizer, corroborating the idea that positional information directly contributes "massively parallel (distributive) gene regulation."

In cephalochordates (amphioxus), the notochord runs through the dorsal side and reaches the anterior tip of the body. It is reported that *gsc* is not expressed in the head but expressed in the notochord. This fact suggests that the change in the expression pattern of *gsc* led to the acquisition of the vertebrate-type head. However, the molecular mechanism of the head organizer evolution involving *gsc* has never been elucidated. In Chapter III, I show the conservation and diversification of gene regulation through CRMs for *gsc*, *lim1* and *chordin* between amphioxus and vertebrates. The data suggest that the regulation of *lim1* by Nodal signal and that of *chordin* by Lim1 are evolutionarily conserved in chordates but the regulation of *gsc* by Lim1 and

Otx are not conserved in amphioxus. Furthermore, the 5' region of amphioxus *gsc* recapitulated amphioxus-like posterior expression of the reporter gene in transgenic reporter analyses with the *Xenopus* embryo. Therefore, I propose a model, in which evolution of CRMs for *gsc* occurred in the vertebrate lineage, resulting in inhibition of trunk genes and the formation of the head organizer and its derivative prechordal plate.

Thus, my study sheds light on the molecular mechanism of evolution of the gastrula organizer in eumetazoans.