ホヤ初期胚における遺伝子発現の時間的な調節の解析

京都大学大学院理学研究科　生物科学専攻　動物学教室
池田　達郎

動物の発生において遺伝子を発現する時間と場所は、ゲノム上に記載されている遺伝子調節ネットワークにより調節されている。遺伝子発現の空間的な調節の理解が進んできた一方で、遺伝子をいつ発現するかという時間的な調節はあまり理解が進んでいない。本研究では尾索動物カタユウレイボヤを用いて、胚発生における遺伝子発現の時間的な調節について、２つのアプローチで研究した。

第一部の研究では、２つの転写因子 Foxa.a および Zic-r.b に注目して、時間的な調節機構を研究した。この２つの転写因子はホヤ胚において、脊索と脳という異なる組織の運命決定に共通して必要である。脊索の系譜では、*Foxa.a* と *Zic-r.b* は同じ細胞で同時に発現し、*Brachyury* 遺伝子の発現を活性化して脊索の運命を決定する。一方脳の系譜では、*Foxa.a* と *Zic-r.b* の発現は時間的に重複せず、まず *Foxa.a* が発現し、その発現の終了後に *Zic-r.b* が発現する。*Zic-r.b* の発現は初期原腸胚期に予定脳/付着突起細胞が分裂して、予定脳細胞と予定付着突起細胞に分離した直後から、予定脳細胞のみで発現する。この発現は Fgf シグナリング依存的であるが、その Fgf シグナリングは分裂前の予定脳/付着突起細胞で 32 細胞期から活性化している。なぜ *Zic-r.b* は初期原腸胚期まで発現を開始しないのだろうか。

この疑問を起点に申請者は、脳の系譜における *Foxa.a* と *Zic-r.b* の発現が、3 つの転写抑制因子 Prdm1-r.a、Prdm1-r.b および Hes.a により時間的に調節されていることを明らかにした。この 3 つの転写抑制因子は *Zic-r.b* が分裂前の予定脳/付着突起細胞で発現することを抑制していた。この発現開始の遅延が、*Zic-r.b* の発現を予定脳細胞に限局し、予定付着突起細胞で *Zic-r.b* が発現しないようにするために必要であった。加えて、*Prdm1-r.a* は *Foxa.a* の発現を抑制し、32 細胞期で速やかに発現を終了させていた。このようなメカニズムのために、*Prdm1-r.a*、*Prdm1-r.b* および *Hes.a* の同時機能阻害胚では予定脳細胞で *Foxa.a* と *Zic-r.b* の発現が時間的に重複し、その結果異所的に *Brachyury* 遺伝子が発現して脊索のプログラムが活性化されてしまうことがわかった。このように、*Prdm1-r.a*、*Prdm1-r.b* および *Hes.a* による *Foxa.a* および *Zic-r.b* の時間的な調節は、予定脳細胞で脊索のプログラムが異所的に活性化しないようにするため、また付着突起細胞が分化するために必要である。

第二部では、申請者は包括的なアプローチによって、胚発生における転写の時間的調節の問題に取り組んだ。ホヤの胚性の遺伝子の転写は 8 細胞期ごろから開始することが示唆されていたので、その時期を含むホヤ初期胚における胚性の転写の時間的な動態を RNA ポリメラーゼ II（Pol II）に対するクロマチン免疫沈降-ディープシーケンシング法により調べた。その結果、4 細胞期以前には遺伝子は転写されず、8 細胞期および 16 細胞期から 239 の遺伝子が転写開始されることを示した。またこれらの最初期に転写される遺伝子の 71%は、転写開始前の発生段階から転写開始領域に Pol II が結合していた。このような動態の観察結果は脊索動物では初であり、類似の研究がおこなわれているハエの場合とは大きく異なっていた。本研究の成果は、胚性の転写の開始期において、異なる動物間で、転写のグローバルな時間的調節の機構に違いがあることを示唆している。

Analysis of temporal regulation of gene expression in early ascidian embryos.

Tatsuro Ikeda

Department of Zoology, Graduate School of Science, Kyoto University

Background

In animal embryos, gene regulatory networks, which are encoded in genomes, spatially and temporally control gene expression. Although spatial controls of gene expression have been extensively studied and well understood, temporal controls of gene expression has not been understood sufficiently. In the present study, I explored how gene expression is temporally controlled in early embryos of the ascidian *Ciona intestinalis* with two different approaches.

In Chapter 1, I focused on two regulatory genes, *Foxa.a* and *Zic-r.b*. In *Ciona* embryos, *Foxa.a* and *Zic-r.b* are required for specification of the notochord and the brain, which are derived from distinct cell lineages. In the notochord lineage, *Foxa.a* and *Zic-r.b* are expressed simultaneously and activate *Brachyury* expression, which is essential for notochord fate specification. In the brain lineage, *Foxa.a* and *Zic-r.b* are expressed with no temporal overlaps; *Foxa.a* expression begins at the 8-cell stage and is terminated at the 32-cell stage, and *Zic-r.b* expression begins at the early gastrula stage. This *Zic-r.b* expression begins immediately after bi-potential progenitors of the brain and palps divide into brain progenitors and palp progenitors at the early gastrula stage. Although this expression depends on Fgf signaling, Fgf signaling is activated in the bi-potential brain/palp progenitors continuously from the 32-cell stage. It was not uncovered how *Zic-r.b* expression is not activated at the 32-cell stage and starts at the early gastrula stage.

In Chapter 2, I examined temporal dynamics of RNA polymerase II in early embryos in a genome-wide scale. Zygotic gene activation (ZGA) in most animal embryos occurs in several hours after fertilization. In *Ciona* embryos, it has been considered that the ZGA occurs between the 8- and 16-cell stages, because zygotic transcription of 34 genes begins at the 8- and 16-cell stages and no genes have been reported to be expressed before the 8-cell stage. However, there is no global view on how RNA polymerase II is recruited to genes during the ZGA, and how many genes begin to be transcribed.

Methods

In Chapter 1, to analyze temporal control of *Foxa.a* and *Zic-r.b* expression, I performed gene knock-down and overexpression experiments by microinjecting specific morpholino antisense oligonucleotides, mRNAs or DNA constructs into *Ciona* embryos. Then, I analyzed effects of such perturbations on spatial and temporal gene expression by in situ hybridization or reverse-transcription followed by quantitative PCR (RT-qPCR).

In Chapter 2, to comprehensively understand temporal dynamics of transcription genome-wide in early *Ciona* embryos, I performed chromatin immunoprecipitation followed by deep-sequencing (ChIP-seq) using an antibody against RNA Polymerase II. Zygotic transcription of several genes, which was identified by the ChIP-seq experiment, was confirmed by RT-qPCR.

Results

In Chapter 1, I found that three transcriptional repressors, Prdm1-r.a, Prdm1-r.b and Hes.a, temporally regulated the expression of *Foxa.a* and *Zic-r.b* in the brain lineage. Prmd1-r.a, Prdm1-r.b and Hes.a prevented *Zic-r.b* from being expressed before the division of bi-potential brain/palp progenitors. Without this repression, not only the brain progenitors but also the palp progenitors were specified to the brain fate. This delay of initiation of *Zic-r.b* expression was essential for *Zic-r.b* to be expressed only in the brain progenitors, but not in the bi-potential brain/palp progenitors or palp progenitors.

Prdm1-r.a also repressed *Foxa.a* expression. This repression is required for terminating *Foxa.a* expression at the 32-cell stage, which began at the 8-cell stage. Consequently, in the brain lineage of triple morphants of *Prdm1-r.a*, *Prdm1-r.b* and *Hes.a*, *Foxa.a* and *Zic-r.b* were expressed simultaneously, which led to ectopic activation of *Brachyury* and its downstream pathways for notochord differentiation.

In Chapter 2, by ChIP-seq analysis using an antibody against RNA Polymerase II, I revealed a global view on how RNA polymerase II is recruited to genes during the ZGA in early embryos. It showed that no genes were transcribed at the 1- and 4-cell stages, and 239 genes start to be transcribed from the 8- and 16-cell stages. RNA polymerase II was found to be bound to the transcription start sites (TSSs) of a majority (71%) of these genes before their transcription begins. My results are the first instance that determined such dynamics of Pol II in early chordate embryos. The dynamics was very different from that in *Drosophila* in which similar studies have been performed.

Discussion

The results shown in Chapter 1 raised two important points. First, my finding indicates that addition of these repressors to the gene regulatory network responsible for brain formation might represent a key event in the acquisition of the primitive palps/placodes in an ancestral animal. Second, because temporal controls by transcriptional repressors are essential for specifying the two distinct fates of brain and notochord by *Foxa*.a and *Zic-r.b*, such a mechanism might enable the repeated use of a limited repertoire of transcription factors in developmental gene regulatory networks. In Chapter 2, I showed that the dynamics of RNA polymerase II during ZGA in *Ciona* embryos is different from the dynamics in *Drosophila melanogaster* embryos . These results shed new light on gene regulatory network analysis; temporal changes of gene expression might have played important roles in animal evolution.