Toward understanding what might have caused the diversification of transcriptional repression mechanisms in urochordate early embryonic germlines.

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Germline formation is an essential and common process during embryogenesis for sexually reproducing animals. However, mechanisms of germline formation are known to be diversified even between closely related species. For example, in a wide range of animals, RNA polymerase II (RNAPII)-dependent global transcription is repressed to protect germline cells from somatic differentiation, and this transcriptional quiescence is established by taxon-specific molecules, such as Pgc in *Drosophila*, PIE-1 in *C. elegans* and Pem in ascidians, with no orthologs outside of each group. The addition of new molecules to the mechanisms contrasts with many other developmental programs known to have changed without altering the outcomes, in which such changes are often achieved by alterations of enhancer sequences and thus of gene regulatory networks. How might the molecular mechanisms regulating the germline formation have been able to diversify so easily?

Closely related urochordate species could be ideal model organisms to address this question for the following reasons. First, embryonic-developmental processes including cell fate maps and germline formation are similar among some urochordate species. Second, Posterior end mark (Pem) has been identified as a molecule responsible for transcriptional repression in several ascidian species, *Halocynthia roretzi*, *Ciona intestinalis* and *Ciona savignyi* but we have revealed that the functional domains required for transcriptional repression are different between Pems in *H. roretzi* and *C. savignyi*, suggesting that molecular mechanism can be diversified within the molecule among different ascidian species. Finally, *Oikopleura dioica,* a larvacean belonging to Urochordata and taking the preformation mechanism for early embryonic germline formation as ascidians, shows transcriptional repression in the germline cells but does not have *Pem* in the genome. Therefore, comparative analyses on germline development among those urochordate species could lead us to understand detailed molecular processes of such diversification during evolution.

We are currently conducting the following experiments. First, we are carrying out cross-species microinjection of *Pem* mRNAs as well as functional domain swapping experiments to determine whether the different functional domains found between Pem orthologs act on different mechanisms leading to the transcriptional silencing or they, despite their amino acid sequence differences, have a same function acting on the same mechanisms in different species. We are also analyzing to look for common properties in the amino acid sequences between the different Pem functional domains. Finally, we are conducting a functional screening using *O. dioica* embryos to identify a molecule(s) that regulates transcriptional repression in the germline. In this presentation, we would like to introduce to you some of the results obtained so far and discuss them with you.