Recent Research Topics in the Laboratory of Bioindustrial Informatics

Tohoku Journal of Agricultural Research
Vol. 59 No. 3-4, March 2009
Printed in Japan

Recent Research Topics in the Laboratory of Bioindustrial Informatics

Tohru SUZUKI and Yoshifumi SAKAI

Laboratory of Bioindustrial Informatics, Graduate School of Agricultural Science, Tohoku University

(Received, March 2, 2009)

Summary

Laboratory of Bioindustrial Informatics is established in 2004, and now intends to reveal to the molecular mechanisms that control early development of teleost fish and also to develop computer programs for DNA sequence analyses. We currently have three main research projects. In the first project, we reveal the developmental mechanism that controls external left-right asymmetry of flounder. In second, we recently started a research project aiming at establishment of technique to prepare induced pluripotent stem (iPS) cells from fish cells. The third project aims at developing efficient computational methods for comparing biological sequences. Here we briefly summarize recent progress of our research projects.

Metamorphic Development of Flounder

In the members of the Pleuronectiforms, including flounder and flatfish species, both eyes locate on a single lateral of the head, and dark chromatophores distribute only on the ocular side. Thus, they show marked asymmetry in their external structure, in addition to the asymmetry of the internal organs and epithalamus (a part of brain composed of pineal complex and habenulae) commonly seen in the vertebrates. During embryogenesis, eyes and pigments develop in bilateral symmetry even in flounder as in other vertebrates. Flounder larvae is transformed into external asymmetric shape during the metamorphosis by emigrating one of paired eyes into other lateral of the head, and then by differentiating melanophores in the skin of ocular side (Watanabe *et al.* 2008). Flounder asymmetry is one of well-known mysteries in animal development. We have been trying to uncover the mechanism that controls flounder external asymmetry.

The mechanism to control the sidedness of the heart, gut and epithalamus has

been understood in details by a series of studies in zebrafish, a model organism for vertebrate development. In brief, the Nodal-Lefty- Pitx2 (NLP) pathway is expressed in the embryos specifically in the left side of these organs primordia to lateralize their asymmetric development (Hashimoto *et al.*, 2004 ; Hashimoto *et al.* 2007b). The expression of the NLP pathway is switched off before the end of embryogenesis in both zebrafish and flounder (Hashimoto *et al.*, 2007a). We have found that in flounder *pitx2*, a transcription factor that functions as the final L-R determinant of organ asymmetry, is re-expressed in the left habenular at the early stage of metamorphosis. This left-sided *pitx2* re-expression in the brain seems to lateralize eye sidedness of flounder, because suppression of *pitx2* re-expression results in randomization of eye sidedness, including left-sided, right-sided and bilateral eyes (manuscript in preparation).

Out recent topic on the pigmentation of flounder is discovery where the precursor cells of adult type chromatophores, which form asymmetric skin coloration, exist (Watanabe *et al.*, 2008). The precursor cells were found to distribute as stem cells at the basal parts of the dorsal and anal fins. In addition, the precursor cells migrate into both lateral of skin from the fins in a bilaterally symmetric manner. This presumably means that asymmetry of flounder skin color is formed at the step of proliferation of the precursor cells at the skin or final maturation into chromatophores. We are now investigating the mechanism whereby the chromatophores differentiate only at the ocular side.

In aquaculture of flounder, abnormal metamorphic development during seed production, such as reversal of eye sidedness and mal-pigmentation, remains as problems to be dissolved. Elucidation of the mechanism of metamorphic development is helpful for identifying causative factors that affect the larva development in seed production.

Pluripotency of fish embryonic cells

In marine aquaculture, species of relatively large body size, such as yellowtail, flounder and tuna, are targets of production. Selective breeding needs maintenance of parental fish with heredities better for efficient production. However, in the case of large marine fish, it is nearly impossible to maintain large number of families due to the problems of cost and space. In mice, induced pluripotent (iPS) cells, with potential to differentiate to all kinds of cell species as known for embryonic stem (ES) cells, can be prepared from fully differentiated somatic cells by inducing expression of four transcription factors, *Oct3/4*, *Sox2*, *Klf4* and *cMyc*. Our idea is that it becomes possible to keep selected families as frozen cells when the technique to prepare fish iPS cells is developed. We are now investigating the features of pluripotency of fish embryonic cells using zebrafish. To assess the pluripotency of cells, we have established a transgenic

zebrafish line carring *Oct3/4* promoter-*GFP* gene. In mouse and human iPS preparation, *Oct3/4* is used as a marker gene to judge whether cells have acquired the pluripotency. The embryonic cells of this transgenic fish emit GFP fluorescence only at early development until gastrulation, and stop emission thereafter. It is expected that somatic cells prepared from this transgenic fish start to emit green fluorescence when they acquired pluripotency in the tests of iPS cell preparation. In addition, we can monitor the pluripotency of the embryonic cells during development. We have found that injection of *sox2* and *klf4* mRNAs into fertilized cells prolongs the pluripotency of embryonic cells. We plan to prepare iPS cells directly from the embryonic cells by microinjection of cocktail of *sox2* and *klf4* mRNAs and antisense morpholino-oligos of genes that induce differentiation in the embryonic cells. To develop culture system for fish iPS cells, we are also preparing recombinant proteins of fish leukemia inducing factor (Lif) and fibroblast growth factor (Fgf) (Abe *et al.*, 2007).

Efficient sequence comparison

Sequence comparison has become essential in modern molecular biology. The longest common subsequence (LCS) problem and the related problems have been studied extensively, because the length of the LCS can be thought of as the similarity between the input sequences. In addition, the LCS can help us find out the regions of the sequences with biological meanings such as motifs. It is known that the LCS problem for two sequences can be solved in quadratic time and linear space.

The longest common increasing sequence problem consists in finding the longest increasing subsequence common to all given sequences of integers, which can be applied when computing the alignment of whole genomes. We have shown that this problem for two sequences is also solvable in quadratic time and linear space (Sakai 2006).

The spliced alignment problem is another problem related to the LCS. The goal of this problem is to find the chain of candidate exons that is most similar to a known related gene sequence, which is one of successful approaches to assembling a gene from candidate exons. Although the number of candidate exons may be quadratic in the length of the target DNA sequence, good filtration is crucial for exon assembly, especially if the target DNA sequence and the related gene sequence are from distant taxa. With no filtration, this problem is known to be solvable in cubic time. On the other hand, in the case where the number of candidate exons is linear in the length n of the target DNA sequence after adequate filtration, the running time of the fastest algorithm known so far for solving this problem is $O(n^{2.25})$. We try to improve this running time to almost quadratic by developing an $O(n^2 \log n)$-time algorithm.

We are also interested in efficient methods for constructing multiple alignments of three or more sequences. It is known that any multiple alignment of sequences specifies the trace layout graph, which represents a class of multiple alignments of the same sequences with gap flexibility. Each topological sort of this graph corresponds to a specific multiple alignment in the class. We are developing efficient algorithms for merging two trace layout graphs under the LCS criterion, which has an application to merging multiple alignments in the progressive alignment method widely employed by practical computer software such as Clustal-W.

References

Abe, T., Mikekado, T., Haga, S., Kisara, Y., Watanabe, K., Kurokawa, T., Suzuki, T., Identification, cDNA cloning, and mRNA localization of a zebrafish ortholog of leukemia inhibitory factor. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **147**, 38–44 (2007).

Hashimoto, H., Aritaki, M., Uozumi, K., Uji, S., Kurokawa, T., Suzuki, T., Embryogenesis and expression profiles of charon and *nodal*-pathway genes in sinistral (*Paralichthys olivaceus*) and dextral (*Verasper variegatus*) flounders. *Zool. Sci.* **24**, 137–146 (2007a).

Hashimoto, H., Rebagliati, M., Ahmad, N., Muraoka, O., Kurokawa, T., Hibi, M., Suzuki, T., The Cerberus/Dan-family protein Charon is a negative regulator of Nodal signaling during left-right patterning in zebrafish. *Development* **131**, 1741–1753 (2004)

Hashimoto, H., Uji, S., Kurokawa, T., Washio, Y., Suzuki, T., Flounder and fugu have a single lefty gene that covers the functions of *lefty1* and *lefty2* of zebrafish during L-R patterning. *Gene* **387**, 126–132 (2007b).

Watanabe, K., Washio, Y., Fujinami, Y., Aritaki, M., Uji, S., Suzuki, T., Adult-type pigment cells, which color the ocular sides of flounders at metamorphosis, localize as precursor cells at the proximal parts of the dorsal and anal fins in early larvae. *Dev. Growth Differ.* **50**, 731–741 (2008).

Sakai, Y., A linear space algorithm for computing a longest common increasing subsequence, *Inf. Process. Lett.* **99**, 203–207 (2006).