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CHRONICLES

Evolutionary Embryology Resurrected in Japan with a New Molecular Basis—Nori Satoh and the History of Ascidian Studies Born in Kyoto in the 20th Century[1]

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Abstract—This paper was written in honor of Dr. Noriyuki Satoh who was awarded A.O. Kowalewsky medal, an international prize of the St. Petersburg Society of Naturalists. We congratulate and thank this eminent zoologist for his outstanding scientific achievements, for his life's work in the discipline founded by A.O. Kowalewsky.

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BEGINNING—NORI SATOH MEETS THE ASCIDIANS

Nori Satoh's scientific activity appears always to have been motivated by his drive to understand the dynamics of development, especially the dynamism associated with certain strictly maintained 'time tables' in animal development. Nori Satoh, Professor of Department of Zoology, Kyoto University, Japan, and the winner of Kowalevsky Medal 2005, is now recognized as the founding father of the study of ascidian developmental biology at the molecular level, and remains even to this day one of the acknowledged leaders in this field. Even now, he continues to work to open up new perspectives on evolutionary developmental genomics, through studies of his favorite animals, the ascidians.

As first demonstrated by Kowalevsky (1886, 1871), ascidian larvae possess a notochord and gills, and develop according to a basic embryonic body plan similar to that of vertebrates. For these reasons, ascidians, once grouped with the molluscs, are now classed as chordates (specifically, as protochordates, a paraphyletic group). It was also these vertebrate-like characters that caught Nori's attention when he began his research.

It was nearly 30 years ago, when he was a young Assistant Professor in the Kyoto University Department of Zoology, that Satoh initiated the study of ascidians, an organism that no one at the time imagined might one day become one of the most popular model species in developmental biology. The first step was the

[1]The text was submitted by the authors in English.

discovery of the molecular clock Nori found at work in the ascidian egg, whose timing is based on a factor activated in a manner dependent on the number of cleavages the cells have gone through. To identify this machinery, he used HRP to perform cell lineage analysis of ascidian larval muscles in the early 1980's with one of his graduate students, Hiroki Nishida, who is now a Professor at Osaka University (Nishida and Satoh, 1985; Nishida, 1987). Nori also searched for that maternal factor molecule by using monoclonal antibodies (helped by Takahito Nishikata, currently an Associate Professor in Konan University), an approach that was still quite new and challenging at the time. Kazuhiro Makabe (currently a Professor in Tokushima University) followed up on that work, attempting to capture the molecule as an mRNA (Sasakura et al., 1998). Although an another lab reported it had identified MyoD as the muscle determinant in that animal (Tapscott et al., 1988), one of Nori's students determined that this molecule does not function in ascidians soon thereafter (Isato Araki, currently a Lecturer in Tsukuba University), which encouraged Nori's lab to continue its unique research strategy (Araki et al., 1994). Yusuke Marikawa and Shoko Yoshida also tried to isolate the factor as cytoplasmic fraction (Marikawa et al., 1994; Yoshida et al., 1996). This series of studies finally saw its completion by the research group led by Nishida (who represented the first generation of Kyoto Children, so to speak) with the discovery of the maternal factor, Macho (its mRNA is localized as the determinant within the egg cytoplasm), which was published in Nature (Nishida and Sawada, 2001).

DEVELOPMENTAL CLOCK

Nori Satoh's first scientific achievement was to elucidate the regulatory mechanism behind the unique timing of the expression of acetylcholine esterase (AChE) by muscle differentiation in ascidian embryos. Having gained some insight by reading of Whittaker's discovery that inhibition of cell division by cytochalasin B does not alter the timing of AChE expression (Whittaker, 1973), he tried to identify the clock that regulates this timing in ascidian embryonic cells. Nori found that neither cytoplasmic nor nuclear division was necessary for AChE expression, but that DNA replication was a prerequisite and was likely to be the basis for the AChE clock. Specifically, he found that inhibition of DNA replication after the 6th cell division prevents AChE expression, whereas when subsequent replication is inhibited after the 7th DNA replication, AChE is expressed normally. This study, together with that by Newport and Kirschner (1982), who found that midblastula transition (MBT) in frogs is regulated by the ratio of cytoplasmic volume to nuclei, should be remembered as one of the most important contributions to our knowledge of regulatory clock mechanisms in animal developmental processes.

After his success in the study of this clock mechanism, Nori Satoh began to bring molecular biological techniques to bear on the study of ascidians. This undertaking also met with great success and indeed largely as the result of his work, *Ciona intestinalis* stands as the third invertebrate animal whose entire genomic sequence draft has been obtained. We should keep in mind, however, that Nori started the ascidian study by testing embryonic cell lineages that had already been described by Conklin (1905). Before ascidians came to be recognized as a popular organism in the field of developmental biology today, Nori conducted a series of steady basic researches, typical of comparative zoology. The fate and lineage map of ascidian embryos without a doubt serves as the basis of the modern-day research by the Kyoto Ascidian Group; we can see this scheme in almost all of the talks presented by both Nori himself and by his students, including Hiroki Nishida. Our understanding of dynamic mechanisms of spatiotemporal gene regulation in embryos with a small number of cells also stems from the same fate mapping. This work served to demonstrated how these cells make their way to the notochord, and that the whole pattern is highly similar to that in vertebrates, which further motivated the drive to sequence this animal's genome (Dehal et al., 2002). This entire range of endeavor was born from the ascidian cell lineage work first done by Nishida and Satoh two decades ago (1985).

EVO-DEVO PUSHES GENOMICS FORWARD

In the 1990s, Nori's scientific interests were focused even more clearly on evolutionary questions. Looking back, it is easy to conceive how his profound knowledge and understanding of ascidian developmental mechanisms could have enhanced this curiosity. He was now peering into dynamism in the timeline of evolution, or on the phylogenetic tree, asking how developmental mechanisms might be changed to give rise to a new morphology.

It was also around that time that he began to promote molecular phylogenetic studies to re-evaluate the phylogenetic positions of various ascidian species, mainly by use of sequences of 18S rRNA and muscle actin genes (Wada and Satoh, 1994; Kusakabe et al., 1997). In fact, Nori was one of the first to realize the importance of coupling phylogenetic systematics with evolutionary developmental biology. In addition to ascidian species, he also incorporated other animals, including amphioxus, acorn worms, sea urchins and sea cucumber, into his work as a means of extending his molecular phylogenetic research to address the question of the origin of vertebrates, as viewed from the entire deuterostome lineage. He also paid special attention to the notochord, a synapomorphy of the chordates and the most fundamental element in the chordate body plan. What repertoire of genes has come into which genetic cascades, to play what developmental roles in the differentiation of this specific structure? Needless to say, questions of this sort are worthy of support by all scientists who are more or less interested in the origin of our own vertebrate lineage. Nori settled upon a gene called *Brachyury*, the master control gene for the ascidian notochord (Yasuo and Satoh, 1993), and started to search for the bases of this evolutionary novelty at the mechanistic and molecular levels. Nori surveyed comparative expression analyses of *Brachyury* homologues in other invertebrate embryos, including those of echinoderms, and comprehensively searched for genes downstream of *Brachyury* in ascidians by a unique differential screening strategy (Takahashi et al., 1999). With these data, he showed that notochord evolution appears to have co-opted several gene cascades downstream of *Brachyury* (Hotta et al., 2000; conducted by Hiroki Takahashi, who is currently an Assistant Professor in NIBB). We still do not know the entire background for the evolution of the notochord; the network upstream of *Brachyury* remains particularly enigmatic. Still, there seems no doubt that this study of Nori's was one of his first projects that involved a genome-wide, comprehensive search of genes, and must have greatly motivated him to move into the ascidian EST and genome projects (mainly conducted by Yutaka Satou, Associate Professor in Kyoto University) (Satou et al., 2001; Dehal et al., 2002). In purely scientific terms, his achievement in this work is to have provided answers to some long-standing, classically important questions in the clear terms of molecular biology, as we have already seen in his discovery of the molecular clock mechanism of muscle differentiation. We now stand poised on the verge of being able to see the entire scheme of genetic cascades that form the ascidian notochord, and in the near future it should also become possible to compare that scheme with those for echinoderms and vertebrates, allowing us finally to grasp the molecular and developmental scenario of notochord evolution.

THE ZOOLOGIST IN SOCIETY

Not only a veteran in the battlefields of science, Nori Satoh has also been a very enthusiastic educator, a hallmark of the well-rounded scientist, and he has been extremely successful in this capacity as well. As should by now be clear from the account of his career above, many of his students have gone on to become PIs in labs throughout Japan, such as Hiroki Nishida in Osaka, and a number of them even head their own their research programs in foreign countries. His contribution has not been limited to the Japanese scientific community; Nori was a major influence on a number of students and young investigators from all over the world who attended the Embryology Course at Woods Hole Marine Biological Laboratory, MA, USA, where Nori served as a lecturer until 1996. It should also be remembered that, as Editor-in-Chief of Zoological Science in 1999 and 2000, Nori helped to increase the quality of that publication enormously, bringing it recognition as a truly international journal. Based in Kyoto, the most beautiful and best known historical city in Japan, Nori Satoh has endeavored to create truly a new field of ascidian developmental biology. Herein we can see how exquisitely Nori's genuinely dedicated approach to comparative zoology and his courage and talent for incorporating modern biological concepts and technology, have been blended. This article was written to announce Dr. Noriyuki Satoh as the winner of the 2005 Alexander Kowalevsky International Prize and to congratulate him on and thank him for his scientific endeavors and achievements, for the life's work of this zoologist represents the field at its very best, the true realization of Kowalevsky's dream.

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