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Special Contribution

Evolutionary embryology resurrected in Japan with a new molecular basis: Nori Satoh and the history of ascidian studies originating in Kyoto during the 20th century

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ABSTRACT This article briefly summarizes the scientific contributions of Nori Satoh, the winner of the 2005 edition of the Kowalevsky Medal, to Developmental Biology and especially to Evo-Devo with his 30 years of research on tunicates - a primitive chordate species. His research began with his pure developmental interest in the clock mechanism of cell differentiation and later expanded into various aspects of evolutionary and developmental phenomena. He is not only known as a founder of molecular biology-based tunicate studies, but also for his world-wide service to education and his prestigious publications in international scientific journals.

KEY WORDS: *Nori Satoh, Kowalevsky Medal, ascidian, developmental biology*

Nori Satoh, Professor of the Department of Zoology, Kyoto University, Japan and the winner of the 2005 edition of the Kowalevsky Medal[1] (Fig. 1), is known as the leading scientist in Japanese developmental biology, especially in the field of tunicate development, which typifies one aspect of developmental biology in Japan today. Nori's scientific activity extends over about 30 years. His output includes more than 270 original papers and reviews, 4 books (including three Japanese editions) and numerous book chapters during his working career. A remarkable diversity of interests is certainly one of Satoh's strengths; his range of pursuits spans genetics, evolution, embryology, developmental biology, biochemistry, comparative zoology and education. He has explored these remarkably diverse interests in a truly liberal atmosphere, which is one of the traditions of the Kyoto University Faculty of Science. The fields of evolutionary developmental biology (Evo-Devo) and comparative molecular embryology owe a great debt to the contributions of Nori Satoh, especially in the understanding of chordate species. Naturally Nori is now recognized as the founding father of the study of ascidian developmental biology at the molecular level and remains 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. Importantly, Nori's scientific activity had started well before the rise of Evo-Devo, as a purely developmental study motivated by his unique insights.

Nori Satoh meets the ascidians

It is nearly 30 years ago, when he was a young Assistant Professor at the Kyoto University Department of Zoology, since Nori Satoh initiated his studies 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 (Fig. 2). As first demonstrated by Kowalevsky (1886, 1871), ascidian larvae possess a notochord, a dorsal hollow nerve chord and branchial openings 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 Urochordates. Nori's attention was also caught by these vertebrate-like characters when he began his research. Nori'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.

The first step was the discovery of the molecular clock that 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 undergone; ascidian embryos develop in a mosaic fashion regulated by localized cytoplasmic factors and the devel-

Abbreviations used in this paper: AChE, acetylcholinesterase; EST, expressed sequence tag; Evo-Devo, evolutionary developmental biology.

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Note 1: For further information on the Kowalevsky Medal, see Mikhailov and Gilbert (2002) and Mikhailov, 2005.

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Fig. 1. Dr. Nori Satoh with his Kowalevsy Medal *in Kyoto (2006).*

opmental fates of cells do not change even if the cells are isolated. To identify this cytoplasmic machinery, or the maternal factor called 'myoplasm', he used horseradish peroxidase tracer techniques to perform cell lineage analysis of ascidian larval muscles in the early 1980s 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). Another lab had identified MyoD as the muscle determinant in that animal (Tapscott *et al.,* 1988). However, one of Nori's students (Isato Araki, currently a Lecturer in Tsukuba University) later found that this molecule was not the determinant, although it did function late in the muscle determination pathway, which encouraged Nori's lab to continue its unique research strategy (Araki and Satoh, 1994). Yusuke Marikawa and Shoko Yoshida also tried to isolate the factor as a 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 gene, *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 principle scientific achievement was to elucidate the regulatory mechanism behind the unique timing of the expression of acetylcholinesterase (AChE) by muscle differentiation in ascidian embryos. Having gained some insight by reading 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 the mid-blastula transition in frogs is regulated by the ratio of cytoplasmic to nuclear volume, 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 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, Nori conducted a steady series of basic research, 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 schematic 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 clearly 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 dynamics of 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 system-

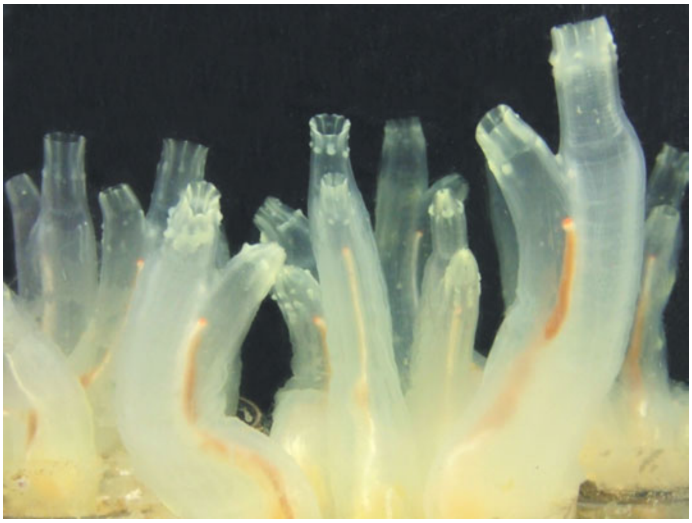

Fig. 2. An adult form of *Ciona intestinalis,* *a tunicate species studied in Nori Satoh-s laboratory.*

atics with Evo-Devo. In addition to ascidian species, he also incorporated other animals, including lancelets, acorn worms, sea urchins and sea cucumbers 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 genes and what genetic pathways play 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 the master control gene for the ascidian notochord, *Brachyury*, (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 at the National Institute for Basic Biology, Japan). 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 ascidian Expressed Sequence Tag (EST) and genome projects (mainly conducted by Yutaka Satou, Associate Professor at Kyoto University) (Satoh *et al.,* 2001; Dehal *et al.,* 2002). In purely scientific terms, his achievement with 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 regard as well (Fig. 3). As should by now be clear from the account of his career above, many of his students have gone on to become principal investigators in labs throughout Japan, such as Hiroki Nishida in Osaka and a number of them even head their own 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 Marine Biological Laboratory, Woods Hole, MA, USA, where Nori served as a lecturer until 1996. It should also be remembered that, as Editor-in-Chief of *Zoological Science* from 1996 to 1998 (Fig. 4), Nori helped to increase the quality of that publication enormously, bringing it recognition as a truly international journal. Currently, he is serving as an Editor for *Development, Genes and Evolution*, an Associate Editor for *Journal of Experimental Zoology* (*Molecular and Evolutionary Development*) and his name is found on editorial boards of many other international journals such as, *Development*, *Developmental Biology*, *Mechanisms of Development*, *Differentiation* and *Evolution & Development*.

Based in Kyoto, probably 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

Fig. 3. Nori Satoh at the bench with his students. *Kyoto (2006).*

Fig. 4. A cover of the journal *Zoological Science.* *The photo from Vol. 19, No. 10 (2002) illustrates doliolaria larva of the Japanese sea cucumber from a paper by Dr. Sato´s laboratory (for details, see Harada* et al., *2002).*

scientific endeavors and achievements, since the life work of this zoologist represents the field at its very best, the true realization of Kowalevsky's dream.

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References

ARAKI, I., SAIGA, H., MAKABE, K.W. and SATOH, N. (1994) Expression of AMD1, a gene for a MyoD1-related factor in the ascidian *Halocynthia roretzi*. Roux's Arch. *Dev. Biol.* 203: 320-327.

CONKLIN, E.G. (1905) The organization and cell lineage of the ascidian egg. *J. Acad. Natl. Sci.* 13: 1-119.

DEHAL, P. *et al.,* (87 authors) (2002) The draft genome of *Ciona intestinalis*: insights into chordate and vertebrate origins. *Science* 298: 2157–2167.

HALL, B.K. (1998) Evolutionary Developmental Biology, 2nd Ed., London: Chapman & Hall.

HARADA, Y., SHOGUCHI, E., TAGUCHI, S., OKAI, N., HUMPHREYS, T., TAGAWA, K. and SATOH, N. (2002) Conserved expression pattern of *BMP-2/4* in hemichordate acorn worm and echinoderm sea cucumber embryos. *Zool. Sci.* 19: 1113-1121.

HOTTA, K., TAKAHASHI, H., ASAKURA, T., SAITOH, B., TAKATORI, N., SATOU, Y. and SATOH, N. (2000) Characterization of *Brachyury*-downstream notochord genes in the *Ciona intestinalis* embryo. *Dev. Biol.* 224: 69-80.

KOWALEVSKY, A.O. (1866) Entwickelungsgeschichte der einfachen Ascidien. *Mem. Acad. Sci. St. Petersbourg* (7)10, no. 15: 1-19 (cited in Hall, 1998).

KOWALEVSKY, A.O. (1871) Weitere Studien über die Entwicklung der winfachen Ascidien. *Arch. Mikrosc. Anat.* 7: 101-130 (cited in Hall, 1998).

KUSAKABE, T., ARAKI, I., SATOH, N. and JEFFERY, W.R. (1997) Evolution of chordate actin genes: evidence from genomic organization and amino acid sequences. *J. Mol. Evol.* 44: 289-298.

MARIKAWA, Y., YOSHIDA, S. and SATOH, N. (1994) Development of egg fragments of ascidian *Ciona savignyi*: the cytoplasmic factors responsible for muscle differentiation are separated into a specific fragments. *Dev. Biol.* 162: 134-142.

MIKHAILOV, A.T. (2005). Putting Evo-Devo into focus. An interview with Scott F. Gilbert. *Int. J. Dev. Biol.* 49: 9-16.

MIKHAILOV, A.T. and GILBERT, S. (2002). From development to evolution: the re-establishment of the "Alexander Kowalevsky Medal". *Int. J. Dev. Biol.* 46: 693-698.

NEWPORT, J. and KIRSCHNER, M. (1982) A major developmental transition in early *Xenopus* embryos: I. characterization and timing of cellular changes at the midblastula state. *Cell* 30: 675- 686.

NISHIDA, H. (1987) Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme. III. Up to the tissue restricted stage. *Dev. Biol.* 121: 526-541.

NISHIDA, H. and SATOH, N. (1985) Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme. II. The 16- and 32-cell stages. *Dev. Biol.* 110: 440-454.

NISHIDA, H. and SAWADA, K. (2001) macho-1 encodes a localized mRNA in ascidian eggs that specifies muscle fate during embryogenesis. *Nature* 409: 724-729

SASAKURA, Y., OGASAWARA, M. and MAKABE, K.W. (1998) Maternally localized RNA encoding a serine/threonine protein kinase in the ascidian, *Halocynthia roretzi*. *Mech. Dev.* 76: 161-163.

SATOU, Y., TAKATORI, N., YAMADA, L., MOCHIZUKI, Y., HAMAGUCHI, M., ISHIKAWA, H., CHIBA, S., IMAI, K., KANO, S., MURAKAMI, S.D., NAKAYAMA, A., NISHINO, A., SASAKURA, Y., SATOH, G., SHIMOTORI, T., SHIN-I, T., SHOGUCHI, E., SUZUKI, M.M., TAKADA, N., UTSUMI, N., YOSHIDA, N., SAIGA, H., KOHARA, Y. and SATOH, N. (2001) Gene expression profiles in *Ciona intestinalis* tailbud embryos. *Development* 128: 2893-2904.

TAKAHASHI, H., HOTTA, K., ERIVES, A., DI GREGORIO, A., ZELLER, R.W., LEVINE, M. and SATOH, N. (1999a) Brachyury downstream notochord differentiation in the ascidian embryo. *Genes Dev.* 13: 1519-1523.

TAPSCOTT, S.J., DAVIS, R.L., THAYER, M.J., CHENG, P.F., WEINTRAUB, H. and LASSAR, A.B. (1988) MyoD1: a nuclear phosphoprotein requiring a Myc homology region to convert fibroblasts to myoblasts. *Science* 242: 405-411.

WADA, H. and SATOH, N. (1994) Details of the evolutionary history from invertebrates to vertebrates, as deduced from the sequences of 18S rDNA. *Proc. Natl. Acad. Sci. USA.* 91: 1801-1804.

WHITTAKER, J.R. (1973) Segregation during ascidian embryogenesis of egg cytoplasmic information for tissue-specific enzyme development. *Proc. Natl. Acad. Sci. USA.* 70: 2096-2100.

YASUO, H. and SATOH, N. (1993) Function of vertebrate *T* gene. *Nature* 364: 582-583.

YOSHIDA, S., MARIKAWA, Y. and SATOH, N. (1996) posterior end mark, a novel maternal gene encoding a localized factor in the ascidian embryo. *Development* 122: 2005-2012.

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