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OBITUARY

Obituary: Tokindo S. Okada (1927-2017)

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Hisato Kondoh and Harukazu Nakamura look back at the life and career of their mentor Tokindo S. Okada, a pioneer of Japanese developmental biology.

Image courtesy of Kiyokazu Agata

Tokindo S. Okada (here referred to as TSO) was one of the leaders who steered developmental biology in new directions when this field was at its turning point around 1980. He also made invaluable contributions to the creation of a global forum for developmental biologists. He died on January 17, 2017, two weeks short of his 90th birthday; his name, Tokindo, stands for his birth on New Year's Day of the Asian lunar calendar. Among developmental processes, two of his major interests were in the flexibility of the differentiated state, and in tissue organization from different cell types with different cell-adhesion properties. His own studies and those of researchers from his school created new directions for modern developmental biology in ways reflecting those interests.

TSO was born in Itami in Hyogo as a son of Rihei Okada, an owner of an old sake warehouse, collector/researcher of rare birds and authority on the Haiku poet Basho Matsuo. He thus grew up in a highly cultural environment. During his Konan High School days, he was exposed to contemporary developmental biology using amphibians, as conducted by Hiroshi Takaya. This inspired him to study developmental biology at Kyoto University. The most important elements of his undergraduate and graduate periods were his meeting and marriage to Ei Waki, who stimulated and supported TSO in all aspects throughout the rest of his life.

TSO received his PhD with a focus on tissue interactions in endodermal organogenesis in amphibians (Okada, 1960). Around this period, TSO, together with Ei Okada (by then his wife), visited the laboratories of Conrad H. Waddington at the University of Edinburgh, Department of Genetics (1957-1959), and of James Ebert at the Carnegie Institute of Washington in Baltimore (1964). These visits had a tremendous impact on his life. Studying with Waddington must have broadened TSO's scope, and working with Ebert made him – in his own words – 'learn from a wide perspective to organize people'.

As a result of these visits to British and American institutions, TSO was acquainted with leading developmental biologists, and he himself became highly recognized. This also promoted visits from many developmental biologists to TSO in Kyoto, resulting in the formation of an international forum centered around TSO, including Nicole Le Douarin, John Gurdon, Anne MacLaren, Lauri Saxén, Alberto Monroy, Walter Gehring, Volker Schmid, Aron Moscona, Jim Weston and others. TSO took advantage of this forum to help strengthen global liaisons among developmental biologists beyond personal ties. He served as the president of the International Society for Developmental Biologists (ISDB) from 1982 to 1986, and was awarded the Ross Harrison Prize in 1989 for his work on transdifferentiation. In the Asian sector, he also made a great effort to create China-Japan and India-Japan collaborations among developmental biologists.

His long friendships with John Gurdon and Nicole Le Douarin were particularly special. Gurdon first visited TSO in 1962 on his way back to Oxford from the USA via Japan; TSO had already told Ei then that John would eventually be awarded the Nobel Prize. Later, a student from the Okada school, Kazuto Kato, did a post-doc with Gurdon. TSO and his family spent a summer at Woods Hole with Nicole Le Douarin when she had started using chick-quail chimeras, which had a revolutionary impact on developmental biology using avian models (Le Douarin, 1973). TSO and Le Douarin developed a mutual respect and friendship. One of us (H.N.) was the first Japanese postdoctoral fellow (1978-80) to work at Le Douarin's institute in Nogent-sur-Marne and many Japanese students followed, including Hirohiko Aoyama and Yoshiko Takahashi from TSO's lab – the latter of whom united many of the ideas of Le Douarin and TSO (e.g. Sato et al., 2002).

TSO was promoted to a full professor at the Department of Zoology in Kyoto in 1967; during this period, he investigated organ reconstitution from dissociated kidney cells (Okada, 1965). This work could be considered a prototype of the currently popular organoid models. However, a real turning point for TSO came when he founded a new laboratory as a professor in the newly launched Department of Biophysics at Kyoto University in 1968. We were the first graduating class of the department. Japanese laboratories at the time were organized by a full professor, an associate professor and a few assistant professors, adopting a style analogous to that of German laboratories. TSO invited Goro Eguchi, who was working

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on lens regeneration in the newt iris, and Masatoshi Takeichi, who was then working on the lens, to join him as associate and assistant professors, respectively. TSO started investigating cell differentiation and flexibility of the differentiated state.

TSO was fond of the color contrast of dark green and red; his office furniture in the new Biophysics building bore this contrast. His green jacket was his trademark. He once owned a red Alfa Romeo. When TSO appeared at Le Douarin's institute, they were very impressed by the color combination of his dress as it surpassed their expectations for a Japanese scholar. Thus, his life was rich in dandyism, and its combination with his clairvoyant science charmed his students and many other people.

In the classroom, TSO's favorite teaching subjects strongly reflected his interests in topics such as transdetermination (e.g. the serial imaginal disc transplantation experiments of Ernst Hadorn; Hadorn, 1968) and tissue segregation (e.g. Malcolm Steinberg's differential adhesiveness hypothesis; Steinberg, 1970). TSO also wrote many introductory books on developmental biology in Japanese for nonprofessionals, students and professional biologists. These were easy to read, inspiring and fascinating, and spoke of the beauty and mystery of developmental processes. They were rich in new and forward-looking conceptual frameworks. Of course, the flexibility of differentiation and cell-cell interactions for organogenesis always formed the basis of his books.

Inspired by his books, many talented students gathered at the TSO lab. The 10-year period from 1975 to 1984 was the highlight of Okada's group, not only because of scientific productivity but also in terms of training the next generation of developmental biologists to develop their own unique characters; this became referred to as the 'Kyoto School of Developmental Biology'. Although only chicken and mouse embryos, and some amphibians, were used in the TSO lab, his broad interests also encompassed areas as diverse as plant development. Graduating students went on to use various organisms in their subsequent careers: cats (Masami Watanabe), zebrafish (Kohei Hatta), medaka (late Kenjiro Ozato), newts (Mitsumasa Okamoto and Shin-ichi Abe), *Drosophila* (Shigeo Hayashi and Akinao Nose), butterflies (Kazuo Watanabe), nematodes (Kazuya Nomura and Shin Takagi), oligochaetes (Chikako Yoshida-Noro), cellular slime molds (Hideko Urushihara) and *Arabidopsis* (Koji Goto). Some of his students turned to cell biology (Yasuhiko Tsunematsu, Kei Takahashi, Masamichi Ueda, Kenji Ueda, Kenji Okazaki, Yasuji Ueda, Yasuaki Shirayoshi and Akira Nagafuchi). This diversity reflects the school's culture that promoted individual interest-oriented choices of organisms and strategies.

TSO devoted himself to the study of the flexibility of differentiated states. As a student, Yoshiaki Ito observed a mass of lens cells that developed in a long-term culture of chicken embryonic neural retina. TSO immediately realized that this represented transdifferentiation from the retina into the lens and started an in-depth analysis of this phenomenon (Okada et al., 1975). He and his student Masasuke Araki identified two different mechanisms by which lens can form from neural retina culture (Araki and Okada, 1977). At early stages (around E3.5), before neuronal differentiation, neural retinal cells behaved like stem cells of all ocular tissues (Okada et al., 1979), whereas at later stages, after retinal cell differentiation (around E8), generation of lens appeared to be genuine transdifferentiation – re-fating of differentiated cells. TSO himself performed many experiments involving retinal cultures and immunohistochemistry. His last series of experiments dealt with the mechanism of lens transdifferentiation from the E8 retina. He found that an approximately 10-day period of spreading culture was required for lens transdifferentiation to occur (Okada et al., 1983). Thirty-five years later, it was shown that the spreading culture condition results in reduction of Notch signaling, which otherwise inhibited the intrinsic lens-generating potential of the neural retina (Iida et al., 2017).

TSO also routinely used mouse teratocarcinomas as a model with which to investigate his interest in the concept of flexible differentiation; this then permitted our use of embryonic stem cells (ESCs) shortly after they were first reported by Martin Evans in 1981 (Evans and Kaufman, 1981). Yoshio Hamada and others from the school made full use of ESCs to knock out their favorite genes. Tadao Atsumi established a monolayer culture line from the embryoid body cell line OTT6050, and this facilitated the discovery of E-cadherin by Masatoshi Takeichi. Although induced pluripotent stem cells were only produced many years later by Shinya Yamanaka, the ideas underlying their isolation had already been introduced to developmental biologists in Japan under the prevailing influence of TSO.

When the cloning age arrived in the late 1970s, TSO was eager to introduce molecular biology to the study of developmental biology. He invited Kunio Yasuda and one of us (H.K.) to join his group as assistant professors, asking us 'do anything challenging, with the condition that it involves the keywords "genes" and "lens"'. Yoshiro Shimura provided technical supervision during the cloning of crystallin genes. We were given tremendous liberty, but were subject to monitoring by TSO's extraordinarily sharp eyes, being told 'Stop it, it's trivial', as soon as we developed irrelevant ideas. One successful outcome was the demonstration that the chicken δ-crystallin gene is correctly regulated in a lens-specific manner in mouse cells, indicating the existence of evolutionarily conserved lens-specific gene regulatory mechanisms (Kondoh et al., 1983). This study developed further, leading to the discovery of Sox2 and Pax6 as interacting transcription factors for the initiation of lens development (Kamachi et al., 1995, 2001), and the identification of the Maf family of transcription factors as essential regulators of lens maturation (Ogino and Yasuda, 1998). The electroporation technique for gene manipulation in chicken embryos was also developed along this line (Nakamura, 2009).

Masatoshi Takeichi, who was then an associate professor, set forth to characterize Ca^{2+}-dependent, trypsin-sensitive adhesion molecules and discovered the cadherins (Takeichi, 1986, 1988), while Hajime Fujisawa (who left the group at an early stage of the 10-year period) later discovered neuropilin and plexin (Satoda et al., 1995; Takagi et al., 1995). Although TSO did not participate directly in the molecular characterization of cell-cell interactions, he successfully furnished his laboratory with an environment to encourage such investigations.

The flexibility of the differentiated cell state is perhaps best manifested during tissue regeneration. Thus, modern studies of regeneration using planarians, pioneered by Kiyokazu Agata and Kenji Watanabe (Agata and Watanabe, 1999), can be regarded as a direct reflection of TSO's interests. In a similar vein, many researchers who joined the TSO school have developed their individual talents and have been successful in various branches of developmental biology.

TSO had planned to keep the laboratory in Kyoto for several more years, but this did not happen. Haruo Kanatani, the Director of the National Institute for Basic Biology (NIBB) in Okazaki, who had also been a friend of TSO at Konan High School, died an untimely death, and TSO was asked to succeed him. He accepted the NIBB Director position and left Kyoto in 1984. He re-formed a tag team with his former colleague Goro Eguchi, who was by then a professor

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there investigating pigment cell-derived lens development. TSO compiled studies on transdifferentiation and related phenomena in a volume of *Current Topics in Developmental Biology* (Okada and Kondoh, 1986), and summarized his work in the book *Transdifferentation* (Okada, 1991).

During his six years in Okazaki, TSO further promoted international collaborations among developmental biologists; he organized many international meetings on different themes in Japan and other Asian countries. These meetings provided hubs for the interaction of developmental biologists on a global scale during the period when international meetings were less frequent than they are today. The small scale of these meetings facilitated trans-generational discussions among participants from different backgrounds. His dedication toward forming global links presumably compensated for his loss of laboratory activities during the period.

For 10 years from 1993, TSO was the Director of the Biohistory Research Hall in Takatsuki, a newly opened private museum owned by Japan Tobacco, which was located midway between Kyoto and Osaka. TSO, together with Vice Director Keiko Nakamura, enjoyed operating this research museum. The research section covered the embryonic and phylogenetic development of various non-mammalian animals, while the museum section aimed to expose a wide audience – ranging from elementary school pupils to nonprofessional biology lovers – to the wonder and beauty of developmental processes. Different types of exhibitions and small concerts were part of the museum's events and were an amalgamation of his enthusiasm for science and music. This was a joyful period for TSO, allowing him to fully express his esthetics. In 2007, TSO received the Order of Cultural Merit, the most prestigious award in Japan.

TSO had various and serious interests in subjects other than developmental biology. One example was his collection of longicorn beetles. His most profound interest was in Western classic music, and he wrote many critiques on 20th century compositions. His son, Akeo Okada, is a professor of musicology at Kyoto University. In the same way that various elements of his broad scientific interests were elaborated by his colleagues and students, one of TSO's talents was clearly passed on to his son.

The life of Tokindo S. Okada was rich, influential and joyful. He was an exceptionally attractive and great mentor. We miss him, but he lives vividly in our memories.

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