

Spemann's influence on Japanese developmental biology

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Introduction

Japan was a closed country until the middle of the 19th century, so it was not until the late part of that century that modern biology and embryology started at the Tokyo Imperial University (TIU). At that time American professors came to Japan to help advance the nation in modern science. Charles Otis Whitman (1842-1910, Fig. 1) in particular, was a pioneer of embryology in Japan. One of his students, Naohide Yatsu (1877-1947, Fig. 1) went to Columbia University (USA) where he studied marine invertebrate development. In Nov. 1888, at the suggestion of Edward Sylvester Morse, members of TIU and its colleagues published an article in the academic "Zoological Magazine" (Dobutsugaku-Zasshi) (Fig. 2). This journal continues today under the name of "Zoological Science". After returning to Japan in 1907 as professor of Zoology at the TIU, Yatsu tried to incorporate his new ideas and approach into experimental embryology. However at the time members of the Japanese Biological Society did not agree with his approach (Okada, T.S. 1994).

Immediately after the German embryologists, H. Spemann and Hilde Mangold, published the first paper on the organizer in 1924, studies on embryonic induction were initiated in Japan. In these early days and indeed subsequently, not a few students from the Departments of Science and Medicine at Tokyo Imperial Univer-

sity and Kyoto Imperial University, which are now known as the Tokyo and Kyoto Universities respectively, studied in American and European countries. A number of the students from these universities later studied at German and American universities allowing them to have relatively early access to information on studies concerning the organizer by Spemann *et al.* For example, Tadao Sato who later became a Professor at Nagoya University, and Hidemichi Oka who became a professor at the Tokyo University of Education, studied in Spemann's laboratory.

There are several more examples. Tsuneo Yamada who was later professor at Nagoya University, Oak Ridge National Laboratory in the USA and at the Swiss Institute for Experimental Cancer Research in Lausanne, studied in the laboratory of Dr Vogt. Dr Vogt introduced the vital dye method of tracing cell lineages allowing the fate map of the amphibian gastrula embryo to be made. Katsuma Dan who became a professor at the Tokyo Metropolitan University studied marine invertebrate development at the Wood Hole Marine Biological Laboratory in the USA. His major focus at this time was on the initial stages of development, which included cell division and fertilization. Yo Kaname Okada who later became a professor at the Kyoto and Tokyo Universities (Fig. 3 left) also studied abroad for six years in numerous European countries including France, England and Germany. He initially returned to Japan as professor of Kyoto Imperial Univer-

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C. O. Whitman



Fig. 1. (Left) Charles Otis Whitman (1842-1910). (Right) Naohide Yatsu (1877-1947).

finding that the neural inductive power differed between the dorsal and ventral sides of newts. In addition, T. Fujii showed that para-quinone had a strong neural inductive power.

At Kyoto University, kaolin and other inorganic substances were identified in the laboratories led by Yo Kaname Okada. Later, influential investigators in this field, such as Hiroshi Takaya, Osamu Nakamura, Izumi Kawakami, Yoshio Masui and Shin-ichi Asayama, and later, Tokindo S. Okada and Yoshio Masui, were trained at the laboratory established by Y. K. Okada. It should be noted that these investigators sought inducers (organizers), but could not identify any substance whose activity was higher than the activity level of the organizer region *in vivo*.

It is important to look at the outcomes of early Japanese studies in this field. Firstly, there is the double potential hypothesis proposed by Tsuneo Yamada (Fig. 4; 1949).

Yamada carefully performed surgery on embryos, comparing the anatomical findings to the fate map. He showed through implantation to the original and other sites, that two factors are needed for animal morphogenesis to proceed. An essential point of his theory is that each area of the body is developed by the gradient of two factors, the Mcc (cephalocaudal mediator) and the Mdv (dorso-ventral mediator). This theory was first presented in the monograph titled "New Biology" published after World War Two (1949). In 1958 his theory was published in English. This indicates that Yamada, had developed the double potential theory, based on his unique experiments, considerably earlier than Toivonen and Saxén who published it in 1955 and are now well known worldwide. Yamada's double potential hypothesis can be deemed quite important when combined with the Vogt's fate map, and his hypothesis greatly affected subsequent studies in Japan.

city. His school strongly influenced the field of embryology and experimental morphology in Japan. Later, these students worked at universities and research institutes and played significant roles in advancing embryology and developmental biology in Japan.

First stage

The return of these students led to active pursuit of studies into the organizer in Japan in the 1930s, primarily in the departments of experimental morphology, histology and morphology at Imperial Universities (Tokyo, Kyoto, Tohoku, Hokkaido, and Nagoya). At Tokyo University, T. Gohda and T. Fujii (Fig. 3 right) identified pyrrole from the frog skin as a neural inducer, based on the

organizers, comparing the anatomical findings to the fate map. He showed through implantation to the original and other sites, that two factors are needed for animal morphogenesis to proceed. An essential point of his theory is that each area of the body is developed by the gradient of two factors, the Mcc (cephalocaudal mediator) and the Mdv (dorso-ventral mediator). This theory was first presented in the monograph titled "New Biology" published after World War Two (1949). In 1958 his theory was published in English. This indicates that Yamada, had developed the double potential theory, based on his unique experiments, considerably earlier than Toivonen and Saxén who published it in 1955 and are now well known worldwide. Yamada's double potential hypothesis can be deemed quite important when combined with the Vogt's fate map, and his hypothesis greatly affected subsequent studies in Japan.

Osamu Nakamura (Fig. 5) studied the newt's fate map in detail and modified the fate map proposed by Vogt (Fig. 6). At the same time, Pasteels also modified Vogt's fate map. These modifications primarily pertained to the formation of the tail in the mesoderm at the lateral margins of the neural plate. Modifications of this region largely affected subsequent studies. Hiroshi Takaya examined the inductive power of the notochord and somites. By the modified transplantation method, Y. K. Okada and Takaya (1942) found that before invagination, the anterior part of the organizer induces only the trunk and tail, whereas after invagination it induces only the head. They confirmed this change of inductive specificity by isolating this part of the organizer and cultivating it in Hiltfreter's solution for 12 hours, which is the time required for invagination of this part in the normal embryo.

This phenomenon was also confirmed 53 years later using an *in vitro* system incorporating presumptive ectoderm treated with activin A (Ariizumi and Asashima, 1995). Tadao Hama studied invagination

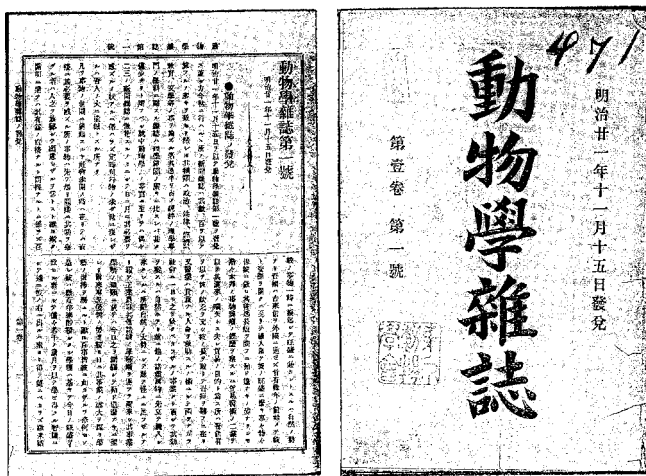


Fig. 2. The first issue of the Japanese "Zoological Magazine" in 1888. (Left) First page of this issue. (Right) Cover illustration.

of cells during gastrulation in detail, including an analysis of changes in the external shape and internal cells (Hama, T. 1949, 1950). Hama was aware that young, recent investigators in Japan were often unable to read German papers, so he translated the whole of Vogt's paper on vital dye tissue staining techniques (a 320-page paper) into Japanese. He spent the ten years after heretired from Nagoya University at the age of 65 completing this work.

Second stage

In 1950 the journal for embryology, "Embryologia", was first published under the leadership of Tadao Sato, who was a professor at the Nagoya University and member of the Embryologia Society. At that time, the Japanese economy was poor, however T. Sato, T. Yamada, G. Eguchi and their colleagues encouraged Japanese embryologists to publish original papers in English. In the first issue of "Embryologia" Yamada wrote an original paper about embryonic induction (Fig. 7; Yamada, T. 1950)

In 1968, the Embryologia Society and the Japan Society for Experimental Morphology, which was founded in 1942, were united to form the "Japan Society of Developmental Biologists". This Society has developed into the main society in the field of developmental biology. The Society replaced "Embryologia" with the International journal, "Development, Growth and Differentiation" (Fig. 7). Both authors of the present article have been closely involved with this journal for some time, as Editor-in-Chief.

At Nagoya University T. Yamada, Y. Hayashi and K. Takata (1955) extracted ribonucleoprotein (RNP) from the liver and kidney

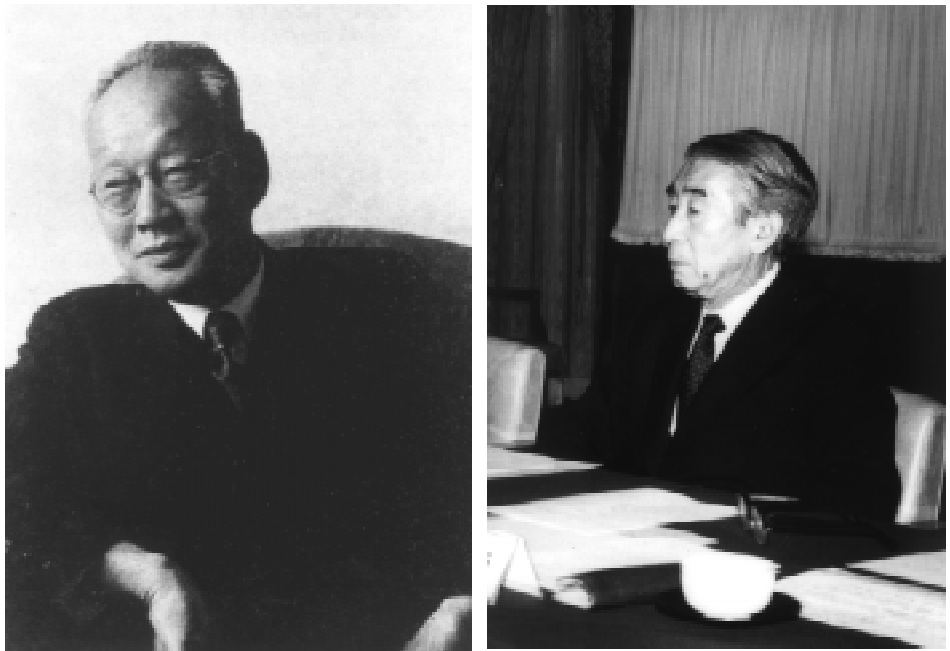


Fig. 3. (Left) Yo Kamame Okada (1891-1973; photo taken in 1961). **(Right) Takashi Fujii** (1909-2000; photo taken in 1983).

of guinea pigs. The RNP isolated from liver showed strong archencephalic induction activity, while the RNP from kidney induced spinocaudal and mesodermal structures. They concluded that RNP is a highly purified inducing substance. Yamada (1958b) also isolated RNP by exactly the same method from two different tissues, kidney and liver, and obtained two different types of RNP.

According to Hayashi (1959), treatment with ribonuclease did not significantly alter liver RNP inducing activity, however when treated with a protease, such as trypsin, chymotrypsin or pepsin, the same samples of RNP gradually lost their inducing activity. As a working hypothesis Yamada proposed the double potential hypothesis described earlier. He regarded the RNPs described above as the factors that mediated the rise in potential.

At Kyushu University, I. Kawakami, K. Yamana, N. Sasaki and co-workers examined the inductive activities of cell components. They found that the ribosome fraction from guinea pig liver and chick embryos had the capacity for neural induction, while other fractions containing nucleus, mitochondria, membranous structures and soluble proteins had no inductive activity (Kawakami, *et al.*, 1961). Finally, they attached importance to the intercellular matrix. They later showed that the matrix of the swimbladder was a highly efficient mesodermal inducer. They found that when the swimbladder had been treated with collagenase, the NaOH-soluble fraction had almost as much mesoderm-inducing capacity as the original tissue, whereas the urea-soluble fraction contained a vegetalizing factor that induced only primordial mesodermal and endodermal cells. Later, Kawakami and Asashima (1993) using the carp swimbladder concluded that this tissue contained an activin-like substance and a basic fibro-growth factor (unpublished data).

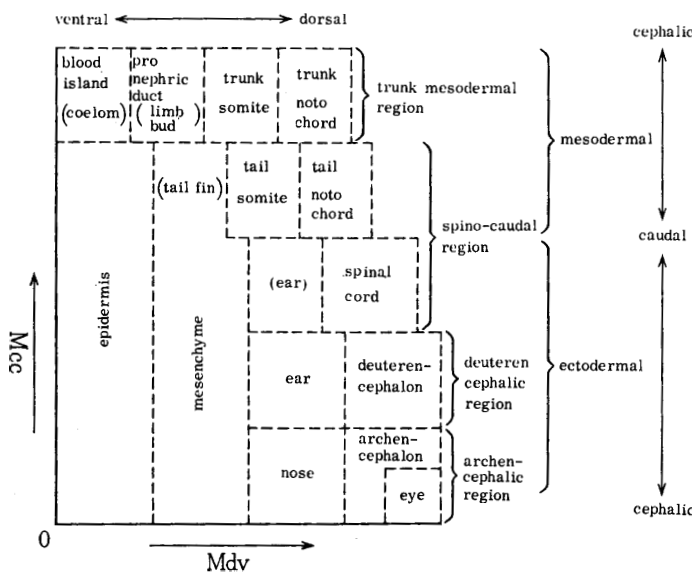


Fig. 4. The Double Potential Hypothesis proposed by T. Yamada. The original legend read: "Diagrammatic expression of the double potential hypothesis; differentiation caused by Mdv and Mcc when they act on the presumptive ectoderm."

Third stage

With the advent of molecular biology, which began to be applied to experimental embryology in the 1960s, the embryologist's interest



Fig. 5. Osamu Nakamura (left), Makoto Asashima (middle) and Tsuneo Yamada (right) in the year 1994.

moved to the subcellular and molecular mechanisms of differentiation. In line with this change in focus, the name of this scientific field was changed from experimental embryology to developmental biology. Since these changes occurred, fewer studies have concentrated on the mechanisms of induction by the organizer. However, since the organizer regulates the determination of undifferentiated cells in the first half of embryonic development, there is a need for renewed efforts to study embryonic induction originating from the organizer. These could be done in parallel with studies investigating the mechanism of differentiation at a subcellular level.

In the 1960s and 1970s, studies into embryonic induction were pursued in two main directions in Japan. The school of Nakamura tried to determine the origin and formation of the organizer. The question of how the organizer arises in the early stage of development was the most fundamental problem in embryology. In addition, Kawakami and colleagues concentrated on the competence of the reacting tissue and tried to clarify what changes are caused by induction in the reacting system. These studies that were performed in a slower period in the history of experimental embryol-

ogy, have in fact provided solid groundwork for researchers investigating developmental biology today.

The question of whether the organizer is formed epigenetically or is preformed at the beginning of development is a key question in embryology. Nakamura and his collaborators grappled with this theme from 1962. Formerly, many investigators thought that the organizer was present in the dorsal part of the marginal zone from the beginning of development. Famous experiments such as Spemann's constricting experiments on newt's eggs and Curtis' cortical grafts seem to have supported this idea. However, these experiments only demonstrated that some of the causal factors giving rise to the organizer are localized in the dorsal marginal zone.

The organizer, the dorsal marginal zone of the gastrula, has two distinct characteristics that are not found in other areas of the embryo: the capacity for self-differentiation into axial mesoderm and neural induction from the presumptive ectoderm. From their extensive isolation or recombination experiments on the presumptive organizer of preblastula-stage embryos, Nakamura concluded that the essential characteristics of the organizer are gradually acquired epigenetically from the morula to blastula stages. The presumptive organizer region acquires its capacity for self-differentiation into the axial mesoderm between the 32-cell stage and late morula (Nakamura and Matsuzawa, 1967; Nakamura and Takasaki, 1970; Nakamura *et al.*, 1970) and the same region becomes capable of neural induction during the blastula stage (Nakamura *et al.*, 1971a). They postulated that these characteristics, which are peculiar to the organizer, are established by coordination of the vegetal-animal gradient of vegetalization and dorso-ventral polarity of the embryo. From the beginning of development the yolk-mass around the vegetal pole contains vegetalizing factors which diffuse towards the animal pole resulting in a vegetal-animal gradient of vegetalization. The dorso-ventral polarity is established soon after fertilization and causes a regional difference in the capacity of the marginal zone for mesodermal differentiation.

Nakamura *et al.* (1971b) and Ogi (1967, 1969) concluded that the phenomenon in which various mesodermal tissues are formed by the recombination of the presumptive ectoderm with endoderm (not containing presumptive mesoderm) was "regulation of a vegetal-animal gradient". In contrast, Nieuwkoop (1969) interpreted this phenomenon as "mesoderm induction by endoderm", which means the induction of mesoderm formation from the presumptive ectoderm. According to Nieuwkoop's theory, the marginal zone is converted to mesoderm from ectoderm under the

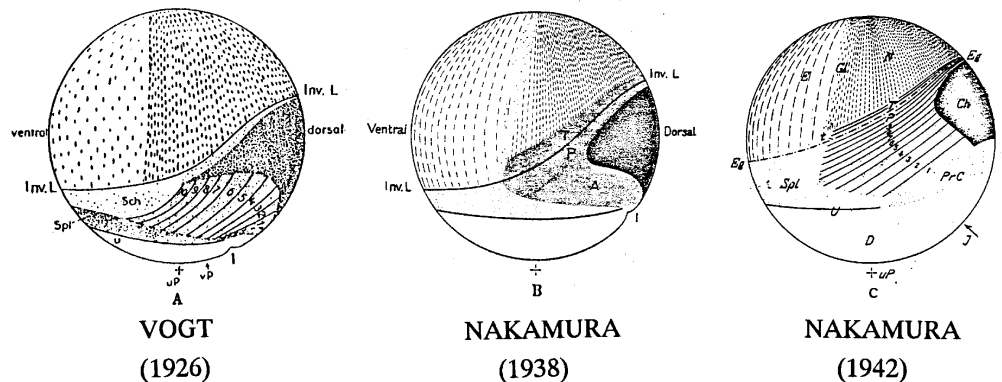


Fig. 6. Fate map of newt embryo by Vogt and Nakamura.

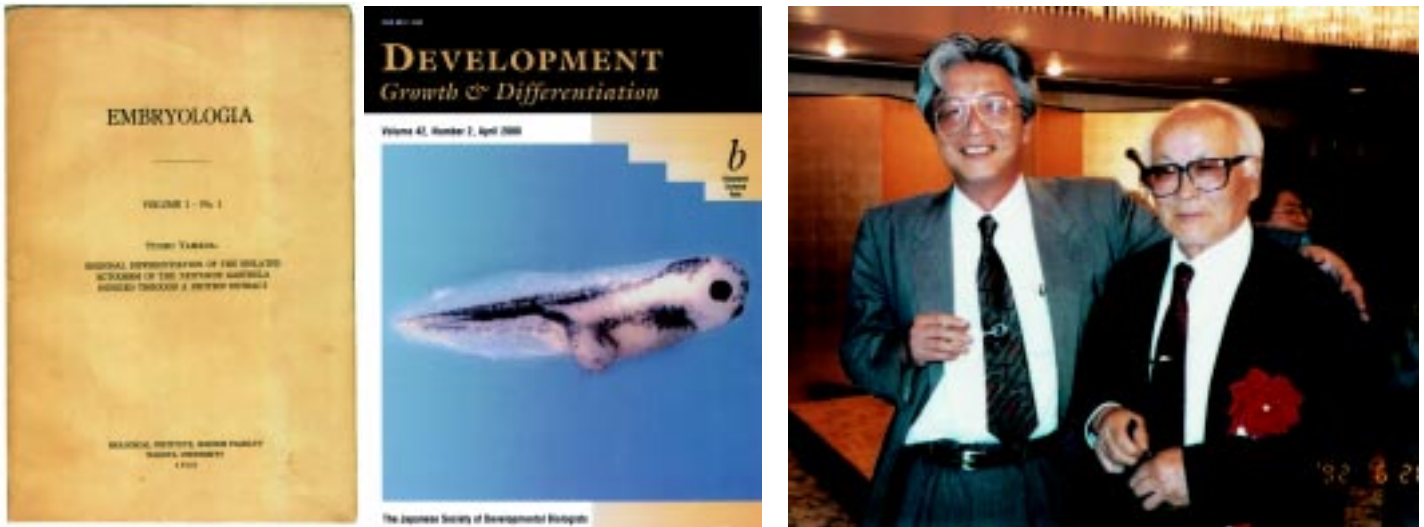


Fig. 7 (Left). Developmental biology journals in Japan. The first issue of "Embryologia" collected the original papers for developmental biology written in English in 1950. "Embryologia" changed its name to "Development, Growth and Differentiation", which continues to this day.

Fig. 8 (Right). Izumi Kawakami and Naoi Sasaki (left) at Kyushu University in 1992.

inducing effect of the endoderm. In normal development, mesodermal tissues develop from the presumptive mesoderm of the marginal zone. Nieuwkoop later recognized the concept of vegetalization. Nakamura *et al.* also demonstrated the vegetalizing effect from the vegetative blastomeres. Recently, certain peptide growth factors have been reported to have mesoderm inducing activity on presumptive ectoderm. As Nakamura pointed out, it is now necessary to determine whether this phenomenon observed *in vitro* actually occurs during mesoderm formation in normal development (Asashima, 1994).

The presumptive ectoderm of amphibian embryos has the capacity for self-differentiation into epidermis. However, it can also differentiate into mesoderm and endoderm. Using the presumptive ectoderm as a reacting tissue, and extract from the carp swimbladder as an inducing substance, Kawakami proposed a hypothesis for tissue differentiation in relation to the competence of reacting tissues. On the assumption that extracellular substances play a major part in the inducing activity, Kawakami successfully showed that the carp swimbladder had a potent mesoderm-inducing activity (Kawakami, 1976; Fig. 8). The fraction extracted with urea induced the presumptive ectoderm to form spherical masses of undifferentiated mesodermal cells, whereas the same fraction after heat treatment induced almost entirely mesodermal tissue. The former fraction also had the capacity to induce masses of yolk cells suggesting endodermal induction. Thus, the urea-soluble fraction has a vegetalizing effect and induces primordial mesodermal and endodermal cells. Heat treatment of the urea-soluble fraction may

result in the appearance of some factor that can induce differentiation of the primary induced primordial mesodermal cells. Primordial endodermal cells were thought to differentiate into endodermal tissues by the same mechanisms as mesodermal tissues.

Kawakami and his colleagues concluded that primary induction of mesodermal and endodermal tissues involves two factors, a vegetalizing factor and a differentiation activator. The differentiation activator is considered to be identical to the neural inducing factor, inducing presumptive ectoderm to form neural tissues. He also proposed that differentiation is dependent on both quantitative differences in the effects of differentiation activators and qualitative differences in competence at the time when the differentiation factor becomes effective.

On the 50th anniversary of the discovery of the organizer by Spemann and Mangold, Nakamura and Kawakami (1997) edited and published a monograph titled: «Organizer: A Milestone of a Half-Century from Spemann». It was first published in Japanese in 1977, and the English version edited by Nakamura and Toivonen (1998) was published the next year (Fig. 9). This monograph is not just confined to a historical review of the organizer research during the half century. The authors point out problems that remain to be



Fig. 9. Monumental books for Organizer works:

- (Left) written by H. Spemann,
- (Middle) "Organizer" edited by O. Nakamura and I. Kawakami in Japanese in 1977
- (Right) "Organizer" edited by O. Nakamura and S. Toivonen in 1978.



Fig. 10. Tokindo S. Okada and M. Asashima.

solved and discuss the prospects for further research. This book will serve as a guide for developmental biologists who study the organizer and primary embryonic induction.

In the 1960s, studies of the classically well-known phenomenon of lens regeneration started a new approach for analyzing cell differentiation in Japan. Goro Eguchi has precisely defined the events of lens regeneration in the newt. After pushing the lens into the eye cavity with a stick, he found that the lens regeneration started from the retina when the lens was lost from the retina (Eguchi, 1961). From this he established an *in vitro* lens regeneration system (Eguchi *et al.*, 1974). At the same time T.S. Okada was also studying lens regeneration using chick embryos, and successfully devised a cell culture system for dissociated retina cells (Eguchi and Okada, 1973). In this system, pigmented or neural retina dissected and dissociated from the embryo had the ability to form lens not only from the dorsal side of the cells, but also from the ventral side. When separated from the whole embryo, the retina cultured *in vitro* has regeneration activity that is independent of the locus of the retina. Finally, Okada and

Eguchi demonstrated that the pigmented retina can be induced to differentiate into the neural retina *in vitro*, and vice versa, and that these cells could also differentiate into lens cells. These experiments introduced the concept of “transdifferentiation” of cells (Okada, 1991), in which differentiated cells of the embryo can be driven to differentiate into different cell types. This has become known as one of the important regulatory systems in embryonic development.

Tokindo S. Okada (Fig. 10) has been a very active and influential investigator in the field of developmental biology for a long period of time in Japan and worldwide. He reported early in his career that determination of endoderm during embryonic development occurs later than that of the other germ layers. Then his studies turned to the regeneration of the lens where he proposed the pioneering work of “transdifferentiation”. The school of T.S. Okada introduced molecular techniques into classical embryology. Many active scientists developed their skills at his school. Masatoshi Takeichi (Kyoto University), Hisato Kondoh (Osaka University), Hajime Fujisawa (Nagoya University), Kenji Watanabe (Himeji Technical University), Kiyokazu Agata (Okayama University) and Kunio Yasuda (Nara Institute of Science and Technology) were among these students. He also organized international symposiums that were supported by the government and other foundations, and which have aided the growth of the young generation of scientists in the field of developmental biology all over Japan.

One example of Taniguchi leadership is his organization of Symposiums into Developmental Biology (I-IV) (T.S. Okada, 1997; Fig. 11).

After the discovery of the inducing phenomena by Spemann, the study into epithelio-mesenchyme interaction was expanded. They tried to culture “organ” or “tissue” *in vitro*, independent of the living embryo. These studies were done using not only amphibian embryos, but also avian and mammalian embryos. Etienne Wolff and K. Haffen (1953) had established the organ culture method. Using this new technique they examined and clarified the epithelio-mesenchymal interaction during organogenesis *in vitro* (examples; testis and Mullerian duct, skin and stomach). At the school of T. Fujii at Tokyo University, they focussed the epithelio-mesenchymal interaction for organogenesis. Yoshihiro Kato (1969) demonstrated the function of

Fig. 11. T.S. Okada organized numerous times the international congress and meetings.

(1997. Taniguchi Symposium, Kyoto). There are many developmental biologists. From right to left (standing person), T.S. Okada, T. Ariizumi, V. Schmit, J. Grudon, V. Nanjundial, A. A. Moscona, J. R. Coleman, J. D. Ebert, M. Okada, K. Nakamura, A. McLaren, S. F. Gilbert, L. Saxen, W. Gehring, K. Kato. (Sitting person) A. Galcia-Bellido, M. Asashima, N. M. Le Douarin, G. V. Lopashov.



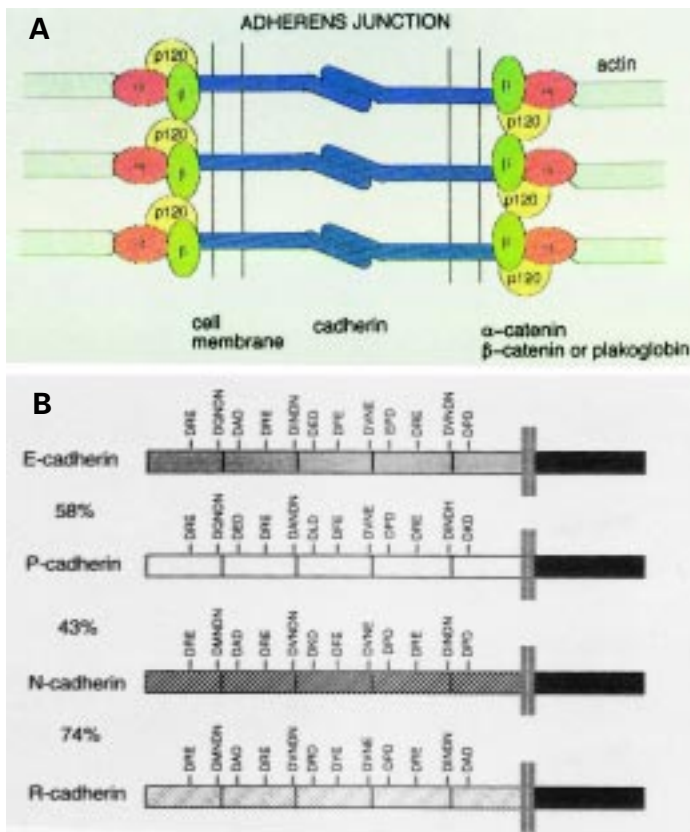


Fig. 12. Cadherins are cell adhesion molecules. (A) The complex of cadherin and catenins that form adherens junctions. (B) Comparison of amino acid sequences of four kinds of cadherins with their conserved domain structures.

mesenchyme during the formation of scale, bill and skin. Takeo Mizuno and his colleagues (1972, 1990) also took the experimental embryology approach to organogenesis *in vitro* in such organs as skin, digestive tube and liver. They proved that the organogenesis of these tissues also involved an epithelio-mesenchymal interaction. Sadao Yasugi (1990) investigated the molecular mechanism of regional differentiation of the digestive tube. He reported that the Shh (Sonic hedgehog) gene is important in the process of controlling the specificity of regionality of the digestive tube.

Fourth stage

In early studies, extracts from various vertebrate tissues such as guinea pig bone marrow (Toivonen, 1953), chick embryo (Geithe *et al.*, 1975) and carp swimbladder (Kawakami, 1976), were found to have a mesoderm-inducing activity. Although these "heterogeneous" inducers could convert the presumptive ectoderm to mesoderm experimentally, the molecule that induced the mesoderm in normal amphibian embryos remained unknown.

Since the mid-1980s, research into a mesoderm-inducing factor (MIF) has taken a new direction. In England, Smith (1987) identified a factor capable of inducing dorsal mesoderm from the culture medium of a *Xenopus* tadpole cell line and designated it XTC-MIF. XTC-MIF was believed to be a natural mesoderm-

inducing factor because of its origin. The most remarkable achievement in MIF research has been the identification of several peptide growth factors belonging to the fibroblast growth factor (FGF) and transforming growth factor-beta (TGF-β) families as MIF candidates. In 1987, Slack *et al.* reported that mammalian basic fibroblast growth factor (bFGF) has mesoderm-inducing activity.

Asashima, in collaboration with the Tiedemann group in Germany (Tiedemann, 1990, Grunz, 1996) first tried to isolate the MIFs from chicken embryo extract. After returning to Japan, Asashima and his collaborators independently attempted to isolate MIFs from carp swimbladder and mammalian cell lines for ten years. Finally they succeeded in isolating several peptides from a conditioned medium from the human K-562 cell line that had mesoderm-inducing activity (Asashima *et al.*, 1990). These peptides were shown to be closely related to the TGF-β superfamily protein, activin A. Activin A was originally identified as a gonadal hormone that promoted follicle stimulating hormone (FSH) production from the anterior pituitary gland. Subsequently, some of the MIFs derived from different sources (eg., XTC-MIF, vegetalizing factor from calf kidney and chicken embryo, PIF from a mouse macrophage cell line) were shown to be identical to activin A or were activin homologues. Furthermore, an activin homologue was confirmed to be present in early *Xenopus* eggs (Asashima *et al.*, 1991). The apparently ubiquitous nature of this factor promised a wide range of experiments that may not have been possible had there been the numerous mesoderm-inducing substances in different species and tissues, as many investigators had predicted.

The discovery in the late 1980s that growth factors may be MIF candidates led many researchers who had previously had little interest in embryonic induction, including those from molecular biology and the medical sciences, to enter the field of embryonic induction and cell differentiation in early development. Recent work is well-known internationally and publications by Japanese authors are easily accessible to researchers all over the world. Here we have briefly reviewed the contributions of Japanese experimental embryologists in their studies into the mechanisms of embryonic induction.

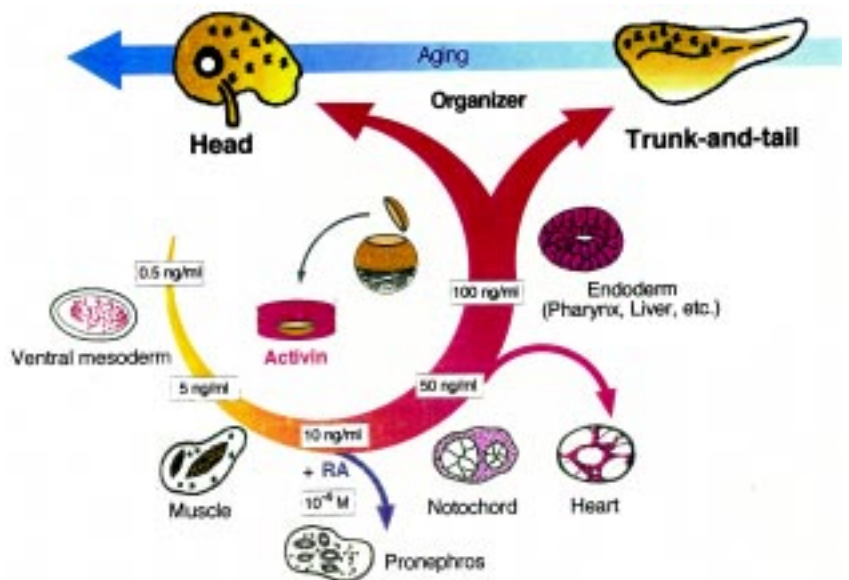


Fig. 13. The Activin In Vitro system. Activin can induce many types of organs and tissues, and regional structures (head, trunk-tail structure) into the undifferentiated cell mass (animal cap) of amphibian blastula.

Ueno (National Institute of Basic Biology) studied the function of bone morphogenetic proteins (BMPs) and their receptors in early development (eg., Suzuki *et al.*, 1994, 1997). The roles of BMPs in embryonic induction were also studied by Wilson and Hemmati-Brivanlou (1995), who showed that the default BMP function is induction of neuralization. Our understanding of the functions of bFGF also progressed. Kengaku and Okamoto (1993) showed that bFGF is not only mesoderm-inducing, but can also induce neural tissues. They dissociated the gastrula ectoderm and treated these dissociated cells with bFGF. After aggregation of these cells they found neural tissues and expression of neural marker genes.

Taira (University of Tokyo) *et al.* (1992) and Dawid at the National Institute of Health (NIH) in the USA, investigated Xlim-1, which is expressed in the organizer region. Sasai. (1994; Kyoto University), who was the post-doctoral researcher with Eddy De Robert in the USA, identified the chordin gene. Chordin is expressed at the organizer region of the upper blastopore lip. This gene is a candidate mesoderm-inducing factor and neuralizing factor, in addition to follistatin and noggin. These findings have stimulated the interest of young researchers in investigating embryonic induction, axis formation and signal transduction. Signal transduction factors such as Smad, IP3, MAPK, TAK, GSK-3 β , and β -catenine have been analyzed not only in embryogenesis, but also in many other fields of biology such as neuroscience and molecular biology. Our understanding of the functions of homeobox genes and Wnt signaling analysis has also vastly increased.

N. Satoh and H. Nishida made the fate map of the ascidian embryo, which is the mosaic egg. They also revealed some maternal genes with localized mRNA. In particular they showed that cell to cell interaction via signal molecules is involved in notochord formation (Nishida, H., 1987; Satoh, N., 1994).

Cell-to-cell communication is also important in embryogenesis. Townes and Holtfreter (1955) performed disaggregation experiments with ectoderm, mesoderm and neurula-stage endoderm using newt embryo. After these disaggregated cells were mixed, they sorted and reaggregated into the same original germ layers. This means that cells dissociated from the whole embryo retain their own characteristics and cell affinity properties. Takeichi (Kyoto University)(1987) isolated the cell adhesion molecule cadherin, which is now known to be part of a large family of factors that are very important not only for cell adhesion, but also for cell-to-cell communication and interactions (Fig. 12). Without these molecules, the embryo cannot make the organism as a whole.

Asashima's group has succeeded for the first time to induce blood cells, muscle and notochord *in vitro* using only activin. Induction occurred in these tissues in a dose-dependent fashion (Ariizumi *et al.*, 1991). In subsequent experiments whole organs including the kidney, heart and pancreas were induced *in vitro* with activin (Moriya *et al.*, 1993; Ariizumi *et al.*, 1996). They further succeeded in creating aggregates of these organs, such as head and trunk/tail structures (Ariizumi and Asashima, 1995; Fig. 13). Using these *in vitro* induction systems, they analyzed genes specific to individual organs and began to unveil the cascades of gene expression that mediate organ development. This success in creating organs *in vitro* has triggered rapid advances in the field (Asashima, 1994, Asashima *et al.*, 1999).

Asashima's group (Chan *et al.*, 1999) attempted to transplant an *in vitro*-induced kidney into embryos from which the site reserved for renal development had been removed. In this experiment, all of

the control embryos died of edema, while the embryos with the kidney transplants survived for more than one month. No other investigator has been able to obtain such success. This technique suggests future possibilities for creating organs from embryonic stem cells (including human ones) and using them for transplantation. The technique has been highly acclaimed as a significant landmark for future medical engineering.

Summary

The discovery of the organizer by H. Spemann and Hilde Mangold, prompted a number of studies of embryonic induction in Japan. C.O. Whitman, N. Yatsu, T. Sato, H. Oka, T. Yamada, and Y.K. Okada were the pioneers in the field of embryonic induction. T. Yamada postulated the double potential theory for embryonic induction. O. Nakamura has modified the fate map of Vogt using newt and *Xenopus* blastulae. T.S. Okada and G. Eguchi proposed the new concept of "transdifferentiation" based on *in vitro* experiments in the retina and lens. T.S. Okada is not only an excellent scientist, but he has also nurtured many active developmental biologists. M. Takeichi, from his school, discovered the cell adhesion molecule, cadherin. Nakamura and colleagues tried to determine the origin and formation of the organizer. They performed recombination experiments using the ectoderm, endoderm and mesoderm, and concluded that the phenomenon in which various mesoderm tissues are formed by the recombination of the presumptive ectoderm with endoderm was "*regulation of the vegetal-animal gradient*". Some groups have also tried to purify specific inducing factors. T. Yamada and colleagues isolated two different types of ribonucleoproteins. I. Kawakami and colleagues showed that the ribosome fraction has neural inducing capacity, and that the extracellular matrix contains mesodermal inducing factors. Finally Asashima and colleagues isolated and identified activin A as a MIF factor. This finding had a great influence not only in the field of developmental biology, but also in molecular biology. Using activin, Asashima's group has successfully generated various organs, tissues, trunk-tail and head structures *in vitro* using animal caps (undifferentiated cells). Some other important molecules such as BMP, chordin and bFGF are also being studied by young Japanese scientists.

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