A view of amphibian embryology research in Japan through the scientific biography of Professor Osamu Nakamura

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Professor Osamu Nakamura has been a pioneer and a leader in the field of amphibian embyology for more than a half century. He was born on March 3, 1911, in Osaka, Japan.

He entered the Zoological Institute of Kyoto Imperial University in 1930 and immediately began his study in the field of experimental morphology under the direction of Prof. Yô Kaname Okada, who had just come back from France to establish a new department of embryology and experimental morphology. After graduating in 1933, he continued his research in the laboratory of Prof. Yô K. Okada. Thereafter, he centered his efforts on the problem of tail formation and further on the development of the posterior half of the body.

His most important contributions to the subject are as follows:

1. He presented a new fate-map of urodele gastrula in 1938, revising the classical map of Vogt in essential parts.
2. He made clear that the presumptive rudiments originally located around the blastopore of the neurula are brought to the final position to form organs in the caudal region by morphogenetic movements quite similar to those that bring about the formation of organs in the anterior part of the body, in contradiction to the old theory of additional growth such as budding from the "tail-bud".

He himself was called away to the war in 1940, leaving a great mass of manuscripts with many figures and notes for an accurate report of his work. It was published in 1942, in his absence, through the friendly help of Prof. Yô K. Okada and Dr. Tsuneo Yamada.

A year later, in 1943, the Zoological Society of Japan awarded its Prize to Nakamura for this work. And then, in 1944, the Minister of Education awarded him the degree of D. Sc.

3. He also demonstrated that differentiation of these organs is induced by a chain reaction emanating from the caudal portion of notochord as the 'tail-organizer'. Accordingly, he presented a new comprehensive diagram of the chain system of induction initiated by the action of the organizer in head, trunk and tail regions (refer to Fig. 1.9 in his book "Organizer — A Milestone of a Half Century from Spemann", 1978).

In 1945, Dr. Nakamura was released from military service. In 1946, he received the appointment of Professor of Biology of the Teachers' College of Osaka.

The college was incorporated into Osaka Gakugei University, which later changed its name to Osaka Kyôiku University.

Just after the war, laboratory conditions at newly built Japanese universities prevented them from carrying out separate scientific research: there was not enough space, equipment was deficient and working members were few and far between. So Prof. Nakamura called together embryologists from six universities in the Kansai district and regrouped them to make a joint study of "Tissue Differentiation and Organ Formation in Early Development", receiving funds from the Ministry of Education.

During this period, he himself did a vital staining study in collaboration with Asst. Prof. Y. Tahara on the development of the digestive organ in amphibia, which proved a fact not in accordance with the theory of classical embryology. According to them, the main portion of archenteron, i.e. the mid-gut, does not form the intestinal cavity, but is closed and obliterated at a later stage, while the definite cavity of intestine is newly formed by perforation through the yolk mass.

Professor Nakamura also noticed the importance of studying the development mechanism at molecular level, and wrote monthly lectures from 1965 to 1966 titled "New Aspects of Developmental

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Fig. 1. Professors Toivonen, Takaya and Nakamura (from the left) in Osaka (1974).

Biology", intending to stimulate researches in a new direction based on a concept of molecular biology. His lectures were the first ones published in Japan on "Molecular Embryology" (as he named it).

Meanwhile, Prof. Nakamura and his collaborators at Osaka Kyôiku University concentrated their energies on an inquiry into the epigenetic origin of the organizer. The main points of their findings can be summarized as follows:

1. The dorsal marginal zone acquires its capacity for self-differentiation into the axial mesoderm at a stage between 32 cells and late morula.
2. The same zone becomes effective in neural induction during blastula stage.
3. 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.
4. The dorso-ventral polarity of the amphibian egg is established soon after fertilization. It causes a regional difference in the capacity of mesodermal differentiation of the marginal zone.
5. The self-differentiation capacity and the inductive activity peculiar to the organizer are established by coordination of the vegetal-animal gradient of vegetalization and dorso-ventral polarity of the embryo.

Recently, the vegetalizing factor has been substantially identified as the protein activin A by Asashima *et al.* (1989, 1990). Dorso-ventral polarity may be associated with a gradient in oxygen consumption, reported by Landström and Løvtrup (1975). It is likely that such a physiological gradient causes dorso-ventral gradient in quantity of activin A transferred to the marginal zone from the vegetal pole area. Thus, Prof. Nakamura's study on the causal factors of epigenetic formation of the organizer paved the way for the amazing progress of experiments, named 'mesodermal induction', and the discovery of the vegetalizing substance.

Finally, he published an authoritative monograph titled 'ORGANIZER —A Milestone of a Half-Century from Spemann', on the 50th anniversary of the discovery of organizer by Hans Spemann and Hilde Mangold (1924). It was first published in Japanese (1977), and later in English (1978).

One of the great merits of this book is that it is not confined to a historical review of research on the organizer carried out during the half century. Various fundamental problems in this field are discussed in separate chapters. Further, the author points out problems that remained to be solved and discusses the prospects for future research. This monograph not only marks a milestone in the history of research but also serves as a guide for developmental biologists who come back to the problem of primary embryonic induction.

Prof. Nakamura was elected President of Osaka Kyôiku University in 1969 and also occupied the Presidency of Kôshien University for 5 years from 1983 to 1988. In 1987, he was decorated by His Imperial Majesty, Emperor Shôwa.

At present, he enjoys good health. He is still active in his scientific life, speaking, writing and usually attending biological meetings.

Dr. Osamu Nakamura, today I want to ask you to review your scientific life in retrospect. First, thinking back to when you entered the University, could you comment on the atmosphere, the situation, and the level of developmental biology in Japan?

I entered the university in 1930 and Dr. Kaname Okada (who used the pen-name Yô K. Okada) came back to Kyoto University from France in 1929 to serve as a professor and to establish the 3rd section of the Zoological institute, and he initiated studies not only of embryology but also experimental morphology in a broad sense. In Tokyo, in that period, Dr. Hidemichi Oka was studying amphibian limb regeneration. Dr. Sato went from Tokyo to Spemann's laboratory in Germany and subsequently returned to become a professor at the department of Biology of Nagoya University. He was studying Wolffian Regeneration; regeneration of the lens. In Sendai, Dr. Isao Motomura published a book about animal embryology which included descriptions of experiments, and he himself studied fertilization and also conducted experiments comparable to those

Fig. 2. Prof. Nakamura in his laboratory *(in the back, from the right, Dr. Takasaki and her 2 students, 1987).*

I later did for my fate-map. In Hokkaido, I remember that Dr. Inukai was studying the development of *Hynobius*, one kind of amphibian. Anyway, this area of study was just opening up in those days, and only a few experiments on regeneration were being done, so we called it experimental morphology rather than developmental biology. Dr. Dan was experimenting with sea urchins. And, a few years later, Dr. Tokio Yamamoto in Nagoya was studying fertilization using fish such as *Oryzias*. But virtually nothing of this sort was being done with the eggs and embryos of amphibians because we didn't know how to keep the eggs sterile. As culture medium, we used Ringer's solution at a half concentration. But most of the eggs we manipulated died. So it was very difficult in the beginning to do experiments in early embryonal stages. In comparison, it was easier to do regeneration or transplantation experiments using adult newts, and we got some good data in this way. That is why this was our primary form of study. Dr. Masao Sugiyama was also in Nagoya at that time, studying fertilization and artificial parthenogenesis with Echinoderms. I think the professors at the Department of Biology in Nagoya University were very shrewd back in those days. They themselves advocated being in charge, in each section, of their own areas of strength. But they were all developmental biologists and cooperated with each other in their research. That was an excellent idea. Dr. Tomojiro Kawamura, in Hiroshima, was also a developmental biologist, who started his study a few years later, if my memory is correct.

When did you begin to use amphibians for your investigations? What was it that first made you think of doing research with amphibians?

From when did I begin to study amphibians? It was soon after I had entered Kyoto Imperial University in 1930. Though I was a freshman, Prof. T. Komai, the chairman of our institute, especially permitted me to assist with the experimentation in the laboratory of Prof. Yô K. Okada, because of the absence of a formal assistant in this section which was still being set up. Under Prof. Okada's instruction, I was engaged in experiments on limb regeneration in newt, cooperating with Mr. Toyomasu who was a post-graduate student.

In the same postgraduate course, Mr. H. Sugino, who was later appointed professor of Osaka Kyôiku University, was studying on regeneration in Planaria. In the undergraduate course, Mr. Tanaka and Mr. Komori were doing regeneration experiments, the former with an actinia and the latter with an earthworm. Afterwards, a regeneration experiment with earthworm was done by Miss Itsue Katô (later Mrs. Kawakami), a private assistant of Prof. Okada.

It cannot be said that in those days I began to work by myself postulating my future course theoretically from a broader perspective. I studied mechanism of development with amphibians under the direction and guidance of my respected teacher, Prof. Yô K. Okada.

After my graduation, I continued to study in the same laboratory. Prof. Okada assigned a theme for my research as given in Fig. 4. My classmate Mr. H. Takaya (later Prof. Takaya in Kônan University) was also doing experimental studies on limb-asymmetry in newt embryos. He and I were the first students instructed by Prof. Okada to make experiments with amphibian embryos.

At that time, Mr. Y. Mikami and Mr. S. Asayama studied in the undergraduate course, the former on lens induction in amphibia and the latter on sex determination. (See Fig. 1 in Tokindo S. Okada's paper in this issue).

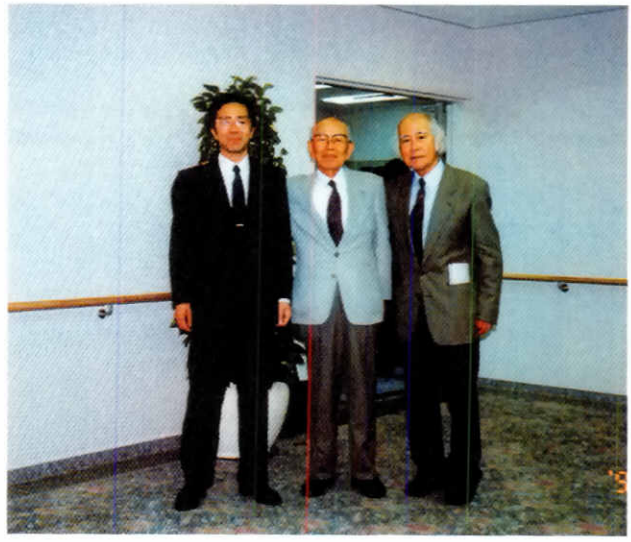

Fig. 3. Professors Nakamura (center) and Yamada (right) with the author (left) in 1994.

Mr. Izumi Kawakami (later Prof. Kawakami who cooperated in editing my book 'Organizer') arrived 2 years later to experiment on the development of sense organ in amphibia.

You have drawn up the fate map of blastula of Japanese newt, *Cynops pyrrhogaster*. How different is it from the one presented by Vogt or that of Pasteels? When you devised an improved fate map, what sorts of difficulty did you experience?

The inspiration for making the fate map was an argument between Vogt and Bijtel. This, I will say, was the main discourse on the fate map. These two authors differed as to the original site of presumptive material of the tail mesoderm. At first sight, the issue seemed very simple, but eventually it contained a serious problem. Vogt himself said that if Bijtel's data were correct, Vogt's Fate Map needed to be modified, though he believed this was simply not possible. I expected that investigation of tail formation would be linked with an issue of primary importance as a subject of the research system.

I think that it is useful to mention the center of the argument between Vogt and Bijtel and the distinction between their results and mine. Vogt said that the neural plate is entirely devoted to the formation of neural tissue while a small amount of tail mesoderm remained in the ventrolateral lip of the blastopore and this area invaginated later into the embryo. Dr. Bijtel on the other hand said that the hind fifth of the neural plate forms tail somites rather than neural tissue. This idea was truly epochal. My results were that the hind portion of the neural plate forms tail somites as Bijtel stated, and not only tail but also the hind somites of the trunk. The presumptive somites in the hind part of trunk were located on the dorsal margin of blastopore, occupying the hind end of the neural plate, while those of the tail were situated more dorsaly beyond the limit of trunk somite region (Fig. 6).

This made me see that Vogt's Fate Map really needed to be modified, as he himself had declared, although he was against it. Then, I attempted to redraw Vogt's Fate Map for the gastrula and

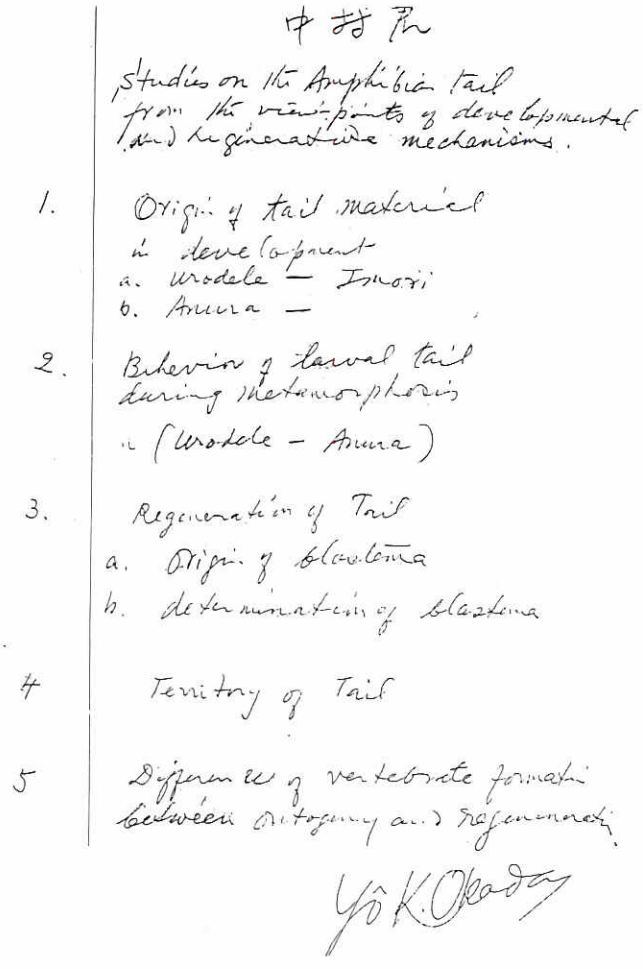

Fig. 4. Instruction of theme given to Nakamura by Prof. Y.K. Okada *(written by himself with signature) (1933).*

blastula. In preparation, I investigated presumptive rudiments around the blastopore in neurula stage and drew that fate map (Fig. 6). The presumptive area of each organ concerning the formation of the hind part of the body was assigned definitely around the blastopore.

Next, I investigated the accurate position of presumptive rudiments on the surface of gastrula, with improved vital staining method (Fig. 8), and also drew a fate map (Fig. 7). To make this intelligible, I assigned three divisions in the somite area; anterior trunk (A), posterior trunk (P) and tail regions (T). The arrangement of presumptive areas in gastrula stage was in good accordance with that in neurula stage.

On the other hand, it is easy to see that this map is quite different from Vogt's (compare two maps in Fig. 7). Vogt described that the tail somites are derived from ventrolateral marginal zone, designated by 'Sch' in his map, while, in my map, the same organ is shown to originate from a dorsomedian area marked 'T' which will be transferred later to the hind end of neural plate.

There remains another important problem I must tell you. It is the morphogenesis of tail mesoderm. In classical embryology up to the 1930s, it was generally believed that the anterior half only of the body develops from germ layers while the posterior half is formed by additional growth such as budding from a growth center, called "tail bud". Both Vogt and Bijtel intended to disprove additional growth theory. But, they did not succeed, because they still believed that the blastopore is 'closed' before the formation of neural plate, though Vogt found 'post-invagination' of ventro-lateral blastoporal lip of neurula.

I myself discovered invagination of the hind part of neural plate through the dorsal margin of blastopore. Keeping pace with it invagination goes on from all sides of blastopore. Thus, all material of trunk mesoderm is rolled in, and that of the tail following it attains the margin of blastopore. At the moment, the neural folds fuse with each other. Thenceforth we cannot observe invagination from outside. Here I must point out a distinction between the meaning of limit line of invagination (Eg line) in Vogt's map and in mine. Eg line in Vogt's map coincides with the blastopore at the time of its "closure" before neurulation, with the exception of its ventro-lateral portion, while the same line in my map means the blastoporal margin at the stage when the neural folds fuse.

In addition, I proved by tracing displacement of stained marks that formative movements quite similar to those before the fusion of folds continue further, to bring the constituents of tail to their destined place. Then, development of both anterior and posterior half of the body can be explained by the same principle of morphogenesis.

I published these results in 1938, as a quick report of 5 pages written in English. Dr. Pasteels read my paper, and tested in the

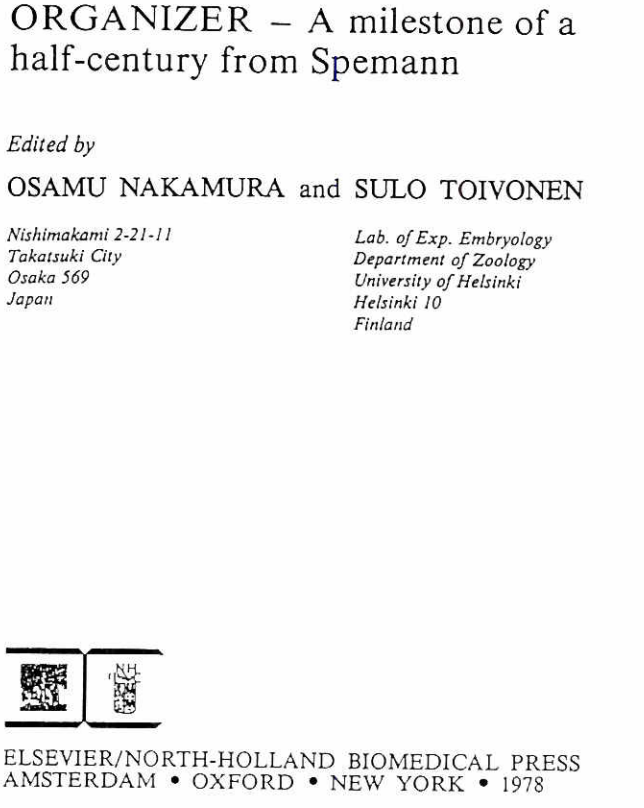

Fig. 5. Cover of *"Organizer — A Milestone of a Half Century from Spemann"*. (1978).

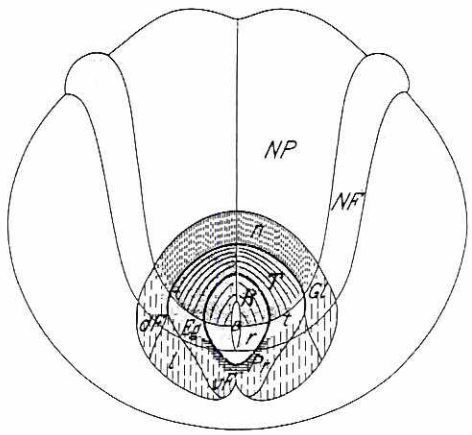

Fig. 6. Fate map of the presumptive regions around blastopore (B) in neural stage of *Triturus pyrrhogaster*. *R: trunk-somite. r: trunk-lateral plate. Eg: limit of invagination. T: tail-somite. t: tail-lateral plate. n: tail-neural tube. Gl: tail-neuralcrest. NP: neural plate. NF: neural fold. dF: epidermis of dorsal fin. vF: epidermis of ventral fin. Pr: proctodeum (anal tube) l: lateral epidermis of the tail.*

following year, 1939, whether my results were correct using European materials, and his results supported mine. Then he introduced my paper to Europe. He himself drew a fate map, applying my theses to European amphibia. Pasteels (1942) wrote "my map is quite similar to Nakamura's version". But in Europe, his fate map was used more than mine, because it represented European material.

A full report of my work was published as an article of 69 pages in German with 35 figures in 1942, thanks to the kind help of my mentor Prof. Yô K. Okada and my closest friend Dr. Tsuneo Yamada, when I was called away to World War II. I owe them my warmest and unending thanks.

You continued to do your amphibian research even during World War II, when it was very difficult to study in Japan. You received the Japanese Zoological Society Prize for amphibian embryology in 1943. Could you describe for me the embryology atmosphere in Japan in those days?

Dr. Tsuneo Yamada (Fig. 3), who had studied under Prof. Vogt, came back to Japan from Germany in 1938. There were a great number of newts in Kyoto, so you could say that Kyoto is a newt paradise. That is why Dr. Yamada came to Kyoto University for his research. He taught us the sterile method for very early embryos then used in Germany. It was a method using Chloramine T devised by Holtfreter which made it possible for us to use young embryos such as the gastrula and neurula used by Spemann. Prof. Yô K. Okada soon began such experiments, implanting various materials into blastocoeles. Dr. Yamada also showed us a new method of explantation and a solution for culturing embryos both devised by Holtfreter, in combination with the sterile method in 1929. Dr. Yamada himself conducted explantation experiments to ascertain the differentiating capacity of various areas of mesodermal mantle. He isolated the presumptive somite and cultured it in Holtfreter's saline. The explanted cells differentiated not into somite, but into pronephric tubules which are an intermediate tissue between muscle and blood cells. When the presumptive pronephric area was explanted, it differentiated into blood islands. On the basis of many experiments yielding such data, he developed a feasible hypothesis of gradient of morphological potential along with the dorso-ventral axis.

This hypothesis provided the basis for his famous "double potential theory" which he established after the war to explain the entire embryogenetic process, including neural induction as well as mesoderm differentiation (Fig. 9). (For a more detailed description of Dr. Yamada's experiments and his theory, the reader is referred to Toivonen's classical book "Primary Embryonic Induction" and Nakamura's recent book "ORGANIZER — A Milestone of a Half Century from Spemann" as well as the article by Prof. Tokindo S. Okada in this issue.)

The most important work in this period was the discovery of progressive change in inducing activity of the anterior part of organizer reported by Prof. Yô K. Okada, Dr. H. Takaya (Fig. 1) and Dr. T. Hama (1942, 1943, 1944).

This fact is supremely important even for present day researchers. Nevertheless, it has long been unnoticed, because it was reported shortly before the end of the World War II. So it is necessary, I think, to review their results here.

For a long time (even today?), many people have believed that the dorsal lip of an early gastrula, i.e. the anterior part of the organizer, was the head organizer. But a piece of the dorsal blastopore lip, brought into contact with the presumptive ectoderm

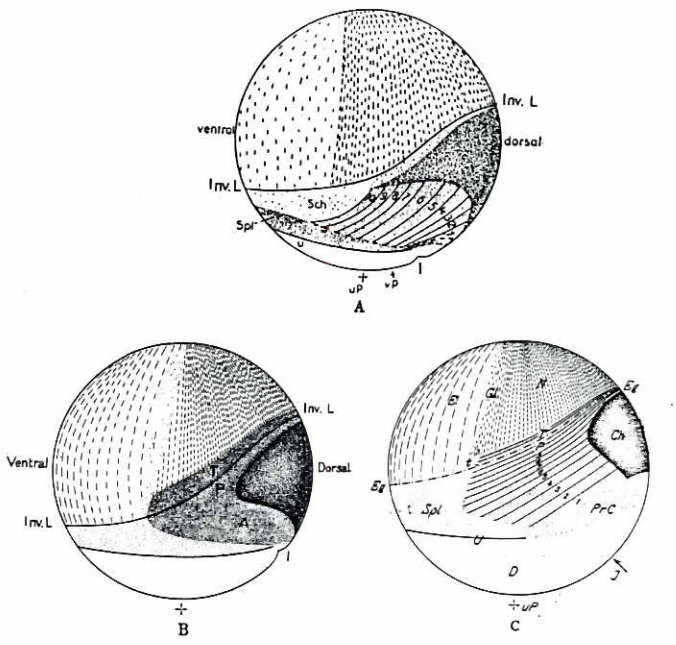

Fig. 7. Fate maps of the presumptive regions. (A) *Vogt's fate map (1926).* **(B)** *Nakamura's fate map of gastrula (1938).* **(C)** *Nakamura's fate map of blastula (1942). A: Somites in the anterior part of the trunk (1-9) 1-9, numbering of trunk somites: P. posterior trunk somites: T, tail somites; t, lateral mesoderm in the tail. Eg=Inv.L: the limit of the invagination. E, epidermis; Gl, neural crest; N, neural plate; Ch, notochord; Prc, prechordal mesoderm, Sch; tail bud somites (Vogt). Spl, lateral plate mesoderm in the trunk. D, endoderm of archenteron; I=J; starting point of invagination; U, lateral lip of the blastopore.*

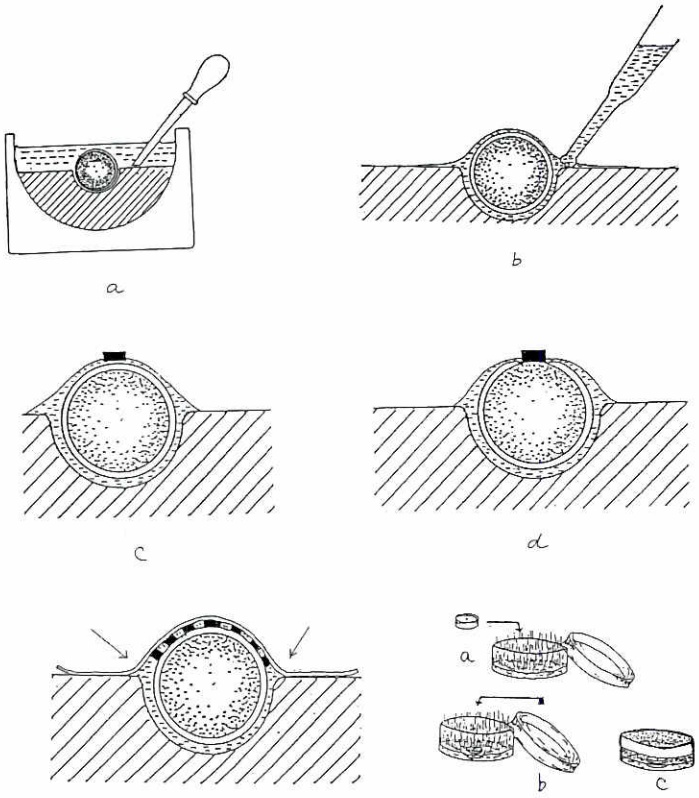

Fig. 8. Vital staining method improved by Nakamura for marking a small area definitely. (a,b) *Removal of the water (leaving the vitelline membrane wet).* **(c)** *A piece of dyed agar applied to the membrane.* **(d)** *The agar swelling promptly by absorbing the water between the membrane and the embryo.* **(Bottom right)** *The dish containing the embryo is transferred into the moist chamber.* **(Bottom left)** *A series of dyed agar pieces inserted between a transparent sheet of cellophane and the embryo.*

just after cutting, did not induce a head structure, either in the sandwich culture or in "affixed" transplantation. The dorsal blastopore lip of the gastrula can induce only the trunk and tail, not the head structure. The head structure can only be induced by mesodermal areas located at the foremost part of archenteron roof. The ability to induce a head can thus be obtained after invagination. Drs. Yô K. Okada and H. Takaya have isolated the dorsal blastopore lip from an early gastrula and left it in Holtfreter's solution overnight. The explant subsequently showed neural inducing activity for head formation. This means that the dorsal blastopore lip had turned into a head organizer as time went on, independently of invagination.

In the previous experiments in Spemann's school, an excised piece of the dorsal blastoporal lip was inserted in the blastocoel. After a time, the inserted piece makes contact with the presumptive ectoderm. During that time, the inducing activity might transform from that of trunk-tail to head. I hope present-day researchers pay special attention to this shift of inducing activity.

At the same age I wrote a manuscript for a paper entitled "Determination and differentiation in amphibian tail development". Then I got another call into the army. Dr. I. Kawakami helped me to publish this paper, which appeared in Japanese in 1947, 2 years after the end of World War II. In our book, "Organizer", I presented a diagram of the induction chain based on this paper (Fig. 10).

I believe that some cities in Japan had the lead in the study of embryonic induction. Could you comment on the circumstances after World War II?

After the war, it was planned that we would establish universities in each prefecture in Japan, and we made efforts to set up our laboratory in new universities. Study was thus maintained by people free of such trouble. For example, these people included Dr. Tadao Hama who was studying in Tokyo University under Dr. Yô K. Okada who had transferred from Kyoto, and, in Kyoto University, Dr. Izumi Kawakami who was a student under Dr. Mamori Ichikawa, the professor who succeeded Dr. Okada. Such people managed to do induction experiments despite difficulties. Dr. Tsuneo Yamada, who came back from the war in New Guinea, accepted a professorship at Nagoya University in 1946 and was absorbed in his research. Then he started a new study on inducing factors, working with excellent people such as Dr. Yujiro Hayashi and Dr. Kenzo Takata. Dr. Yamada's research in Nagoya University was surely the most important in Japanese embryology. So, in the 1950s, the university was regarded as the leader in this field. In the 1960s, when Dr. Yamada left Japan for America, this leadership seemed to have moved to Kyushu University, where Dr. Kawakami was appointed as professor.

I think Dr. Yamada's double potential theory was expressed in 1950, Dr. Toivonen's gradient theory in about 1955, and Nieuwkoop's activation-transformation theory in 1952-1958.

To overcome the difficulty in conducting research in newly built universities, I called together embryologists from six universities in the Kansai district, around Osaka and Kyoto, and realigned them to a common study entitled "Tissue Differentiation and Organ Formation in Early Development".

Meanwhile, I saw the importance of studying differentiation on molecular level and I wrote a series of monthly lectures, partly assisted by members of this working team, for 12 months from 1965 to 1966, in the biological magazine "Iden". This was the first review in Japan in which DNA, RNA and genetic code were discussed in close relation to embryology. Books by Ebert (1965)

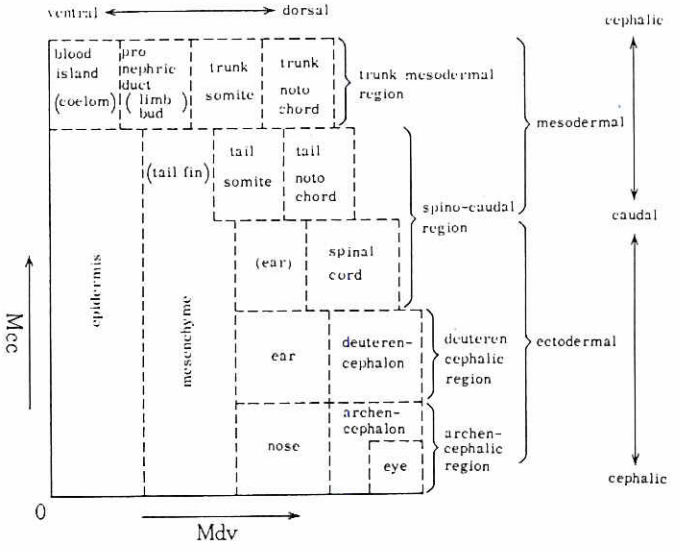

Fig. 9. Diagrammatic expression of the double potential hypothesis; differentiation caused by Mdv and Mcc when they act on the presumptive ectoderm. (After Yamada. 1958).

and Waddington (1966) were imported into Japan after finishing my lectures.

In 1965 I read the book "Experimentelle Beiträge zu einer Theorie der Entwicklung", written by H. Spemann, when I was at Tokyo University, and I was engrossed by it, so I wanted to study primary embryonic induction by the time I was graduating in 1967, and looked all over the country for places where such research could be done, but no one was studying embryonic induction any longer in the laboratories of major universities. Especially, research on inducing substances decreased suddenly, even though it had previously been very popular. What were the circumstances surrounding research conducted in Japan at that time?

In the 1960's, many people studying embryonic induction left the field. Only a few groups continued their research. As I wrote in my book 'ORGANIZER', 20-year cycles have occurred in this field. From 1940 to 1960 many scientists throughout the world tried to isolate and identify inducing substances using biochemical techniques. On the other hand, there was other research on the regionalization of the embryo during development concerned with inducing factors. Though some scientists had tried to isolate the inducing substances from the eggs or embryos themselves, these starting materials were too small to isolate such factors. Many scientists had tried to isolate the inducing factors from various sources such as the liver or kidney of the guinea pig, chick or calf. These materials have the same inducing activity as the blastoporal lip in the embryo. It is possible to get a lot of starting materials from these sources.

What kind of substances in embryonic cells change after induction? This question has the same meaning as cell differentiation. But, at that time, biochemical research on the embryo was very scarce.

Many researchers have thus tried to isolate the inducing factor from adult tissues, or have observed the embryo or tissues by electron-microscopy. While no beautiful electron microscopic pictures were made using embryos or eggs, it was easy to make nice pictures using adult tissues. So many people tried to study at the microbiological level by electron-microscopic or biochemical techniques using adult tissues or cells. In the 1960s, the study of cell differentiation gained popularity. Researchers wanted to determine the general principles of cell differentiation using not only embryos, but also cells of animals and plants as experimental materials. Throughout these studies many people became interested in cell biology, biochemistry and morphology, leading the way from classical experimental embryology or experimental morphology to what they wanted to call "developmental biology", as a new term.

At this time Dr. Yô. K. Okada and Dr. T. Fujii were attempting to identify inducing substances by the insertion method into blastocoeles. They concluded that all things, including inorganic materials such as kaolin and quinone could induce neural induction. This neural induction was caused by destruction, which affected ectodermal cells secondarily to differentiate into neural tissues.

On the other side, we have examined the effect of ions causing cell differentiation. Dr. Masui in our team has tested some kinds of ions for inducing effect on isolated presumptive ectoderm, and found that the application of Li ion to ectoderm induces mesodermal tissues.

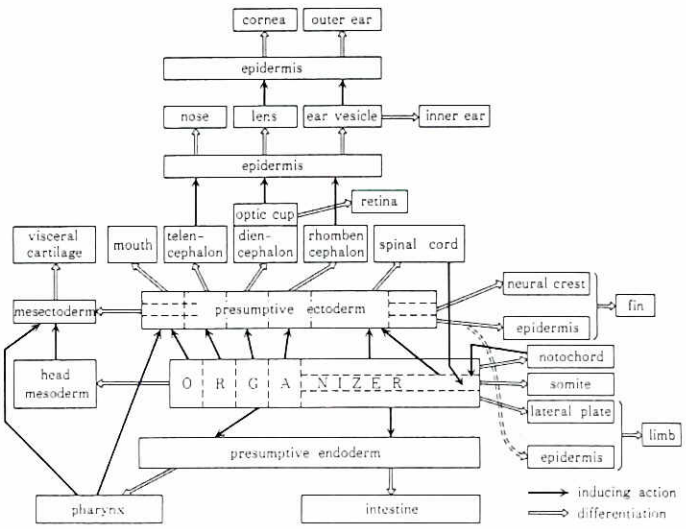

Fig. 10. Diagram of the chain systems of inductions initiated by the action of the organizer.

As a result, scientists who wanted to identify the special inducing factor for cell differentiation lost interest in the idea, and many people left classical embryology to go into modern biochemistry or cell biology.

I subsequently entered the laboratory of Prof. Dr. H. Tiedemann in Berlin, Germany. What was the situation of developmental biology in the world at that time?

I studied embryonic induction problems concerning how the organizer itself acquires its inducing activity and self differentiation capacity. This problem has also been studied by Dr. Nieuwkoop's group in Europe. Dr. H. Tiedemann's group approached induction at the molecular level. He succeeded in purifying the mesodermal inducing or vegetalizing factor as a protein from chicken embryo extract. You yourself were a member of his group, which was the first to succeed in identifying Activin A as the mesodermal inducing factor. As a consequence of his studies and other investigations, it is now possible to do research at the molecular level. In the 1970s there were only a few groups working on embryonic induction. These were the groups of Drs. Nieuwkoop, Toivonen, Tiedemann, and mine. The major people in the fields of developmental biology worked on cell differentiation and gene expression at the cellular or at molecular level. They did not return to experimental embryology which is the origin of developmental biology itself.

You have researched in great detail the inducing activity and self-differentiation capacity of the organizer during early development. The data is just as accurate as that of recent research. What was clearly proven in your experiments?

According to Prof. Yô. K. Okada *et al.*, in the early gastrula with a crescent-shaped blastopore, the so-called head organizer has the properties of a trunk organizer and differentiates into notochord. However, the region which is presumed to form the notochord itself, termed the trunk organizer, does not yet have enough inducing activity. It eventually comes to possess the ability to both differentiate into notochord and induce the trunk region.

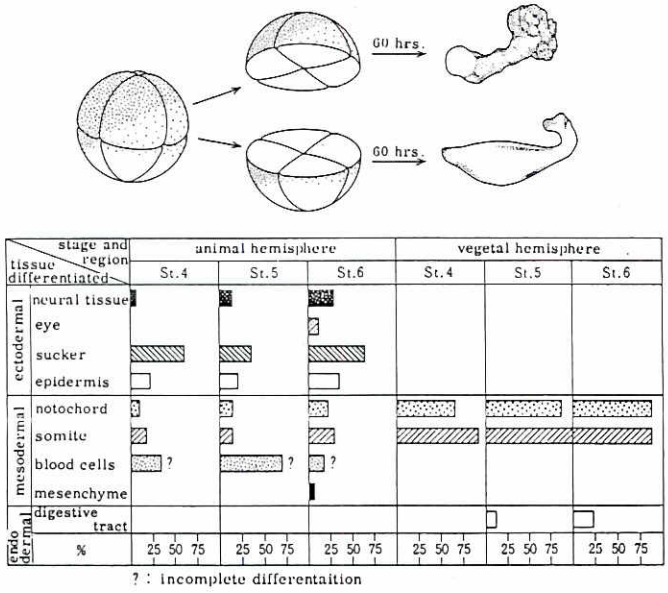

Fig. 11. Isolation of animal and vegetal hemispheres at stage 4 (8-cell stage), stage 5 (16-cell stage) and stage 6 (32-cell stage) in *Xenopus laevis*. (Above) *Changes in external shape.* **(Below)** *Frequency diagram of occurrence of tissues (Nakamura et al., 1971).*

Furthermore, after invagination, the so-called head organizer changes to acquire the ability to induce the head region.

It is clear from these observations that the inducing activity of the organizer changes with the passage of time. Then the following questions arise as to what kind of inducing activity the organizer has before the gastrula stage and also how it acquires this activity during early development.

I would like to go back to the story of how the organizer is formed.

As a result of our investigation, the presumptive region for the organizer was shown to acquire the differentiating capacity for axial mesoderm at stage 6 1/2 (in *Xenopus*) and stage 7 (in *Cynops*) and that same regions became effective in neural induction at stage 9.

Thus, it was evident that the organizer is not preformed but rather is formed epigenetically and gradually from the morula to blastula stages. We also examined electron-microscopically what kinds of changes were observed in the cells of the marginal zone at the different stages.

The most remarkable event was the appearance of prenucleolar bodies in the nucleus. These resembled the nucleolus but were very small and appeared from stage 7 to 12, before the nucleolus did.

According to cytochemical studies, the prenucleolar bodies include RNAs at their center surrounded by proteins and resemble masked mRNAs in structure. While in the cytoplasm, the collapse of the margin of the yolk platelet and changes in the structure of the Golgi apparatus and endoplasmic reticulum were observed. Such changes were observed just in these stages, but it was unclear whether or not these phenomena relate directly to organizer formation.

There are several phenomena which appear likely to be related to organizer formation. We conducted experiments in which RNA synthesis was blocked with actinomycin. On the basis of the results obtained, we went ahead and presented a hypothesis. At first, RNAs synthesized from stage 7 to 8 begin to synthesize neural inducing agents at stage 9 to 10. The second, unique differentiation of each tissue proceeds on the basis of information from mRNAs which are synthesized after the onset of gastrulation. Finally, the neural inducing agents or mesoderm inducing agents exert control at the transcription level or regulate at the translation level of these mRNA synthesis. Since the hypothesis described above is a working hypothesis, further examination is needed.

The suggested experiment, showing the mechanism of organizer formation, is one in which the presumptive ectoderm is cultured in combination with the presumptive endoderm.

These recombinants do not absolutely include the dorsal marginal zone. They formed the axial mesoderm which is normally derived from the marginal zone, accompanying neural tissues and sense organs. It is reasonable to consider the marginal zone as being newly recovered by regulation, and that it acts as the organizer and differentiates into mesoderm itself since the recombinants are eliminated the marginal zone from the whole embryo.

If an animal hemisphere at the beginning of stage 4 is cultured, mesodermal tissues are formed in it (Fig. 11). The blastomeres of the animal side has not yet received the induction from vegetal blastomeres because the egg doesn't divide into animal and vegetal hemispheres at the end of stage 3, that is, 4 cell stage. But, since each blastomere includes animal and vegetal poles, there is a possibility that the vegetalizing agents diffuse more or less from the vegetal pole towards the animal pole. Therefore, it is reasonable to guess that the vegetalizing agents are diffusing to the upper region of the equator. I think the circumstances can explain the vegetalization but not the induction.

Another experiment is one in which the dorsal blastomeres in the animal hemisphere are combined with the ventral ones in the vegetal hemisphere in a 32 cell stage embryo. All recombinants which developed well formed the notochord and, in some of them, differentiation of neural tissues was observed.

Therefore, I think that the marginal zone itself possesses dorsoventral polarity, independently of the influence from the blastomeres in the endodermal area.

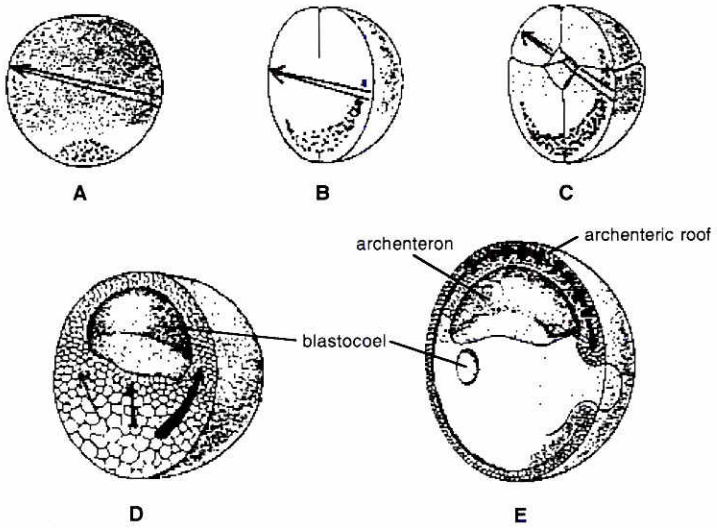

Fig. 12. Hypothetical model of inductions. (A-C) *Vegetalization by activin.* **(D)** *So-called mesoderm induction.* **(E)** *Neural Induction.*

I met you for the first time just after returning to Japan following my studies on embryonic induction at Dr. Tiedemann's laboratory in Germany. You asked me then to help you in writing a book titled "Organizer". What made you write this book?

Under such circumstances we welcomed the year 1974. It was exactly 50 years after the discovery of the organizer by Hans Spemann and Hilde Mangold in 1924 and thus an anniversary year. I had planned to publish the book to summarize all the organizer research done during those 50 years. My aims in writing this book were not only to commemorate or review past accomplishments, but also to renew interest and make places for the developmental biologists who had studied the cell differentiation, biochemistry or molecular biology. This book was conceived also as an aid to scientists wanting to study embryology or embryonic induction. I poured all my energy into editing the book with emphasis on the following items (Fig. 5). What is the sum total of the work accomplished in the 50 years since discovery of the organizer? What data are clear? If molecular biologists want to work in the field of embryonic induction, what problems are they likely to face from the molecular side? What important problems have remained unsolved over the 50 year period? In the future, after 1974, what kinds of studies must be done for issues to be resolved in the field of developmental biology? I have always believed that in the future many developmental biologists will return to the challenge of embryonic induction. So I wanted to show them where to start and provide a starting point for coming back from the field of molecular biology to classical embryology.

Your book, "Organizer" shown in Fig. 5, was first published in Japanese, and then in English by Elsevier. Although it was not much appreciated at that time, it enjoys a good reputation today. Can you comment on this?

About 15 years have passed since its initial publication. Over the years, a lot of data about the organizer and embryonic induction have accumulated. We must think about the organizer in light of these new data, adding them to the content of the book. As far the book is concerned, I want to emphasize the contribution of Dr. T. Yamada. He wrote a small chapter in the book. Though his chapter is short, the content is very important and suggestive. When young people want to start investigating embryonic induction problems, they should read through this book, especially Dr. T. Yamada's chapter. They can ascertain the essential points, as well as obtaining important guidance and advice on attitude.

You say that research on the organizer comes every twenty years. We are now in the fourth period of the cycle you've described. Can you comment the direction of today's organizer research?

At present, as you know, study of the organizer has been gaining force with the concept "mesoderm induction". It has been carried out at the molecular level and, as a result, inducing factors were identified, such as the activin reported by Prof. Asashima *et al.* This is exciting, but we need to observe great caution when applying these results to formation of the organizer in embryos. Especially, I want to emphasize that this term, "mesoderm induction" ought to be used only in an experimental *"in vitro"* system. Prof. Nieuwkoop was the first to use the term "mesoderm induction". But he subsequently amended his opinion. According to one set of results, on induction of not only mesoderm but also endoderm in the recombinant, he stated that it was better to use the word "vegetalizing" than the term "mesoderm induction". Prof. Toivonen said that a more concrete term such as "mesoderm-endoderm induction" ought to be used. Prof. Nieuwkoop expounded in his early works that the embryo was made up of an animal hemisphere having the properties of ectoderm and a vegetal hemisphere having the properties of endoderm, and the mesoderm is newly formed by induction from the vegetal endodermal hemisphere to the animal ectodermal hemisphere. That is to say, ectoderm changed into mesoderm. This explanation appealed to young biologists and was accepted as if it were a completely established fact, because it was easy to understand and attractive. Owing to this outcome, it seems to me that studies progressed toward proof of mesoderm induction using a molecular level approach. I think there are problems with this trend, however. The most important point is that the dorsal marginal zone already has the differentiation capacity of mesoderm before becoming a blastula, as reported by our group in the 1960s. I would like to urge young investigators to use the term 'mesoderm induction' carefully. It is necessary that the events seen in experimental systems be recognized as not necessarily occurring in normal development. During normal development, neural tissues are formed from the presumptive ectoderm. However, the mesodermal organs develop from the marginal zone, which has acquired self-differentiation capacity as early as morula stage, and not from the presumptive ectoderm. Therefore, even if the mesodermal organs are formed from the presumptive ectoderm as a result of inducing process in experiments, it is an abnormal event in which the presumptive ectoderm is artificially converted into mesoderm. The term 'mesoderm induction' is used by analogy with 'neural induction' only in experimental conditions. We also tested the combination of presumptive ectoderm and presumptive endoderm of the blastula. This explant formed mesoderm, but we thought this was a situation in which mesodermal region was removed from the whole embryo, and this result was caused by recovery of the marginal zone owing to regulating ability in the embryo without mesoderm. We explained the embryo as having a vegetalizing gradient from the vegetal to the animal pole, the highest part turning into endoderm, the lowest into ectoderm and the middle into mesoderm, but with the loss of the middle part, the embryo should be able to remake this part and recover its entire gradient. I hope that the vegetalizing gradient will be demonstrated to be a density gradient composed of a concrete substance like activin.

Mesoderm induction is followed by neural induction. Please give us your opinion on the latter.

I think the truth is that neural induction is the most important and typical phenomenon of the inducing action exerted by the organizer. In the morphological experiments, and also in the work of biochemical research on induction, the phenomenon of neural induction has been a primary focus of study.

I think that there are considerable differences between neural induction and mesoderm induction. I'll try to point out some of the differences. The first is the relationship of space and site of action.

Mesoderm induction is an action between the cell populations of neighboring regions in a continuous layer.

Therefore, I think that this action could be called vegetalization because it acts from the vegetal pole to the equatorial region.

This can explain how vegetalizing agents, such as activin, diffuse to the animal pole via the equatorial region starting from vegetal pole in the blastomeres of a 4 cell stage egg (Fig. 12).

However, as you know, neural induction is an action exerted from layer to layer, that is, from the mesodermal layer which has already invaginated to the ectoderm overlying the mesodermal layer.

Prof. Gurdon et al. described mesodermal induction as being necessary to act on cell populations and not effective on single cell in a mono-layer.

For all that, in normal development, the wall of cleavage cavity is not more than a few rows of cell populations in morula and also in blastula stage. We can guess that the vegetalizing substances reach as far as the outer region through intercellular spaces. But, in the case of neural induction, its action is plane to plane, that is between compact cell layers.

Secondly, we attempted to consider the inducing agents or the ionic circumstances causing the induction. A long time ago, when Dr. Yamada was actively working, it was concluded that the substance causing neural induction was the protein fraction of nucleoprotein and that mesoderm was induced by a non-nucleoprotein.

Adding to Dr. Yamada's work, in that of Prof. Tiedemann's group, Kawakami's group and others, some inducing agents were isolated from various tissues. These researchers found differences in nature between neural inducing agents and mesoderm inducing (vegetalizing) agents in terms of stabilities against heat and several chemical treatment.

Dr. Masui, a member of our research group, and Dr. Barth, both of whom have studied ionic circumstances, described the presumptive ectoderm as differentiating into neural tissues when it was treated by acid or alkali, while mesodermal differentiation was caused by Lithium ion alone.

A fundamental problem relating to neural induction is when and how the organizer acquires this inducing activity. It remains to be elucidated. I and my collaborators studied these problems about twenty years ago. We reported that the inducing activity of the organizer is not preformed at the beginning of development, but acquired epigenetically around the blastula stage. So, I want to clarify how the organizer acquires its inducing activity. We have to examine whether the acquisition of inducing activity is due to vegetalizing agents such as activin, as we had expected, or some other absolutely different mechanisms.

If we attempt to study the phenomenon of neural induction at the molecular level, we must first start with studies that look for the peculiar substances which are synthesized in cells during neural formation and determine markers of these substances.

Next, we must examine how the substances are synthesized by whatever processes involved, for example, what kind of genes activate the process, whether the inducing agents control the activation of these genes, and any materials controlling such mechanisms.

I presume that the inducing agents are proteins. Therefore, if we can clarify when the inducing agents are synthesized in response to the actions of any of the genes involved in the organizer and by which mechanisms these genes are activated, we can definitely understand how the inducing activity is acquired. Before anything else, however, I hope to know how the organizer acquires its inducing activity, but I think that this has come last in the order of our priorities.

I have heard that N-CAM genes or homeobox genes, involved in axial formation, were activated for a limited time after activin treatment. This activation may afford a clue for the solution of the problem.

There was a model of neural induction that Dr. Otte *et al.* were advocating. This model is very easy to understand; PKC is activated by the first step signal and weak neural differentiation occurs, then, by the time of the second signal, AC is activated strongly and more definite peculiar genes for neural differentiation is activated.

I would like to be absolutely clear about which regions these signals described in this model exist in, in the normal embryo, what kinds of substances they are, and how these substances move and how they move to their sites of action.

According to my interpretation, the first step signal corresponds to neuralization or activation and the secondary signal corresponds to caudalization or transformation.

The most important problem of developmental biology is how an egg develops into an adult. Can we completely explain this phenomenon using molecular techniques?

In 1961, Jacob and Monod advocated the theory of regulation on gene activity, and in 1964, Nierenberg et *al* began to decode the genetic cord. On the other hand, the results of electrophoretic study of isozyme were reported by Markert *et al.* in 1963.

In such an atmosphere, I noticed the importance of new research on molecular level in the field of developmental biology. So I wrote the monthly lectures to which I have already referred, intending to stimulate and promote study in this direction.

In the lecture I emphasized how to interpret on molecular level the main results obtained in experimental embryology, and how to study on molecular level the mechanism of induction and differentiation. I also showed a diagram of supposed courses of regulation by inducing substance emanated from the organizer. It was cited in my book "ORGANIZER".

As study has progressed, however, researchers have studied only at a molecular level and ignored morphologic changes in the embryo. Indeed, developmental biology offers indispensable molecular techniques, but these techniques alone cannot make clear all developmental phenomena. For example, changes at the molecular level cause the production of specific materials which bring about the differentiation of the cell. Next, the cells aggregate and form tissues, and then organs. These phenomena are problems of morphogenesis. Thus, intercellular distribution of substance and control at a molecular level cannot alone explain these phenomena. Finally, to understand development, studies at the molecular level are absolutely necessary. This is a necessary, but not sufficient, condition. I say "One who examines each tree cannot understand the forest".

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