

Experimental Embryology in Japan, 1930-1960

A historical background of developmental biology in Japan

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Introduction: Preparatory Period before 1930

Japan opened its doors to internationalization in the middle of the 19th century, with the Meiji Restoration in 1868 being the turning point in the country's history. Studies of natural history had existed, though in primitive form, before this period in Japan. They were mostly done under the influence of western science from Holland, the only western country that had diplomatic contact with Japan. Although recently renewed attention is being paid to them, the attention is generally from the standpoint of either historical curiosity or artistic appreciation. The opening of the first Japanese university, the Tokyo Imperial University (T.I.U., to be called Tokyo University after 1946), in 1877 marked the start of in-depth academic activities on biology.

Studies in embryology started in the late 19th century at the T.I.U. This is largely credited to American professors who took up teaching posts in the University, including such names as Edward Sylvester Morse, Bashford Dean and others. Particularly, Dr. Charles Otis Whitman (1842-1910, Fig. 1) should be remembered among them as the grandfather of embryology research in Japan (cf. Yo K. Okada, 1954).

Embryology seems to have quickly risen in rank in terms of education and research in both biology and medicine. It is worth noting that the first textbook published in Japanese appeared as early as 1906. The book consisted of 300 printed pages (Fig. 2), and its author was Kei Iizuka (Professor at the Gakushuin High School). Here, the development of frogs and chickens was described with a number of figures.

From my point of view, however, research in embryology was subsidiary and could not be considered as a major field in biology, like taxonomy, systematic biology and physiology, in Japan at least

until the 1930s. The biography of Naohide Yatsu (1877-1947) may give credence to this. He was a graduate of the T.I.U. (Zoology) and went to the USA (Columbia University) in 1901 after spending several years studying the development of various marine invertebrates. His six years' stay in both the USA and Europe was extremely fruitful. He published several important papers on cyto-embryological studies using experimentally anucleated eggs. For instance, his experiments on the developmental potency of different parts of the egg cytoplasm of *Cerebratulus (Nemertinea)* are exemplary even in this day. He dissected the eggs into six parts and examined the developmental potency of each after inseminating each part individually (Yatsu, 1910; Fig. 3). His work was undoubtedly at the forefront of that period. It is to be regretted that after returning to Japan in 1907 as a professor of Zoology at the T.I.U., he did not continue this line of work. Yo Kaname Okada, a great pioneer of experimental embryology in Japan, commented in his article in memory of Yatsu (1948) that Yatsu was way ahead of his time and Japanese biology had not matured enough to accept his new (even revolutionary) approaches for analyzing embryonic development through experiments, not simply by observation.

In my view, such a negative attitude toward experimental analysis through artificial manipulation of living organisms is not particularly associated with traditional Japanese mentality or philosophy. Senior professors of that period seemed to have focussed their interest on taxonomy and systematics. Perhaps they felt that static studies would be more authoritative than the new trend of analyzing the dynamics of living organisms by experiments.

C.O. Whitman came to Tokyo as a professor of Zoology at the T.I.U. in September, 1879. He studied zoology under L. Agassiz and later under K. Leuckart (in Germany), and came to Japan to stay until 1881. Later, he became the first director of the Woods

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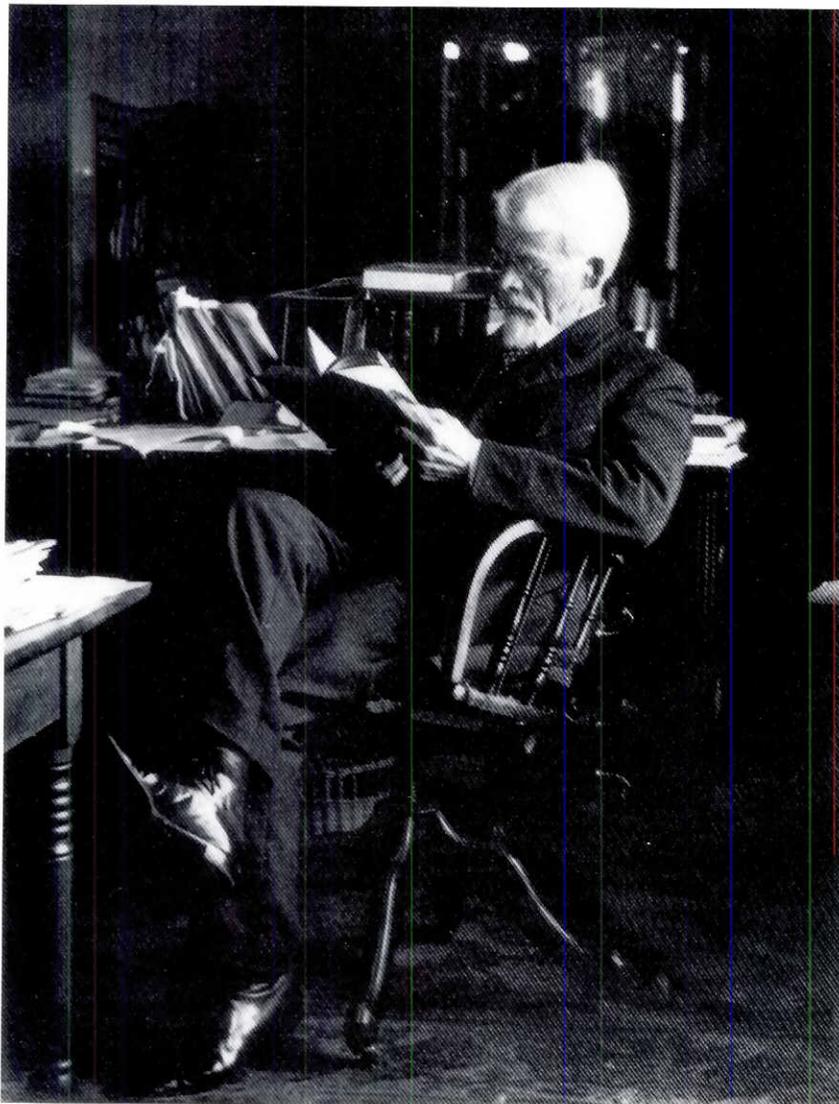


Fig. 1. Charles Otis Whitman (1842-1910). Courtesy of the Marine Biological Laboratory, Woods Hole, USA.

Hole Marine Biological Laboratory as well as a professor at Chicago University. Under his initiative, Kakichi Mitsukuri (1858-1909), the first Japanese professor of Zoology at the T.I.U., became interested in embryology. Naturally, all studies concerned were descriptive. Various marine invertebrates were chosen as subjects, but the development of *Trionyx* (*Criptodira*) was also actively investigated. Probably one of the pioneering studies in this period was on the cell-lineages of blastomeres of *Aplysia* (*Pleurocoela*) (T. Fujita, 1895).

The purpose of my present article is not to trace the studies in that era in detail. My concern is to write a historical review of *experimental embryology* in Japan from the 1930s, which was about the time when this new field was introduced to Japan. Studies performed from 1930 to 1950 are my chief concern. More recent works are already well-known internationally and publications by Japanese authors are easily accessible to researchers all over the world. Thus, I believe that it is essential to introduce the work that took place during the approximate twenty-year period including World War II. This will help readers to see that the high

activity of researchers in Developmental Biology in present-day Japan is based on the tradition created throughout this period.

Included here are some notes on my policy in preparing the present manuscript.

(1) Japanese names are not easily remembered by foreign readers, and, even worse, there are so many namesakes. To avoid confusion, I have tried as much as possible to write out given names in full when they first appear in the text, and also to include initials throughout the text for discrimination.

(2) Citation of references is incomplete. Based on the nature of the present article, only selected papers are listed in the bibliography. Many of them are written in either English or German. Some papers written in Japanese which I consider to be of great importance are also listed with my own translation of the titles. In principle, papers by foreign authors are not included in the list, except for those with special importance for reviewing the history of experimental embryology in Japan.

(3) Developmental Biology is a new term created around the mid-1950s and was not popular among Japanese scientists in the

span of the two decades discussed here. On the other hand, even at that time, many Japanese considered that "Embryology" was already an old-fashioned term, since it denotes only the morphological description of normal development. They preferred to call the new science of development "Experimental Embryology and Experimental Morphology". The latter includes endocrinology, which was gaining much interest during this period in Japan. To avoid such a clumsy expression, "Experimental Embryology" is adopted as the title of this article. Since the influence of German schools was great in this field from the 1930s to the 1940s some people preferred the use of the German word (again considered by me to be very clumsy and too conceptual) "Entwicklungsmechanik" sometimes with the literally translated Japanese terminology (Hassei-Rikigaku). I was impressed by the fact that Emperor Showa (the previous Emperor of Japan, widely known as an expert in the taxonomy of certain marine invertebrates) used this word in a private interview with me. He must have learned it from Tadao Sato (see later), who was his personal friend.

The selection of materials from extensive sources as well as their treatment in the text is entirely my own responsibility. I gave preference to works which developed further and thus may attract the interest of modern-day readers. For instance, the discovery of homeotic mutants in silkworm is one such case. The works cited here are mostly those published before 1950. Of course, I will also discuss the present situation of works which were started in this period, if they have continued up to now; i.e., from the classical studies of Wolffian lens regeneration to the discovery of cell transdifferentiation *in vitro*.

The blossoming of experimental embryology

As I mentioned earlier studies on normal development of various animals were constantly done in Japan after the end of the last century. They were, however, a mere description of the development of individual species and more or less related to taxonomy and systematic zoology, which were the major and exclusive concerns of biology in Japan before 1930. Nevertheless, it cannot be ignored that these rather primitive efforts provided (although not always) a basis for welcoming the introduction of true experimental embryology in the following era.

For instance, in the case of amphibians which would be a major subject of study of this new field, publications concerning their embryonic development can be traced back to as early as 1897. According to Yo K. Okada's review on the history of experimental morphology published in 1964, a paper entitled "Relation of embryonic axis and closure of the blastopore in amphibian eggs" was published in 1901 by S. Ikeda. However, this study never developed into a new approach. Studies which paralleled those of Yatsu done abroad were never performed in Japan.

As long as studies remained descriptive, they seemed to be comfortably accepted in the then conservative atmosphere of Zoology. The new stream of experimental embryology came from different origins.

Studies of experimental embryology in Japan blossomed in 1930. This could be credited to two outstanding figures, Yo Kaname Okada (1891-1973, Fig. 4) and Katsuma Dan (1904-, Fig. 5). K. Dan graduated from the T.I.U. (Zoology) in 1929 and left Japan to study in the USA. His stay was mostly at the University of Pennsylvania and, in particular, at the Woods Hole Marine Biological Laboratory where he met his future wife. K. Dan returned to the

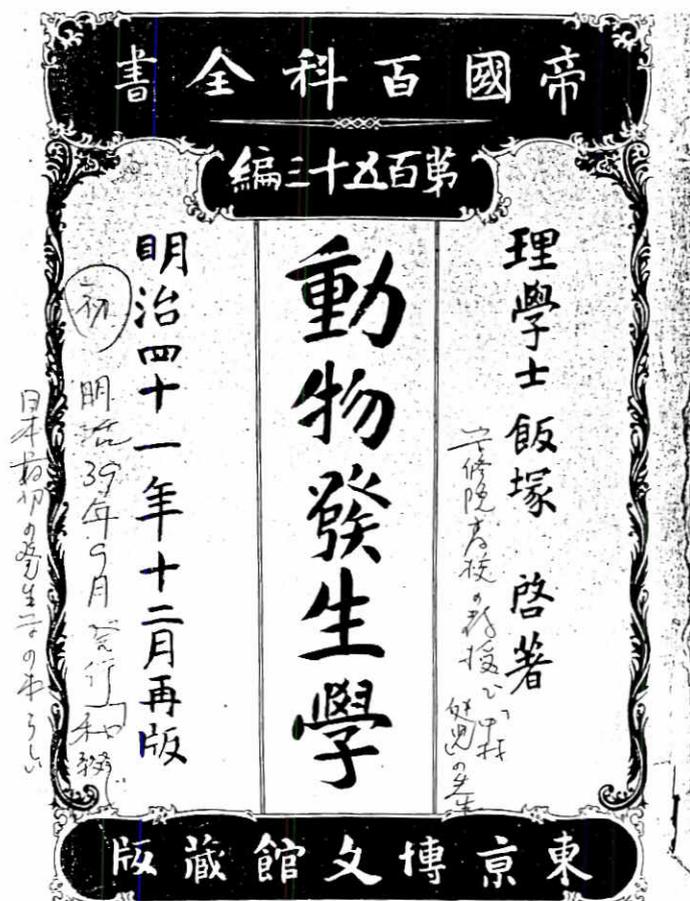


Fig. 2. Title page of the second edition (1908) of *Animal Embryology* by Kei Iizuka published by Hakubun-kan Publ. Co., Tokyo. It states that the book is volume 153 of the Imperial Encyclopedia (Japan). Courtesy of Ken-ichi Kato with his note in the margin.

T.I.U. and started to work at the Misaki Marine Biological Laboratory, which belonged to the T.I.U., with his wife, Jean Clerk Dan, who was also an esteemed cyto-embryologist, after his long stay in the USA. Yo K. Okada, after having stayed in Europe for 6 years, came back to the Kyoto Imperial University (K.I.U., to be called Kyoto University from 1946). Not a few young students joined them, being attracted by the new frontier in biology. However, the central issues of the research subjects (and perhaps philosophy) of these two pioneers were conspicuously different, so two major streams were established in experimental embryology and have continued in Japan since then.

K. Dan and his followers utilized marine animals, and their research was mainly conducted in marine laboratories, with Misaki being the pioneering center. Sugashima and Asamushi Marine Biological Laboratories, which belong to the Nagoya Imperial University and the Tohoku Imperial University, respectively were established later. They dealt mainly with the initial stages of developmental events including fertilization.

On the other hand, the main subjects of Yo K. Okada and his followers were amphibians, though Okada himself published a number of works on the regeneration of marine invertebrates

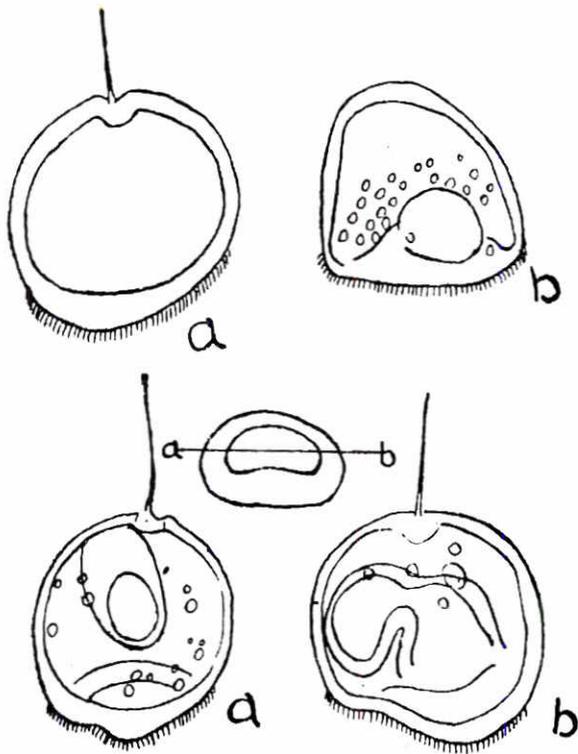


Fig. 3. Development of the isolated parts of *Cerebratulus* egg. (Upper, **a**) from the upper 4 blastomeres and (upper, **b**) from the lower 4 blastomeres of the 8-cell stage egg. (Lower, **a**) from the upper half and (**b**) from the lower half of the blastula separated at the level a-b. (Yatsu, 1910).

carried out at the Seto Marine Biological Station, which belongs to the K.I.U. Their main interest was the problems related to organogenesis (including embryonic induction) and regeneration. K. Dan is always straightforward in the analysis of rather simple, or at least apparently so, events in development. Yo K. Okada's interest was in more complicated issues of "regulation" in developmental phenomena, being sometimes slightly conceptual. It is interesting to note that such differences in character of research between these two lineages from these two origins can still be conspicuously recognized among present-day researchers, even after such a dramatic change in the social and economic structure of the country after World War II.

Studies of early development using marine invertebrates enjoyed international acclaim quite early. One of the main subjects of the school from Dan's group has long been the mechanisms of cell division of cleaving sea urchin eggs. It is of little doubt that papers on tracing cell surface movement by an ingenious technique utilizing kaolin particles are a classic in the field (Dan *et al.*, 1937; cf. also Dan, 1943).

A notable contribution of Jean C. Dan was the discovery of the acrosome reaction at the site of sperm insemination in echinoderm fertilization (1950). Already a Japanese citizen, J.C. Dan went back to her mother country immediately after the restoration of peace and brought back a precious gift to biology in Japan. It was a phase-contrast microscope. Careful observation utilizing this new instrument led to her this important discovery.

K. Dan (and his American colleague, Daniel Mazia) assumed that cell surface movement for division is motivated by an internal

force, the movement of mitotic apparatus (Mazia and Dan, 1952). Later, however, Yukio Hiramoto (1956) disproved their assumption by demonstrating that cell division can continue even after removal of the mitotic apparatus by microsurgery.

Thanks to the leadership of the Dans, people in this school have continued to publish their papers in international journals since the early post-war period. Thus, these are easily accessible to foreign scientists and have been widely known among experts in related subjects since a rather early period.

Studies of the early development of echinoderm eggs, under the Dans' influence, were very active and popular in many places in Japan. Isao Motomura at the Tohoku Imperial University in Sendai (Toh. I.U., Tohoku University after 1946) contributed to this field, in particular to studies of the formation of the fertilization membrane, around 1940-1960. Masao Sugiyama of the Sugashima Marine Biological Station, belonging to the Nagoya Imperial University (N.I.U., presently Nagoya University), worked very actively on a similar subject.

Embryology at the Toh. I.U. bears some historical interest. Biology at this university was closely associated with American universities. Sometime after its opening in 1928, the Toh. I.U. regularly invited distinguished American scientists as special lecturers. In 1930, a famous scholar in experimental embryology, C.M. Child, then a Professor at the University of Chicago, came to Toh. I.U. Upon his departure from Toh. I.U., one of his students, Isamu Watanabe, accompanied him to Chicago. The latter contributed much to the proposal of Child's gradient theory through a number of experiments on the regeneration of *Planaria*. Although lineages of Child and Motomura have been completely disconnected in Sendai, Developmental Biology there is still active today, as seen in the article of Hiroyuki Ide in this volume. He himself used to work on the differentiation of pigment cells with Tadao Hama (see later).

K. Dan called himself most comfortably a "cyto-embryologist" (cf. Dan, 1951). However, this is not the main reason for my omission of more detailed descriptions of this field in this present article. It is due to my inability to provide a comprehensive review of this field, since I have worked neither on early development nor on marine animals, and my own research is in line with what Yo K. Okada initiated. Readers will refer to the recent situation of research in this field through the papers by M. Hoshi and I. Yasumasu in this issue.

Amphibians in the old capital

In 1930, a completely new section (called *Koza* in Japanese, which is difficult to translate literally into English and probably corresponds best to "Abteilung" in traditional German academies) was created at the Kyoto Imperial University (K.I.U.) for exclusive research and education in embryology and experimental morphology. Yo K. Okada (Fig. 4) was the first professor of this new section (Fig. 6).

Yo K. Okada (1891-1973) was, no doubt, a great leading figure of zoology in Japan and exerted a strong influence, whether directly or indirectly, on embryology for a long period of time. He graduated from the T.I.U. (Zoology) in 1918 and left for France, England and Germany for the period 1924-1929. During this period, he carried out a number of experiments on reproduction, regeneration and other problems using a large variety of invertebrate animals. He was also fortunate enough to visit research laboratories in Ger-

many, where the researchers in embryology were enjoying a golden period of "Entwicklungsmechanik". After returning to Japan, he took up the professorship of a new section in the Zoological Institute of the K.I.U. (1930). This opened a long series of productive research on experimental embryology, particularly in amphibians (Fig. 7). Although there had been several descriptive studies on the development of Japanese amphibians in the K.I.U. even before, the establishment of this new section marked the start of a novel field, i.e., experimental studies of the mechanisms of development by means of microsurgery of embryos.

Embryos of the Japanese newt, *Triturus* (presently *Cynopus pyrrhogaster*) are well suited for such studies, because its eggs are large enough for microsurgery. Manipulation of embryos such as the separation of each germ layer as well as the removal and transplantation of specific organ rudiments can be neatly carried out. Above all, the animals were abundant then in Kyoto. It may be of interest to foreign readers that a number of newts used to live in ponds in the gardens of historical shrines and temples as well as those in suburbs in this old capital of Japan.

However, I heard of a few difficulties which faced my senior colleagues for establishing standard conditions of experiments. One concerned *water*. Holtfreter's saline, which was designed for the culture of amphibian embryos, was not suited for experiments in Kyoto since water in Kyoto contained much less calcium than water in Germany, with which the original formulation of the saline was prepared.

Studies on the Organizer

I would like to start my review on the studies conducted at the K.I.U. by summarizing the papers of Yo K. Okada concerning embryonic induction published in 1938 and 1939, because these papers gained international attention immediately after their publication and are of historical importance in the history of studies on embryonic induction in general.

By the early 1930s, a race had started among several laboratories to identify the chemical nature of the presumed inductive molecules which were supposed to reside in the organizer region of amphibian embryos. However, it was soon disclosed that a number of tissues of wide varieties of organisms and several organic substances were capable of inducing neural and other tissues from the competent ectoderm of urodelean gastrulae. Thus, the situation made some researchers suspicious as to whether or not there would be a specific (and perhaps a single) substance responsible for embryonic induction by the organizer.

Yo K. Okada approached this problem in a straightforward manner by the introduction of inorganic minerals into the blastocoel of early *Triturus* gastrula. The inductive effect of Fuller's earth, silica and calcium carbonate was tested. In a large number of specimens which survived after the operation — i.e., 4 out of 24 with Fuller's earth, 36 of 69 with silica and 17 of 30 with calcium carbonate — various responses of the competent ectoderm ranging from outer pigmentation to a partial formation of the neural plate were observed (Fig. 8). The results strongly suggested that neural induction is not necessarily elicited by a specific organic substance, but is due to an injury of the competent ectoderm by non-specific agents.

This work became internationally known immediately after its publication (1938). Waddington (1940) proposed two separate aspects of induction; i.e., evocation to bring about some sort of

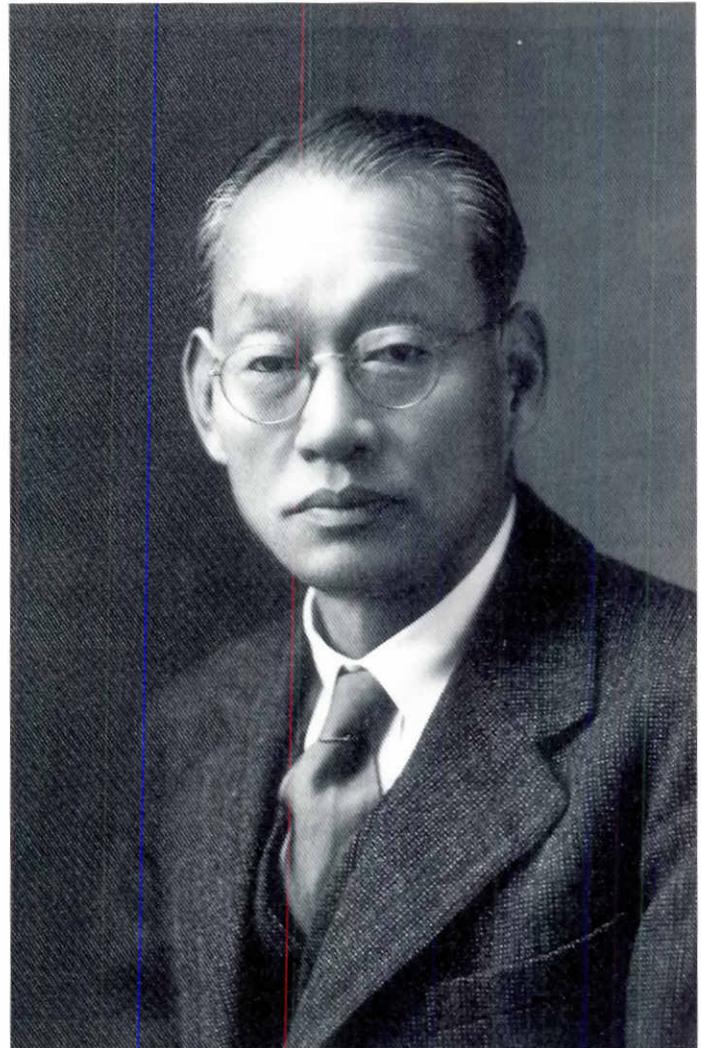


Fig. 4. Yo Kaname Okada (ca 1935). Courtesy of Osamu Nakamura.

induced differentiation, and individuation to form an organized structural entity. His idea seemed to be highly influenced by this work of Yo K. Okada, which, indeed, reported the case of evocation without leading to individuation (cf. Waddington's *Organizers and Genes*, 1940, Cambridge University Press).

It was the pioneering insight of Yo K. Okada pointed out that "induction was realized through observations only when the introduced material injures the internal tissue, destroys it and eventually extrudes the cellular contents" (cited from Okada, 1938). Although Holtfreter (1948) later discovered that neural tissue is formed simply by dissociation and reaggregation of the presumptive ectoderm and claimed that *sub-lethal cytolysis* causes the formation of neural tissue without any specific inductor, it seemed that a similar concept was already formulated by Yo K. Okada about ten years earlier.

Although he made a number of important contributions to experimental embryology *per se* from his school at the Kyoto Imperial University, as will be described later, he seemed to prefer to call his field *experimental morphology*. This was probably due to



Fig. 5. Katsuma (right) and Jean C. Dan (photographed by T.S. Okada in 1957).

his own main interest in the regeneration and reproduction of the adult form of various animals, and not in embryogenesis. The journal (in Japanese) which he initiated in Japan (1944) devoted to embryology (in its broadest meaning) was not named "Experimental Embryology", but *Annual Journal of Experimental Morphology*. He organized a society for Japanese scientists in this field, which was called the "Japan Society for Experimental Morphology" (1942). These were the initial activities, however small, towards organizing a community for exchanging research information in the field of embryology. The activities did not cease during the war and continued into the post-war period. Yo K. Okada himself left the K.I.U. in 1939 to assume a professorship in Zoology at the T.I.U. He discontinued his work on embryos and his main interest there was more or less related to endocrinology together with some work on regeneration.

In 1950, the Embryologia Society was launched under the leadership of Tadao Sato, then a Professor at the Nagoya University (formerly the N.I.U.). The aim of the Society was to publish in Japan an international journal of original papers on embryology written in any western language. This was a challenging job, particularly during the time of tremendous economic difficulty in post-war Japan.

In 1968, both societies were united, forming the "Japan Society of Developmental Biologists". The Society has been extending its activities since then. An international journal to succeed *Embryologia* was named *Development, Growth and Differentiation* (DGD). This united Society continues to bear responsibility for editing the journal, which is currently enjoying a prosperous life.

A long series of works related to primary induction by an organizer were carried out by researchers coming from Yo K. Okada's school until about 1960. The main results of experiments obtained after 1950 were reviewed by their respective researchers and assembled in the form of a monograph entitled *Organizer — A Milestone of a Half-Century from Spemann*, edited by O. Nakamura and S. Toivonen (1978), which is accessible to international readers. Therefore, I would like to mention mainly the works done during the 1940s, as they remain virtually unknown outside Japan until now and have been seldom referred to by foreign authors even

in the closely related field. As one exception, Waddington (1956) wrote: "Extensive studies on regional specificity during gastrula stages have been made by Okada (Yo K.) and a group of Japanese workers, but seem to suffer from lack of adequate statistical evaluation (from *Principles of Embryology*, 1956, George Allen & Unwin, London, p. 458). This is true, indeed. From their papers, we do not know how many embryos were operated on or the percentage of the operated embryos which were really positive. All were short papers written in a rather old-fashioned style. Moreover, the quality of printing of both text and photographs was extremely poor. Economic poverty during and after the war is clearly seen from these publications.

Nevertheless, a series of studies contains quite a bit of interesting information which should be re-examined by present-day researchers. The most important of these is the discovery of a shift of the action from the trunk-tail organizer to the head organizer of the region with the same prospective fate in the process of invagination. At that period, the distinction of two organizing activities was clearly defined, as shown in the paper of Mangold (1933) and others. The later report then that two different tissues (heterogenic organizers) exert (mimic) head and trunk-tail organizer action distinctly reinforces the concept of the predisposition of these two organizers in different regions of embryos (for instance, Chuang, 1939).

Papers by Yo K. Okada and Hiroshi Takaya (1942) and Okada and Tadao Hama (1943, 1944) reported a conversion of trunk-tail organizer action to head organizer action in the same embryonic region. The experiments were done on *Triturus* (now *Cynopus pyrrhogaster*). The dorsal blastopore lip of the early gastrula had been known to develop into foregut and prechordal plate, and to be provided with a head-inducing activity. The dorsal lip of the late gastrula, which develops into the notochord and somites, in contrast, induces trunk-tail. These were the conclusions universally accepted during that time (and even now!).

The novel discovery of Okada and Takaya (1942) is that the *uninvaginated* part of the dorsal lip of the early gastrula, in contradiction to their expectation, induces the formation of the trunk-tail structure and not the head structure. This particular embryonic

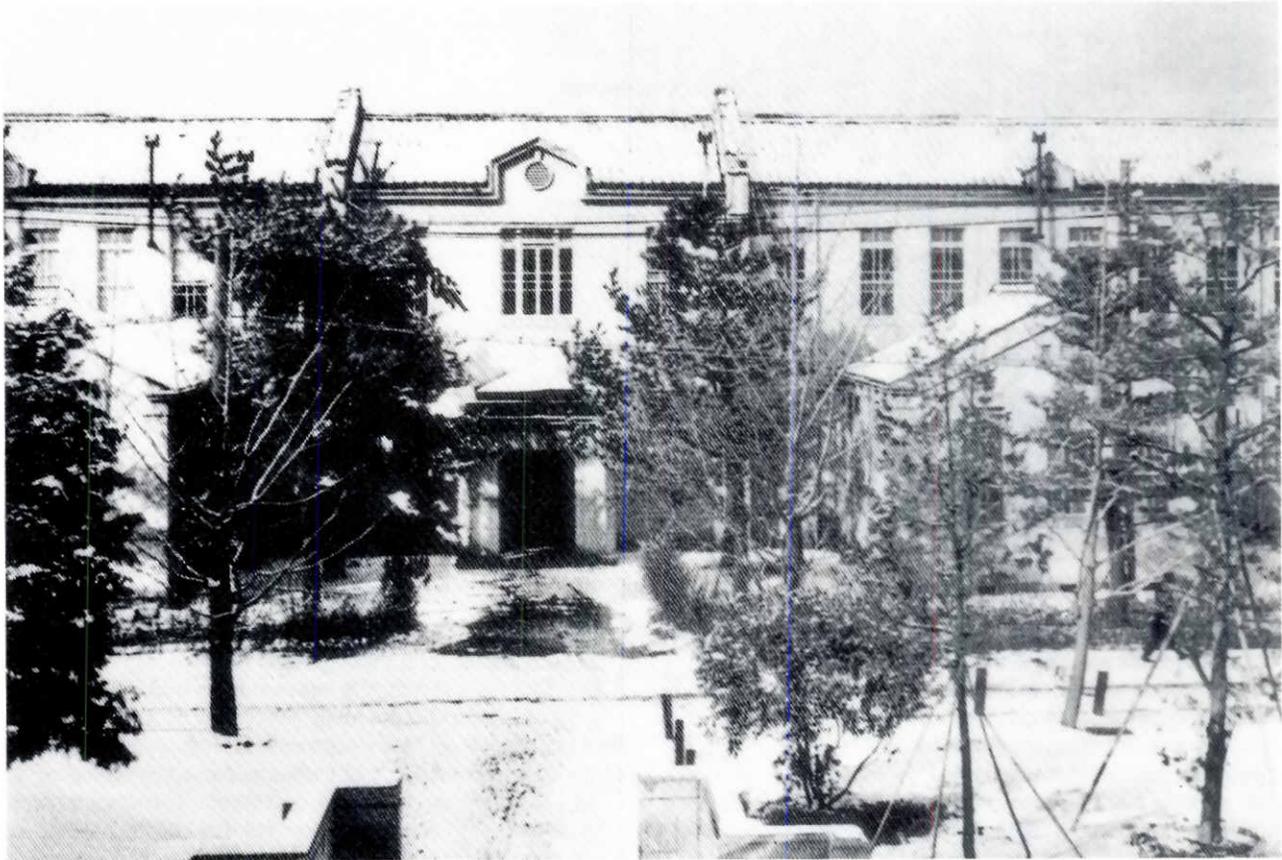


Fig. 6. Zoological Institute, Faculty of Science, the Kyoto Imperial University in the 1930s. Yo K. Okada's group worked on the second floor of the right wing. Courtesy of Noriyuki Satoh.

region induces the formation of the head structure *only after invagination* through the blastopore. The results demonstrated that the region acting as the head organizer originally induced trunk-tail, but shifted to induce head after invagination.

It is noted that they did not examine the organizer action by inserting the test piece into the blastocoel ("Einsteckungsmethode") as routinely utilized by other workers, but by the so-called "affixation method" introduced by Schechtman (1938). In this method the test piece is affixed to the excised piece of the presumptive ectoderm and then this recombinant is transplanted into the ventral ectoderm region of the early gastrula.

Yo K. Okada and T. Hama (1944) showed that the uninvaginated piece of dorsal lip of early gastrula induced trunk-tail by explantation of the piece sandwiched by the presumptive ectoderm. However, the same piece converted from its trunk-tail-inducing activity to evoke the formation of the head, when it is cultured alone *in vitro* for 10 h. A series of these extensive works was reviewed by Hama (1949, 1950, in Japanese).

We wonder why the previous workers failed to discover the trunk-tail organizer activity of the presumptive head region before invagination. T. Hama (1950) assumed that the use of a different method would bring about a discrepancy in the results (Table 1). In the affixation method or the sandwich method, the test piece will come into close contact with the competent ectoderm quickly,

whereas in the "Einsteckungsmethode" it may take some time before the contact, during which the inducing activity will transform from that of trunk-tail to head.

A similar experiment was performed to examine the inducing activity of *Rana* organizer, using *Triturus* as the competent system. In Anura, head induction occurred using the uninvaginated portion of the early gastrula. The determination of regional differences in inductive action seems to occur earlier in *Rana* than in *Triturus*.

In spite of the results of *Rana*, the basic concept of these authors is that the head organizer action should appear first, with a particular portion of the organizer (anterior portion of the archenteric roof) converting its action to induce head activity later after invagination. This is an interesting statement, which is, to me, still very meaningful even at present. I may be led to think that the conversion from trunk-tail to head induction of the organizer is worthy of confirmation. As such we must not miss a chance to investigate a molecular change associated with this conversion. With present technology is it highly possible to detect which set of genes would be activated (or inactivated) in the process of invagination. This may hopefully be a novel system to analyze the most intriguing nature of the organizer.

H. Takaya was one of the main figures in Yo K. Okada's school. I will mention later some of his interesting work in other subjects also. One day in 1950, I asked H. Takaya privately why he chose

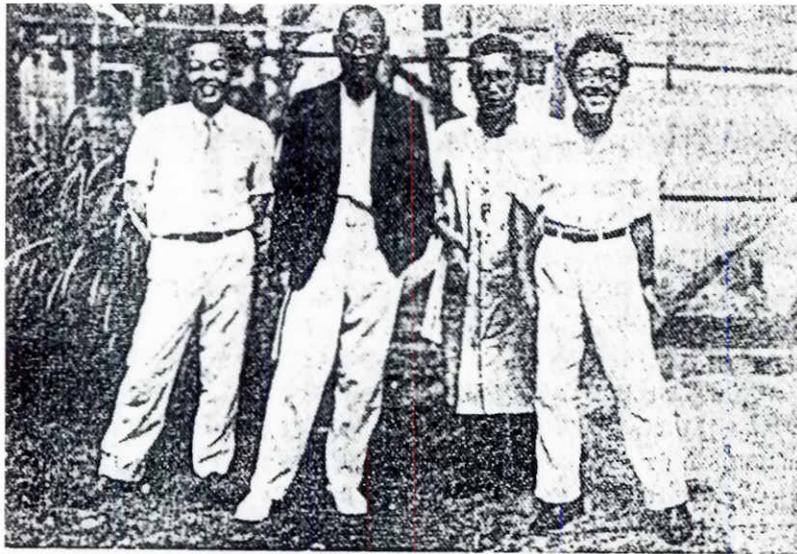


Fig. 7. A group of the first Kyoto School in 1936. The photograph was taken near a small pond for newts at the Zoological Institute of the Kyoto Imperial University. From left: Osamu Nakamura, Yo K. Okada, Tateki Mikami and Shin-ichi Asayama (who studied the development of gonads of newts). Courtesy of Osamu Nakamura.

the affixation method, which is more elaborate technically than "Einsteckungsmethode". He simply answered, as I still clearly remember, that the induced structures are always more beautifully organized in the former method. Esthetic choice seemed to have led to a new discovery in science! T. Hama was a graduate of the T.I.U. (Zoology), but conducted most of his experiments in Kyoto, being attracted by the abundance of newts there. His work after 1950 mainly concerned the biochemistry of pigment cells at Keio University (Tokyo) and, later, at the Nagoya University.

Quite a number of studies in experimental embryology from Kyoto were conducted, owing much to the ease of obtaining experimental materials. Newts and other amphibians thrived in abundance, and it was quite easy to collect many eggs from areas near the university. Thus, one of the basic tasks was to establish a standard table of the normal development of *Triturus pyrrhogaster*.

In the case of the Japanese newt, there had been a table prepared earlier by Junji Oyama (1930). Unfortunately, the sketches were not always correct and the stages were not properly divided. An extensively revised new version appeared in 1947 authored by Yo K. Okada and Ichikawa (Fig. 9). Although the legends were written in Japanese, the table has often been referred to by international researchers, due to its reliability in providing information on urodelean development.

Nowadays, studies on primary embryonic induction have been revived in Japan. Undoubtedly, it was the discovery of activin as a molecule to cause mesoderm induction by Makoto Asashima and others which motivated this revival. Recent results are reported by M. Asashima in this issue. M. Asashima is a graduate of Tokyo University and is quite independent of the lineage from the Kyoto School.

Productive years of amphibian experimental embryology in Kyoto

Hereafter works on experimental embryology other than primary induction from the Kyoto School will be summarized in chronological order.

Studies to examine the differentiation potency of the neural crest by means of transplantation and other experiments were

initiated around 1930 in Europe and America. Mamori Ichikawa published results of transplantation experiments of neural crest in 1937. The work reported a wide repertoire of differentiation from the neural crest experimentally, which was not particularly novel. At that time, however, there seemed to remain some controversy as to the neural crest origin of cartilage. Now, the neural crest origin of some cartilages was well confirmed. The experiments were carried out using *Rana japonica*, not *Triturus* as in other works of the Kyoto School. Modern-day workers on amphibian neural crest may be interested to know that the neural crest of neurula of this species appears as a big bulk of compact cell mass under the neural fold, and thus a neat separation of this tissue from others is well guaranteed.

H. Takaya, before dealing with the problem of primary induction as mentioned above, studied the polarity and asymmetry of the limbs of urodeles. This problem was initiated by R.G. Harrison (1918) and pursued by his students including S.R. Detwiler and F.H. Swett. A very extensive paper by H. Takaya published in 1941 is no doubt a milestone of its kind in the research of this interesting subject. Waddington wrote in his book, *Principles of Embryology* (1956), that "the main contribution to this subject since the most

TABLE 1

DIFFERENCES IN THE REGIONAL INDUCTION OF THE DORSAL BLASTOPORAL LIP OF EARLY GASTRULA* USING DIFFERENT TECHNIQUES

Techniques	No. of cases	Head	Head+trunk	Head+ Trunk-Tail	Trunk- Tail
"Einsteckungs- methode"	12	2	7	3	0
Sandwich	6	0	1	5	0
Affixation	10	0	0	3	7

* comprising both invaginated and uninvaginated portions. According to T. Hama (1950).

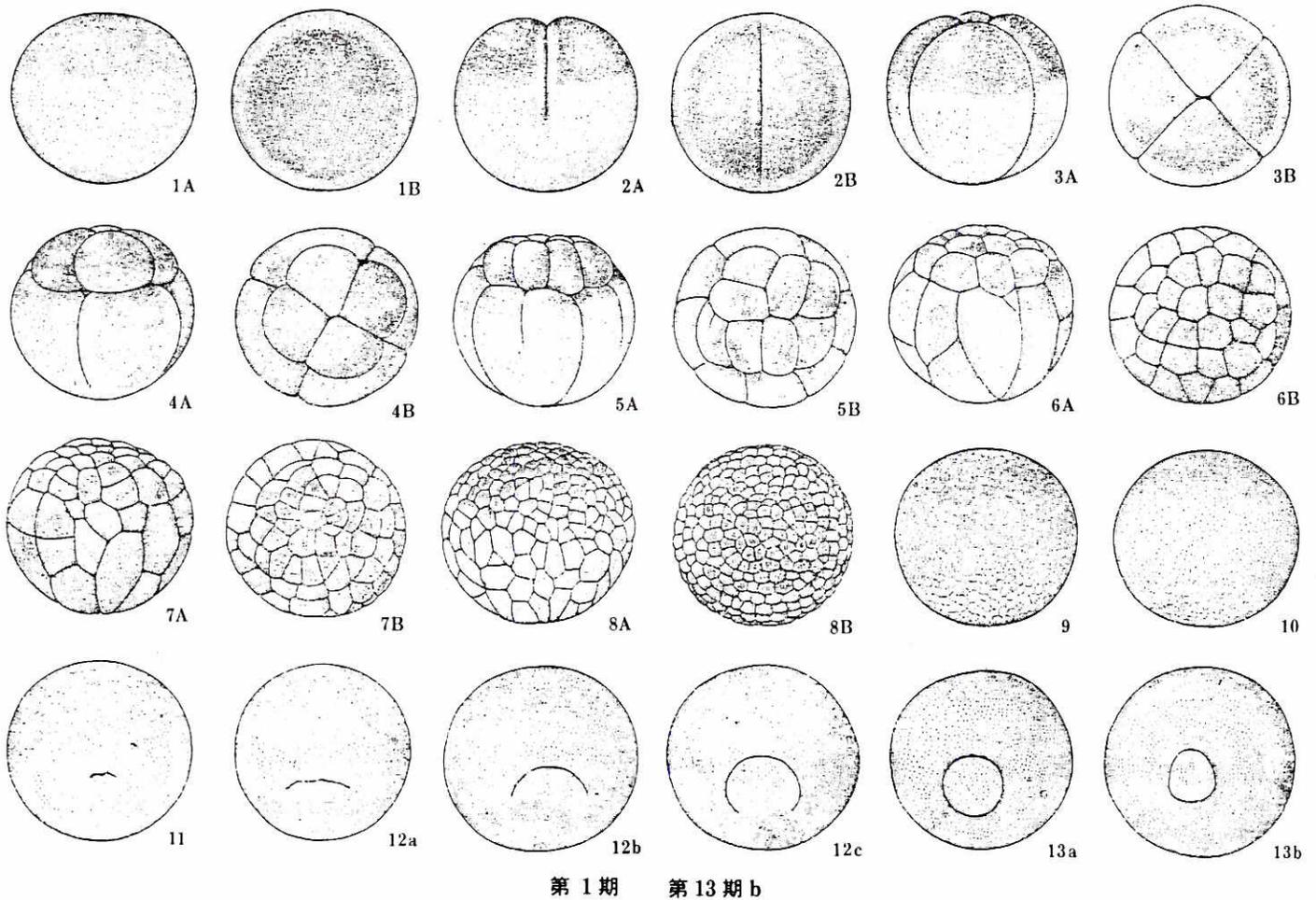


Fig. 9. An illustration of the early stages of the revised standard table of normal development of *Triturus pyrrhogaster* by Yo K. Okada and M. Ichikawa (1947).

These results were published in a short report in 1938, and were immediately confirmed by a Belgian embryologist, J. Pasteels (1939). A full paper appeared later, in 1942. It was rather exceptional that the "Vorwort" (preface) of this paper (in German) was written not by the author himself but by the author's mentor, Yo K. Okada. It stated that after the completion of all experiments, the author was called to military service and the German text was written by Tuneo Yamada.

Later, O. Nakamura examined the developmental potency of the presumptive materials of each constituent of the tail at the neurula stage of *Triturus* by transplantation experiments. The determination of respective materials for the future development seemed to have been accomplished. However, in the final limitation of the presumptive fate, the influence from the notochord is essential. Regrettably, the work was published only in Japanese (Nakamura, 1947; Fig. 13).

O. Nakamura continued to be active in his research until the 1970s. After the war, his main material became *Xenopus* at the laboratory of the Osaka University of Liberal Arts, and his works on inductive interactions between different parts of pre-gastrula (even pre-blastula) stages were reviewed in chapter 6 entitled "Epigenetic formation of the Organizer" in the monograph which he edited with

S. Toivonen (1978). Readers may refer to the bibliography of the book for an original report.

In another system, Izumi Kawakami studied the induction of head sensory organs using *Triturus* embryos (1938-1945). The induction of nose and ear development by the brain was studied extensively. The regions inducing nose and ear development were respectively mapped in the brain. Both regions were somewhat overlapped. The archenteric roof can also induce these sensory organs directly, indicating a double assurance in the mechanisms of the development of these organs. His papers were written only in Japanese. An extensive review summarizing all his results was not published officially due to the war-time conditions and was circulated only privately (1945); however, it later appeared in printed form in Japanese (Kawakami, 1948). I. Kawakami moved to Kyushu University in Fukuoka after the war and established a new group there. His main interest was the isolation and identification of specific molecules with the activity as an organizer. The discovery of the highly specific activity of a purified protein isolated from swim bladder of a crucian carp fish, *Caracius auratus*, which induced only mesodermal tissues was one of the outcomes of his efforts (cf. Kawakami and Sasaki, 1978, chapter 5 of the monograph edited by Nakamura and Toivonen). It remains to be seen

which of the inductive molecules discovered later is identical with this protein. Kiyotaka Yamana (determination of embryo axis in *Xenopus*) and Koichiro Shiokawa (currently at Tokyo University working on molecular embryology of *Xenopus*), whose names are internationally known in the field, came from the group of Kyushu University.

Studies of development by means of microsurgery of embryos only through transplantation and explantation basically came to an end in 1950. It was a general trend then throughout the world to think that since enough basic concepts and information obtained through these classical techniques had been accumulated, researchers should start to look for a new perspective to explore the biochemical background of developmental processes.

Nevertheless, it is rather curious to know that the thorough investigation of the development of the endodermal digestive tract remains to be accomplished by the classical technique. In extensive studies of isolation of the different parts of amphibian gastrula, Holtfreter (1938) arrived at the important conclusion that development from the presumptive endoderm was exclusively due to "Selbstdifferenzierung" (self-differentiation), and never to tissue interactions. The future pathway of each part of the presumptive endodermal region is predetermined rather rigidly to develop into a particular segment of digestive tract and into a particular accessory organ such as liver or pancreas. The development of the endoderm would not be influenced by the organizer action or by any other parts of embryos. This rather curious conclusion was only challenged once until the end of World War II. B.I. Balinsky (1938) in a paper published in a local journal written in his native language, Ukrainian, suggested on the basis of transplantation experiments the pluripotency of the parts of the endoderm.

It was left for Japanese researchers to complete the study of the subject. In an experiment using Japanese salamander, *Hynobius nebulosus*, I demonstrated that after the removal of the whole anterior or posterior half of the endoderm of the neurula, the regenerative development of a complete digestive tract occurred, which indicated a highly regulative capacity in the development of the endoderm (1953). Next, I carried out a series of extensive experiments (1954-1957) mostly by means of explantation of a number of recombinants of small pieces from different parts of the neurula endoderm with different mesodermal pieces. General conclusions were stated in a paper published in 1960. These are: (1) different parts of the endoderm are not yet predetermined at such a late stage as neurula, being pluripotent to develop into any segments of the digestive tract; (2) future pathways of each part can be shifted by the mesodermal pieces to be recombined; and (3) the shift occurs more easily toward the posterior direction, the foregut piece often giving rise to the intestine, after recombination with the presumptive lateral plate mesoderm of the trunk region. My study was immediately followed by a similar experiment by Chinami Takata (1960), which basically confirmed these conclusions.

At the time of these studies, a precise fate map of the neurula endoderm was not known. Yutaka Tahara and O. Nakamura successfully achieved this using *Rana nigromaculata nigromaculata* (Tahara and Nakamura, 1961). Similar studies were carried out also in *Triturus* (Nakamura and Tahara, 1966). Through these studies, they disproved the archenteric origin of the intestine, which was accepted since the report of Goette (1875). The entire endoderm originally lining the midgut is incorporated into the dorsal wall as a consequence of the closure of the archenteron, and the perforation of the definitive cavity of the intestine occurs through the yolk mass.

All these studies already sounded old-fashioned and even

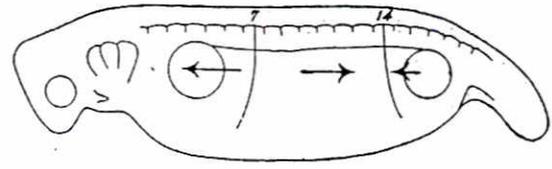


Fig. 10. Direction of the anterior-posterior limb field in different flank regions. Circles mark the normal position of the limb buds (from Takaya, 1941).

anachronistic in 1950. However, they were considered to be reliable works and were immediately cited in some standard textbooks (cf. Balinsky's *An Introduction to Embryology*, Saunders Co. 1960). Thus, endodermal development should not be separated from other regions in its mechanism, as once proposed by Holtfreter.

The studies on endoderm development led by chance to an unexpected outcome which was related to the basic theory of modern Developmental Biology. In the nuclear transplantation experiments using *Rana pipiens* conducted by T.J. Kings and R. Briggs in the mid-1950s, the potential of the transplanted nuclei of the endoderm to support development is shown to be more limited than nuclei of other regions. This finding was interpreted *a priori* as being associated with the erroneous conclusion by Holtfreter of the early predetermination of the endodermal region. My announcement of the flexibility in the further development of neurula endoderm was one of the motivations for J.B. Gurdon to challenge his celebrated experiments on the transplantation of *Xenopus* endoderm nuclei (1960). His experiments were a milestone in terms of demonstrating the multipotency of the differentiated cell nuclei to support various types of cell differentiation.

Though not having been officially stated, my interest in the problem of tissue affinity (Gewebeaffinität by Holtfreter, 1939) was awakened through observations of tissue behavior in the recombined explants. This initiated my studies of the mechanism of cell contact since 1960, which led to an announcement and discovery of the adhesion molecule, cadherin(s) in the late 1970s by Masatoshi Takeichi in my group of the second Kyoto School.

Before closing this section on the activities of the Kyoto School, I would like to mention that a book introducing this up-to-date field of experimental embryology for general readers (not for experts) was published in Japan as early as 1939 (naturally in Japanese). The author was M. Ichikawa and the publisher was Kobundo (Kyoto).

Experimental embryology from other lineages

In the 1940s, studies on experimental embryology gained prosperity in Japan, and researchers apart from those originating from two pioneering figures, K. Dan and Yo K. Okada, also worked very actively in several laboratories.

Quite independent of the workers of the Kyoto School, Takashi Fujii at the T.I.U. published an important paper related to the chemical nature of the organizer in 1944. He was originally a physiological biochemist who investigated the biological function of compounds containing pyrrole. Among a number of subjects, inductive action was the most attractive and up-to-date target to examine the possible biological activity of the compounds.

T. Fujii examined the inductive action of frog skins by inserting them into *Triturus* gastrula. Results were interesting: the dorsal

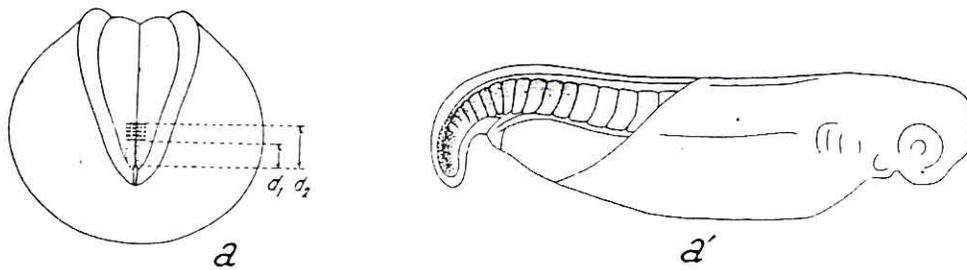


Fig. 11. Two portions of the dorsal lip of the blastopore of *Triturus neurula* were vitally stained (a). Development of marked portions into the tail somites in the tail-bud stage embryos (a'). From O. Nakamura (1942).

skin induced the development of neural tissues, sometimes with notochord and somites, whereas the ventral skin induced the development of mesodermal tissues exclusively, without accompanying neural structures. He assumed that the presence of pyrrole-containing compounds in the dorsal skin and not in the ventral skin might be responsible for the difference. Later, mesoderm induction by heterogenic inductors was demonstrated by S. Toivonen, T. Yamada, I. Kawakami, among others in the late 1950s and their discovery was highly acclaimed. T. Fujii's result was the pioneering one published before these well-known reports. His intentions of unveiling the molecular mechanism of the organizer were clearly seen in his paper. For him, compounds having the organizer action seemed to be those of purine derivatives. T. Fujii, then a professor of Zoology at Tokyo University (formerly the T.I.U.), maintained a continuous interest in Developmental Biology. The late Haruo Kanatani (the celebrated discoverer of 1-methyl-adenine as an inducer of egg maturation), Takeo Mizuno (currently at Teikyo University), Sadao Yasugi (currently at the Tokyo Metropolitan University) (both working on epithelio-mesenchymal interaction in organogenesis in chicken embryos), and others came from his school. These people have contributed to this special issue in terms of their own research, although H. Kanatani has been represented by his academic successor, Yoshitaka Nagahama.

In Tokyo University, Juro Ishida and his students were active in conducting biochemical studies of development. One of his notable works was on the studies of hatching enzymes of fishes (1944). Furthermore, the molecular nature of the enzymes has been investigated continuously for a long period of time, resulting in the isolation of a gene coding for the hatching enzyme by Shigeki Yasumasu and others (Yasumasu *et al.*, 1992). Among Ishida's group, Hideo Mohri (currently at the University on the Air) is well known in the field of spermatology and is also remembered as the first person to apply the name "tubulin". Ikuo Yasumasu (Waseda University) is actively pursuing the biochemistry of sea urchin development, as seen from his contribution to this volume.

Both T. Fujii and J. Ishida were students of the late Tokusuke Goda, Professor of Zoology at the T.I.U., who was a pioneer in physiological biochemistry. Their interest in embryological work came from biochemistry and not from embryology *per se*, and the different view from that of the Kyoto School is immediately noticeable.

Tuneo Yamada, after having graduated from the T.I.U. (Zoology), spent a considerable period in Zürich and later in Munich to study experimental embryology under W. Vogt. His stay abroad ended with a fruitful outcome as a very extensive paper of 120 printed pages in the journal, *Roux' Archiv für Entwicklungsmechanik der Organismen* (1937). He investigated the state of determination of different regions of the neurula mesodermal mantle using

European newts. At that time, not much serious attention had been paid on the development of mesodermal organs, the rudiments of which constitute a mantle in the trunk region of the neurula. By transplantation experiments, T. Yamada showed that except for the presumptive notochord situated at the median top strip, no part of the mesodermal mantle is predetermined to follow its own presumptive fate.

T. Yamada continued his study on the mesoderm after returning to Japan. Though the T.I.U. was his research base, he spent his time in Kyoto during the breeding season of newts to conduct experiments at the K.I.U. His 1940 paper on the determination of the mesodermal mantle by means of explantation is now one of the celebrated classics of experimental embryology.

The differentiation of presumptive somites, pronephros and blood island is not in accordance with the presumptive fate when explanted alone. If each of these is recombined with the notochord, the differentiation shifts stepwise and becomes almost in line with the presumptive fate. The notochord added did not exert a specific inducing activity, because the response was different depending on which part of the mesoderm is recombined.

T. Yamada interpreted the results as indicating that the qualitative differences in the differentiation of the mesoderm originating from the mantle were due to the quantitative differences inherent to the mantle along the dorso-ventral axis. The differences can be represented as different morphogenetic potentials with the value arbitrarily assigned. There is a threshold value which separates each primordium, thus resulting in the qualitative difference. I consider that this explanation is similar to the modern positional information in the logical framework.

After the end of World War II, T. Yamada moved to Nagoya University in 1946, where he established a very strong group for modern experimental embryology armed with biochemical equipment. He was ambitious enough to extend his potential hypothesis from the mesodermal mantle to the explanation of the entire pattern of embryonic morphogenesis.

His hypothesis was called "the double potential theory". Before T. Yamada, A. Dalcq and J. Pasteels (1937) had proposed the double gradient theory, which is synonymous with that of Yamada. However, they are basically different. The Belgian authors tried to explain embryonic morphogenesis by assuming two gradients, one concerning cortical factor and the other vitelline factor, to be present in fertilized eggs.

T. Yamada tried to explain the entire embryogenetic process in terms of a shift of a value of the potential with time characteristic of each location within embryos. There are two gradients of potentials, one along the dorso-ventral axis (d-v), and the other along the anterior-posterior or cephalo-caudal axis (c-c). In the mesodermal mantle, the notochord can shift the d-v potential to dorsalize. In the presumptive ectoderm, a shift of the d-v potential causes the

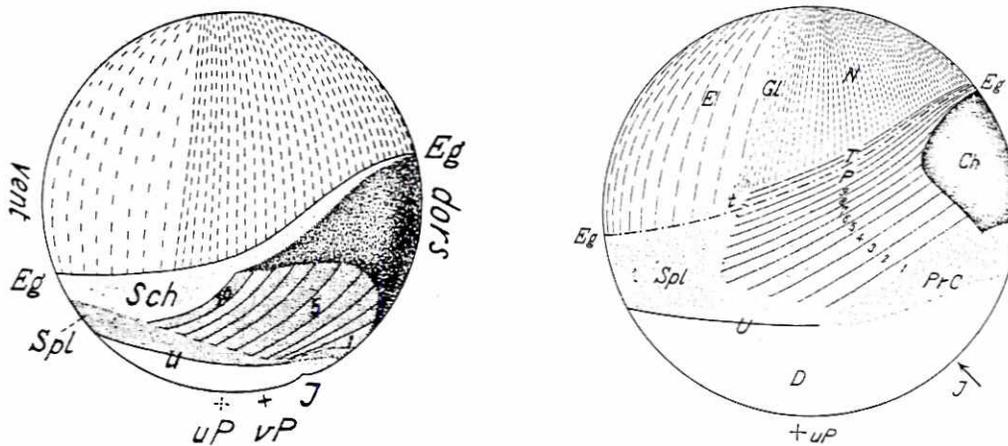


Fig. 12. Two anlagen plans of the urodele gastrula, just at the start of invagination: left, presented by Vogt (1926); right, a revised new version by O. Nakamura (1942). Ch: presumptive notochord; E: presumptive epidermis; Eg-Eg: boundary of invagination; Gl: presumptive neural crest; N: presumptive neural plate; P: presumptive somites of the caudal part of trunk; PrC: presumptive prechordal plate; Spl: presumptive lateral plate; T: presumptive tail somites; U: presumptive blastopore groove; Numbers indicate the trunk somites. From O. Nakamura (1942).

differentiation of neural tissues. A shift of the c-c potentials is caused by stretching and convergence activities of the embryonic region. T. Yamada interpreted the trunk-tail induction of the *uninvaginated* dorsal lip (by Yo K. Okada, H. Takaya and T. Hama) based on this assumption.

Unfortunately, Yamada's hypothesis with comprehensive discussions appeared in publication only in Japanese (1947, 1949). It is of little doubt that T. Yamada proposed quite an interesting logical framework for the unitary interpretation of embryonic morphogenesis. Although not much evidence seems to have been obtained in the shift of the c-c potentials, an extension in the shift of the d-v potential as a means of determination and differentiation of the mesodermal mantle to the presumptive ectodermal area seems to gain experimental support.

T. Yamada explained neural induction in terms of the quantitative change due to a shift from a lower potential (presumptive epidermis) to a higher one (presumptive neural plate) in the presumptive ectodermal area. His idea seemed to be very much influenced by the discovery of Hisao Tada (1944), who suggested the transformation of presumptive melanophore (neural crest cells) into neural plate by the influence of the notochord. Thus, there seems to exist a shift of the d-v potential in the ectoderm, similarly to that existing in the mesodermal mantle, which brings forth the differentiation of the epidermis to the neural crest and finally to the neural plate in a stepwise fashion.

H. Tada was a graduate in Zoology of both the Taihoku Imperial University in Formosa (presently the University of Taipei) and the K.I.U., and then continued research in the latter institution. His two short publications seemed to predict a productive future as an experimental embryologist. However, he was drafted for military service and, most regrettably, was killed in action in the Philippines without seeing his papers in printed form. Since I did not refer to Tada's work when I reviewed the works of the Kyoto School, to which he belonged, I would like to mention his achievements here in memory of this then promising biologist.

T. Yamada reported one successful experiment which indicated that a shift of the d-v potential from a lower to a higher value (dorsalization) could be brought about by the same agent both in the presumptive ectoderm and mesoderm. Using *Triturus* gastrula, he showed that the differentiation of dorsal tissues of the mesoderm from the medio-ventral marginal zone and that of neural tissues from the presumptive epidermis were elicited by ammonia

treatment (1950). Dorsalization as a key issue for patterning in embryonic morphogenesis seems to gain support from recent studies using *Xenopus* (cf. Sive, 1993).

According to Yamada's double potential hypothesis, however, the factor causing a shift along the d-v axis is different from that responsible for the shift along the c-c axis. It had been previously known that there existed two groups of heterogenic inducers, one which induced the head structure (such as guinea pig liver) and the other which induced the trunk-tail structure (such as guinea pig kidney). T. Yamada considered the first group as the "dorsalizing" factor (influencing the d-v potential) and the second group as the "caudalizing" one (influencing the c-c potential). He started an effort to isolate and identify each factor with his colleagues, including Kenzo Takata and Yujiro Hayashi, among others. The results were published in international journals which are easily accessible and the reviews of his long series of works appeared several times by his own hands (cf. 1958, 1961).

T. Yamada left Japan in 1961 for the Oak Ridge National Laboratory, Tennessee, USA. There, he departed from studies related to embryonic induction and shifted his research subject to Wolffian lens regeneration of newts. No doubt, T. Yamada was one of the very few Japanese embryologists who achieved international fame before 1950. His laboratory in Nagoya University was exceptionally well equipped for biochemical work compared with any of the other biological laboratories in Japan in the 1950s. Naturally, it attracted pioneering young students. Such highly acclaimed Japanese molecular biologists like Shozo Osawa (in the field of molecular evolution) and the late Reiji Okazaki (discoverer of Okazaki fragments in DNA replication) started their scientific careers in Yamada's laboratory. In fact, R. Okazaki published quite an interesting paper on embryonic induction (1955). It is well known that when the organizer is treated by heat, alcohol or other forms of treatment, it loses its capacity to induce the development of the trunk-tail, leaving only the inducing activity of the head. In this paper, R. Okazaki showed that if the organizer is devitalized without denaturation, its activity to induce the trunk-tail remains. It was unfortunate that due to his early departure from Japan, T. Yamada missed an opportunity to establish, so as to say, the Yamada school of Developmental Biology in Japan.

Not many studies were done in experimental embryology of vertebrates other than amphibians. In the period of 1930-1950, Japanese embryology seemed to be mainly comprised of amphib-



Prof. Nakamura decorated by
His Imperial Majesty (Nov. 1987)

Fig. 13. Professor Emeritus Osamu Nakamura, 1987 (then the President of Koshien College).

ians (experimental embryology) and sea urchins (dealing solely with the cyto-embryological study of initial stages of development). In teleosts, there is a truly Japanese species, *Oryzias latipes*, a small fish which can be cultivated easily in the laboratory. The fish which is called internationally by its Japanese name, Medaka, is becoming popular now as one of the good systems together with Zebrafish for studying molecular developmental biology. A table of the normal development of Medaka was made by Kizo Matsui (1949). The table appeared with detailed text in Japanese.

Tokiō Yamamoto, a protagonist of Medaka research, published a number of studies relating to fertilization. Later, he shifted his main interest to genetics, and studied the sex reversal and genetics of this lovely fish at Nagoya University. Among them, the functional sex reversal in genotypic male of the Medaka was one work which attained great international fame (1954).

Regeneration is one of the traditional topics in Japan

As described before, important contributions came from Yo K. Okada and his school on amphibian experimental embryology. However, his own interest, which he originally gained from his stay in Europe, seemed to remain in the regeneration and reproduction of invertebrates.

Of a series of papers related to reproduction, I mention here one paper describing spectacular observations of the formation of stolons, each of which gives rise to a new organism. This was observed in two species of Japanese Polychaeta, *Antolytus purpureimaculata* and *Trypanosyllis asterobia*. As shown in Fig. 11, the pattern of assembly of new stolons is conspicuously different between the two. In relation to planarian regeneration,

Hisao Sugino and later Tetsuji Kido made some significant contributions.

In the 1930s, Wolffian lens regeneration, lens regeneration from the dorsal iris in newts, was one of the major issues in experimental embryology that paralleled embryonic induction. There are a number of specific problems in relation to this phenomenon, many of which still attract our renewed interest nowadays.

One of them was (and still is, as discussed by Eguchi in this special issue) concerned with the question of why lens regeneration occurs *only* from the dorsal iris. An experiment to solve the problem was carried out by Tadao Sato (1930), who started the work in Freiburg, Germany, in Hans Spemann's laboratory. He dissected the entire iris of adult newts into 6 sectors and examined lens potency by implanting each piece into lenticotomized adult newts. Similar experiments were repeated by Tateki Mikami (1941) from the Kyoto School, who divided the entire iris into 9 sectors. The results of both workers showed that lens potency was not strictly confined to the most dorsal part of the iris; rather, it was distributed diffusely with diminution over the dorsal half of the iris.

Tadao Sato (Fig. 15) was a graduate of the T.I.U. (Zoology) and studied under H. Spemann in Freiburg. Spemann did not give T. Sato a subject related to the organizer; instead, he suggested lens studies, which H. Spemann himself investigated extensively before turning his main interest to the organizer. T. Sato, after returning to Japan, was appointed as the first professor of the newly established Biology Institute in the Nagoya Imperial University in 1943. He was an absolutely sound experimenter with scrupulous skills in operation and an uncompromisingly keen observer.

He successfully extended his examination of lens potency from the iris to the pigmented epithelium of the retina (RPE) (1951). Lens potency was found to be distributed over the tissue with the gradient diminishing along the ventral direction and the dorsal iris being the peak. The same results were also obtained in *Anura*, *Rana temporaria*, tadpoles (1953).

It was already known at that time that Anuran RPE can regenerate excised neural retina. After implanting fragments of RPE into the posterior chamber of lenticotomized tadpole eyes, T. Sato observed the differentiation of lens as well as neural retina, showing the bi-potential nature of tadpole RPE.

T. Sato established a unique, though small, school for lens studies in Nagoya. Michio Hasegawa attempted a complete removal of both the lens and the neural retina of adult *Cynopus* (*Triturus*) eyes with a very elaborate surgical procedure, and showed the regeneration of both neural retina and lens from the remaining RPE (1958). In 1961, T. Sato himself announced that lens regeneration from the dorsal iris occurred in a teleost fish (Cobitidae, *Misgurnus caudatus*), in exactly the same manner as that in *Triturus*. This is a very important discovery, because it is the only reliable example of Wolffian lens regeneration in adult animals, besides newts.

Prior to these works, Yoshito Ikeda conducted interesting research on lens regeneration at the Anatomy Institute of Medical School of the Toh. I.U. (in Sendai) quite independently of both the Nagoya and Kyoto Schools. He was one of the rare persons who worked on the subject of experimental embryology at medical school in Japan. Needless to say, in European and American countries, Anatomy Institutes were places for active research in experimental embryology, represented by such big names as R.G. Harrison (Yale University) and W. Vogt (Zürich, later Munich). In fact, several Japanese anatomists in the early 20th century went to western countries to learn experimental embryology. For instance,

Mutsunosuke Ogawa from Kyoto went to Yale under the tutelage of R.G. Harrison (1919-1921) and Shigetake Suzuki from Sendai went to Munich and studied under W. Vogt (1924-1927). Although they continued research in this line after returning home for a while, no school was established and there was not much to tell of their work here. I guess that they were not comfortable at their home laboratories, because many of their colleagues might, at that time, consider the work using frogs and newts as inappropriate in the Anatomy Institute belonging to a Medical School. Thus, experimental embryology missed an opportunity to establish its roots in medical schools in Japan. These circumstances seem to remain essentially unchanged even now. One of the exceptional cases was Junnosuke Nakai, a former Professor at the Anatomy Institute of Tokyo University, who has contributed greatly to neuro-developmental biology. His article also appears in this volume.

Yoshito Ikeda was considered to be another exceptional figure due to his novel contribution. After graduating from the Medical School at the Toh. I.U., he studied in Berlin-Dahlem under O. Mangold from 1936-1938. After coming back to Japan, he conducted extensive studies on the loss of the lens regeneration capacity with development in Japanese salamander, *Hynobius unnanaso* at the Department of Anatomy of the Toh. I.U. This species cannot regenerate lens from the iris in the adult, but can do so in the larval stages. He reported an interesting case of lens regeneration from the cornea in this species (as known in larval *Xenopus* today) at embryonic and larval stages. It is very sad that Ikeda was one of the victims of the atomic bomb in August, 1945 in Nagasaki, where, at that time, he had just been appointed as a professor in anatomy.

Goro Eguchi, the editor of this volume and associate editor of this Journal, came from Sato's school. Among many works of lens regeneration which he had done in Nagoya, his extremely precise observation of the process of Wolffian regeneration by means of electron microscopy has been highly acclaimed since its publication (1963, 1964). In particular, the finding that an interaction of macrophages and the iris melanophores facilitates the latter's discharge of melanosomes was novel and impressed many researchers interested in this subject. This was later confirmed by T. Yamada and J.N. Dumont (1972).

Eguchi joined my group at Kyoto University (formerly the K.I.U.) in 1968, and this new (the second) Kyoto School enjoyed international fame in work related to lens. In some sense, this subject is thought to have quite a long tradition. It was an amalgamation of works starting from Spemann(!), to Sato and Eguchi, on the one hand, and those starting from Yo K. Okada's Kyoto School, on the other hand. The discovery of transdifferentiation of eye cell types *in vitro* by this second Kyoto School was based directly on the history of regeneration studies. This discovery was made in 1973 and is outside the era to which this article is confined. However, in consideration of the fact that modern transdifferentiation studies have such historical roots, allow me to discuss their present situation. This tradition continues in Eguchi's group at the National Institute for Basic Biology, Okazaki, (see Eguchi's paper in this volume) and in groups of Kunio Yasuda at the Nara Institute of Technology and of Hisato Kondoh at the Research Institute of Cell and Molecular Biology at Osaka University. Recent discoveries by Yasuda's group seem to be a modern version of this traditional subject. A transcription factor called $\alpha E1$, which is specifically responsible for the lens-specific expression of the α -crystallin gene, is transcribed only in such non-lens tissues that are transdifferentiable into lens (Okada and Yasuda, 1993). H. Kondoh,

formerly an active molecular biologist in my group in Kyoto, continues to study the molecular mechanism of lens-specific expression of α -crystallin genes together with a new subject of the function of oncogenes (mostly *myc*) in embryonic development, at Osaka University.

Since studies on lens regeneration have a long history, important monographs have appeared time after time, for instance, those of Lopashov (1968) and Yamada (1977). My recent review monograph entitled "Transdifferentiation" (1991) also includes works related to lens regeneration. All publications by Japanese researchers cited in relation to lens regeneration in this article are listed in the bibliography of this monograph, to which international readers may refer.

Eguchi published an important book entitled *Regeneration of Lens* in 1980. It is unfortunate, however, that the publication is written in Japanese. He also made a number of interesting discoveries which have not been published in the properly printed form. Among these, I would like to refer to one exciting finding with his permission. In the long history of study on lens regeneration, the researchers wonder if newts run the risk of losing their lens in nature. If not, then lens regeneration would not be a beneficial phenomenon in the life of these animals. Eguchi (1969), by means of electron microscopic observation, observed that parasites (probably *Nematode*) live in the "normal" eyes of newts collected from the field. Moreover, he discovered that the parasites ate lens (only!). Thus, newts often became lens-less for a while, but regenerated it before another invasion of the parasites.

Before moving to the next section, I mention here two important facts in relation to embryology in Japan. The first is the publication



Fig. 14. Two patterns in stolon formation (Yo K. Okada, 1933).



Fig. 15. Tadao Sato (1902-1977). Courtesy of G. Eguchi.

of a comprehensive monograph on *Invertebrate Embryology* by Baifuukan Publ. Co., Tokyo. The first edition appeared in 1957, with Matazo Kume and Katsuma Dan as editors. The book consists of 452 printed pages with 385 figures. Later on, the publication of its extended version (consisting of two volumes) was successfully realized. The first volume appeared in 1983 with 400 printed pages, and the second one appeared in 1988 with 592 printed pages, under the editorship of K. Dan and others. The book is exclusively concerned with descriptive and normal development. But I am quite sure that these publications will be highly acclaimed on an international level, since they are monumental achievements in this subject. In fact, I know for a fact that the late J.C. Dan attempted to translate the volume (the first version) into English. Due to her untimely demise, this job was unfinished and remains as a challenging task to be completed.

The second fact concerns an existing tradition in the studies of experimental embryology using ascidians. Hidemichi Oka (1902-1982) was a graduate of the T.I.U. and studied "Entwicklungsmechanik" under O. Mangold in Berlin-Dahlem. Sometime after returning to Japan in 1930, he became a professor at the Tokyo Bunrika University (Tokyo University of Education) and studied limb morphogenesis in urodeles. However, more interesting are his works on the regeneration and reproduction of

ascidians which were carried out at the Shimoda Marine Biological Station belonging to his university.

It was thought that a compound ascidian, *Botryllus primigensis*, propagates by either only stolonial or by stolonial and pallean budding. With his junior colleague, Hiroshi Watanabe, H. Oka reported a new type of budding, which they called vascular budding (1957). Here, new buds are formed from aggregates of blood cells (lymphocytes) at the base of ampullae. They were able to show that the lymphocytes themselves are capable of organizing the development of new individuals. In modern terminology, lymphocytes are provided with the nature of multipotential stem cells.

H. Watanabe continued the work on ascidians with his mentor, H. Oka, and studied the colony formation of a Japanese compound ascidian, *Botryllus*. The first paper of this important series of works appeared in 1957 with H. Oka. Fusion occurs between colonies having the same genetic constitution. There is rejection between colonies lacking common genes, but fertilization can readily take place (cf. an article by H. Watanabe in this issue). This work became internationally acclaimed among the various areas of biology, because it reported the most primitive type of discrimination of self and non-self prior to immune mechanism. Immunologists considered that the *Botryllus* histocompatibility system in colony formation reflected the adaptive function of ancestral MHC genes, (cf. Burnet, 1971, *Nature* 232, pp.230-235; Scofield *et al.*, 1982, *Nature* 295, pp. 499-502).

Works on ascidians by researchers belonging to Oka's lineage have been continuous at the Shimoda Marine Biological Station (presently Shimoda Marine Research Center belonging to the University of Tsukuba) and at Kochi University. At the latter, Mitsuaki Nakauchi reported a discovery of basic importance: the first successful development of functional adults from the ascidian one-half embryos (Nakauchi and Takeshita, 1983).

At present studies on molecular and cellular mechanisms in the early development on ascidians are very active in Japan, such as those of Noriyuki Satoh (Kyoto University) and Kunitaro Takahashi (Brain Research Institute, Tokyo University), though they were independent of Oka's school.

The insect which supported the national wealth of Japan

In the past, the survival of Japan as a nation was highly dependent on a silkworm species, *Bombyx mori*. It was true that silk continued to be the top export for nearly a century after Japan began to engage in foreign trade. Therefore, the science of sericulture was truly of national importance. Quite a large number of works on the genetics of the silkworm have been done since the last century, and Japan was already one of the leading countries in this field before World War II. Naturally, some of the works were relevant to development.

As early as 1920, an experimental procedure to study the genetics and expression of eye color of the silkworm was established by transplantation of the ovary. Based on this, Hideo Kikkawa (1937) showed that the transplantation of the ovary of larvae with genetically red eyes into a larval strain with colorless eyes colored the latter's eyes as well as its eggs. He successfully elucidated the chemical nature of the factor secreted by the transplanted ovary, and found it to be a derivative of kynurenic acid. This work can be regarded as one of the pioneering studies on an international level in the then new field of biochemical study of gene expression.

Nobukazu ITIKAWA 1943. Genetical and embryological studies of a dominant mutant, the "new additional crescent" of the silkworm, *Bombyx mori* L. Report I. *Jap. Jour. Genet.* 19: 182-188. (With English résumé, p. 187).

新重「い形」蠶の遺傳と異常胚子に就て¹⁾ (第I報)

市川 信一

農林省蠶絲試驗場綾部支場

昭和17年10月17日受理

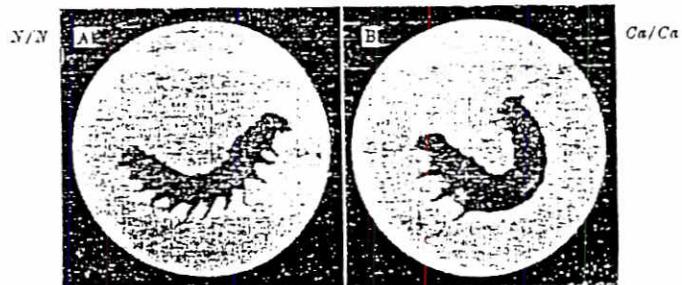
I. 緒言

家蠶の斑紋遺傳子 C_a (重「い形」) は E (翅過剩肢), EI (過剩半月紋過剩肢), E_p (過剩肢), D (過剩半月紋), H (い字形), 及び EI^{**} (無半月紋過剩肢) 等に對し重複對立遺傳子である (5) (9)。

1943年8月

市川: 新重「い形」蠶の遺傳と異常胚子に就て (第I報)

183



第2圖 同型複合型の胚子の形態 (×30)。

Fig. 15. Upper half shows the title page of the paper by N. Itikawa announcing the discovery of the homeotic mutants of silkworm. Lower half shows copies of photographs in the original paper. In spite of poor reproduction, a homeotic change in C_a/C_a mutant (a defect of the abdominal legs) is clear. From Itikawa (1943).

One important discovery was announced from the Imperial Sericultural Experiment Station (Ayabe Branch, Kyoto). Nobukazu Itikawa (1943) discovered mutant silkworms with peculiar features. Mutant embryos homozygous for E^N (new additional crescent) express many thoracic-type appendages in the would-be abdominal segment (Fig. 16). He also reported that the embryos homozygous for E^{Ca} (additional crescent) did not express abdominal legs in the abdominal segments. Itikawa studied their genetic background and reached the conclusion that such an enormously dramatic change in macroscopic morphology was caused by a single mutation of the gene responsible for morphogenesis. This was, as all modern readers can instantly recognize, a celebrated discovery of homeotic mutants for the first time besides *Drosophila* (together with an earlier report by S. Hashimoto, 1940).

This finding of utmost importance did not seem to be welcomed by sericulturists at that time. They considered that the mutants were not beneficial for production, and the phenotypes too complex to analyze! Itikawa did not pursue the study any further, but fortunately the strains were kept.

Studies of these E homeotic mutants, as they are called today, have been revived after a time lapse of nearly half a century by Koji Ueno and Yoshiaki Suzuki and their colleagues at the National Institute of Basic Biology, Okazaki. Homeotic genes responsible for this homeotic transformation were cloned and sequenced, and their expression in development was extensively studied (Ueno et

al., 1992). Suzuki is well known internationally as a pioneer in the isolation of the fibroin gene in 1979 and also for a series of works on the transcription mechanisms of the gene. It is very good to see this active revival by modern Japanese researchers utilizing the most traditional materials in Japan.

Studies of insect metamorphosis constantly remain as interesting and major problems throughout the entire history of the biology of developmental process. The use of silkworms in these studies is of great advantage due to their abundance as well as the establishment of a standard method for their culture. The work of historical importance in this field was done by Soichi Fukuda using silkworms (1944).

S. Fukuda was a graduate of the T.I.U. (Zoology) and worked at the Research Laboratory of Katakura Sericultural Experimental Station, Matsumoto, where he conducted large-scale experiments. At that time, a hormone secreted from *corpora allata* was known to induce molting. S. Fukuda noted that the thorax was very critical for both molting and metamorphosis, in addition to the head where *corpora allata* could be found. Then, he successfully demonstrated that the prothoracic gland in the thorax secreted a hormone essential for insect development. He concluded that the transformation from larvae to pupae, and from pupae to adults occurred under the control of the hormone which was *only* secreted from the prothoracic gland. On the other hand, molting requires both hormones from the prothoracic gland and from the *corpora allata*. In



Fig. 17. Modern Japanese developmental biologists in the international community at the 1985 Congress of the International Society of Developmental Biologists (Los Angeles, USA). Left, T.S. Okada in national dress (then the President of ISDB), and right, J.B. Gurdon (Cambridge).

fact, if the *corpora allata* was removed from the 3rd instar larvae while leaving the prothoracic gland intact, a small adult metamorphosed, skipping further moltings.

The hormone secreted from the prothoracic gland is now known as ecdysone. Isolation of ecdysone-like steroids from plants, with the same function as ecdysone, was done by the Japanese biochemists, Koji Nakanishi and others (1966) and Sutematsu Takemoto and others (1967). Evidence of the biosynthesis of ecdysone was also later provided by a Japanese biochemist, Haruo Chino (1974).

The successful work of S. Fukuda was confirmed immediately by C.M. Williams in the USA (1946). I am curious to know how he came across the paper by S. Fukuda (published in 1944!) so soon. It seemed as though American secret agents had been assigned to obtain even basic scientific information from the enemy! At any rate, Fukuda's work achieved international fame. In this paper by C. Williams, using the *Cecropia* moth, *Platysamia cecropia*, the presence of neurosecretory prothoracotopic hormone from brain was also shown.

The latter finding was followed up by Mamoi Ichikawa, who succeeded Yo K. Okada as a professor of embryology and experimental morphology at the K.I.U. in 1943. He turned his interest from his previous subject of amphibian neural crest to insect metamorphosis. His group conducted a series of works particularly of brain function in metamorphosis by establishing a very sensitive assay system to directly detect the activity of candidate molecules of the brain hormone. The headless *Philosamia* pupae acted as an ideal host to test the activity of samples prepared from silkworms.

M. Ichikawa's group already started efforts to identify and isolate the brain hormone in the 1950s, and announced its protein nature in 1961. However, most active and laborious work toward the goal was started when Hironori Ishizaki, who had been a collaborator of Ichikawa in Kyoto, moved to the Institute of Biology at Nagoya University in 1973. S. Fukuda was a professor there at that time, and the tradition is kept alive. Finally, H. Ishizaki and his group reached their goal in 1990 after nearly 30 years of untiring effort (Ishizaki and Suzuki, 1992). They isolated and determined the

structure and function of all three brain prothoracotopic hormones of the silkworm (cf. an article by H. Ishizaki in this issue). Surprisingly, one of them, called bombyxin, was found to have a very common structure as human insulin, to which the nucleotide sequences of the gene coding for this insect hormone or their amino acid sequences were compared (1989).

Retrospect from the present

It was indeed fortunate that universities were never closed even during the most difficult times of World War II in Japan. Although research conditions were miserable and a number of students and staff were drafted for military service, some basic research, albeit small in scale, continued.

T. Hama (1948) made a very meaningful survey of all papers dealing with experimental morphology (in its broadest meaning, including descriptive embryology, experimental embryology and endocrinology) published in Japan during the period from 1942 to 1947. He listed about 400 papers, 72 of which were original reports written either in English or German. Looking at Hama's report today, I am quite surprised, and am very proud of such activities on basic science during the most difficult time in the history of Japan. In this context, I would like to impart to readers that theoretical physics in Japan was particularly fruitful and creative in the late 1930s to 1940s. Hideki Yukawa and Shinichiro Tomonaga were awarded with the Nobel Prize after the war, but their works were actually done during the war. Though not as notable as theoretical physics, biology, and in particular, experimental embryology, also remained active in this period. The recent great advances in scientific technology in Japan would not be occurring without such a historical background.

Activities in Developmental Biology in Japan today are well known internationally. Aside from research, Japanese scientists are contributing much to organizational activities in this field. Domestically, the Japan Society of Developmental Biologists has about 1,000 subscribing members. Internationally, most international journals specializing in this field have Japanese members in

their editorial group. I myself assumed the Presidency of the International Society of Developmental Biologists from 1982 to 1985 (Fig. 17), and received the honor of being awarded the Harrison Prize by the Society in 1989.

Thanks to Goro Eguchi, the editor of this special volume as well as a current representative of the Japan Society of Developmental Biologists, most of the esteemed Developmental Biologists in Japan have contributed articles to this special volume. Many names of the present contributors appear in this review as they continue and develop the interesting works started, so to speak, one generation ago. This tells us that there is already a *tradition*, albeit a short one, of Developmental Biology in Japan. Of course, nowadays, many scientists in this field come from such diverse fields as genetics, biochemistry and, in particular, molecular biology.

However, there is one thing which is regrettable for our field. In academies of Japan, research and education of Developmental Biology are *officially* done only in the Faculty of Science and its related organizations. Although many researchers who are active in this field belong to medical or paramedical areas, there are still (except for Kumamoto University) no official organizations or groups for Developmental Biology *per se*. In Japan, generally speaking, Developmental Biology does not yet seem to have achieved recognition as one of the important centers of biological and life sciences. I sincerely hope that the tradition of Developmental Biology, which was imparted to the readers by this article, will continue and develop into a more solid academic basis.

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