Perspectives

Lens studies continue to provide landmarks of embryology (developmental biology)

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1. Introduction

The lens is one of the tiniest independent tissues in the vertebrate body, but is in itself, a beautiful entity. Its uniqueness lies not only in the transparency that rivals a shining jewel, but also in the perfect crystal-like geometrical arrangement of constituent cells. Such remarkable characteristics have continuously attracted the keen interest of a number of embryologists to the problems of how the lens is formed, in addition to studies in the domain of vision research. Studies of lens development have provided significant landmarks in the history of modern embryology* in both methods and concepts. It should be remembered, that historically, the first announcement of the "interaction of different parts of embryos" or of the inductive relationship as a major principle of animal development was revealed through studies in the lens system (mostly in amphibians) around the turn of the last century.

Since then, insights into the intricate nature of lens development have been reported in a number of papers and future challenges still await us now. Some problems have their roots in biological fields of wide scope, such as the relationship between ontogeny and regeneration, evolution of the basic mechanisms of development and others, from which arise important conceptual arguments.

In this article, I do not attempt to cover the vast amounts of information available in references published throughout the 20th century. Instead, I will try to emphasize that lens studies have been deeply related to a conceptual history of embryology, and vice versa, information gained by study of the lens sheds light on past concepts beyond the practical value of individual experiments. Therefore, rather than referring to past literature exhaustively, I only cite papers reporting results deemed crucial in my (subjective) eyes.

In recent years, a bulk of information has accumulated with regard to the molecular changes observed in both the development and regeneration of the lens. But this information has not yet been strongly nor directly linked to concepts. In the mainstream of evolutionary developmental biology today, we are now in an ideal situation to tackle old topics, like the evolution of inductive interaction or that of regeneration, with a completely new approach. Having stood the test of the past century, I believe that the lens still stands out as an ideal subject for doing so. This makes it timely to present here a rather subjective review, looking back over the history of the embryology (or developmental biology) of the lens.

2. Finding embryonic induction through the lens

At around the turn of the last century, the first predictions that interaction between different tissues was necessary for the differentiation of particular tissues were made by two investigators, Theodor Rabl (1898) and Hans Spemann (1901), through lens studies. The fact that the lens almost always appears in association with tissues derived from the optic vesicle led them to speculate the presence of a causal interaction between the two in development. In the famous ligation experiments in urodele embryos, Spemann was impressed by the observation that in anomalous eyes sometimes formed in ectopic sites, a lens developed together with the optic vesicle if the normal anatomical

*Presently, developmental biology has almost replaced the term embryology. But, in terms of history and concept, I do not think both are completely synonymous. In the present article, I mostly use "embryology", without a strict definition, because what I am often concerned with are historical issues before developmental biology came to be known as such.

relationship prevailed. The prediction of a causal relationship between these two tissues was subsequently challenged through experiments by Hans Spemann. These experiments certainly constitute a major landmark, introducing the method of microsurgery of embryos for the first time in the history of embryology. They also provided a typical model of how one could address embryological problems by experimental means: we can see here perfect realization of the programme for "*Entwicklungsmechanik*" (causal study of development), which had been proposed just prior to this by Wilhelm Roux (1895). The proposal had been mostly philosophical and I suspect that even Roux himself could not have expected that it could be so impressively realized within only a few years.

Experimental proof of causal association in the development of lens and optic vesicle was obtained by removing the prospective optic vesicle area, leaving the future lens portion intact in the neurula of *Rana fusca* (the two prospective areas are located separately in this stage). No lens developed, in spite of the fact that its prospective material was left undisturbed. Here, I reproduce a typical case of lens defect resulting from removal of the optic vesicle from Spemann's (1901) original and seminal paper (figure 1).

The subsequent path of lens studies after such an illuminating opening was by no means straight, but rather complicated. Table 1 summarizes the diversities in the dependency of lens on optic vesicle. It tells us that the dependence of the lens on optic vesicle is far from a unifying principle; even among species belonging to the same genus, *Rana*, species diversity is conspicuous. In 1927 Spemann himself encountered contradictory results when he shifted his experimental material from *R. fusca* to *R. esculenta*. With *R. esculenta*, he observed free lens in the absence of association with the optic vesicle, after removal of the latter at the neurula stage (see reviews: Spemann 1938; Saha 1991).

The situation became even more intriguing, when the

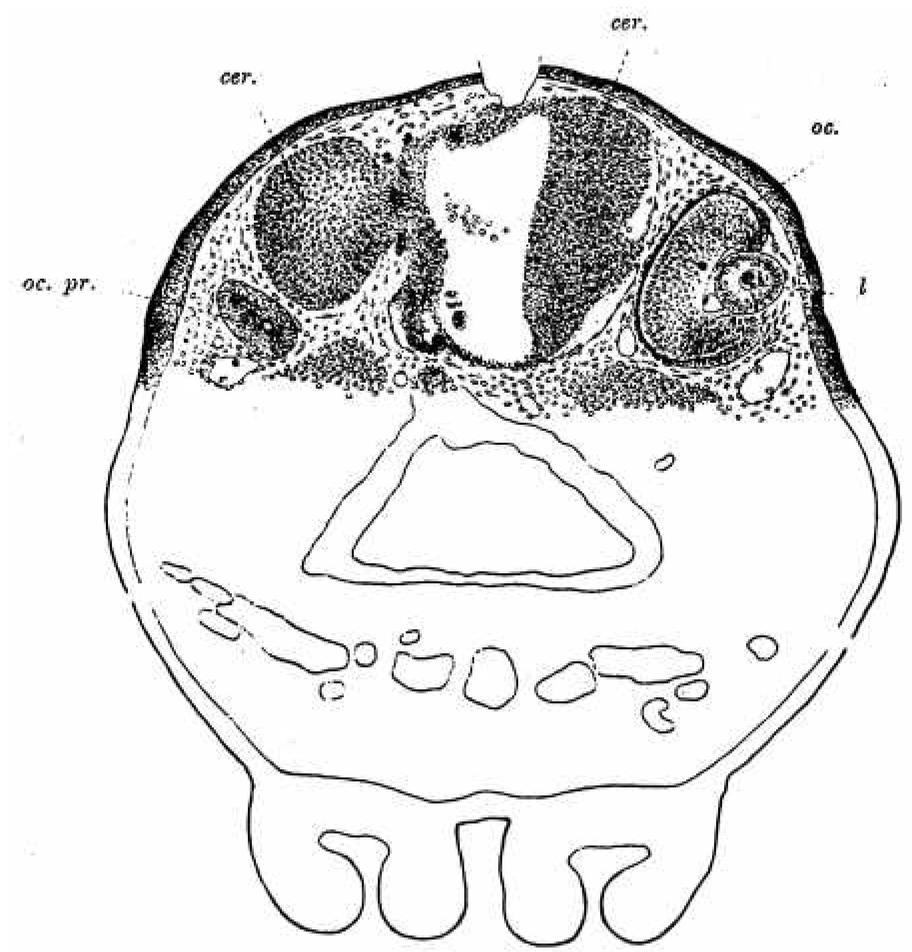

Figure 1. A section of the head of *R. fusca* larva after a removal of anlage of optic vesicle of the left side of neurula; no lens formation on the operated side. Formation of a normal lens (*l*) on the control side (from Spemann 1901).

ability of the optic vesicle (the inductor tissue) and the lens competence of the epidermis were tested separately between different species. For instance, the optic vesicle of *R. esculenta*, which is not needed in lens formation *in situ* in this species, can nevertheless induce lens formation when transplanted heterotopically under the epidermis. Therefore a simple induction model of lens formation by the optic vesicle cannot serve as a unifying and a sole principle. There seems to be a dual nature, dependence and independence of lens on optic vesicle. To explain this, Spemann introduced the term "double assurance" (*doppelte Sicherung*). The term was borrowed from the vocabulary of engineers where it is used to denote a synergetic principle of safety devices which can support a load far in excess of the normal demands of their constructions. Before Spemann, an anatomist, Braus, introduced this term to explain the fact that the opening of the operculum in frog embryos which is normally caused by the pressure of the forelimbs, was still able to take place even when the forelimb rudiments were removed in the early stages of development.

It is apparent that in lens development the situation which can be stated as double assurance is present. But, we should be aware that double assurance is neither a functional concept nor a working hypothesis, because it does not stand as a basis to open the way for the next step of analytical studies. Nevertheless, we know empirically that phenomena fitting the term double assurance are widely found in most instances of developmental regulation. As a metaphor, if I dare to call it so, this term is very useful to characterize the essential nature of development. I will return to the estimation of this metaphor later, after the discussion of lens regeneration.

3. Is a rendezvous with the optic vesicle necessary for the lens? A proposal for double assurance

In more recent years, the too simple interpretation of lens formation as one-step induction by the optic vesicle and the exclusive necessity and sufficiency of the latter is no longer generally accepted. Instead, a multi-step fixation of the stability of lens anlage determination is emphasized. Each step does not proceed independently, but requires interaction with other embryonic regions to reach its goal. The role of the optic vesicle has been demoted from its once almighty rank to being considered necessary only for supporting the final step of the growth and maturation of lens fibres (Grainger 1996).

I consider that this lowered valuation of the role of the optic vesicle in lens formation was brought about by recent trends to use mainly so-called "model" species, i.e. *Xenopus laevis* in this case. We have recently carried out experiments to re-evaluate the role of the optic vesicle using *Cynopus* (previously *Triturus*) *pryrrhogaster*, a Japanese newt, in comparison with *Xenopus* (Mizuno *et al* 1998). Urodele embryos had enjoyed star status as the material of choice in the past golden age of experimental embryology during the early half of the 20th century. I have had personal experience in looking at the prime importance of the optic vesicle in newt lens formation, not in serious scientific research but as a part of laboratory practice in the classroom in Kyoto University.

Our recent investigations using *Cynopus* embryos demonstrated clearly the necessity of the optic vesicle for lens formation in this species (Mizuno *et al* 1998). As shown in table 2, as well as in figure 2, acquisition of the ability of independent formation of a free lens occurs at stage 27. The contact of lens anlage with the optic vesicle up to this stage is a necessary condition to guarantee free lens formation. In this investigation, the expression of three major crystallin genes (a–A, bB- and g) was examined by *in situ* hybridization using the three *Cynopus* cDNAs as templates for probes.

Results obtained utilizing molecular techniques revealed that free lenses which were formed independently after ablation of the optic vesicle at later embryonic stages differed in an interesting manner from the normally developing lens with the optic vesicle; the onset of the expression of aA crystallin was greatly delayed and no expression

Table 1. Dependence or independence of lens formation on optic vesicle reported in classical papers.

Lens formation is dependent on optic vesicle in:	*Rana fusca*, *R. catesbiana*, *R. palustris**, *R. sylvatica**
Independent lens formation occurs in:	*Salmo*, *R. esculenta**, *Bombinator***

*Elicitation of lens by transplantation of optic vesicle heterotopically.
**Only incipient lens.

Table 2. Stages of embryos referred to in the left column are as according to Okada and Ichikawa (1949). The numbers in the middle column represent the total number of operated embryos excludiτng those found with contaminating neighbouring retinal tissues at stage 40. The right column shows the number of cases with lens-like structures which express bB1-crystallin on the operated side (from Mizuno *et al* 1998).

Stages of optic analage removal	Number examined	bB1-crystallin-positive
17–18	18	0
22–24	26	3
25–26	30	3
27	14	14

of γ occurred in free lenses. Therefore, we can speculate that if the rendezvous of lens anlage with optic vesicle does not occur at the proper timing during embryonic development, and if apparent lens formation occurs as free lens, then the programme of sequential and coordinated expression of major crystallin genes is likely to be distorted.

According to a series of extensive studies by Spemann leading to the metaphor of "double assurance" of lens formation however, the degree of dependence of lens on optic vesicle is determined in a species-specific way. It is important to note that the observed variability is not merely a statistical phenomenon within individuals belonging to the same species. Naturally, we should ask if it is possible to alter this dependency by experimental manipulations. One factor that was suggested was temperature. In the results communicated in Woerdeman (1939), an experiment by Ten Cate reported that the effect of ablation of the presumptive optic vesicle from neurulae of *R. esculenta* differs between operated embryos previously reared at 10°C and at 25°C. In the cold-treated group, independent lens formation occurred at high frequencies. The results were interpreted as showing the advancement of lens determination without any apparent morphological changes. This result, although interesting, has not been re-confirmed in more recent years.

4. Multiple origins of the lens in regeneration

The problems of lens formation are even more intriguing, when we consider lens regeneration. It is not only complicated, but also relevant to the fundamental and universal understanding of development, because in regeneration, the lens often originates from various sources such as the pigment epithelium of the iris (PIE), the inner epithelium of the cornea and pericorneal epidermis and, rarely from neural retina (NR), pigmented retina (PR) and others. Among these, only corneal tissues share a common origin with the normally developing lens. PIE and NR (PR) are derivatives of the optic vesicle, and although are often necessarily correlated with the lens, as its inductor, are usually segregated from the anlage of the lens very early in normal ontogeny. From the view of cell lineage, therefore, PIE and NR (PR) are totally unexpected origins for the lens. We can emphasize here that lens regeneration is often not a simple repetition of the ontogenic process.

In addition to regeneration, it has also been shown that in cell culture systems these non-lenticular cells can transdifferentiate into the lens at high frequencies, as extensively reviewed by Okada (1991). Another remarkable fact is that in cell culture, retinal cells of almost all vertebrates (including adult humans) can switch differentiation paths to acquire the characteristics of the lens.

There is little doubt that in some amphibian (urodele) species, completely different methods of lens formation are chosen among members of the same species and sometimes even in the same individual, during ontogeny and regeneration as well as in cell culture. Here seems to be a clear example of the presence of double (or even triple) assurance. The most striking example is found in a classical work by Yoshindo Ikeda (1936) using the Japanese salamander *Hynobius unangso*. Indications of lens regeneration after its removal were observed in late embryos and larvae. At younger stages, however, the lens arose most frequently from the cornea (or the ectoderm that healed over the eye after the operation). Within a

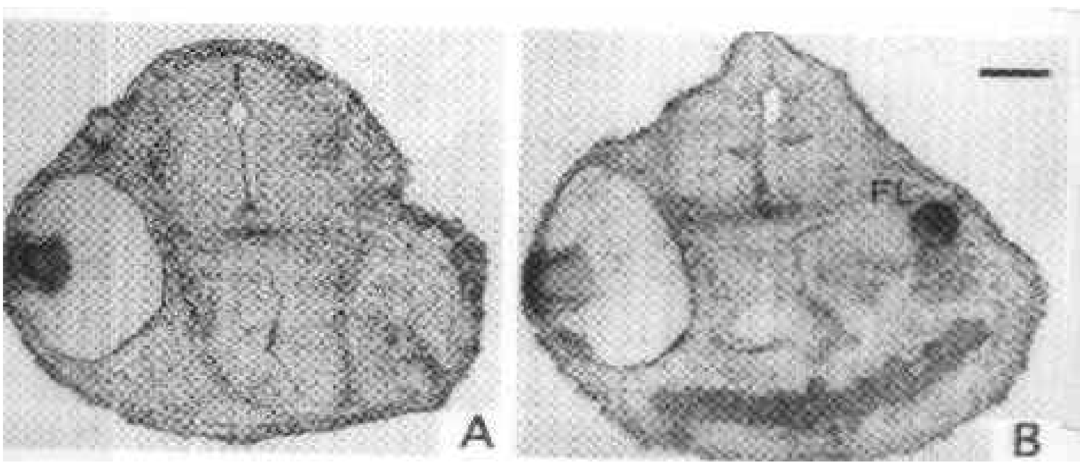

Figure 2. Typical *Cynopus* embryos fixed at stage 40 without (**A**) and with (**B**) free lens formation. (**A**) Operated at stage 18. (**B**) Operated at stage 27. FL, free lens; Bar = 100 µm (from Mizuno *et al* 1998).

short period in later embryos, regeneration of the lens from the iris (and perhaps tapetum and neural retina) occurs also.

In his intensive study of lens regeneration, Ikeda operated on and examined over 1700 specimens. He found that the switch in the choice of regeneration pathway was clearly associated with development. Yoshindo Ikeda had started his work on the lens in Berlin-Dahlem under Otto Mangold, then continued his studies in the Anatomy Institute of Tohoku University, Sendai, Japan. He then assumed the position of Professor in Anatomy at the Medical University in Nagasaki, but unfortunately became a victim of the atomic bomb shortly after.

Hamburger (1988) wrote: "Together with more recent announcements of transdifferentiation of vertebrate eye tissue cells in culture, the original idea of double assurance, which seemed to be a teleological fantasy, can claim to be the progenitor of a very productive train of thoughts which contributed substantially to the elucidation of embryonic induction and related phenomena."

Now I would like to look far into the past to recall briefly the history of lens regeneration studies. There seems to be still a little confusion about its first discovery, since lens regeneration of amphibians is popularly known by the name of Wolffian regeneration. Charles E Dinsmore (1992), a modern champion of studies of the history of regeneration, tried to clarify this historical issue and described a very attractive story of how Charles Bonnet (1720–1793), a Geneva-born great natural historian of the French School, discovered lens regeneration in the head of a poor salamander that had been accidentally injured. The discovery by Collucis (Emery and Vincenzo 1891), whose names are also sometimes referred to as discoverers of lens regeneration, followed, and then Wolff's rediscovery came around the turn of the last century (1894).

Gustav Wolff was like the Pope in the field of zoology in Germany in those days and had much influential power. It was he who designed the experiments of intentional lens removal and microscopically observed the process of regeneration. He succeeded in suggesting the PIE origin of new lenses. This was a most unexpected finding that has continued to puzzle scientists for over a century, because this is one among a small number of convincing facts to denote that the same product – the lens – can be derived from an origin separated at a much earlier stage of ontogeny from the normal anlage. In the modern view, here lies a serious fact that contradicts the rationale of cell lineages in development since the formation of lens in regeneration as well as in cell culture systems can arise from multiple sources, using different pathways. In this article I will call only lens regeneration from PIE (particularly from its dorsal part) as Wolffian (lens) regeneration.

Thus, a very basic problem for comprehending the nature of development is raised here through the specific discovery of lens regeneration in salamanders (newts). Already in 1901, Curt Herbst, a very brilliant embryologist in the early 20th century whose name is strongly associated with the discovery of the remarkable effects of lithium ions on the development of sea urchin embryos, commented on Wolff's discovery and considered it the most puzzling in the field of morphogenesis in general. He declined to attempt to answer the enigma of lens regeneration that occurs entirely differently from the normal path of lens formation and seemed inclined towards Driesch's vitalistic view, which he had earlier abandoned as a true pioneer of chemical embryology.

With such a profound historical background, it is natural that over the past century the problem of lens regeneration has periodically resurfaced at frequent intervals. An important monograph was written by Goro Eguchi (1980); it was indeed of great importance, because it was not just a review, but described quite a few of his own experiments, which were otherwise unpublished. Unfortunately, this monograph was published only in Japanese and remains therefore inaccessible to most international readers. With the permission of Eguchi, it is my pleasure to introduce some of his internationally unknown work later on in the present article.

A review by Randall Reyer published in 1954 is an excellent key to survey the works in the first half of the 20th century, when the trend of experimental embryology was at its height. The references cited, numbering about 150, are exhaustive. Even the papers published by Japanese and Russian authors in local journals did not escape the alert eyes of the author.

It is interesting to see the geographical distribution of the authors appearing in the references of this review: USA 32, Japan 32, Russia 25, Germany 21 and Italy 20. The abundance of Japanese scientists is not surprising. Many influential Japanese researchers at early periods in their career had stayed in what was then the very central German laboratories (Sato with Spemann in Freiburg, Ikeda with Mangold in Berlin-Dahlem) and they established their own schools of lens research after returning to Japan. A Russian tradition of the embryology of eye research was opened by Filatov and has continued until very recently under the leadership of Lopashov. What is surprising is that there was no contribution from the United Kingdom. Might this have been due to the lack of or difficulty there in obtaining urodele species which can regenerate lens for the experiments? The review by Reyer is not only a reliable source of historical materials on the subject, but was written with a logical framework. The reviewer's proper comments on each cited paper can be well appreciated even today.

When people started to think of the biochemistry of development, they considered lens, in parallel with muscle, a favourite tissue for this line of study, because the abundant accumulation of proteins named crystallins rather specifi cally in the lens was already known. In the embryological discipline, they hoped that it might be possible to explain the invisible process of determination in terms of the gradual restriction of specific substances (crystallins in the case of the lens) to characterize a given differentiated tissue, which may be widely distributed earlier. This was in the 1950s and, for instance, in the textbooks of Waddington (1956) these expectations were clearly stated in the cases of development of the heart and lens.

My motivation to start lens studies in the 1970s was the desire to introduce biochemical (or molecular) techniques to unveil the long-standing enigma of lens regeneration. After our own success of transdifferentiation of non-lenticular tissues to lens in cell culture, we tried to identify the molecular characteristics of trans-differentiable cells by now asking whether these lens-potent cells might transcribe (though not translate actively) the genes of lens-*specific* molecules (Okada 1991). Earlier studies using δ-crystallin genes seemed to substantiate this expectation. More recently, the groups of two of my previous colleagues, Kunio Yasuda and Hisato Kondoh, were successful in proceeding one step forward in this problem and showing that transcription factor genes that promote the expression of crystallins are closely related to the lens potency of trans-differentiable tissues (review by Kondoh 1999).

In a recent paper, we have examined the expression of *Pax-6* and *Prox-1*, which are master genes of eye development, in *Xenopus laevis* and *Cynopus pyrrhogaster* lenses (Mizuno *et al* 1999). In parallel with ontogenic observations, lens regeneration was studied. Since *Xenopus* lens is regenerated from the cornea, a tissue that shares a common origin with the lens in ontogeny, whereas *Cynopus* lens is derived from the dorsal PIE cells, it allowed the interesting comparison of two different patterns of regeneration.

The results demonstrated a universality in the pattern of expression of these transcription factor genes in lens regeneration irrespective of the source of the regenerating tissue. In both species, *Pax-6* is expressed soon after lentectomy in a region broader than that giving rise to the regenerating lens. The expression of *Prox-1* begins within the *Pax-6*-expressing tissue, and *Prox-1*-expressing cells give rise to the regenerating lens. This sequence of events of gene expression is also common to lens formation in the normal development of both groups of amphibians.

A conclusive statement to be drawn from these observations is that the genetic programme leading to lens formation appears conservative and well preserved in both ontogeny and in regeneration in two different groups of amphibians. On the other hand, we should remember that patterns of lens regeneration are greatly divergent in different groups.

5. Implication of comparative studies

Knowing that both universality and diversity coexist in lens regeneration, we have to realize that comparative studies need to be seriously considered in the problems of experimental embryology. Of course, comparative methods were of prime importance in the era of descriptive embryology, but ceased being so after Roux's introduction of *Entwicklungsmechanik*. Neglect of comparative studies was a logical outcome in view of the philosophy of "*Entwicklungsmechanik*". Repelling excessive and sole interest in the diversity in development, where embryology is almost synonymous with comparative embryology, a new discipline was established to look for universality by using only selected ideal experimental animals (corresponding to the use of model animals in recent times).

The birth of experimental embryology (*Entwicklungsmechanik*) was to liberate embryology from the tedious description of comparative embryology and make it free for the establishment of a universal discipline of causal analysis. This made us forget the presence of topics such as the evolution of inductive interactive systems, evolutionary meaning of pluripotentiality of embryonic cells and others. There have been numerous studies in comparative embryology, but few in *comparative experimental embryology*. This trend seems to still continue even in the present golden age of evolutionary developmental biology (evo-devo) researches. At times I have the impression that the most recent trends are a mere transcription of classical comparative embryology in the terminology of gene expression, by-passing the conceptual evaluation of experimental embryology.

The Russian embryologist, Dmitri P Filatov (1876–1943), must be one of the very few champions to advocate "The Comparative-Morphological Trend in Developmental Mechanics". Except for some papers in German, almost all of Filatov's publications were written in Russian. We should thank Dettlaff and Vassetzky (1997) for their recent review which allowed Filatov's works and philosophy to become accessible to us. Unlike W Roux, who when making comparison, sought only to confirm a fact previously established on another object, Filatov was concerned mainly with the differences, and he saw in the comparative method a mean for detecting variability in morphogenetic apparatuses. According to Dettlaff and Vassetzky (1997), Filatov in 1934 defined the comparative method as "*a method of putting the static into motion and thus revealing its hidden properties*", and Filatov states that ". . . in a comparative morphological [should be read as morphogenetical in the present author's eyes] analysis, one's approach to the object is largely determined by the evolutionary concepts and rests upon the fact that every morphogenetical (modified by

T S Okada) phenomenon of morphogenesis has not only the present but also the past and the future. The historical method sheds light on those sides of a phenomenon which otherwise would have remained in obscurity" (from Dettlaff and Vassetzky 1997, slightly modified by T S Okada).

After half a century, Gardiner and Bryant (1996) wrote: "As has so often proven to be the case, comparative studies are necessary to observe the full range of developmental potential: investigating only one "ideal model" system is self limiting if that system is derived, specialized or developmentally restricted". Here, we can meet the same vein as Filatov's.

Presently, urodeles are, as a whole group, endangered and include many species to be protected carefully for their preservation. More than that, *urodele embryologists* look to be an endangered group of biologists facing extinction in the near future. We should recall that without using these animals, studies of regeneration, particularly of the lens, even the discovery of this remarkable phenomenon itself, might not have appeared in the history of science.

Here I will mention a precious report surveying very extensively the ability of lens regeneration in 29 urodele species in adult and larval form. This survey was carried out by one of the most authoritative and eminent biologists in this field (Stone 1967). Of 21 species (11 American, 8 European, 2 Japanese) covering 7 genera, all could regenerate the lens by the Wolffian process (i.e. from the dorsal PIE). All 15 species belonging to *Triturus* (some having been known as *Diemictylus* and as *Taricha* in the past and as *Cynopus* now) had this ability. No ability was observed in the 4 species of *Ambystoma.* Very intriguing cases were seen in two closely related species belonging to the genus *Eurycea*, *E. lucifera* and *E. longicauda melanopleura*. The former can regenerate the lens but the latter cannot. Stone suggested an interesting experiment. Since these two species are closely related, cross-breeding might be possible, and what would happen after removal of the lens from the hybrid offspring?

In Stone's reports, the results were not different within species, but were essentially species-specific. This situation urges us to conduct hybridization studies, before extinction of these species in nature forever precludes this opportunity.

6. Towards the general biology of lens regeneration

In this last section, I will offer some comments related to the general biology of lens regeneration in nature, not to analytical studies as model systems of present day developmental biology. How do particular animals acquire such a specific ability of lens regeneration? Is it a kind of adaptation? If so, to which conditions are animals adapting? Is lens regeneration really beneficial for their survival? In nature, is there a high risk for these animals to meet mishaps in which they would lose their lenses?

These questions had already been raised by Wolff himself immediately after his discovery of regeneration. His discussion was not only very lengthy, but extremely philosophical or even metaphysical, perhaps influenced by the German philosophers of his period. In summary, his attitude was essentially teleological and vitalistic, and a rejection of Darwinian selection as an underlying cause for the ability of lens regeneration.

The ability for regeneration in the animal kingdom is often summarized based on a very simple thought: a linear decrease from lower to higher animals. Indeed there appears to be such a trend, but when distribution of this ability is examined more closely, we find the situation to be much more complicated. Even at the species level, we can see extreme diversities.

Let us consider an example in nemertine worms mentioned in an article by Newth (1958) written for the general public. Two nemertine species, *Lineus ruber* and *L. viridis*, are taxonomically so closely related and so much alike in structure that only an expert can distinguish the two. Nevertheless, in regenerative properties, they differ conspicuously. When cut transversely, *L. ruber* can form a new head and a tail, but no regeneration occurs in *L. viridis*. I have already mentioned the differences in the ability of lens regeneration between closely related urodele species of the North American genus that were reported by Stone.

We should pay attention to the fact that besides newts and salamanders, only one reliable case with irrevocable evidence of Wolffian lens regeneration in the adult form exists. This was reported by Tadao Sato (1961) in the Japanese freshwater bony fish, *Misgurnus anguillicaudatus*, Cobitidae. This fish, called "Dozyo" in Japanese, is usually 7–10 cm in length and of not so elegant an appearance. The fish are offered as a delicacy by gourmet restaurants mostly in the eastern part of Japan and can be obtained live through dealers. Thus, Sato, having had the opportunity to examine lenses in many of these fish, discovered individuals whose lenses were in the process of regeneration. He observed that these lenses were smaller than normal ones and interestingly accompanied by a narrower dorsal rim of IPE, a definite sign of Wolffian regeneration.

The observation prompted Sato to conduct experiments removing the lens of this fish. The surgical operations and histological observations were carried out with the perfect craftsmanship that Sato had directly inherited from Hans Spemann during his earlier stay in Freiburg. The results were a beautiful demonstration of Wolffian regeneration in *Misgurnus*, with regeneration occurring in 100% of the operated specimens.

In this paper Sato briefly described the presence of parasites (perhaps trematodes) in eyes with regenerating lenses and suggested the possibility of interaction between the parasites and regeneration. This fact was not only confirmed in *Misgurnus*, but was beautifully extended to *Cynopus* by Goro Eguchi, so as to persuade us that this interaction does exist. Eguchi was Sato's student and later was his research associate in Nagoya before joining me for studies of transdifferentiation of ocular cells in culture, and is a representative of a strong lineage of lens studies in Japan, Spemann-Sato-Eguchi. He described the possible interaction of lens regeneration and trematode-like parasites in his important monograph on lens regeneration (1980). This publication was written in Japanese, and Goro Eguchi was too modest to write any article describing the interaction for international readers, giving as the reason that the species name of the parasites had not been identified. In the turn of this century, the relationship of trematode parasites and amphibian morphogenesis has been spotlighted with respect to anomalous limb morphogenesis (Sessions and Ruth 1990; Johnson *et al* 1999). So I thought it very timely and important to include Eguchi's discovery in this article.

Eguchi noted that newts collected from natural environments often have smaller lenses that are connected with the dorsal iris (suggesting strongly the occurrence of Wolffian regeneration in nature). In eyes with such lenses, degenerating lenses are also often present and parasites (Trematoda) were found in these degenerating lenses (now cataract-like) (figure 3). The parasites live in the cortical fibre region of the lens and may dissolve and absorb the fibres. When we learn of these phenomena, we cannot help but think of lens regeneration as a kind of physiological turnover in nature.

Eguchi made a similar survey of *Misgurnus* fish living in the same area where the *Cynopus* studies were conducted (Fukui prefecture; the middle part of Japan's main island facing the Japan Sea). Very similar results to those of *Cynopus* were obtained with even higher frequencies, although the parasites found in these fish seem to be a different species from those in the *Cynopus* eyes.

These extraordinary facts are the first suggestion of lens regeneration as a kind of adaptive phenomenon. Taking a broader view, this is one of the first examples indicating the interaction of two different species in controlling the development and differentiation of a particular organ system, and thus implies a link between the disciplines of developmental biology (embryology) and ecology.

Figure 3. *Left*: A parasite (Trematoda) found in the eye of the Japanese newt, *C. pyrrhogaster* (ca × 150). *Right*: A lens of the adult *Cynopus*. On ventral side a degenerating lens is seen, while a regeneration of the normal lens from the dorsal part of the iris is in progress (from Eguchi 1980; reproduced by courtesy of the author and publisher).

Over the past few years, we have been struck by the high frequency appearance of frogs with serious limb anomalies in ponds in several US States (Johnson *et al* 1999). The cause(s) of such occurrences are still not well known, as only very recently have biologists started to pay attention to these striking facts. Among numerous candidates, a relationship between the anomalously developing limb and parasitic trematodes living in the morphogenetic field of such limbs is, though not the sole reason, almost certain to exist.

The history of science always teaches us the lesson that scientific progress is not always linear in one direction. In the present time, we are experiencing a great tide of developmental biology in terms of the function of genes, but these studies seem to open a totally unexpected direction through a link of developmental biology with ecology in both phenomena and concept. Together with limb studies, the problems of lens regeneration should be seriously re-examined in such directions. Thus, lens studies still continue to provide landmarks in developmental studies *over three centuries*.

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