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A brief history of regeneration research—For admiring Professor Niazi's discovery of the effect of vitamin A on regeneration

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Abstract. Studies of regeneration research has a very profound historical background, longer than genetics and embryology *per se.* In this article, I have tried to make clear the importance of the discovery of Niazi on the effect of vitamin A on amphibian limb regeneration in the long tradition of regeneration study. His discovery is truly one of the main milestones in recent era of the field. It revitalized the study of regeneration, in particular the problem of replication of pattern. A more recent discovery of heteromorphic regeneration in tadpoles elicited by vitamin A by Mohanty-Hejmadi is also of a great historical significance. It has opened the way to investigate the most mysterious phenomena known from early 20th in modern eyes, most probably in terms of the epigenetic switch of homeotic genes.

Keywords. Regeneration; vitamin A; heteromorphic regeneration history (embryology).

1. Regeneration studies: the origin of developmental biology

In a book review to the volume, *A history of regeneration research: Milestones in the evolution of a science*, edited by Dinsmore (1992), Mittwoch (1992) wrote that genetics (the term having been first used by Bateson in 1905) was a child of embryology, the study of which evolved in the 19th century as an offshoot of the regeneration studies that flourished in the previous century. Thanks to Dinsmore's book, we can now easily survey the vast contributions by such people as Reaumur, Spallanzani, Bonnet, Trembley and others in studies of regeneration in the 18th century, without having the ability to read the French, Latin or Italian of that period.

There is sufficient reason to regard the rise of experimental embryology in the turn of this century as a descendant of earlier regeneration studies. Experiments for testing the developmental potency of one half of two-cell stage embryos as performed by Roux, Driesch, Spernann and others using amphibian's and sea-urchins around the turn of the century were nothing but a logical extension of the regeneration experiments to earlier developmental stages. Thus, regeneration studied commencing in the 18th century are the origin or the stem of the whole field of developmental biology including embryology *per se.*

There is another main contribution to the present-day developmental biology which was also directly originated from the regeneration studies. In 1907, H V Wilson who was a marine biologist, not E B Wilson famed by the book "The cell in development and inheritance (1896)", described that (isolated) viable sponge cells in suspension could be recombined to reform a mini functional sponge. This study opened the way for a long series of cell adhesion studies as a foundation of animal morphogenesis and cancer

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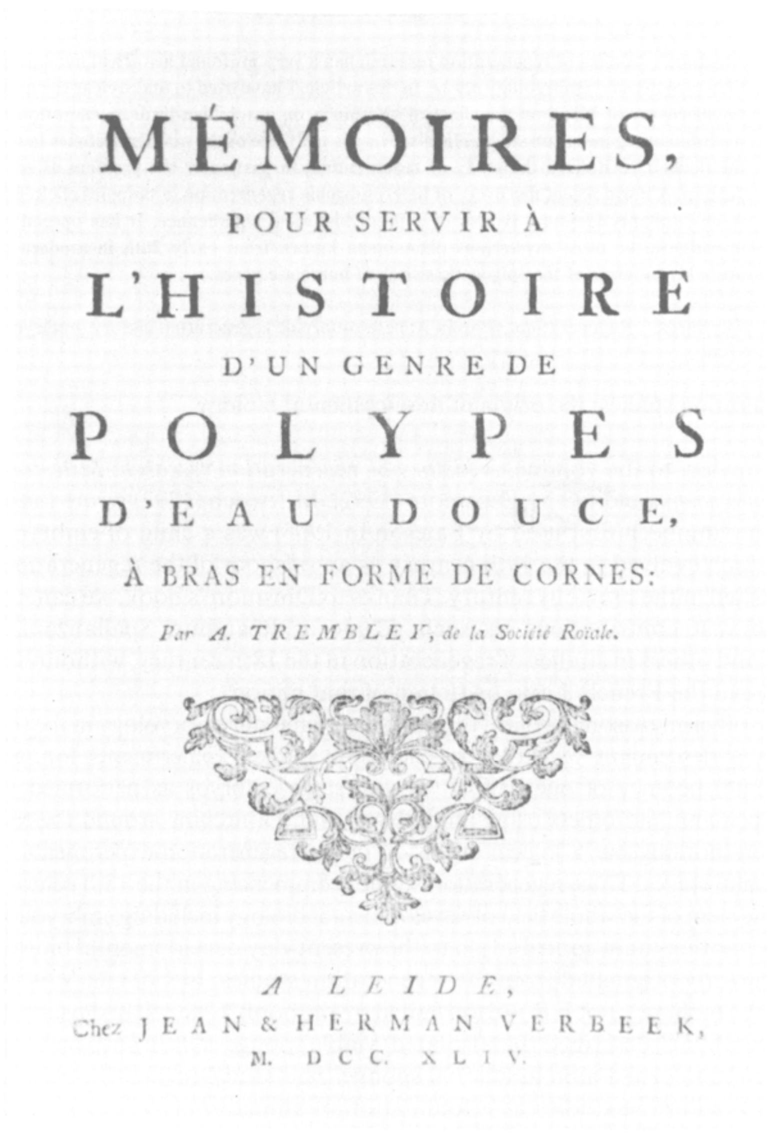
metastasis, as represented by works of Holtfreter, Moscona, Okada *et al.,* Edelman, Takeichi and others (reviewed by Takeichi 1988; Grunwald 1991 and others). The original study of Wilson should see the ability of regeneration from dissociated cells. In fact, his first paper was entitled as "On some phenomena of coalescence and *regeneration* in sponges (see also a title of Galtsoff's paper published in 1931, *Regeneration* after dissociation, an experimental study of sponges).

The historical implication of regeneration studies is even broader, because they initiated the experimental or analytical approach to questions raised from mere observation of living organisms. Following, is a typical example in studies of regeneration of Hydra by Trembley in 1744. A motivating force for his regeneration studies was the question whether


MÉMOIRES,
POUR SERVIR À
L'HISTOIRE
D'UN GENRE DE
POLYPES
D'EAU DOUCE,
À BRAS EN FORME DE CORNES:
Par A. TREMBLEY, de la Société Roïale.

A LEIDE,
Chez JEAN & HERMAN VERBEEK,
M. DCC. XLIV.

Figure 1. A title page of Trembley's monograph published in 1744.

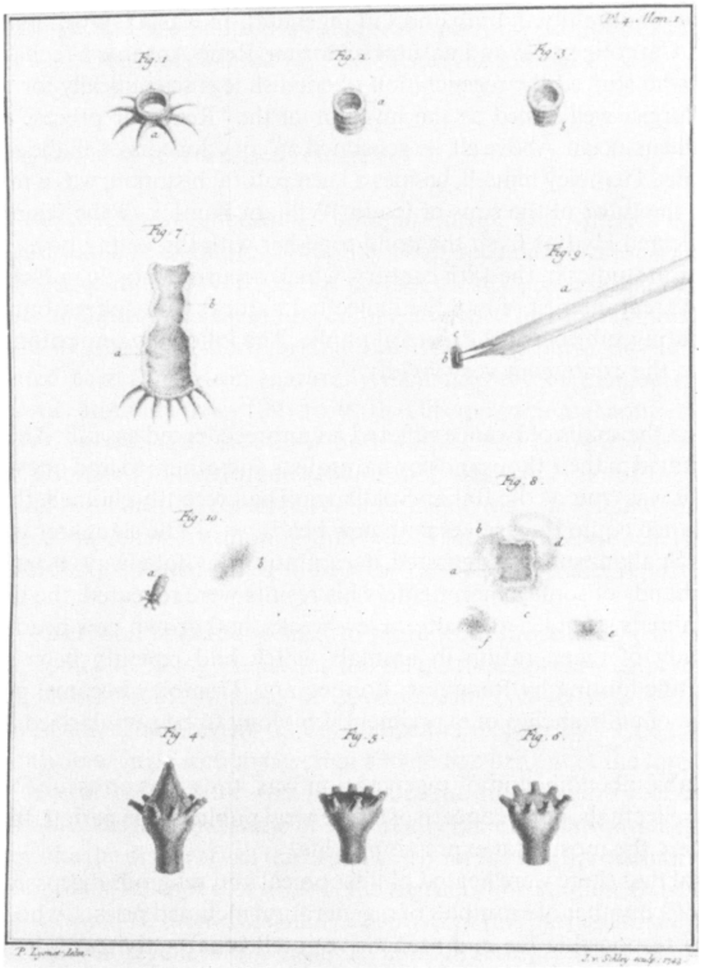


Figure 2. A plate reproduced from Trembley's monograph showing sketches of some of his ingenious experiments on *Hydra* regeneration.

Hydra was a plant (which regenerates) or an animal (which may not regenerate) by the *experimental manipulation* of living organisms (figures 1 and 2). Trembley, after observing the regeneration, should have reached the conclusion that Hydra was a plant. But, it was not the case in view of other important characteristics of this organism.

Roux's experiment of pricking one blastomere of two-celled frog embryos is appreciated as the first example to introduce experimental and analytical method in developmental biology. But, it is Trembley who established the experiments of manipulating living organisms as the methodology (not synonymous to technology in philosophical meaning) for answering biological problems. Therefore, regeneration studies in the 18th century can be regarded as the origin of all experimental biology, including developmental biology and genetics, which followed in the next centuries.

Distinguished scholars who joined in regeneration studies in that period were truly multitalented with Renaissance character. We have to realize that it was a time well before the establishment of science as a profession. Lazzaro Spallanzani (1729-1799), the discoverer of amphibian limb and tail regeneration was a lawyer, mathematician, philosopher, Catholic priest and natural historian. Rene-Antoine Frechault Réaumur (1683-1757) who studied the regeneration of crayfish legs scientifically for the first time, was a metallurgist well famed as the inventor of the "Reaumur process" for the steel industry, mathematician. Above all, he generated an encyclopedia of all the arts, industries and professions. Trembley himself, besides a keen natural historian, was a mathematician and lived as the tutor of the sons of Count William Bentick of the Hague, on whose estate he collected Hydras from the pond together with the young boys.

Regeneration studies in the 18th century, which occurred mostly in France, not only attracted the great interest of highly intellectual natural philosphers, but also became a very fascinating subject for the general public. The following quotation from Newth (1958) tells us the excitement very vividly.

> "In 1768 the snails of France suffered an unprecedented assault. They were decapitated in their thousands by naturalists and others to find out whether or not it was true, as the Etalian Spallanzani had recently claimed, that they would then equip themselves with new heads. ...... The slaughter went on. But, as Spallanzani had suggested, decapitation did not always bring death. In the hands of some experimenters his results were repeated, the unfortunate animals survived and, after a few weeks, had grown new heads. Thus the study of regeneration in animals which had recently been put on a scientific footing by Reaumur, Bonnet, and Trembley became, perhaps, the first of all branches of experimental biology to be popularized."

A remarkable phenomenon of regeneration was, thus, not an issue for specialized experts or intellectuals, but a concern of the general public in the period. Endeed, people should see here the most vital expression of life.

It is natural that there were heated philosophical and religious discussions, since the discoverers of a number of examples of regeneration included priests, who, at that time, served to be responsible for comprehensive intellectual activites, For instance, the philosophers asked, if each part of an animal could regenerate the entire animal (as shown by Trembley in Hydra), where was the residence of the 'soul'? Some of them used the Hydra to argue against the notion of the soul as an organizing principle. In order to see how deeply people were impressed, it may be pertinent to cite the reaction of such a distinguished philospher like Voltaire after the regeneration experiments by his own hands.

> "Voltaire marveled briefly: he saw at once that the loss and replacement of one's head presented serious problems for those who saw that structure as the seat of a unique 'spirit' or soul: and thought of the possible consequences of the experiment for man. Writing at this time to poor blind Madame du Deffand, he lamented that for snails but not for her the replacement of bad eyes by good was a possibility. Later he expressed confidence that men would one day so master the process of regeneration that they too would be able to replace their entire heads. There are many people, he implied, for whom the change could hardly be for the worse (From Newth, 1958)."

If we delve into the background of regeneration studies, we are struck with the knowledge of how deeply developmental biology is rooted in the hostory of intellectual activites in Europe and are amazed by its great and long past. As in the caption of Maden's review to Dinsmore's book (1992), "History is not bunk".

2. Repressing period of regeneration research

In the turn of the century, regeneration studies in Europe and America increased very rapidly. The catalogue of examples of regeneration became enormously extensive by a number of researchers who were, in this period, mostly professional natural historians, zoologists and antomists including embryologists. The most important archives published were written by a German anatomist Dietrich Barfurth. He reviewed all literature related to regeneration (naturally including the experiments on early or cleaving embryos) annually from 1891 to 1901. This monumental contribution is well summarized by Churchill (1991). Several monographs were also published in the early 20th century. Thomas Hunt Morgan (1866-1945), who was one of the most active experimenters of regeneration research at that time, wrote one volume on this subject (1901). A very comprehensive monograph was written by Korschelt (1927), which consisted of about 300 printed pages and covered almost all studies reported to date at that time.

Later on, regeneration research seemed to retire from the leading part in the recent history of developmental biology, replacing its role to studies of early embryonic development. If you take a look at "Analysis of Development" (Willier *et al* 1955) which was perhaps the most important review on developmental biology by 1950th, you will find regeneration only in a single section covering a mere five percent of the total 720 pages.

There are several reasons for the recent decline in popularity of regeneration research. Probably, the fact that none of such really major scientific issues in developmental biology like the discovery of the organizer, or of the multipotentiality of somatic nuclei in development, has not come out of the regeneration studies, may have resulted in the failure to attract the interest of many scientists to this particular subject (table 1). The phenomenon of regeneration itself is in many respects too complex to be accommodated well in the modern trends in developmental biology.

As already pointed out, the results of regeneration research were a favourite subject in religion and philosophy in the 18th century. Even after the accumulation of vast information by the early 20th century, a basic process of regeneration, replication of the pattern of the lost structures, needed a somehow organismic viewpoint for understanding. Morgan's life was symbolic. After having started his brilliant scientific career as a regeneration researcher, he left this subject and turned his interest to genetics.

Regeneration was, and still is, a tremendously difficult subject to grasp in terms of entity or of element. An introduction of some recent techniques at that time like biochemistry, electron microscopy and others, did not help much. Furthermore, there was no possibility of a genetic approach, since no mutation was known (in principle, even now), which affects regeneration.

I consider the discovery of dedifferentiation of mesodermal cells in blastemae of the urdele regeneration limb as an important milestone for understanding this complex process at the cellular level. Even an apparently simple question of where do cells in the regenerate come from, has remained strongly controversial. Allthough the dedifferentiated cell origin was clear in, some cases, the dichotomy of *dedifferentiation versus neoblast formation* (raised in cases of planarian regeneration by Baguna. 1981) continuously remains as a central issue.

Table 1. Selected major issues in regeneration studies in their history of longer than two centuries.

Abraham Trembley (1744):	First scientific discovery of regeneration by using Hydra.
Lazzaro Spallanzani (1765):	Announcement of regeneration of tails and limbs in Salemander; in "Prodromo".
Charles Bonnet (1762):	Reporting several regeneration systems in Salamander (including the lens regeneration).
Tweedy John Todd (1823):	Discovery of "neurotrophic effect" on the limb regeneration.
Gustav Wolff (1894):	Announcement of the regeneration of the lens from the dorsal iris in newts (Wolffian Linsenregeneration).
Elmer Grinshaw Butler (1933):	Establishment of dedifferentiated mesoderm as a source of regeneration blastema.
Goro Eguchi and T S Okada (1973):	Demonstration of cell transdifferentiation *in vitro* systems.
I A Niazi and S Saxena (1978):	Induction of abnormal limb regeneration by vitamin A in anuran tadpoles.
Susan V Bryant and others (1981):	Interpretation of the pattern formation in the regeneration in terms of positional information.
Priyambada Mohanti-Hejmadi and others (1992):	Induction of heteromorphic regeneration by vitamin A and retinoids in tadpoles

The cases of regeneration of ocular tissues provides a unique opportunity for studying the process at the cellular level. Here, we regard the regeneration in a confined world, the eye, and the changes occur in very limited number of cell or tissue types. The cellular origin of regenerates as well as the transdifferentiation of progenitor cell types into others have been documented (Okada 1991). However, still more complicity remains in other systems. A comprehensive view of the cellular events in regeneration cannot be obtained by a simple choice of two alternatives; *neoblast formation versus dedifferentiation* or *transdifferentiation versus differentiation of stem cells,* as extensively discussed in my above-cited monograph. In summary, cellular analysis, though valuable in its domain, failed in approaching the classical and essential problem of regeneration, i.e., the mechanisms of replication of organized pattern.

3. Milestone in recent history of regeneration research

In biological sciences, a key break-through is brought about by either the theoretical proposal of a new hypothesis or by an unexpected discovery through experiments. Progress in regeneration studies is not an exception.

An introduction of the hypothesis of "positional information" was certainly an event (Wolpert 1971). Its logical framework is clearly reminiscent of the theory of gradients and is with a long and deep historical root. But the hypothesis was presented in a more rigorously defined concept to meet with the analytical minds of modern biologists. Thus, we can interpret the process of regeneration as the re-establishment of positional value.

Almost concurently, totally unexpected results were reported by Niazi in the regeneration of tadpole limbs treated by vitamin A. Now, allow me to write some of my personal retrospectives.

From 23 to 26 November 1982, a very unique international meeting entitled, "An Afro-Asian perspective of Developmental Biology" was held in Poona, India, organized by the late Srush Goel. The meeting was sponsored by the Indian Society of Developmental Biologists and assisted by the International Society of Developmental Biologists (both being abbreviated as ISDB!). Since I had assumed the Presidency of the latter organization at that time, this meeting gave me the pleasure to visit India for the first time in my life.

I have to confess my ignorance of Niazi's work until that occasion. He spoke of his very extensive series of experiments on the effect of vitamin A on limb regeneration of tadpoles and frogs of Indian species. So, it was even a shock for me to learn that limb regeneration was not simply a mere replication of the original pattern, but resulted in the formation of abnormally long limb after treatment with vitamin A (Niazi and Saxena 1978). An experimental revival of the lost regenerative ability of the post-metamorphic frog by vitamin A was equally a surprise (see Niazi 1983). It was unfortunate that this series of his important papers appeared in local Indian journals and other journals which do not have wide international circulation.

The remarkable effect of vitamin A on limb regeneration was soon confirmed by Maden (1982, 1983), who made this very important phenomenon internationally known. By "international" I mean not only the publication of Maden's report in the most internationally popular journal, *Nature*, but also the reproducibility of the experiment using popular species of Amphibians (both urodela and anura) that are common for laboratory use at the international level.

The impact which the vitamin A experiment gave was immediate, great and very broad. In regeneration studies, for the first time in a very long history, we created an experimental system which enable the manipulation of pattern by a mere extrinsic agent, vitamin A.

Abnormal patterns of regenerated limb induced by vitamin A can now be neatly explained in terms of positional information; vitamin A alters the value so as to let the regenerate become a more proximal structure than would normally arise at a given amputation level. The finding that the proximal regeneration occurs in a dose dependent manner to the administrated vitamin A justifies perfectly the interpretation based on the positional information hypotheses (Stocum and Crawford 1987). Therefore, here two breakthroughs with different nature beautifully came together. The regeneration studies had come into a new era, I dare to say, Investigations on the molecular mechanisms of vitamin A, when conjugated with exploration of the expression of homeotic genes will essentially contribute to the understanding of positional value in terms of gene expression. But, we have to remember that the original concept of the positional value can be traced nearly 250 years ago by Bonnet as a rather philosophical issue (Dinsmore 1992).

The effect of vitamin A causing alteration of a given cell or tissue was discovered as early as 1920. Of the works thereafter, the most well-known one was the elicitation of mucus-secreting epithelia by high vitamin A values from chick embryonic skin in organ culture (Fell and Mellanby 1953). But, Niazi's work was the first to demonstrate that vitamin A does not influence only cell and tissue types, but also does so remarkably effect the pattern in morphogenesis.

At present, the interest in vitamin A (now retinoic acid) and its receptors is astonishingly wide. Almost all morphogenesis-related developmental events are studied in connection to vitamin A. The list of pathological phenomena including cancer which are related to vitamin A becomes lengthier year by year. Such a burst would not have occurred, without the very original finding by Niazi.

4. Heteromorphic regeneration. A critical cue for understanding morphogenesis

Following the discovery of induction of abnormal limbs, another startling effect of vitamin A was reported, also from an Indian laboratory (Mohanty-Hejmadi *et al* 1992; see figure 3). This time, not regeneration of the original structure, but transformation of the quality of the regenerated structure was shown. When frog tadpoles with amputated tails were exposed to vitamin A during the first few days of regeneration, legs (hind-legs) were regenerated from the tail blastema.

These results are really amazing. Nowadays, research of developmental biology are more or less sophisticated, and thus we (at least myself) tend to not expect discoveries that are totally unexpected and amazing by Itself.

The original experiment of Mohanty–Hejmadi was done using a local Indian frog, the so-called marbled balloon frog (*Uperodon systoma*). Although not publicly announced, more than few people have tried to confirm the results with other species. Unfortunately, it does not seem to be successful in most of the popular laboratory

Figure 3. A photograph on the occasion of Indo-Japan Seminar on Morphogenesis, Genes and Development held in Mysore, India, on Febroary, 1995. From left to right: K Yasuda, M Maden, I A Miazi, T S Okada and P Mohanty-Hejmadi.

amphibians, including *Xenopus.* However, Maden (1993) succeeded in confirming these most amazing results by using an European frog, *Rana temporaria.* As reported in this issue, Mohanty-Hejmadi and her group has extended their findings to many other Indian species, in addition to the original *Uperodon* (see also Mahapatra and Mohanty-Hejmadi 1994).

Such species difference of this phenomena is presumably related with the different levels of endogenous thyroid hormone receptors, which interact with retinoic acid receptors (Maden 1993). A use of retinyl palmitate by the Endian workers, not retinoic acid as routinely used in many laboratories, may be somewhat meaningful in this respect. A neat theoretical interpretation of the transformation of tails into limbs in regeneration is accomplished by Bryant and Gardiner (1992) in terms of a shift in positional qualities.

It should be recalled that the present system operated by vitamin A is the first irrevocable presentation of homeotic regeneration in vertebrates. Holtfreter (1954) reported two cases of homeotic regeneration in urodeles. Here, the secondary tail-structure induced by the heterogenic organizers, was amputated, and then limb-like structures were regenerated. This was essentially a confirmation of the earlier report of Farinella-Feruzza. (1953), in which the regeneration of limb-like structures was obtained from a tail which was xenoplastically and ectopically transplanted into the flank (limb field).

It should be emphasized here that studies on homeotic regeneration in a number of invertebrates was really prosperous in the early 20th century. Ef you take a look into Korschelt's book (1927), you will find a surprising number of examples of this interesting phenomena. Among them I will mention here only two representative cases: (i) The homeotic regeneration of a walking leg from the amputated antenna of the walking-stick insect (*Dixoppus*)*;* (ii) the regeneration of antenna from the amputated eye stalk in crustaceans (figure 4).

I would like the readers to pay special attention to the fact that these discoveries were made well before the announcement of homeotic mutations in *Drosophila.* Indeed, here

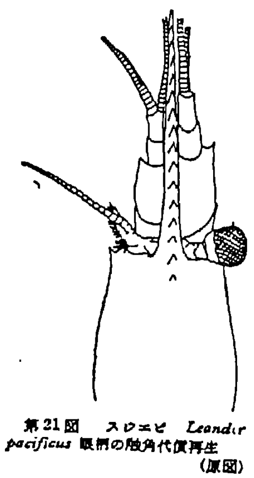


Figure 4. The regenerated antenna-like structure from excised eye-stalk in *Cambarus* (*Crustacia*). From Y K Okada (1944)

also "embryology preceded genetics", but it is very unfortunate that there were practically none to inherit the pioneers's legacy. From the very start, regeneration studies were more or less suffered from too much organismic or totalitarian philosophy and were often incompatible with the stream of analytical-minded biology. The phenomena themselves are already mysterious enough. Among them, homeotic regeneration may be a high-light.

In Japan Y K Okada, a great pioneer in embryology in this country who exerted here a vast influence on zoology in general, kept a keen interest on studies of homeotic regeneration throughout his life, and published a review (in Japanese) on this problem in 1950. I recall him often as a distinguished scholar with too much sympathy to a philosophy of wholism.

Recalling the history of developmental biology, it seems a rule that new major progress was gained through the experimental production of anomalous development. The production of identical twins from separated two celled blastomeres and of double embryos through transplantation of the organizer are two such examples, which led to new trend in developmental biology.

Homeotic regeneration, which is a very interesting issue by itself, should be such a case opening a new approach for investigating the normal mechanisms of regeneration. It is definite that here is a epigenetic (not genetic) regulative change of homeotic genes. Necessary information and techniques to explore the genes involved are ready for our use at present time. Thus, why should we not expect a coming new era for studying this most historical issue in regeneration studies?

Acknowledgements

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