Int. J. Dev. Biol. 33: 1-6 (1989)



# In search of embryonic inductors

## An interview with Sulo Toivonen on his 80th birthday

JUAN ARECHAGA

*Department of Cell Biology. University of the Basque Country. E-48940 Leioa, Vizcaya (Spain)*

Professor Sulo Ilmari Toivonen has been a leading figure in Developmental Biology for more than forty years and is one of the best known European experimental embryologists of the post-WWII period. He was born on January 12, 1909, in Somero, Finland, at a time when this country was still an autonomous Grand Duchy under the rule of the Russian Czar. While studying at the University of Helsinki he was deeply influenced by Gunnar Ekman (1883-1937), Professor of Experimental Zoology, who had been a pupil of Hans Spemann at Freiburg in the twenties. Under the guidance of Dr. Ekman, Toivonen earned his undergraduate degree in 1934, but he had to finish his Ph.D. thesis alone because of the early death of Ekman. The manuscript, entitled «Über die Leistungsspezifität der Abnormen Induktoren im Implantatversuch bei Triton» (a classic for embryonic induction studies), was completed and printed during the difficult times of the so-called «Winter War» against the Soviet Union (Toivonen, 1940). As a teacher Toivonen held several appointments at the University of Helsinki: Assistant in Zoology (1934-45), Lecturer in Experimental Embryology (1945-52), and Guest Professor of Experimental Zoology (1952-73). He was awarded an honorary M.D. degree in 1966 and has received many other national and international honors, prizes and awards. Moreover his bibliography contains over one hundred publications on experimental embryology of vertebrates. But, essentially, Prof. Toivonen's place in the history of modern developmental biology must be remembered as that of an original and creative pioneer who, with his stimulating laboratory research and lectures, tutored a fruitful generation of prestigious scientists —*the Toivonen team*— setting up a network of international contacts for Finnish embryologists after the Second World War.

If we look into the laboratory results and the specific content of the publications of Dr. Toivonen and his group over the years, certain steps in their development become apparent. He began his scientific career as a mammalian embryologist working on kidney development (Toivonen, 1934, 1945), a topic successfully explored later by his senior disciple Lauri Saxén, who with his own group used kidney development as a biological model to study inductive cell interactions (see Saxén, 1987). However, Toivonen's attention was soon attracted by one of the essential problems in developmental biology: the study of primary embryonic induction, mainly in amphibians. The most important results in this field can be summarized in four successive periods:

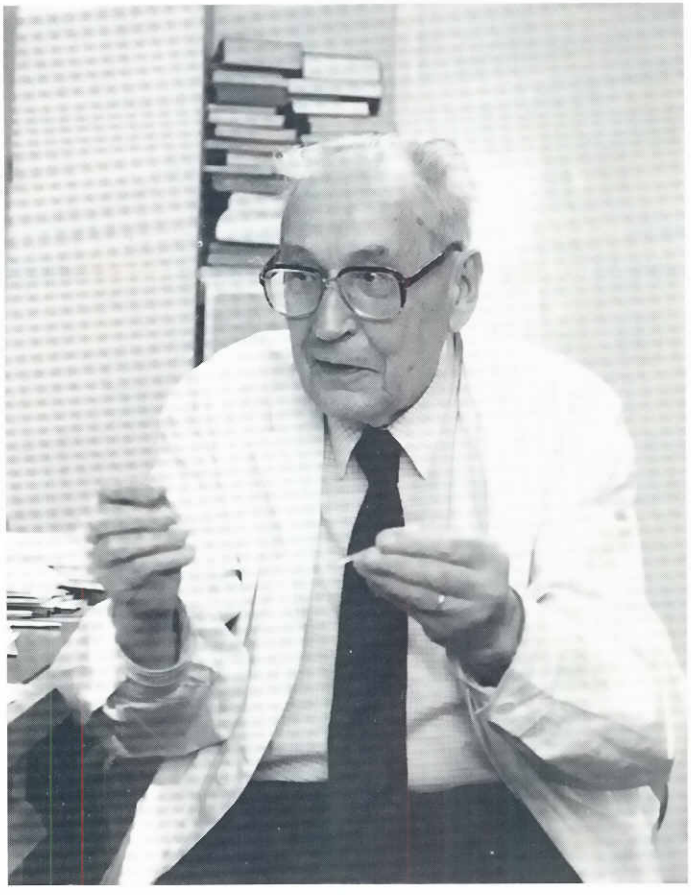
1. The discovery of the inductive action of foreign adult tissues on regional differentiation of the responding ectoderm (Toivonen, 1938a and b, 1940).
2. Attempts at chemical characterizations of determining agents to support a qualitative theory of primary induction (Toivonen and Kuusi, 1948; Toivonen 1949, 1950; Kuusi 1957a and b). The most important of these findings was that of a specific spinocaudal inductor (Toivonen, 1953, 1954) which rendered possible implantation and explantation experiments in amphibian gastrulae with two distinct heterotypic tissues: liver (cephalic and neural inductor) and bone-marrow (caudal and mesodermic inductor) from guinea-pigs (Toivonen and Saxén,

1955). Some years later and stimulated by the experiments of the Toivonen group, several laboratories used chick and *Xenopus* embryos to purify two active proteins generally known as neuralizing and mesodermalizing (or «vegetalizing») factors (see Tiedemann, 1981).

3. The experimental confirmation of the so-called *two gradient hypothesis* combining the effects of heated and unheated HeLa cell suspensions at different ratios as cephalic and caudal heterotypic inductors (Saxén and Toivonen, 1961). Similar results were obtained later with natural inductors (Saxén *et al.*, 1964; Toivonen and Saxén, 1968).
4. Assays to resolve the problem of the diffusibility of inductive agents, using radioactive or flurorescent tracers (Kuusi, 1959, 1960; Vainio *et al.*, 1962) and interposed membranes in different kinds of «sandwich» techniques (Saxén, 1961; Saxén and Saksela, 1971; Wartiovaara *et al.*, 1974; Lehtonen *et al.*, 1975; Toivonen *et al.*, 1975, 1976; Toivonen, 1979).

In addition to these and many other personal research contributions, Professor Sulo Toivonen has been an excellent reviewer and compilator of current literature on embryonic induction. In this regard, two of his books, *Primary Embryonic Induction* in collaboration with Lauri Saxén (1962) and *Organizer - A Milestone of a Half-Century from Spemann* co-edited with Osamu Nakamura (1978), are among the masterpieces of modern monographs on experimental embryology.

The following interview was made in Prof. Toivonen's office at the University of Helsinki Department of Zoology on March 17, 1988. Prof. Saxén kindly helped us to edit the text and took the photographs during the interview.



**After a long career as a developmental biologist, what or who motivated you to study embryology and how did you begin your training in this particular field?**

As a schoolboy I was already interested in biology, but upon entering the university, I was not sure whether I would like to learn biology or medicine. The first year of studies was common for both medical and biology students; hence, I had courses in vertebrate anatomy, plant anatomy, chemistry and physics. After this the decision had to be made, and I chose biology.

My training in embryology began during my biological studies at the University of Helsinki and especially when I met Professor Gunnar Ekman, who had worked with Professor Herman Braus and Professor Hans Spemann in Germany during the twenties and thirties. Dr. Ekman suggested the topic for my undergraduate thesis, which dealt with the early development of the mammalian kidney (Toivonen, 1934). I prepared serial sections and reconstructions from 8 to 26-day rabbit embryos and also dissected the nephric anlagen. The results were published ten years later (Toivonen, 1945).

By the time I had finished my first degree, Ekman was very interested in Holtfreter's experiments in which heterogeneous inductors like foreign tissues yielded isolated lenses in embryonic *Triturus* tissue. This is the reason why he proposed that I study the problem of how it is possible to obtain this kind of «abnormal» induction through the use of foreign tissues. Using this type of experimental approach I was able to repeat Holtfreter's findings, but additionally I soon found that different foreign adult tissues differed in their inductive capacities, since some induced merely cranial neural structures while others induced more caudal formations. Unfortunately, Gunnar Ekman died in October 1937, but he had already encouraged me to write my Ph.D. thesis on the basis of our results. However, I decided to expand the material and continued to collect samples during 1938 and 1939, so that I finally had close to 2000 operated embryos. The manuscript was finished in 1939, but the printing and official presentation had to be postponed until December 1940 (Toivonen, 1940).

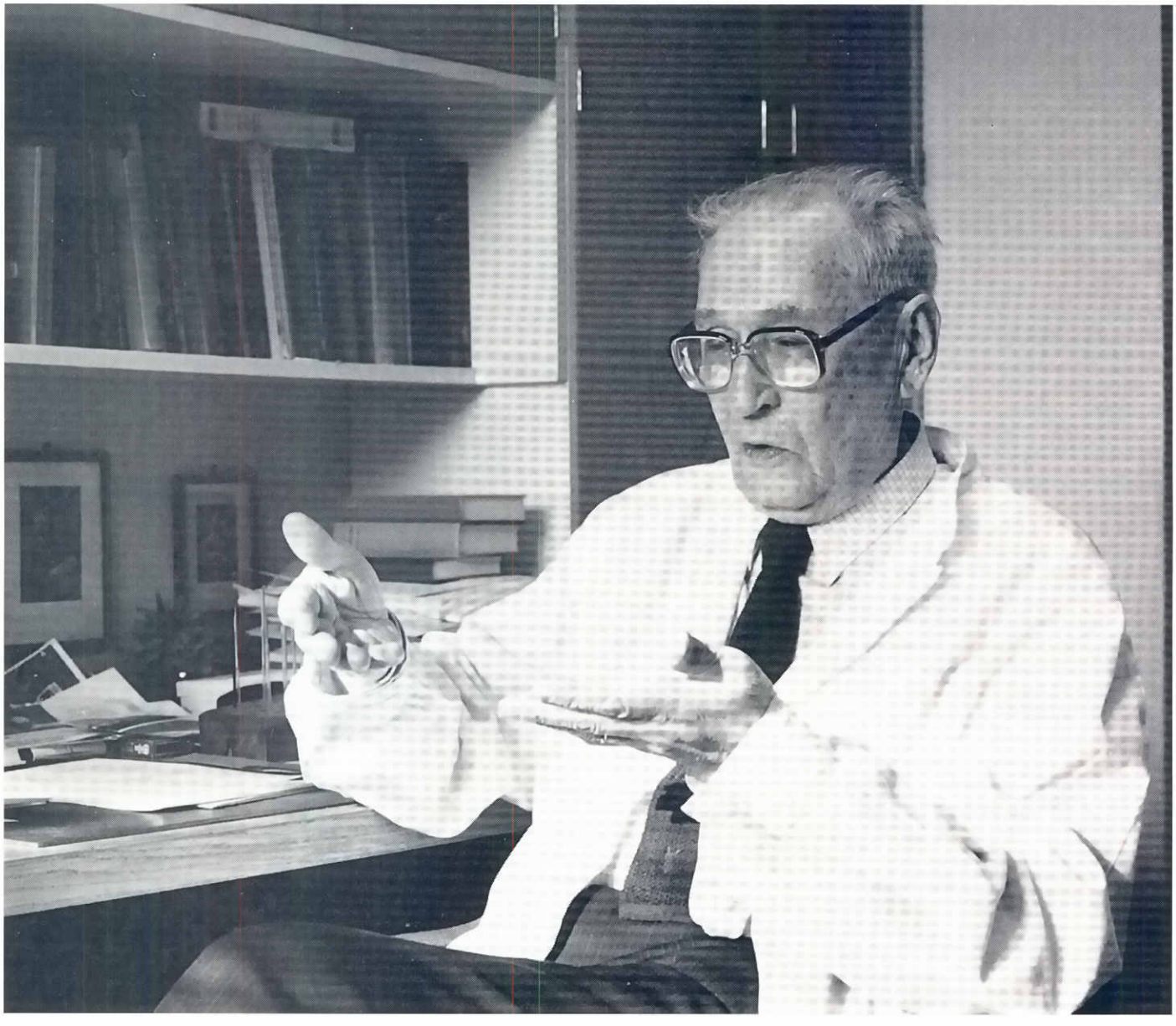
**Tell us about your personal experience at the side of Gunnar Ekman during this period**

Well, an interesting fact that I can relate from that time is the introduction of the aseptic technique for manipulating amphibian embryos. When I started with Ekman, the experiments were hampered by the very high mortality rate of the operated embryos due to infections. As his left-hand instrument Ekman was using the classic hair loop which could not be sterilized, and it carried the infectious agents into the operated embryos. I started to use a more aseptic technique involving thin glass needles drawn through a flame before each operation. The eggs themselves could be cleaned using short-term alcohol treatment. After removal of the membranes, I would expose the samples to 1 promil chloramine solution. Later, during my stay in Utrecht, I learned to use platinum loops and needles instead of fragile glass instruments. The aseptic technique allowed me then to prepare really large series of embryos to explore the inductive capacity of different tissues. The «operative season» in Finland is restricted to May-June, but with the high survival rate of the embryos, I was able to produce more than 1000 successful experiments each year.

**Like so many other young experimental embryologists of the thirties, you were probably fascinated by the Freiburg school. What findings of the Spemann group were most inspiring and stimulating for you at that time?**

As you know, Spemann had started his induction experiments long before I began, and together with Hilde Mangold he had shown in transplantation experiments that the dorsal lip region of an amphibian gastrula can induce a secondary neural plate in another embryo of the same stage. My *qualitative hypothesis,* postulating more than one inductor and different inductive actions, was really a direct continuation of the experiments of Spemann and his school.

**This is a very interesting point. Can you please tell us about the controversy between the quantitative and**



**qualitative hypotheses concerning embryonic induction?**

The *qualitative* hypothesis was not immediately accepted like the prevailing Dalcq-Pasteels theory *(quantitative)* which postulated a gradient of only one active factor. I presented my results and conclusions on several occasions, but it was not until 1954 when they were thoroughly discussed at a symposium in Bern that they began to be more widely accepted. At the symposium, I presented our first fractionation results obtained with my student and collaborator Taina Kuusi. Starting again from heterogeneous inductors, we showed that there is a strong thermo-stable inductor principle producing forebrain structures as well a thermo-labile principle inducing the more caudal structures. These and many solubilization experiments made it clear that there were two inductive principles, and toward the end of the symposium the Chairman, Professor Lehmann, proposed a model based on two opposite gradients of neural vs. spinocaudal inductors. Later, together with Lauri Saxén, we modified Lehmann's proposal and presented the so-called *two-gradient hypothesis* in 1955.

**To my knowledge, the development of this *two-gradient theory* to explain the process of determination of the central nervous system continued approximately until 1968. Could you please summarize the main experimental findings of this hypothesis and their consequences?**

Yes. This series of experiments was started in 1954, but was not really completed until fourteen years later. Without going

into detail, the hypothesis postulates two inductive principles, a «neuralizing» principle and a «mesodermalizing» one acting either separately or jointly in different concentrations along a gradient. The original hypothesis was based on experiments employing different heterogeneous inductors, but later we confirmed the results by using predetermined normal tissues from *Triturus* gastrulae and neurulae. Disaggregated cells from the presumptive anterior neural plate region were mixed with cells from the caudal axial mesoderm in different ratios. A whole array of CNS structures were obtained. The neural determination of the ectoderm is thus still labile and can be «regionalized» by the mesoderm during a second step.

**One more general question on this problem. In your opinion, which is more specific: the action of the inductive agents or the changes in the responding cells?**

I can tell you that the reactivity of the responding cells, their «competence», is of short duration. When isolated gastrula ectoderm is precultivated *in vitro* prior to exposing it to an inductor, the «neural competence» is lost in approximately fourteen hours. The responsiveness to mesodermal inductors seems to last somewhat longer as Anto Leikola in my laboratory has shown. Therefore, I think that both the competence of the target tissue and the type of inductive signal determine the outcome; although, to my mind, the second is the more specific.

**With such great progress in biochemistry during the last twenty years, why can we still not define embryonic induction in molecular terms?**

Let me tell you my personal experience. In 1963 I participated in a Developmental Biology Congress in Copenhagen and discovered that I could understand only about 10 percent of the lectures. People were presenting data on immunology and molecular biology, fields which I had not followed. Therefore I decided that there is no point going to meetings without knowing these disciplines. I started studying books on the two fields, but found learning at this age very difficult. Finally I decided to remain an old-fashioned morphologist and leave immunology and molecular biology to younger people.

I am sure that the final solution to the problem of embryonic induction is in the hands of immunologists and biochemists. My morphological contribution may provide a good basis for such analyses at the molecular level. Today we know that the inductors are proteins, and the mesodermalizing component must be internalized to act properly. The neuralizing protein has been extensively studied by several groups, and this compound acts on the cell surface. The precise mode of action is still not known.

**Finally, how do you see your life as a scientist in retrospect after more than fifty years of work?**

It is hard to say. Naturally, we were lucky to be linked to the Spemann tradition via Ekman who also brought the basic techniques to Finland. It has also been our policy to send our young doctors abroad for post-doctoral training, usually to the United States. Of my students, Saxén, Vainio, Kohonen and Leikola spent long periods in the US and Canada to learn new techniques and ideas. Tapani Vainio unfortunately died in an automobile accident soon after his return in 1965, and Lauri Saxén moved in 1967 to the Medical School in Helsinki together with his medically trained students. Juhani Kohonen accepted an appointment from the University of Turku, and Anto Leikola's interest shifted more toward science history, a field in which he received a professorship at the University of Helsinki. I myself retired in 1974, but have still been active in the spring season and perform some 500 operations on *Triturus* embryos annually with younger students. As a summary, I can tell you that I am very happy to have had very good teachers and excellent students. My personal contributions must be evaluated by others.

## Acknowledgements

We wish to thank the interest and skilled assistance given to us by Ms. Annikki Kaitila of the University of Helsinki (Finland) and Ms. Karen Shasok of the University of Granada (Spain) in the transcription of the tape and the editing of this interview.

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