

## Inductive tissue interactions

An interview with Chancellor Lauri Saxén

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Chancellor Lauri Saxén has been one of the leading figures in developmental biology for more than thirty years and he is one of the best known experimental embryologists of the post World War II period. Lauri Otto Saxén was born on July 27, 1927 in Helsinki, Finland. Studying at the University of Helsinki, he earned a M.Sc. in 1950, M.D., Ph.D. (Faculty of Medicine) in 1954 and Ph.D. (Faculty of Science) in 1962. He had a short Postdoctoral Fellowship in the laboratory of Clifford Grobstein at Stanford University in 1959. Dr. Saxén continued his research at the University of Helsinki, intermittently as a Research Fellow of the National Research Council, as an Assistant (Instructor) and as a Specialist (Consultant) in Pathology until 1965, when he was appointed Associate Professor in Pathology. In 1967, Lauri Saxén was appointed lifetime Professor in Experimental Pathology (invited position) at the University of Helsinki and in 1993 he was elected to the post of Chancellor of this University. After retiring in 1996, Lauri Saxén has maintained a very active national and international role in science.

The main scientific interests of Lauri Saxén have included the development of visual cells, hormonal control of Amphibian metamorphosis, primary embryonic induction and epithelio-mesenchymal interactions. He has also had a major impact in both epidemiological and experimental teratology. Lauri Saxén has published three monographs, approximately 300 scientific publi-

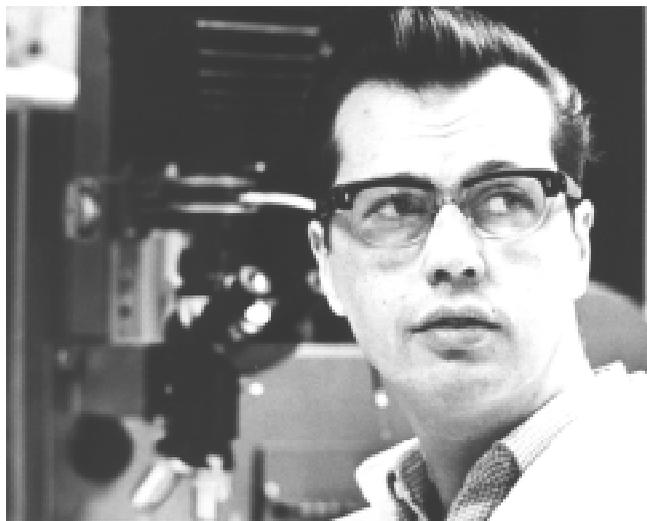
cations and 80 editorials and has held 130 invited international lectures. He has supervised 25 academic dissertations.

The many international positions of Lauri Saxén include the presidencies of the European Teratology Society (1973-74) and the International Society of Developmental Biologists (1973-77). He was the Editor-in-Chief of *Cell Differentiation* from 1981-90 (*Cell Differentiation and Development*, 1988-1990). Lauri Saxén was the President of the Finnish Cultural Foundation (1977-79), President of the Finnish Medical Society Duodecim (1982-85), Chairman of the Section for Medical Sciences of the Finnish Academy of Sciences (1983-92), Chairman of Board of the Institute of Biotechnology, University of Helsinki, (1989-93) and General Secretary of the Delegation of the Finnish Academies of Science in 1993. He acted as Editor-in-Chief of the Finnish Medical Journal "Duodecim" in 1971-72. Lauri Saxén has been the Chairman of the Finnish Institute in Athens since 1995.

The many awards of Lauri Saxén include Honorary Memberships of the Finnish Medical Society Duodecim (1987) and the Finnish Society of Developmental Biology (1987), and the Honorary Presidentship of the Finnish Culture Foundation (1994).

The following interview was held at Lauri Saxén's home on May 25, 1999.

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Lauri Saxén at the Laboratory of Developmental Biology, Third Department of Pathology, University of Helsinki, 1969.

**How does a medically trained student become interested in basic developmental biology which, in principle, has no overt connection to medicine?**

Perhaps you should first find out why a young student chose research instead of practical medicine. The answer is that the choice was easy, as science was already dominant in the family –my grandfather was a mathematician and all his three sons were scientists, as was my elder brother. How, then, the student becomes specifically interested in developmental biology is a somewhat more complicated story and involves a series of coincidences. Starting as a biology student, the theme of my MA-thesis was “photomechanical movements”, a subject of considerable interest in the forties which concerned the spatial shift of the retinal sensory cells as a response to light vs. dark adaptation. Working on three amphibian species, I soon came across some peculiar types of visual cells, the double and twin cells. To clarify the role and function of these elements, I began a systematic study of their origin and differentiation during early embryogenesis. This interest soon reprogrammed the entire project to be focused on the “Development of the Visual Cells”, which subsequently became the theme of my Ph.D. thesis.

During this work I became fascinated by the mysteries of development, differentiation of various cell types and their exact spatial organization during embryogenesis. Hence, when I finished my Ph.D. studies in 1954, I attended a short course on “Techniques in experimental embryology” given by Professor Sulo Toivonen at the Department of Zoology of the University of Helsinki. This exciting experience of early amphibian embryos and their dissection was the ultimate trigger for my scientific career, and I immediately applied for a postdoctoral position in Professor Toivonen’s laboratory. This began our close collaboration which was to last for 15 years until I established my own laboratory in the newly finished facilities of the Department of Pathology. My friendship with Sulo Toivonen lasted until his death in 1995.

**During those years, however, you had obtained special**

**training in pathology (morbid anatomy). Did this affect your research interests or were they still mainly focused towards developmental biology?**

My interests may have somewhat broadened, and many of my MD-students seemed to prefer themes more closely related to medicine and mammalian pathology. But I myself considered developmental biology and the problem of inductive tissue interactions still most exciting. In the late fifties, the original idea of Spemann (Spemann and Mangold, 1924) concerning the determination of the neuraxis had been repeatedly confirmed, but the exact nature of the molecules which apparently emitted the determinative stimuli were still practically unknown and their mechanisms of action obscure. This was an obvious challenge for a young scientist.

**But in the sixties you started to explore another inductive system, the interaction of epithelium and mesenchyme during early development of the mammalian kidney. Why this shift?**

The main reason was a pragmatic one. Traditionally, the exploration of the “primary”, neural induction used *Triturus* eggs and embryos for the dissection experiments. Our local species, *Triturus vulgaris* did not breed in laboratory conditions, and, hence, we were fully dependent on wild material collected in springtime. Consequently, all experiments had to be completed in May-June, whereafter the samples were analyzed in the fall and winter. This meant that both all confirmatory experiments and tests of new ideas had to wait for another year, and this was really too long a process as we were working in a competitive and rapidly developing field. Hence, new model-systems had to be looked for, and the one developed by Clifford Grobstein seemed ideal in many respects. After a short stay in his laboratory, I introduced this system to researchers in Helsinki in collaboration with a former classmate from the medical school, Tapani Vainio. Most unfortunately, our fruitful cooperation ended in 1965 when Tapani was killed in an automobile accident. The kidney model-system has, however, been used in my laboratory since, and many foreign visitors have introduced it to their laboratories. Some of the main results obtained by this technology are presented in this volume. (I also refer to my obituary for Clifford Grobstein in this volume and to my monograph from 1987.)

**What, then, would you consider your most important scientific contribution using these two model systems?**

I am not the one to evaluate the scientific merits of these investigations, but I can certainly discuss the approaches that have given me the most pleasure. As to the *Triturus* work, exploring primary induction was personally most satisfactory. Everything from the collection of the animals, planning and implementing the manual experiments and the final analysis of the samples was done by us without practically any technical assistance. To follow daily the operated embryos under the microscope and detecting the gradual formation of the induced supernumerary structures was truly pleasurable. Sometimes you became so much attached to these little objects that it was almost a pity to fix them for further analysis (I still have somewhere in my

drawers a collection of photographs labeled “pets” representing the most beautiful cases). Due to the short “operational season” mentioned above, the experimentation was extremely intense and each spring we practically lived in the lab for six weeks. This tended to promote close personal bonds between the members of the team and stimulated night-long discussions on science, philosophy and sometimes even politics.

When we shifted to the kidney model-system, and started attracting young colleagues to the team, the pleasure became different. Instead of a personal contribution at the bench, you now enjoyed the efforts and devotion of young, bright people who gradually matured from ordinary medical students to trained, independent researchers. Of those who wrote their dissertations on kidney development, I would like to mention here Jorma Wartiovaara, Olli Koskimies, Eero Lehtonen, Peter Ekblom, Hannu Sariola and Kirsi Sainio. Irma Saxén (Thesleff) modified the technology to be used in studies of the epithelio-mesenchymal interactions in tooth development later adopted by Seppo Vainio. Most of these students later established their own research groups, and their old supervisor follows their careers with satisfaction and pride.



Tapani Vainio, Lauri Saxén and Sulo Toivonen (from left to right) at the Wetterkulla Medical Center in 1964.

**Your name is usually linked to the “double gradient” hypothesis. Could you briefly outline this classic theory and its background?**

As already mentioned, Spemann and his group had convincingly shown in the twenties that determination of the neuraxis is “induced” by the axial mesenchyme underlying the prospective neuroectoderm. The signal molecules were then unknown, and in the thirties the prevailing hypothesis postulated one “organizer” acting in different concentrations upon the competent ectoderm. The hunt for this magic substance failed and, gradually, interest in the problem likewise faded. In Finland, the Spemann tradition flourished after Gunnar Ekman (1893-1937) who introduced it from Germany and convinced his student, Sulo Toivonen (1909-1995) to explore the matter further. Using the so called “heterogeneous” inductors (live and devitalized tissues of various species), Toivonen showed that their action varied qualitatively; some inducing cranial neural structures, while some others converted the responding ectoderm into caudal neural structures and mesodermal derivatives (Toivonen, 1940). He concluded that there had to be at least two inductive principles with different actions, and this was the point where our collaboration started in 1954.

Chemically purified samples or compounds exhibiting such effects to test the postulated action of two inductors were at that time not available, and throughout our experimentation in 1954-68 we had to overcome this by the use of heterogeneous inductors mimicking the effect of our hypothetical factors. We began by using a combination of a neuralizing tissue (liver) and another tissue with an almost pure mesodermalizing effect (bone marrow). The combined effect of these two was not a simple summation of their actions, but produced a completely new array of neural and mesodermal structures. This led us to formulate the first version of the “double gradient” hypothesis (Toivonen and Saxén, 1955). Next, we used another set of heterogeneous

inductors now mixed in different ratios and obtained results well explainable by our hypothesis (Saxén and Toivonen, 1961). The third decisive series of experiments made use of experimentally predetermined cells (neuralized vs. mesodermalized) mixed in a disaggregated state and subcultivated after reaggregation. Briefly, the observations indicated that determination of the CNS was clearly a multistep process where a primary neuralization is followed by regional specification by mesodermalized cells (Saxén et al., 1964). The quantitative nature of these sequential processes was finally proven in experiments where normal embryonic tissues (anterior neural plate and axial mesoderm from early neurulas) were mixed in different ratios. The array of CNS structures developed in these aggregates was exactly that predicted by our “double gradient” hypothesis, which we now considered rather well confirmed (Toivonen and Saxén, 1968). For various reasons we stopped here in expectation of more advanced molecular techniques and a new generation to carry the analysis further. It took another fifteen years until this really occurred, but today our knowledge of “primary” induction has increased tremendously. In my mind, however, the molecular approach has not yielded observations which contrast with our old hypothesis of sequential induction implemented by several factors acting jointly along gradients. Needless to say, I follow these developments with a keen interest and with a certain amount of pleasure.

**You have also shown considerable interest towards teratology, the causes and pathogenesis of congenital defects. Is this because of your medical background?**

In 1961 the world was shocked by the “thalidomide catastrophe” as a seemingly harmless drug which, when ingested by mothers to be, affected thousands of embryos. One consequence



Sulo Toivonen, Bo Thorell, Osmo Järvi, Erkki Saxén, Lauri Saxén, Harri Nevanlinna and Esko Nikkilä (from left) at the Wetterkulla Medical Center in 1970

in Finland was the establishment of a Register of Congenital Malformations, originally planned to function as a warning system for new epidemics or defects. As one of the few medically trained developmental biologists in the country, I became involved and we soon developed the system into a small unit for epidemiological studies. The use of questionnaires and personal interviews led to the development of a data bank which was subsequently analyzed in order to identify potentially harmful factors (Saxén, 1983). Since this approach gave little information about the mechanism of abnormal development, we decided to use the rather advanced organ culture methods available to explore the mechanisms. Several exogenous agents were tested *in vitro*, e.g., polyoma and rubella viruses, antibiotic tetracycline and cortisone preparations. By using tissues from various inbred strains of mice, we also got some insights into the genetic control of susceptibility to these harmful factors added to the culture medium (Saxén, 1988).

These interests also produced a small textbook, written with my former student, Juhani Rapola, for Holt, Rinehart and Winston in New York (Saxén and Rapola, 1969). But all this was more or less a sideline as inductive tissue interactions were still the main theme of our research.

Over the years you have served on committees for various funding and administrative bodies like the Finnish Medical Society, the Finnish Cultural Foundation, the Society for Popularization of Science and many international organs such as the International Society of Developmental Biologists, not to mention the many scientific journals you have edited. Ultimately, you reached the pinnacle of your academic career with your appointment as Chancellor of the

**University of Helsinki. All this must have distracted you from scientific activities. Has it been worth it?**

Indeed, I have divided my time between all these activities, but for the most part it has been enjoyable. Moreover, I believe that it is of utmost importance that such posts are held by experienced scientists rather than by bureaucrats. We must also remember that Finland, with its small population, has probably the same number of such bodies and committees as many larger countries, and hence one cannot avoid being involved.

I especially enjoyed my three years in the Chancellors office. In this post one has a perfect view of the country's entire academic life, one is in the position to make important decisions and initiatives and the chancellor has the opportunity to meet truly interesting new people. The Chancellor of the University of Helsinki has the traditional privilege to attend the Cabinet Meetings (as the only outsider), and this naturally offered me a unique opportunity to become acquainted with the highest level of decision-making. I cannot say that I particularly enjoyed these meetings.

**May I next ask about the "Wetterkulla Medical Center", an institution often mentioned among our scientists but otherwise rather unknown. What exactly is behind this eponym?**

Thank you for bringing up this favorite institution of mine in this interview. I could write a book about this half-serious organization but let us provide only a short summary. In the heart of the Häme province, lies the manor Wetterkulla, settled as far back as the 16th century and later owned by my mother's family. The manor may have found its way into the medical history of Finland by mere



Senior members of the staff of the Third Department of Pathology at the University of Helsinki in their weekly business meeting (1973). From left: Lauri Saxén, Martti Virolainen, Erkki Saxén, Stig Nordling, Jorma Wartiovaara, Pekka Häyry and Eero Saksela.

chance, and remains there as a matter of cultural curiosity. One dark autumn day in 1960, Sulo Toivonen and myself retreated to the peace and quietness of the place to finish our monograph "Primary Embryonic Induction" (Saxén and Toivonen, 1962) which, indeed, was completed in this favorable environment. Inspired by this fruitful example, some of our good friends and colleagues subsequently joined the fraternity which quickly grew to its final strength of 11 members. The unwritten operational principles became defined: to secure tranquil reading and writing surroundings for a scientist, to provide an inspirational atmosphere for the innovative mind, and to offer a moment's relaxation for the frustrated man.

Over the past 35 years the group has met 70 times - one 3-5 days' session in the darkest season (December) and another regular meeting in the spring. More than 100 lectures have been delivered by its members and by distinguished visitors, and many initiatives have been undertaken by the group representing various disciplines in biomedicine and their background organizations. Close bonds between the members have developed and the three small booklets published on the activities are witnesses to the enjoyment, hard work and recreation in the beautiful surroundings.

#### **Finally, what future developments do you predict in your field?**

Looking back to the extremely rapid and unpredictable development of the field over the past twenty years or so, one becomes rather hesitant to forecast events and themes for the next millennium - or even its first decade. In basic developmental biology, we will certainly enter a postgenomic era; as a great number of developmentally regulated genes have already been found and soon the entire genome of various species would have been mapped out, it is now time to use our modern technology to unravel the function and interaction of these genes. Here, sequencing the genes and localization of their gene products might not be sufficient and new functional tests must be developed. This may take us back to classical embryology as we search for better model-systems and organisms to experimentally manipulate. This is going to be an ambitious but not an easy task.

As to the application of developmental biology to medical problems, some recent advances are most remarkable. Successful cloning of several mammalian species might, in the more distant future, offer a means to bypass paternal infertility, and the extraction of pluripotent cells from preimplantation human embryos may provide material for replacement of cells and tissues in diseased organisms - patients with Parkinson's disease or



**Lauri Saxén and Sulo Toivonen at the Wetterkulla Medical Center in 1974.**

juvenile diabetes have been mentioned as possible recipients. Much work in basic developmental biology will be required to find the means to canalize these competent cells to their ultimate stage of differentiation. A challenging line of research was recently outlined by scientists at the new Geron Bio-Med company: to circumvent the use of human eggs and the consequent ethical problems, efforts will be made to create stem-cell donor material by "reprogramming human cells without using eggs or creating embryos" as Ian Wilmut has put it (see Wadman, 1999).



**Spring Symposium of The Developmental Biology Laboratory in Tvärminne Zoological Station, close to the town of Tammisaari on the coast of the Gulf of Finland, 1995. Lauri Saxén is standing second from right**

## References

- SAXÉN, L. (1954). The development of the visual cells. *Ann. Acad. Sci. Fenn. (A)* 23: 1-93.
- SAXÉN, L. (1983). Twenty years of the study of the etiology of congenital malformations in Finland. In *Issues and Reviews in Teratology*, (ed. H. Kalter), Vol. 1, Plenum Press, New York, pp. 73-110.
- SAXÉN, L. (1987). *Organogenesis of the Kidney*. Cambridge University Press, Cambridge.
- SAXÉN, L. (1988). Teratogenesis *in vitro*. *Adverse Drug React. Acute Poisoning Rev.* 2: 75-94.
- SAXÉN, L. and RAPOLA, J. (1969). *Congenital Defects*. Holt, Rinehart & Winston, New York.
- SAXÉN, L. and TOIVONEN, S. (1961). Two-gradient hypothesis in primary induction. The combined effect of two types of inductors mixed in different ratios. *J. Embryol. Exp. Morphol.* 9: 514-533.
- SAXÉN, L. and TOIVONEN, S. (1962). *Primary Embryonic Induction*. Academic Press, London.
- SAXÉN, L., TOIVONEN, S. and VAINIO, T. (1964). Initial stimulus and subsequent interactions in embryonic induction. *J. Embryol. Exp. Morphol.* 12: 333-338.
- SPEMANN, H. and MANGOLD, H. (1924). Ueber Induktion von Embryonalanlagen durch Implantation artfremder Organisatorer. *W. Roux's Arch. Entwmech. Org.* 100: 599-638.
- TOIVONEN, S. (1940). Über die Leistungsspezifität der abnormen Induktoren in Implantationsversuch. *Ann. Acad. Sci. Fenn. (A)*: 55: 1-150.
- TOIVONEN, S. and SAXÉN, L. (1955). The simultaneous inducing action of liver and bone-marrow of the guinea pig in implantation and explantation experiments with embryos of *Triturus*. *Exp. Cell Res.* 3 (Suppl): 346-357.
- TOIVONEN, S. and SAXÉN, L. (1968). Morphogenetic interaction of presumptive neural and mesodermal cells mixed in different ratios. *Science* 159: 539-540.
- WADMAN, M. (1999). US stem-cell pioneers buy "Dolly" cloning company. *Nature* 399: 127.