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The causal analysis of development in the past half century: a personal history

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I feel greatly privileged having been asked to talk to you here and I want to begin by thanking the organizers for this invitation.

My task of preparing this talk has caused me considerable worry. Obviously, I shall not be able to present here a sound and objective history of embryology over the past 50 years. If nothing else, my great admiration for my close friend Jane Oppenheimer would keep me from being bold enough to step onto her territory, and there have been other serious attempts of an analytical evaluation of embryology during the past half century, e.g. the Nottingham symposium in 1983, published in 1986. What I intend to present here are my personal reflections based on reminiscences over the years during which I had the good fortune of seeing our science develop and of getting to know personally many of the scientists actively involved in the causal analysis of development. Because I am convinced that scientists do not operate intellectually or experimentally in a vacuum totally divorced from personal, social and political phenomena in their environment, I have always paid attention to these extraneous factors, and I am glad to have an opportunity to share here some personal and perhaps unorthodox impressions which I have gained over the years. To me, at least, they add to the total historical image of our branch of science.

I came onto the scene of experimental embryology, as it was called at that time, in 1928. I had completed several semesters studying Zoology and Chemistry in Königsberg, my home town in East Prussia, and in Berlin, and in a course in Embryology I had learned of Spemann's work. In Germany at that time students were still encouraged to migrate as much as possible, and so I decided to go and see if Spemann would accept me as a graduate student. Our first meeting made it quite clear that we were not meant for each other, but I suppose Spemann did not have quite enough courage to turn me down outright. Actually, I had come there at the same time as a fellow student, a young man who became an object of Spemann's love at first sight and who remained his favourite pupil. So Spemann decided that I could be of some help by carrying out a rather boring descriptive study of limb development for my own Ph.D. dissertation which would provide the essential basis for the young man's quite exciting experimental problem of the respective roles of ectoderm and mesoderm in limb pattern formation that Spemann had proposed for his doctoral research. There was no doubt about Spemann's prejudice against women, and my own case serves as an illustration.

Nevertheless, the years in Spemann's Institute were stimulating to the utmost. As a graduate student I shared a lab that housed six pre- and post-doctoral students, among them Hall, an American, Schmidt, a Russian, Sato, a Japanese, and Oscar Schotté of hopelessly mixed nationality. Spemann came to our lab every day, stopped at every desk and discussions between him and the rest of us were extremely instructive. Spemann had high standards of perfection, and we had to learn to live up to them. During the 'season', i.e. that of Amphibian egg laying, all of us worked day and night and we shared results, interpretations, etc. Spemann's eminence in his field of experimental embryology attracted visitors from all over the world, and I remember meeting Walter Vogt who introduced Nile blue as a vital stain of Amphibian embryos, Richard Goldschmidt, Paul Weiss and others there. Spemann was very outspoken in his criticisms, and I never forgot the scathing words with which he demolished Paul Weiss after a seminar the latter gave. In contrast to this, Spemann's admiration for Boveri, who had established the individuality of the chromosomes and their role in heredity, knew no limits and to this day I feel the strong influence this had on me. I never met Boveri, but knew his widow who taught Biology at a girls' college in New Haven, and I had the good fortune of being asked some years ago to translate Boveri's 1902 paper into English. While working on that, I experienced some of the excitement Boveri must have felt in the course of interpreting his experimental results with polyspermy in sea urchins and the development of the chromosome theory of inheritance.

In spite of our great admiration for Spemann, some of us in the institute were surprised by his obvious limitations. I was particularly conscious of his total lack of recognizing and taking into account any genetic considerations in the interpretations of his experimental results, in spite of his being a student of Boveri. When Oscar Schotté in xenoplastic transplantation experiments accomplished the demonstration that anuran tissues when transplanted into urodeles were subject to embryonic induction by urodele, i.e. host, tissues but that the developing structures expressed donor, i.e. anuran, specific traits, Spemann used a military analogy in his interpretations: he compared the response of the induced cells to that of a German soldier to the order of 'salute' after having been put into the French army. The soldier understood the order but in response he would salute

the German way. In using this analogy, Spemann totally left out of consideration the possible role of the genetic makeup of the induced tissues. The narrowness of mind expressed in this incident, both on the intellectual and the scientific level, surprised me greatly.

I would like to say a few words about Spemann's political attitudes without analyzing them in detail here. He was a strong German nationalist, full of mistrust towards other nationalities and sharing prejudices of his fellow nationals. I already mentioned his prejudice against women expressed also in his dealings with Hilde Proescholt[1], the discoverer of the 'organizer,' who is reported not to have appreciated it when Spemann added his name to the publication of her thesis while other male students were permitted to publish their work alone. Many years later, the story of the 'dead' organizer provides another example: it was a female graduate student, Else Wehmeier, who first observed embryonic induction with a Bouin-fixed piece of upper blastopore. I was around at that time. Her name did not even appear on the first publication reporting this exciting result!

However, it is not my intention to give you a totally negative picture of Spemann here. I think of him as a very influential and productive figure in 20th century experimental embryology - but I do not worship him as a hero, and in many ways he is a true representative of the German academic atmosphere at that time, which was full of prejudices of various kinds, including anti-semitism. Some of the narrowness expressed in such prejudices extended also into Spemann's science. Conceptually, the 'dead' organizer did not really appeal to him since he was essentially a vitalist whose mysterious concepts actually even served to prevent bold experiments, and he was full of mistrust towards people like Waddington who wanted to explore and identify the chemical nature of the organizer. Waddington came to Freiburg as a young graduate of Cambridge and we became very close friends. He opened our eyes to the biochemical and molecular problems inherent in inductive interactions between cells. He also fully recognized the involvement of genetic mechanisms in developmental phenomena. Waddington remained one of my closest friends until the time of his death.

Among those who influenced me strongly in my scientific development is Viktor Hamburger. He was Spemann's so-called 'assistant' when I became a graduate student in Freiburg. Spemann turned over to him the close supervision of my dissertation research, and Viktor saw to it that I remained on a straight path. At that time, Hamburger's work was concerned with the analysis of the role of innervation in limb development. His approach to science was broad, and he was the only one who provided us students with some introduction to the principles of genetics. He also engaged us in theoretical discussions, and was most likely responsible for arranging the joint seminars with the Department of Philosophy and Heidegger, the phenomenologist, which, though memorable, did not accomplish much. Hamburger also had to leave Germany in 1933, and of course you know of him as one of the founders and leaders of modern Neuroembryology.

In the United States, I had the good fortune of meeting Ross G. Harrison soon after my arrival there in 1933 as a refugee from Hitler. Harrison's dynamic approach to problems of development and differentiation was in many ways no less impressive than that of Spemann, and it seems that he would have deserved to share the Nobel Prize with Spemann. I enjoyed my contacts with Harrison immensely, and I remember particulary his great sense of humour. His early experiment, in which he proved that the nerve fiber arose as an outgrowth of a single neuronal cell, is most impressive. For the purpose of this experiment, he developed a totally new method, i.e. that of tissue culture; however, unlike many scientific technologists, he did not continue to pursue this exciting method for its own sake but only in the service of significant problems.

My own scientific career was decisively influenced by my association with L. C. Dunn at Columbia University who had recognized early the significance of genes in processes of development and differentiation. Furthermore, Dunn appreciated the potential of mutations affecting mammalian development for purposes of identification and analysis of the corresponding normal developmental mechanisms and their genetic control. In particular, Dunn focused his attention on the T-locus and its mutational effects on mouse development. He realized that some knowledge of experimental embryology would be helpful in these studies, and in 1935 he invited me to join his laboratory because of my previous experience. The environment at Columbia University differed greatly from that which I had left behind in Freiburg. Politically, Dunn was extremely progressive. He helped Nazi refugees, fought Fascism and participated in activities of the 1930s, e.g. those of supporting the Spanish Loyalists and the American-Soviet Friendship Committee. In my early years at Columbia, I even met E. B. Wilson who came to the Department on crutches and only rarely, but I still appreciate these meetings. I consider Wilson and his book among the most important milestones in the history of developmental biology.

During the middle and late 1930s and the 1940s, I witnessed the expression of a strong liaison between embryology and genetics. I know that this view contrasts with that held by others, and I believe it may be due to a large extent to my own close contacts with particular people such as Dunn, Goldschmidt, Waddington and Ephrussi. Of course, E. B. Wilson's book also lent strong support to the early and close relationship between the two fields. I got to know Richard Goldschmidt well, after he came to the USA, and I came to admire his breadth of vision, his courage, his imaginative thinking and his knowledge. His well-known and feared arrogance did not bother me, and to this day I appreciate his writings, be it in the book he created for his children, *Ascaris*, his *Physiological Genetics*, his book on 'the material basis of evolution,' or his *Theoretical Genetics*.

Waddington continued his contributions to Developmental Genetics more on the theoretical than the experimental level. His books *Organizers and Genes*, *New patterns in Genetics and Development*, and *The Strategy of the Genes*

[1]Hilde Proescholt is perhaps better known by her married name, Hilde Mangold, co-author with Spemann of their famous 1924 paper. (editors' note)

provided much interpretation and speculation concerning development and its genetic control. As quoted by Needham not long ago, Waddington predicted that for the analysis of development, people would have to come back to experimental embryology since concentration on the genetic code and genetic engineering would never reveal all the secrets of embryogenesis. Waddington's relatively early death prevented him from seeing how this is actually happening now. I would have loved to discuss the current state of affairs with him!

Ephrussi's significant contributions to the causal analysis of development are unfortunately not sufficiently known, e.g. that to the analysis of T-locus effects in 1935 where he used explantation techniques to exclude the possibility that the T mutation acted as a cell lethal. He was a brilliant scientist who perhaps paid too much attention to what he himself called 'l'ordre du jour', and thus changed experimental systems too frequently, instead of pursuing problems in greater detail and depth.

If we return now once more to the question of the Amphibian 'organizer' and ask ourselves about real progress in its analysis since the time of Spemann, i.e. more than 50 years later, answers are restricted largely to the negative category. Many chemical substances have been excluded, and we know to some extent what the organizer is **not**, but we still do not know precisely what it actually represents. I remember a long talk between Spemann, Rudolf Schoenheimer and myself in 1932 in which we tried to design a joint project in order to identify the biochemical basis of the organizer. The project never materialized because of the Nazis. But in the final analysis even now the point has not been reached where the chemical nature of the neural inducing factor, i.e. the organizer, has been determined. Much important work in the years since, on cell and tissue interactions e.g. in the development of the kidney and various glands as studied under in vitro conditions, is in a way an outcome of the pursuit of the question of inductive phenomena during early embryogenesis. I am thinking particularly of the contributions by Edgar Zwilling, a developmental geneticist who worked with Walter Landauer at the University of Connecticut in Storrs, and who made use of methods similar to those developed by Grobstein. Zwilling was a good friend who died quite young.

Among the concepts which have undergone radical changes in the course of the past 50 years is that of the constancy of the genome during development. Whereas the dynamic state of the developing and differentiating cell came to be accepted in the course of transplantation and explantation experiments, the stability of the genes and the constancy and integrity of the DNA during embryogenesis attained the state of a dogma that lasted until not very long ago. Today this dogma is being reevaluated in the light of dynamic progress of modern developmental genetics. In this connection, I remember discussions with Barbara McClintock more than 40 years ago. In my personal history of developmental analysis, these talks with her play an important role, even though Barbara and I disagreed about possible solutions for problems of women in science! I also remember with pleasure discussions with Briggs and King, who presented the first evidence for possible irreversible modifications of the nuclear genome during differentiation. At a meeting many years ago, it was Leroy Hood who first called my attention to the immunoglobulin system and its role in the formulation of the concept of genomic plasticity during development. Of course today the elegant experiments of nuclear transplantation, e.g. those performed by Davor Solter, give the strongest promise of eventually unravelling some of the puzzles of genomic modifications during differentiation.

It is essential at least to make reference to several additional systems of developmental analysis. My personal memories include a meeting with Andrej Tarkovsky, the creator of the chimaera system for studies of development. His first paper on this was published in 1968! Since that time, many variations of experimental chimaera production have been developed, and at this time sophisticated methods for chimaera production are being used by many people, e.g. Jaenisch who injects single transfected cells into blastocysts. These approaches appear most promising in developmental studies and will hopefully be as successful as Jaenisch's earlier experimental design of using insertional mutagenesis for the identification of developmental genes.

In my talk here, I have so far focused exclusively on vertebrate and particularly mammalian embryos, which is appropriate because they are closest to my own scientific activities. In this personal account of the history of causal analysis of development, I must however transcend the mammalian organism and include the recent most exciting work on *Drosophila* by E. B. Lewis of whose fan club I have been a strong member for many years. Lewis's work focuses on the so-called bithorax gene complex and its mutations which disrupt the characteristic segmentation of the fly's body and cause the transformation of particular segments and their specific organs into body segments of different types and with different specific organs, i.e. so-called homeotic mutations. Lewis attempted to correlate these mutational changes of morphogenesis with underlying changes in the relevant genes and their organization. His own classical approach - that of a developmental geneticist who works his way from the mutant phenotype back to the genome - received strong complementation by the interest and active collaboration of molecular biologists such as David Hogness and Welcome Bender and their direct attacks on the DNA level itself with sophisticated molecular genetic techniques. In this way, they succeeded in mapping a considerable stretch of the bithorax complex and locating mutational changes of its DNA and the eventual discovery of homeoboxes. The problems of homeoboxes in various organisms and their developmental effects, are far from solved. But the analysis of the bithorax complex presents a striking example of progress in developmental genetics made possible by the study of the genetic and developmental details of a system with the best tools available and with the imaginative foresight of a classical geneticist. Thus, Lewis prepared the stage for an approach with techniques of molecular genetics that has the potential of promoting the general understanding of the molecular basis of the genetic control of development and differentiation.

I also knew Hadorn well, who actually was the first to make use of homeotic mutations in *Drosophila* in his analy-

sis of the genetic control of cell determination. Hadorn was remarkable in combining a strict and straightforward attitude to life, to science and to all his activities with an unusual degree of imagination in experimental design and interpretation. I remember with great amusement a small meeting in London where Hadorn and Waddington, protagonists of two diametrically opposed Weltanschauungen, clashed repeatedly on issues including science as well as life style.

I am fully aware of many omissions in this account. To mention just one particularly glaring one, it is that of teratocarcinomas and stem cell lines, which offer such promising and potentially valuable systems of analysis.

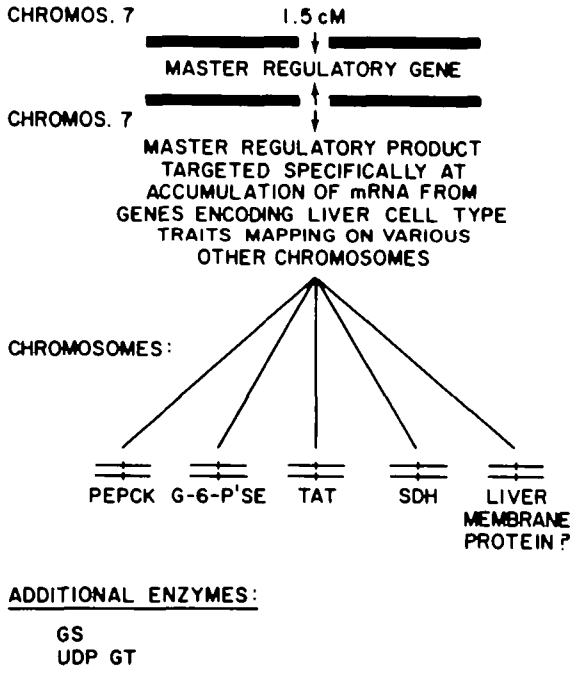
Obviously one of the most exciting areas, in which much progress has been achieved in recent years, is that of growth factors and the analysis of their role in development and differentiation. It all started with the Nerve Growth Factor and Rita Levi-Montalcini. I remember meeting Rita at a Growth Symposium in the USA in the mid 1950s and hearing the exciting story of NGF, the first growth factor to be discovered. We have remained close friends ever since, and I was delighted to hear of the recent Lasker Award that she received. Of course, throughout the years, the number of growth factors has grown astronomically, and it appears significant that the nucleotide sequences of the genes encoding them and their receptors show some homology to those of oncogenes. The prospects of causal analysis of growth factor action are underlined by the finding of DNA sequence homology between the gene encoding EGF and the *Notch* gene in Drosophila. *Notch* is instrumental in the regulation of epidermal versus nerve tissue differentiation of ectoderm. The gene's deletion, analyzed so beautifully years ago by Poulson who failed to receive the well deserved recognition for his discovery, results in overgrowth of nerve tissue at the expense of epidermal tissue. This adds fascination to the sequence homology!

To return once more to a vertebrate species, I want to mention the zebra fish that George Streisinger only a few years ago put on the map in such a brilliant fashion as a potentially very productive system of developmental genetic analysis. I knew George when he used to visit the Zoology Department at Columbia University as an eager young student at the famous Bronx High School of Science in the 1940s. In this connection, I must also mention Joshua Lederberg who as an undergraduate hung around the Zoology Department at Columbia, and whom I supplied with discarded mice even though the departmental chairman had strictly forbidden this. Lederberg used the mice for experiments of parabiosis where he wanted to study the question of whether liver regeneration might be promoted by a substance circulating in the blood. Lederberg later (1966) rewarded me with statements, e.g. "embryology should be studied with embryos" and that "the mouse should be the central material in developmental biology."

If I appear amiss for not even mentioning the elegant developmental analysis of *Caenorhabditis elegans*, it is because I never had the good fortune of personal experience with this worm as an analytical system. The only exception is my acquaintance with nematode cultures which we grew in Spemann's laboratory on potatoes for the purpose of feeding our Salamander larvae. But seriously, there is no doubt that the intriguing combination of apparently rather strictly determinate cell lineage with the discoveries of an ever increasing amount of cellular interactions and regulative phenomena in this nematode, harbours an enormous potential for the identification of general principles of developmental genetics. This potential is underscored by the limited size and cell number of the worm which make possible a complete analysis.

Finally, I must add my personal view of immediate and future concerns of causal developmental analysis. Our ultimate problem remains that of finding out how the zygote, which contains one set of maternal and one set of paternal genes, manages to differentiate into the multicellular organism with its multitude of cells derived by mitosis and therefore presumably of identical genotypes but totally heterogeneous phenotypes. The expression of particular genes in certain cell types, their failure to be expressed in others etc. etc. is the result of strict regulation of gene activity during differentiation. It is the identification of *cis* and *trans* acting regulatory genes, their gene products and the mechanisms by which they affect various cell type specific structural genes that is among important tasks today.

I cannot resist the temptation of showing here at least one slide which demonstrates a system of analysis that we are exploiting in the search for regulatory genes and their mode of action in mammalian development. With the help of a series of overlapping chromosomal deletions, we have identified a chromosomal region that includes gene sequences instrumental in the trans regulation of expression of a cluster of unlinked structural genes encoding liver specific traits. I shall try to summarize the crucial details of this system in the following figure.



The following questions are in the centre of our interest at this time:

(1) The level of regulation. Inducible but not constitutive expression of the structural gene targets seems to be affected by the deletion. We postulate that this is due to the absence of a *trans* acting regulatory factor essential for the induction of the relevant DNA sequences by inducing stimuli, be they metabolic or hormonal.

(2) Identification of the regulatory factor.

(3) Analysis of the molecular defect.

(4) Cloning of the regulatory gene(s). (Schütz et al. in Heidelberg).

Having reached the present, a look into the future seems appropriate. I personally am increasingly impressed with the degree to which molecular developmental biology and molecular genetics are merging into one science. While giving thought to this lecture and the period it covers, I came to realize the extent to which Boveri's concepts of the role of chromosomes in development and differentiation had prepared the ground for the concepts of molecular geneticists in 1986. The list of contents of a recent symposium entitled 'Molecular Developmental Biology' and held in 1985 in the USA includes four headings, all of them dealing with 'Gene Expression in Development'. The unifying concept of the vast majority of molecular approaches in developmental biology is that of gene expression and its regulation by *cis* as well as *trans* acting regulatory factors. Nonetheless, there is no doubt that problems of development extend beyond the level of gene expression and that molecular developmental biology will have to pay attention to mechanisms of differentiation on these additional levels. I have in mind phenomena such as the role of cell surface molecules in cell interactions, the formation of membranous structures, the role of phosphorylation in differentiation, the general problem of form in all its ramifications - I doubt that the molecular analysis of gene expression by itself can provide answers to these and similar questions. I therefore would expect increasing awareness of the need to focus on molecular and biochemical problems of development beyond the level of gene expression, i.e. that of epigenesis.

To quote and paraphrase Ephrussi and Lederberg once more: embryologists may still have to look at embryos in the pursuit of their analytical studies of development and differentiation.