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The long road to chemical and molecular embryology. What amphibians can teach us on differentiation

An interview with Professor Heinz Tiedemann

HORST GRUNZ*

Abteilung Zoophysiologie, Universität GH Essen, Germany

Since the famous organizer experiment of Hans Spemann and Hilde Mangold seventy years ago the search for inducing factors responsible for the programming of the embryonic body plan is one of the most fascinating and challenging topics in developmental biology. In the forties and early fifties the techniques were not available to isolate those factors from the embryonic tissue. However, it could be shown by several laboratories that so called heterogeneous inducers like guinea pig kidney or liver could partially mimic the inducing effects of the dorsal blastopore lip (Spemann's organizer). Several groups (Tuneo Yamada, Jean Brachet, Hsiao-hui Chuang , Sulo Toivonen and Heinz Tiedemann, cited in Saxén and Toivonen, 1962) started experiments to get information about the chemical nature of early embryonic inducing factors.It could be shown that short-term treatment at 70° to 90°C altered the inducing activity of guinea pig kidney or fractions from chicken embryos from a spinocaudal (mainly tail structures) type to an archencephalic (forebrain) one. Furthermore the first crude fractions prepared from heterogeneous tissues lost their inducing activity after treatment with proteolytic enzymes, indicating that the postulated factors may be proteins in nature. By the invention of the phenol extraction method Professor Tiedemann convincingly could show that the inducing activity was found in proteins isolated from the phenol layer and not in the aqueous phase (nucleic acids). Heinz Tiedemann's group succeeded to isolate and purify a factor from 11-13 day's old chicken embryos to homogeneity. Since this factor induced mesoderm and endoderm derived tissues, which are mostly located in the vegetal part of the embryo in normogenesis, it has been named "vegetalizing factor". The biological significance of this factor was no longer questioned when it turned out that the vegetalizing factor is activin A or an activin A homologue, proteins which belong to the superfamily of transforming growth factors ß (TGF-ßs). Activin or closely related substances are assumed to play a central role in mesoderm and endoderm determination during early embryogenesis. They are powerful mesodermalizing factors. Therefore the work of Heinz Tiedemann's group on the isolation, chemical nature and the mode of action of the vegetalizing factor turned out as a crystallization point for recent gold-rush-like activities in many laboratories, since now many new techniques of molecular genetics can be combined with embryological methods for the study of the complex processes during early embryonic development.

Professor Tiedemann was born on February 16th, 1923 in Berlin. He studied medicine in Berlin and Freiburg im Breisgau and since 1943 chemistry. In 1948 he passed the final medical examination in Berlin. In 1949 he earned the M.D. with a thesis, which he had done in the laboratory of Professor Else Knake, and in 1950 the diploma in chemistry. He became research assistant to Professor Otto Warburg at the Kaiser-Wilhelm-Institut für Zellphysiologie (then renamed to Max-Planck-Institut) in Berlin-Dahlem. In 1952 he earned the Ph.D. and in 1954 moved to the Heiligenberg Institut in Heiligenberg (Baden) where he became research assistant in the department of embryology which was headed by Professor Otto Mangold. After the Habilitation he had to give lectures in biochemistry at the chemical institute of the university in Freiburg.

In 1963 Heinz Tiedemann got an invitation as a fellow of the Carnegie Institution of Washington and moved to its department of embryology in Baltimore. In 1965 he became a member of the Max-Planck-Gesellschaft and head of a department at the Max-Planck-Institut für Meeresbiologie in Wilhelmshaven and in 1967

***Address for reprints:** Department of Zoophysiology, University GH Essen, FB 9 (Biologie), Universitätsstr.5, 45117 Essen, Germany. FAX: 201.1833173. e-mail: h.grunz@uni-essen.de

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Fig. 1. Prof. Heinz Tiedemann.

moved back to Berlin, where he held at the Freie Universität a chair at the Institut für Physiologische Chemie in the faculty of medicine (which later became the Institut für Biochemie und Molekularbiologie).

His bibliography contains over 150 publications. He is member of editorial advisory boards of internationally well known scientific journals. Furthermore he is an excellent reviewer of recent literature on embryonic development and molecular embryology. In 1991 he received the Theodor Boveri award of the Physico-Medica in Wuerzburg.

The following interview was held in Prof. Tiedemann's home on February 16, 1994.

When did your interest in embryology arise and how did you come to embryology?

I grew up in Berlin. In the spring of 1942 I came as a medical student to Freiburg im Breisgau. It was not only that I came out of the war into a beautiful and peaceful town which impressed me so much, but also the possibility to learn and to experience so many interesting things. I did not have a special interest in embryology at that time, but fortunately medical students had then to attend a course and a lecture in general Zoology. Otto Mangold had succeeded Hans Spemann (who died in 1941) as professor of Zoology. The lecture in embryology was however given in the institute of anatomy, where Dr. Keller, a lecturer at this institute showed to me histological sections of Triturus gastrulae and neurulae. I became interested in general embryology and did read Hans Spemanns book "Experimentelle Beiträge zu einer Theorie der Entwicklung" and his autobiography "Forschung und Leben" which was just published. This was my first encounter with embryology. In the institute of Zoology I attended seminars given by students working on their thesis and by postdoctoral fellows. Ten years later I was married to one of the students.

My other special interest aimed at chemistry. It was the time when chemical methods were successfully used to uncover more complex vital processes like gene activity and enzyme synthesis (one gene - one enzyme hypothesis, Butenandt and Kühn). Just before the first medical examination I was taken ill with a hepatitis and had to postpone the examination for half a year to the next term. This gave me a chance to start work in the laboratory of organic chemistry, which was generously allowed to me by Georg Wittig, head of the section of organic chemistry in the department of chemistry.

Back in Berlin after the war I studied medicine at the Humboldt Universität in East-Berlin and chemistry at the Technische Universität in West-Berlin and after its foundation at the Freie Universität in West-Berlin. I passed the final medical examination and worked for the M.D. thesis in the laboratory of Else Knake. She was a professor of pathology and an expert in tissue culture. But in postwar Berlin it was not possible to get all the materials needed for tissue culture. Professor Knake proposed to work on another interesting topic, the supply of autologous and homologous skin transplants with blood vessels. This took me on the road to science. I learned how to tackle a problem, not to be (too) impatient and to put the results into a critical context. I owe very much to Professor Else Knake.

After having finished the study of chemistry I had to look for a place to do work for the diploma and the Ph.D. thesis. My first choice was Otto Warburg. At this time Warburg's institute, the Kaiser-Wilhelm-Institut für Zellphysiologie was just given back to the Kaiser-Wilhelm-Gesellschaft (then renamed to Max-Planck-Gesellschaft) after it had served as the American High Command in Berlin. Warburg remarked to this event: "As a replica of the castle of the Prussian-frederician cavalry general Von der Marwitz it fulfilled this purpose with great dignity."

With the help of Else Knake I managed to get accepted by Otto Warburg. This was not an easy task, because Warburg preferred to work with technicians and especially skilled mastermechanics. Fortunately I was not dismissed within the first weeks. This was my start to biochemistry. Otto Warburg was one of the most impressive personalities I have met because of his extraordinary intellect, his originality and last but not least his independence of the main stream thinking. To work in his laboratory was an excellent opportunity to become familiar with the most advanced biochemical methods of this time. But I also learned that the big problems in science are more rewarding. In science you have to work hard anyway, whether the problem is big or small, Otto Warburg told me. At first I had to isolate and crystallize the enzyme aldolase by already published methods, as well as the coenzyme NAD by an improved method. For the Ph.D. thesis I had to investigate mice ascites tumor cells for the inhibition of glycolysis by respiration (Pasteur effect) and for the effect of light on the inhibition of respiration by carbon monoxide. The experiments confirmed the results which Professor Warburg had obtained when he discovered the Warburgsche Atmungsferment (cytochromoxydase) in yeast cells.

This was a rather long detour on the road to embryology. Do you think it was helpful to you?

From my experience, I think that a broad background is very important for an embryologist. Many things I had to learn were of no direct use for me, but the broad spectrum from chemistry to

biochemistry, biology and pathology made it more easy to evaluate new interdisciplinary developments and to find a practicable way to solve a problem. When I look back it also occurs to me that curricula should be more streamlined to the basic principles, reliable new developments and the multidisciplinary character of science. This is not only important for those who will become scientists, but for many academic careers. But unfortunately just the opposite happens. Science gets more and more divided into small territories. With regard to embryology in the sixties Joshua Lederberg stated, referring to the slow progress in molecular embryology, that "embryology suffers from being a traditional science". These days when the molecular biology has grown up, one sometimes has the impression that the embryology got lost.

Fig. 2. Otto Mangold at his laboratory in Heiligenberg.

How did it happen that you joined Professor Mangold's department at the institute in Heiligenberg, a small village in the countryside of Baden-Württemberg?

In 1953 I got married to Dr. Hildegard Waechter and moved to Heiligenberg. Heiligenberg is a beautifully located small village some miles from Überlingen and Meersburg looking over the lake of Constance to the Alps. The Heiligenberg Institute was founded in 1946 in a former hotel when in the war, which at the end of 1944 had reached Freiburg, many institutes were destroyed. The Heiligenberg Institute had a department of embryology, which was headed by Otto Mangold and departments of radiology and chemistry. After having finished her thesis in Freiburg my wife became assistant to Otto Mangold. In 1955 Jochen Born after having finished his studies as a chemical-engineer came to Heiligenberg. This was the beginning of a very effective and harmonious cooperation for more than 35 years. I was lucky to have in Heiligenberg and later in Wilhelmshaven and Berlin very able coworkers. In 1957 Dr. Ursula Becker (now Kocher-Becker) joined our group. The laboratory equipment was very primitive compared to our present standard, the research budget very small. Without the constant financial help of the German research organization (Deutsche Forschungsgemeinschaft) it would not have been possible to do the work. The next libraries were in Zürich and the small university town of Tübingen, also the host of Max-Planck-Institutes. This was of course not very convenient, but had the positive effect that we got in close contact with our colleagues in Zürich and Tübingen. After the Habilitation in biochemistry I had to give lectures at the Chemical Institute in Freiburg.

Upon the arrival in Heiligenberg Otto Mangold converted a saying from the beginning 16th century, the time of the emperor Maximilian: "Bella gerant alii, tu felix Heiligenberg nube" (Originally it reads: "Bella gerant alii, tu felix Austria nube"). After all it was not the only happy liaison at the institute.

But in regard to the scientific success on embryonic induction I was not so sure. We therefore started a second research project on the regulation of glycolysis by tissue respiration and found out that the enzyme phosphofructokinase is activated by ADP and 3', 5'-AMP. Under anaerobic conditions, with no oxydative phosphorylation, the ADP level in the cells increases at the expense of ATP and glycolysis is enhanced. Such enzyme regulations were later shown by the famous work of Monod, Jacob, Changeux and Wyman to be controlled by the interaction of enzymes with small molecules at secondary "allosteric sites" distant from the active (substrate) centre. Cooperative effects of protein subunits are involved in allosteric regulation.

Do you think there are correlations between these research topic and your main research field, the study of early embryonic development?

These experiments seem to be quite unrelated to our embryological work, but only at first sight. They gave me a much better understanding what proteins are like and how they interact. This was helpful when we found out that the inducing factors are protein in nature and when we started to purify these proteins.

How long did you stay in Heiligenberg?

We stayed in Heiligenberg until 1963, when James Ebert invited us to Baltimore to join for one year the Department of Embryology as a fellow of the Carnegie Institution of Washington. This was a very happy time in our life. The atmosphere in the department was very stimulating and generous. We met Johannes Holtfreter in Rochester, Tuneo Yamada at the Oak Ridge National laboratory and Viktor Hamburger, who welcomed us like old friends. I remember very well an evening with Viktor

Hamburger, his students and Carl Cori at his home in St. Louis. Dietrich Bodenstein showed us the quarters of the slaves of the professors on the old campus in Charlottesville. But these times have gone. We would have stayed in the United States or Canada. But unforeseen I got in 1964 an offer from the Max-Planck-Gesellschaft.

Your main research project was the isolation of inducing factors. Can you tell us how it was found out that the inducing factors are protein in nature and how their purification was started?

Professor Mangold was very much interested that the chemical nature of the inducing substances should be cleared up. The first attempts in other laboratories during the thirties had led to conflicting results and more or less to a chaos. Many embryologists and biochemists did not believe that inducing factors could be isolated or they even thought that such factors do not exist at all. Years later Jean Brachet found that the activity of inducers is reduced after treatment with ribonuclease. So we tried to isolate nucleic acids from chicken embryos, which Holtfreter had shown to be a very good source for inducing factors. Because a simple method for the isolation of nucleic acids was not available, we invented the phenol method for the separation of nucleic acids and proteins. The nucleic acids proved to be inactive as an inducer. The reason for the conflicting results was that at that time even highly purified pancreatic ribonuclease was not free from proteolytic enzymes. Later we became friends with Jean Brachet. He was one of the most remarkable personalities I have met, with his broad knowledge, so many good ideas and his very benevolent character. We respect him very much.

Fig. 3. Heiligenberg Institut (the former Hotel Post) in Heiligenberg/Baden (above) Institut für Molekularbiologie und Biochemie der Freien Universität in Berlin-Dahlem (below).

Fig. 4. Viktor Hamburger, Hildegard Tiedemann at the campus of Washington University in St.Louis.

To our surprise the phenol extracted proteins induced secondary tails or hind heads ending in a tail. At the same time it was found out by Tuneo Yamada, Yujiro Hayashi and in Heiligenberg that inducing factors are degraded by proteases. The experiments not only showed that the factors are protein in nature, but that they are chemically related to proteohormones. These relatively small proteins can completely be renatured to biologically active molecules after separation in the presence of denaturing agents like phenol or urea. Extracts from the pituitary anterior lobe had a high inducing activity, but hypophyseal hormones known at this time, did not induce. As a prerequisite for the purification of inducing factors my wife together with Ursula Becker developed quantitative test methods.

Fortunately at that time we did not know that methods for the final purification of the factor were not yet discovered and so continued with our efforts after we had moved to Baltimore, then to the Max-Planck-Institut in Wilhelmshaven and finally to the faculty of medicine at the Freie Universität in West-Berlin. The factor proved to be a dimer of 13 kDa subunits held together by a disulfide bridge. Later we could purify the factor, which induces endoderm and mesoderm, to homogeneity by consecutive reversed-phase-HPLC using different stationary phases.

How did the idea arise that the vegetalizing factor is related to the TGF-ß superfamily of growth factors and how was it finally identified as an activin A homolog?

The vegetalizing factor forms complexes with proteoglycans and can be purified by affinity chromatography on heparin-

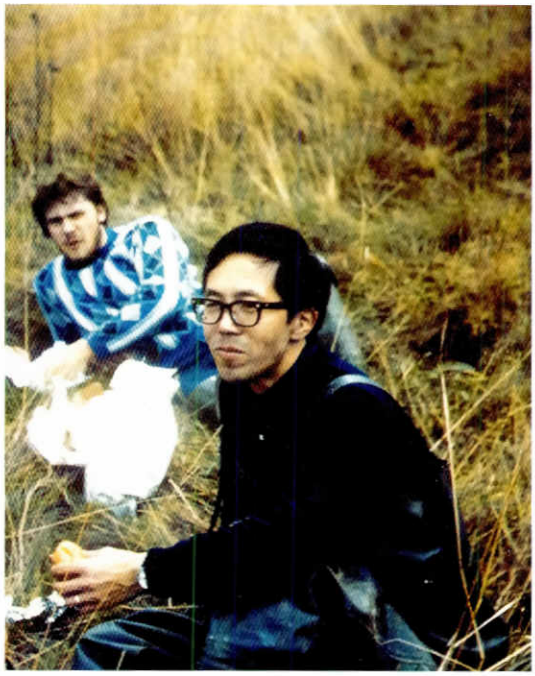
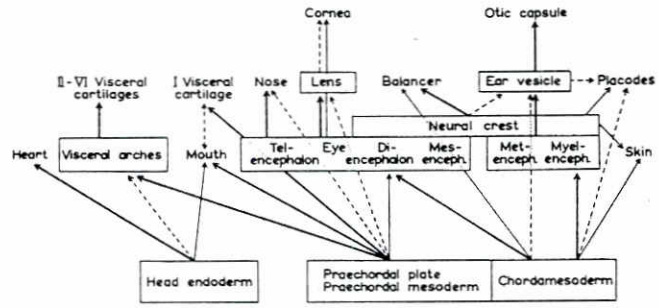
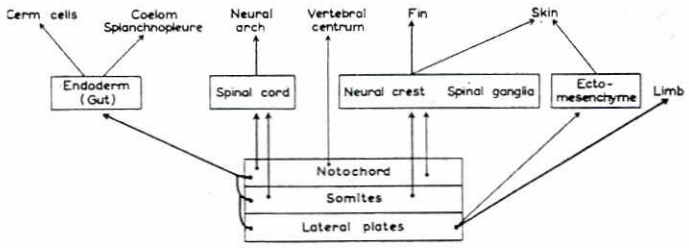


Fig. 5. The breeding season of *Triturus* begins. *The newts and the progeny were returned to their ponds after breeding. (left to right: Hildegard Tiedemann, Peter Hoppe, Heinz Tiedemann, Hans-Jochen Born, Makoto Asashima, Jochen Born, Peter Hoppe, Walter Knöchel).*

sepharose. This prompted us to test together with Horst Grunz in Essen and Wallace McKeehan in Lake Placid heparin-binding growth factors (FGF's), which do indeed induce preferentially ventral mesoderm. However the affinity of the vegetalizing factor to heparin is low compared to the FGF's, and other chemical properties of the factor are also different, but closely related to the transforming growth factors ß (TGF-ßs), which are also dimers of about 13 KDa. Together with Walter Knöchel we could show that TGF-ß1 induces ventral mesodermal tissues, as mesothel, immature blood cells and mesenchyme. Walter Knöchel had as a postdoc in our laboratory, discovered the intron-exon structure of the primary globin transcript before he joined the work on embryonic differentiation and induction. - At the same time Igor Dawid and his group independently found out that TGF-ß2 induces muscle. The tissues induced by TGF-ß1 were identified by histology. I emphasize this, because nowadays some embryologists are antipathetic against histology. Markers are useful for the rapid identification of tissues, for which reliable markers are already available, especially with the whole mount technique. However they can supplement, but not replace histology. It may be remembered that in pathology, diagnosis, often vital for the patient, is mostly made by histology.



A. INDUCTION PROCESSES IN THE HEAD



B. INDUCTION PROCESSES IN THE TRUNK

Fig. 6. Schemata showing the induction process during early urodele development in head (A) and in trunk (B) regions. *The inductive action starts from the archenteron roof (inductor of the first degree) and proceeds by inductors of second, third, etc. degrees. The different strengths of the inductive action are indicated by thick, thin and broken arrows (Mangold, 1961; reprinted from Saxén and Toivonen, 1961).*

In 1989 Makoto Asashima discovered that activin A, another member of the TGF-ß superfamily, which is identical with the erythroid differentiation factor, has a very high mesoderm inducing activity in the lower nanogram range and is much more active than TGF-ß. Professor Asashima, now at Tokyo university had in the seventieths spent two years in our laboratory with a stipend of the Deutsche Forschungsgemeinschaft. On a trip to Europe in the autumn of 1989 he visited his friends in Berlin and gave a seminar. Obviously activin and the vegetalizing factor must be closely related. Professor Asashima and his colleagues then found out in cooperation with our group that the vegetalizing factor has the same erythroid differentiation activity as activin and like activin, it is inhibited by follistatin. A mesoderm inducing factor found by Jim Smith in the conditioned medium of Xenopus transformed fibroblasts (XTC cell factor) and of several other cell lines was shown by Horst Grunz to be related to the vegetalizing factor. The XTC-cell factor of Jim Smith and a mesoderm inducing factor isolated from amniotic fluid by O. Chertov and his colleagues proved to be activin or activin homologues. To get enough vegetalizing factor for sequencing was difficult. The concentration in chick embryo tissue is about 500 times lower than the concentration of TGF-ß in blood platelets, the source for the isolation of TGF-ß. Finally professor Lottspeich at the Max-Planck-Institut für Biochemie in Martinsried (near Munich) could identify a partial sequence near the C-terminal end, which is identical with the corresponding activin A sequence, showing that the factor is activin A or an activin A homologue. Direct sequencing was not possible because the factor is blocked at the amino terminal end. At the time when the mesoderm inducing activity of the TGF-ßs was discovered, Douglas Melton and his group found in Xenopus oocytes the Vg 1 m-RNA and protein. It belongs also to the TGF-ß superfamily and it is preferentially located in the vegetal half. The C-terminal sequence of Vg 1 is closely related to the sequences of the activin A and B monomeres. Whether Vg 1 is not at all or to a very small extent in the embryo processed to a mature dimer with inducing activity is still an open question.

Another member of the TGF-ß superfamily, the bone morphogenetic protein 4 (BMP4) has been shown by Professor Knöchel (now at the university of Ulm) to be involved in ventral mesoderm differentiation. In addition to proteins of the TGF-ß-superfamily additional factors which are involved in the formation of the mesodermal tissue pattern (noggin, Wnt 8) have recently been identified in *Xenopus* embryos. However how the factors interact in the regulation of the gene network is only poorly understood.

The early processes are followed by a series of successive tissue interactions. They have been compiled by Otto Mangold. Many of the factors which are involved in the formation of organ pattern are still not known.

Endodermal and mesodermal tissues can be induced in isolated ectoderm. But how do the determinants cooperate in the embryo, when mesoderm is formed in the marginal zone and endoderm in the most vegetal region?

I think too little attention has been paid to the fact that not only mesoderm, but endoderm can also be induced by the vegetalizing factor or by activin. First let me briefly say what has been found out by T.S. Okada, O. Mangold and others on the state of endoderm determination in the embryo. The endoderm anlage possesses a certain degree of labile determination. But the differentiation of the endodermal tissues depends on the adjacent mesoderm and is not stabilized before the end of neuralization.

The induction of gut in isolated ectoderm was for the first time observed by Tuneo Yamada. This was confirmed in experiments with the vegetalizing factor. For the histological identification of endodermal tissues the explants must be cultured much longer than for the identification of mesodermal tissues. After induction with the vegetalizing factor, followed by dissociation of the explants for 20 h prior to reaggregation, in a high percentage liver and gut were found by Horst Grunz besides ventral mesodermal tissues. Notochord and somites which were found in non-disaggregated controls were not induced, probably because under these conditions cell interactions, which are needed for the differentiation of dorsal mesodermal tissues, can not take place. Elizabeth Jones has recently identified induced endoderm with a gut specific monoclonal antibody by immunohistology. Furthermore Frederick Rosa found that one of the early response genes for activin (Mix 1) is expressed in the embryo in the future endoderm and in part of the future mesoderm (marginal zone).

Now to the problem of mesoderm determination. There is no doubt from the experiments of Ogi, Nieuwkoop, Nakamura and Asashima that presumptive endoderm when combined with presumptive ectoderm in explants induces mesodermal tissue. But it is not likely that in the embryo a vegetal center in the endoderm

induces presumptive ectoderm to mesoderm in the morula to blastula stages. Several lines of evidence rather suggest that determining factors are already prelocalized, probably by cytoplasmic rearrangement after fertilization, in the marginal zone which later gives rise to mesoderm. It is likely that, in their active state some of the factors form in the embryo animal-vegetal and dorso-ventral gradients (or more correctly graded distributions due to the cell borders). The gradients however could so far not be visualized by immunohistological methods because of the very small amount of these proteins.

In the process of mesoderm differentiation the ectoderm in the animal region not only plays a passive role, which was assigned to the ectoderm in classical amphibian embryology. The differentiation of mesodermal organs in the marginal zone depends besides their determination by the vegetalizing factor on the presumptive ectoderm of the animal cap as Hildegard Tiedemann has found out. It seems to be possible that a factor (or factors) from the animal ectoderm are needed for the differentiation of mesodermal tissues. Recently Walter Knöchel has shown that a gene of the forkhead family, which codes for a transcription factor the expression of which is not dependent on activin, is first transcribed in the animal cap of morulae and later in the mesodermal marginal zone. These are interesting aspects for future research.

Fig. 7. First row (right to left): Horst Grunz, Ursula Kocher-Becker, Hildegard Tiedemann; second row (right to left): Wilhelm Minuth, Hans-Peter Geithe; third row (right to left): Walter Knöchel, Christian Finke, Jochen und Edith Born.

You told us about mesoderm and endoderm determinants. It occurs to me that the term induction has changed when in the embryo determinants are already prelocalized in the region where they act via plasma membrane receptors on neighbouring cells. Spemann has defined embryonic induction as the determination of a (multipotent) tissue by a neighbouring tissue. But in cases where the factors are prelocalized they could act in an autocrine like way. There is no doubt that mesoderm and endoderm determinants exist in the embryo. But the whole story started with the famous organizer experiment of Hans Spemann and Hilde Mangold when they showed that the neural plate is induced by the underlying mesoderm. Is this process also mediated by chemical factors?

Neuralizing factors certainly exist. They act at the cell surface via signal transducing mechanisms. Obviously you have in mind that neural differentiation can be brought about in isolated *Triturus* ectoderm without inducing factors, for instance by an increase of the Na^+-concentration in the medium as Hildegard Tiedemann has shown (but not by a transient increase of Na+ in the cells). These and other experiments point to the plasma membrane and may suggest that an inhibition of neuralization signalling is released by conformational changes. In the embryo an inducing factor would release the inhibition - The mimilry of such processes is not exceptional for neural induction. The action of the nerve growth factor for example can also be mimicked.

At physiological conditions the test for neural inducing factors is absolutely safe. Neuralizing factors have been found in several subcellular fractions of the Xenopus embryo homogenate. The factors are protein in nature and not inactivated after treatment with substances which reduce disulfide bonds. Together with Jutta Janeczek a high molecular weight (about 90-110 kDa) and a low molecular weight form (about 14-25 kDa), which prevails, were partially purified from the cytosol. The larger factor could be a precursor of the smaller one. The factors are of maternal origin and mostly present as inactive complexes with other proteins, as it is known of many growth factors. Together with George Lopashov we have partially purified a neuralizing factor from neuroblastoma cell lines. One line secretes a small amount of the factor. A neuralizing factor from brain was partially purified by Alexander Mikhailov and coworkers.

Interestingly in the dorsal and ventral ectoderm, which can be induced to neural tissue, a masked inactive neuralizing factor is already present. The factor is activated and in part released during induction, so that the induced neural plate in turn gets neural inducing activity, a process called homoiogenetic induction. Because the induced tissue (the gastrula ectoderm) contains a neuralizing factor in a masked form, it can at present not be decided, whether a factor acts as a neural inducing factor or as a releasing factor for the neural inducing factor proper and whether these two processes are regulated by different proteins. This can only be resolved, if homogeneous factor(s) are available.

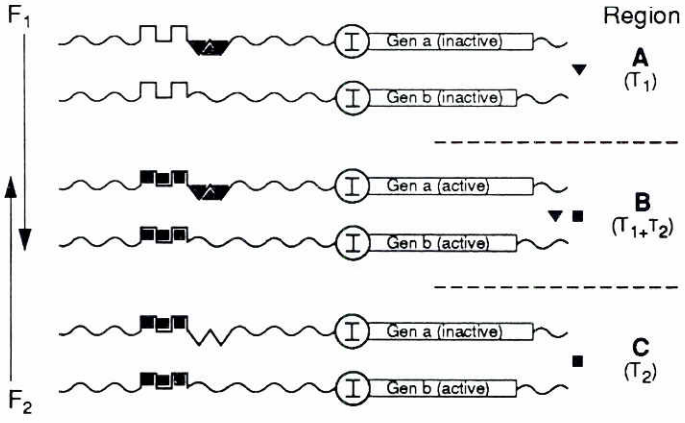


Fig. 8. Schematic diagram of gene activation by two overlapping factors F1 and F2 which in early embryos determine different regions, from which different tissues arise. *Transcription factor T1 (▲) is activated by F1, transcription factor T2 (■) by F2 . Genes are only active, if all sequence motif for transcription factors are occupied, i.e. gene a is activated when the sequence motif for T1 (W) and the sequence motif for T2 (⊓⊔) are occupied (region B), gene b when the sequence motif for T2 is occupied (regions B and C). I, Initiation complex (Tiedemann et al., 1995).*

The neuralizing factor induces forebrain with eyes and noses. When tissues inducing neural or mesodermal organs are mixed, hindbrain and neural tube develop depending on the ratio of the inducers as Lauri Saxén and Sulo Toivonen have found. They have furthermore shown that the isolated forebrain anlage is shifted to hindbrain or neural tube when combined with caudal axial mesoderm in different ratios and concluded that the neural anlage is regionalized along the anterior-posterior axis in secondary steps. When our partially purified neuralizing and mesodermalizing factors were combined, hindbrain and neural tubes were likewise induced, depending on the ratio of the factors.

Together with Michael Davids we found out that phorbolester induces in Triturus ectoderm large complexes of neural tissue. At a higher concentration of phorbolester neural tissue is also induced in Xenopus ectoderm. This suggests that protein kinase C is involved in the signal transduction mechanism. I think one of the prerequisites for the further progress is the identification and characterization of neural inducing factor(s). Several substances have been claimed to be neuralizing factors. But they did not induce neural tissue when tested on competent ectoderm.

There exist certainly quite a number of factors, but on the other hand a single factor as the vegetalizing factor resp. activin can induce many different tissues in competent ectoderm. Is this not a contradiction?

The vegetalizing factor actually induces all kinds of mesodermal tissues. Which tissues are preferentially induced depends as you have shown in 1983 on the concentration of the factor. This was confirmed in experiments with activin. It is not a contradiction. But how can it be explained?

There are several possibilities which are by no means mutually exclusive. There exist several cell surface receptors for activins as well for FGF's. Receptors which may transduce signals to different genes could have different affinities for their effectors, so that they are occupied at different concentrations. In this way signals could be transduced to different genes at different concentrations of activin.

Another possibility results from the mechanism of gene transcription in eukaryotes which is more complicated than in bacteria. At the promoter site, where gene transcription starts several proteins bind in an initiation complex, which directs a basal level of transcription. Mostly similar proteins have been found in the initiation complexes of different genes. Gene regulation is mediated by transcription factors which bind to DNA sequence motifs upstream from the promoter sequence, called enhancers. The transcription rate can increase (positive regulation) but also decrease (negative regulation). There exist distinct structural families of transcription factors, which bind to distinct DNA sequence motifs. Several of them have been detected in amphibian embryos. A transcription factor can exist in a cell in an inactive or active (DNA binding) form. Activin (and this holds also of other factors) is, via signal transduction from the cell surface, involved in the activation of transcription factors. Now it is important for the regulation of gene activity that one gene can contain several enhancer elements of either the same or different DNA sequence motifs. This means that the same enhancer element can be present in different genes and that one transcription factor can bind to different genes which could be involved in pathways leading to different tissues. It has further to be considered that a transcription factor can bind to the corresponding enhancer elements in different genes with different affinities for the following reason: A gene is probably only regulated if all enhancer elements are occupied by transcription factors which mutually interact. In analogy to the interaction of protein subunits of allosteric enzymes, the mutual interaction of transcription factors could modify their affinity to the gene enhancers and hence the concentration at which gene activation occurs. The dose-response curve would become steeper (sigmoidal shape of the curve) so that activation (or inhibition) could occur in a small concentration range. As a consequence activin could at different concentrations, via signal transduction and concentration dependent activation of a transcription factor, activate different genes which finally lead to the formation of different tissues. Genes can also be repressed by other genes (directly or via signal transduction at the cellular level). Together these effects can lead to tissue patterns with sharp boundaries. The differential distribution of overlapping transcription factors, which are activated by different overlapping inducing factors, together with the different combined sequence motifs of the gene enhancers, could be the basis for an additional regulatory mechanism (Fig. 8).

These hypotheses for the regulation of the gene network in embryos will certainly be modified. But again they show, how closely the progress in embryology depends on the progress in biochemistry and molecular biology.

You have used the term organizer only once in this interview. Was this purposely or just by chance?

It was not on purpose. But our goal was to analyse the mechanisms behind the organizer.

It is well to understand that Hans Spemann coined this term, when he and Hilde Mangold saw that a well organized second embryo was induced. But it was also a tribute to the organismic view of this time. Hans Spemann was however never in doubt

that the organizer effect should be analyzed by all available methods, but he also saw that this would be a long way – more than half a century now. Viktor Hamburger in his book: "The heritage of experimental embryology" and Johannes Holtfreter in one of his last papers have considered from their personal knowledge the history of the organizer and the reasons for and against this term. I hope that many embryologists will read them.

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