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Spemann's heritage in Finnish developmental biology

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Introduction

At the beginning of the 20th century, experimental embryology was still a new discipline which slowly entered the small and geographically remote scientific community of Finland. The field was introduced here by single scientists who were stimulated and trained abroad, especially in Germany. Thus it is not difficult to follow the creation of a new school and its early development by these pioneers. Another feature of the Finnish school focusing on embryonic induction, is the easily delineated time period when the new field was introduced, flourishing and thereafter splitting into several new groups with an intellectual heritage from the early German school. These 40 years of active research can be further dissected into three, partially overlapping periods during which embryonic induction was explored at the Department of Zoology at the University of Helsinki.

Introduction of the ideas and technology

Two scholars can be named as the founders of the Finnish school of experimental embryology, both being directly influenced by Hans Spemann already in the early second decade of the 20th century. Aleksander Luther (1877-1970) worked for two years, 1912-14, in Spemann's laboratory, then in Rostock, and learned the techniques used to manipulate early amphibian embryos. Upon his return to Finland, Luther performed some experiments on the development of amphibian limbs and sensory organs but was soon fascinated by the unique fauna of the breakish waters of the Gulf of Finland. Despite this shift in interests. Luther recognized the extraordinary skills and creativity of Hans Spemann, and put him forward to become a Corresponding Member of the prestigious Finnish Society of Sciences in 1922.

Gunnar Ekman (1883-1937), a student of the prominent Finnish zoologists J. A. Palmén became interested in embryology already during his early years as a student at the Department of Zoology. Ekman, too, decided to be acquainted with modern experimental embryology and its technology. He, therefore, spent two years (1912-1914) with H. Braus in Heidelberg where the recent achievements and methods of Spemann were well known and adopted. After this initial stimulus, Ekman visited Spemann's laboratory in Freiburg several times in the 1920´s, and was intimately familiar with the classic work on embryonic induction. Back in Finland, Ekman's main interest, however, became not the induction problem but he focused on experiments related to the development of the branchial arches and the heart primordia (see Leikola, 1989)- His keen interest in Spemann's early work, however, persisted and he published some short reviews on the topic (in Finnish and Swedish). Finally he tackled this fascinating problem by experiments of his own and published in 1936 a 100-page monograph based on studies with Triturus embryos and the inductive action of fragments of the blastopore lip. Gunnar Ekman died suddenly in 1937, but had already passed the baton to his young student, Sulo Toivonen (1909 - 1995) who had recently completed his Masters thesis on the development of mammalian pronephros.

When Toivonen in 1936 started his experimental investigations in Ekman's laboratory, Johannes Holtfreter, a student and

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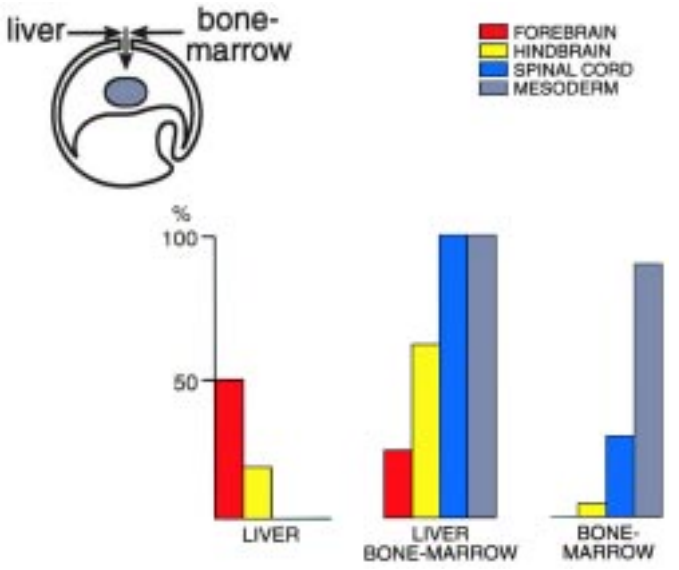

Fig. 1. Recorded secondary CNS-structures induced in implantation experiments by two heterogeneous inductors and their combination. *(After Toivonen and Saxén, 1955a).*

collaborator of Spemann, had published his result on "heterogeneous" inductors. In brief, Holtfreter had shown that many heterologous tissues from both embryonic and adult animals exerted an inductive action when tested against the competent ectoderm of amphibian gastrulae (Holtfreter, 1934). In addition he had shown that not only living tissues carried this unexpected action but devitalized tissue fragments released inductive stimuli as well. Ekman suggested to his student a more extensive and systemic study of such inductors and this was to lead to a series of fundamental findings by Sulo Toivonen over a period of 30 years from 1938 to 1968.

Exploration of heterogeneous inductors: the qualitative hypothesis

At an initial stage of his experimentation, Toivonen improved the classic manipulation and operation techniques of the amphibian embryos by developing strictly sterile conditions for handling the objects. Trivial as it may sound today, this aseptic technique proved pivotal for his subsequent massive studies. Before this, as Toivonen himself used to relate, of the laboriously operated embryos only some 5 per cent survived long enough to be harvested for a meaningful analysis. Now the radically increased survival rate allowed Toivonen to collect annual series of up to 2000 specimens for the analysis of differences in the inductive action of the heterogeneous inductors. Thus, already in 1938, Toivonen could report that the many tissues used by him differed in their inductive action and showed a certain tissue-specificity. This organ- and tissue specific action, "Leistungsspäzifität" was confirmed in the subsequent, extensive series published in 1940. Three main types of inductions where consequently distinguished: the archencephalic, the deuterencephalic and the spinocaudal inductions. Very similar results were obtained independently by H.H.-Chuang in Germany and published likewise in 1938 and 1940,

The main conclusion from these results was that such reproducible, tissue specific differences could be explained only through the existence of several inductive factors. This view, soon to be known as the *qualitative theory* contrasted clearly with the prevailing hypothesis, the *quantitative hypothesis* postulating that the regionally different inductions were determined by a single factor acting along a gradient in different concentrations. Due to the turmoil in Europe in the early 1940´s, an actual debate between the two basic theories had to wait until the end of the decade, and by then Toivonen could provide additional evidence for his hypothesis. Together with a young biochemist, Taina Kuusi (1919 - 2000) he performed extensive separation and fractionation experiments with various heterogeneous inductors showing that there are chemically different molecules (fractions) exerting different inductive actions (Toivonen and Kuusi, 1948 Kuusi, 1951). (For details of these studies as well as of the debate between the two theories, see the monograph by Saxén and Toivonen in 1962.)

Toivonen and Kuusi (op. cit) concluded that there are at least two chemically and functionally different inductive "substances", the neuralizing and the mesodermalizing ones. This seemed to be a vindication of the qualitative hypothesis but left several questions unsolved. First, the exact chemical nature of these signal substances remained open, and the analytical approach had to be continued. Here, especially Heinz Tiedemann and his school were successful as described in detail by Horst Grunz in this volume. Yet, the actual molecular mechanism of these interactive events could not be solved by the restricted methodology of that time - in fact answers to this question had to wait for the more sophisticated methods of molecular biology, and only in the 1980´s did essentially new information start to accumulate (for reviews see: Gurdon, 1987, Gilbert and Saxén, 1993, Gilbert, this volume, Grunz, this volume).

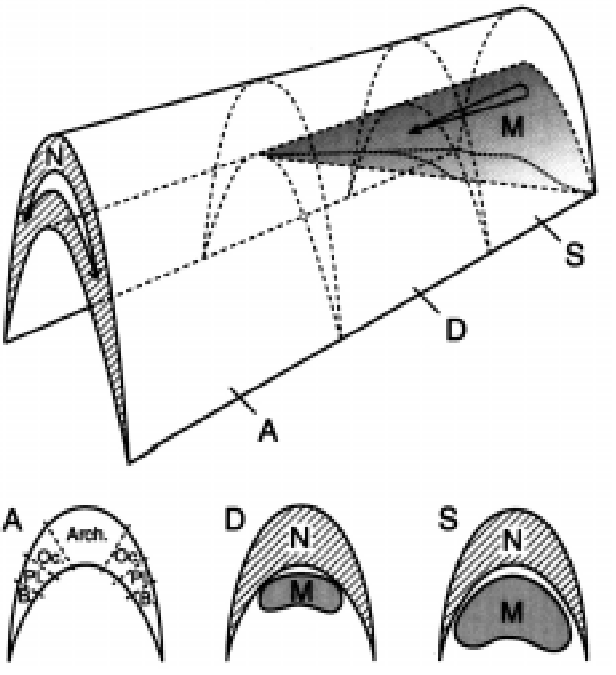

Fig. 2. The original double gradient hypothesis *by Toivonen and Saxén (1955a) postulating a dorso-lateral neuralizing gradient and a caudo-cranial mesodermalizing gradient determining the regionalization of the central nervous system. A, archencephalic (forebrain) induction; D, deuterencephalic (hindbrain) induction; S, spinocaudal induction.*

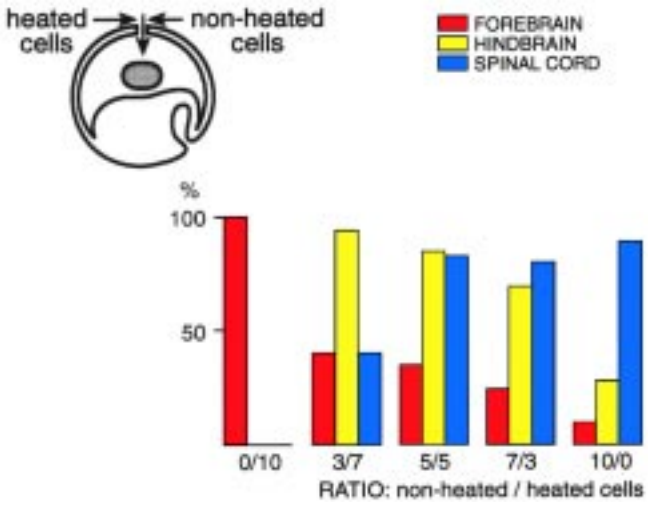

Fig. 3. Vindication of the double gradient hypothesis by implantation experiments *with heated and non-heated HeLa cells mixed in different ratios (After Saxén and Toivonen, 1961).*

The second question related to the postulated co-action of the two types of inductors. As long as no purified samples of such signal substances were available, a direct experimental approach was difficult. A decisive finding was made when Toivonen (1953) detected a practically pure mesodermalizing inductor with only a slight spinaudal, neural "contamination", the guinea-pig bone marrow. This could now be used as a tool in tests for combined inductors.

Experiments with combined inductors: the double gradient hypothesis

In 1954, the author joined Toivonen's small group after presentation of his Ph. D. thesis dealing with the development of the visual cells. The collaboration then started was to last for 14 years and lead to a modified and specified model of the induction process and the regionalization of the CNS.

The first test to explore the postulated co-function of two types of inductors applied two heterogeneous inductors: the practically pure mesodermalizing bone marrow and the guinea-pig liver tissue which after a short-term heat treatment induced exclusively neural structures belonging to the forebrain region (archencephalic inductor). These two inductors were now implanted simultaneously into the gastrula blastocoel to act jointly on the competent ectoderm. The result was, as expected from our hypothesis, that instead of a simple summation of their separate actions, a new type of response was recorded. In addition to forebrain structures and mesodermal derivatives, neural structures belonging to the hindbrain region (deuterencephalic inductions), and spinal cord were frequently detected in the secondary formations (Fig. 1). A more elegant approach applied isolated gastrula ectoderms into which the inductors were wrapped, the so-called sandwich technique. Here all possible influences of the host organism could be eliminated and, yet, the results were practically identical with those obtained with the implantation technique (Toivonen and Saxén, 1955a, 1955b). Based on these results, a modified qualitative hypothesis was formulated suggesting two induction principles, a neural and a mesodermal one, which could act either separately or in different quantitative ratios (Fig. 2). This hypothesis shared, in fact, several features with the "activation-transformation" theory of Nieuwkoop and his school in Utrecht (1952, 1955) which, on the other hand, again reflected the qualitative ideology. This Dutch model will be dealt with in another context of this volume by Gerhart..

Soon the hypothesis of two active principles and their co-action gained further evidence from the fractionation experiments by Tiedemann and Tiedemann in 1956. They showed that a "deuterencephalic" (hindbrain) inducing protein fraction could be further separated by chromatography into an "archencephalic" (forebrain-inducing) and a mesodermalizing component. When these two chromatographically separated fractions were recombined, a preparation exerting hindbrain-inducing activity was again restored (Tiedemann and Tiedemann, 1964).

The decisive role of the different ratios of the two inductors determining the regionalization of the CNS remained, however, still hypothetic and we had to wait another five years for direct proof - again because purified samples of the inductors were not available. To by-pass this obstacle, we used cultivated HeLa cells in the next series of experiments. This continuous line of human cancer cells is kept in laboratories round the world, and by chance we observed that they are potent inductors of predominately mesodermal components and caudalmost neural formations (spinal cord). As expected, a short-term heat treatment of the HeLa- cells produced an inducer of forebrain derivatives without any mesodermal or caudal neural structures. These heat-treated and untreated cells could now be brought into suspension and mixed in precisely determined ratios where the homogeneity of the mixture was confirmed by labeling studies. These different ratios were tested both in implantation experiments and in sandwich-type cultures. Analysis of the samples disclosed a whole array of CNS-structures: a gradual increase in the relative amount of non-heated cells resulted in corresponding caudalization of the CNS-structures (Fig. 3). Thus, these highly artificial inductor preparations closely mimicked the action of the different regions of the normal inductor, the archenteron roof previously

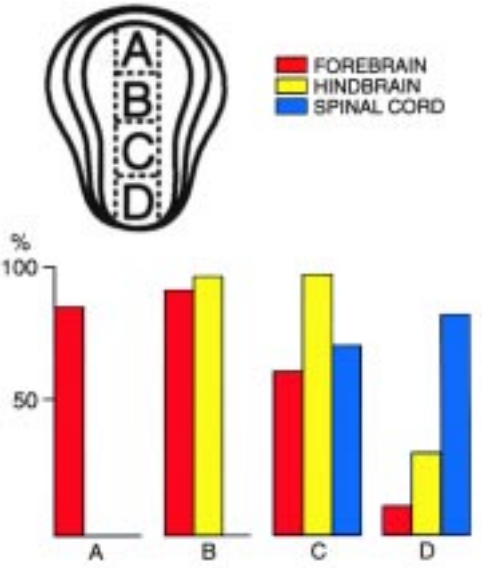

Fig. 4. The regional-specific inductive action of four consecutive segments of the archenteron roof. *(After Sala, 1955). Compare with Fig. 3.*

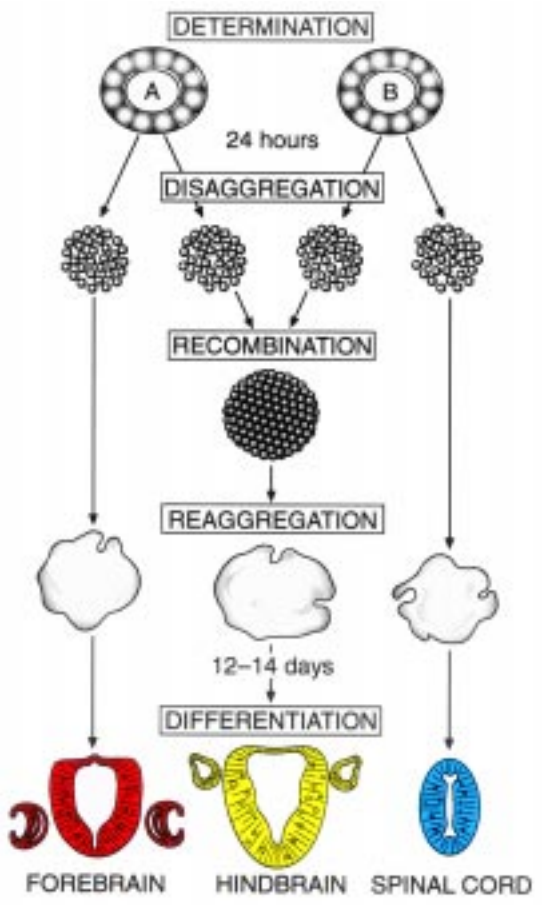

Fig. 5. The experimental protocol for a test of the two-step hypothesis of neural induction. *(After Saxén et al., 1964). A, "Neuralizing" inductor; B, "Spinocaudal" inductor. See text for details.*

mapped out by Otto Mangols in 1933 and subsequently by Sala (1955) (Fig. 4). The results thus corroborated our double gradient hypothesis (Saxén and Toivonen, 1961), The mechanism of the combined action on the competent ectoderm remained still open, however, and clearly invited further analysis.

Following the idea of the Dutch school of a two-step process, with an initial activation followed by a secondary transformation (Nieuwkoop *et al.,* 1955), the following experiment was planned (Fig. 5): Using heterogeneous inductors with know action, isolated ectodermal fragments were induced *in vitro* towards either neural or mesodermal direction, respectively. When the initial induction was completed in 24 h, the inductor was removed, the ectodermal cells disaggregated, washed and subcultivated either as separate reaggregates or as aggregates consisting of both initially neuralized and mesodermalized cells thoroughly mixed in suspension. The results showed that differentiation of the combined reaggregates clearly differed from the separately cultivated aggregates. They showed, in addition to forebrain and caudal neural structures, neural derivatives of the hindbrain region not found in the two types of control aggregates. It was easy to conclude that the final repertoire of the neural structures in the combined cultures was the result of a sequential determination : in the first step cells were induced towards a general neural vs. mesodermal direction, respectively, and in the second step the regionalization of the CNS was determined by the mesodermalized cells, (Saxén *et al,*. 1964). The finding thus showed that the initial neural determination caused by the "primary" inductor was regionally still labile and versatile and could be altered by a second-step influence. This labile period of the determination of the anterior-posterior axis was confirmed 30 years later by Saha and Grainger (1992) who used segment-specific gene markers to characterize the regional structures of the CNS.

So far, all our experiments were performed with foreign, heterogeneous inductors in artificial conditions. Although the response of the target ectoderm in these experiments closely mimicked normal development, the approach could be criticized because of these abnormal conditions and foreign inductors. Hence, the last experiment in this long series was devised to make use of normal embryonic tissue counterparts, the cranial part of the neural plate and the caudal segment of the archenteron roof. In isolate the former would differentiate exclusively into forebrain derivatives and its disaggregation would not alter this mode of development. When the caudal mesoderm of the archenteron roof was added to the suspension in increasing amounts, the neural differentiation gradually shifted towards more caudal structures. Again, a whole array of these distinguishable neural formations were produced (Fig. 6). We concluded that our original hypothesis of two types of inductors acting in different ratios and sequentially was vindicated (Toivonen and Saxén, 1968). Naturally, sophisticated studies using modern technology have since brought details and additions to our crude model for the induction and regionalization of the central nervous system. But as such, it might still be considered a bridge between the classic views of the 1920´s and the present admirable approaches to clarify the induction process at the molecular level.

The end of one school: the birth of new groups

Until the mid - sixties the Finnish group focusing on the induction problem had been working in the Department of Zoology at the University of Helsinki with adequate funding and support. Several coincidences led, however, to the dissolution of this intimate group and the 1968 report was to be the last original paper based on this collaboration. The group first lost its biochemist Kuusi who moved

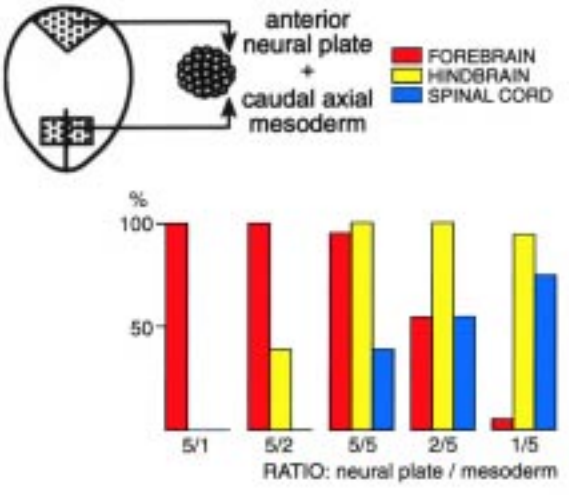

Fig. 6. Experimental evidence for the double-gradient hypothesis within the amphibian embryo. *Suspended cells from the anterior part of the neural plate where mixed with similarly disaggregated cells of the posterior mesoderm. (After Toivonen and Saxén. 1968). Compare with Figs. 3 and 4.*

to administration. Next the brilliant immunologist Tapani Vainio was killed in an automobile accident in 1965, at the dawn of the molecular era in developmental biology. Two years later the author of this review was invited to the chair of Experimental Pathology at the University of Helsinki and was offered excellent facilities at the Meilahti Medical Center. Sulo Toivonen remained active in his old laboratory and was a frequent guest in the meetings and seminars of his former students until his retirement five years later.

A new ambitious generation of developmental biologists took over and created first-rate groups in Helsinki, Turku and Oulu. Inductive tissue interactions still remained a central theme in their experimental work, but for mainly pragmatic reasons the classic "Spemann model" was replaced by model-systems using mammalian organ rudiments. The tradition is now carried on by a constantly expanding number of young scientists supported by a special programme of the Academy of Finland. When giving the Introductory Lecture at the annual meeting of the Finnish Society of Developmental Biologists in 1999, I was pleased to count more than 50 next-generation scientists in the audience. The heritage of Spemann, Ekman and Toivonen is well treasured.

Summary

The Finnish school of developmental biology can be considered a direct descendant of Spemann´s school as both the original technology and the fundamental problems were introduced into Finland by Gunnar Ekman (1883-1937) who had worked for extended periods in Germany. After his early death, the work was continued by Sulo Toivonen (1909-1995), and until 1968 the group explored the mechanisms of primary induction and the subsequent segregation of the central nervous system. The extensive investigations led to the formulation of the "double-gradient" hypothesis and ultimately to its experimental vindication.

KEY WORDS: *Neural induction, mesodermal induction, double-gradient theory.*

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