Classical embryology to molecular biology: a personal view of amphibian embryonic development

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1. Introduction

Much information is available concerning the scientific activities of my laboratory and the establishment of Developmental Biology (including Molecular Developmental Biology) in Germany (*see* reviews in special issues of the *International Journal of Developmental Biology* [*IJDB*]; *Developmental biology in Germany*, 1996, Vol. 40; *Spemann–Mangold organizer*, 2001, *IJDB* Vol.45; *Vertebrate organizer* (ed.) H Grunz, 2004). Therefore, this article deals mainly with my personal background.

The famous Organisator (organizer) experiment of Hans Spemann and Hilde Mangold in 1924 led to Spemann being awarded the Nobel prize for Physiology and Medicine in 1935. Soon thereafter, the search for factors that might be responsible for the organization of body plan during early embryonic development resembled a gold rush. At the time, the findings of Spemann and Mangold with relation to understanding early embryology were regarded as revolutionary. Spemann's school pioneered the use of specially designed micro instruments and sterile methods to dissect 1 mm-sized amphibian embryos under nearly sterile conditions. No antibiotics or sulphonamides were available. The microsurgery method attracted scientists from all over the world and several visited the Spemann laboratory in Freiburg. Most of those early techniques are still used today, at least in modified form, as a prerequisite for further studies at the molecular level. Heinz Tiedemann and I represent this tradition (*see* interview with Tiedemann; Grunz 1996, in *IJDB* Vol. 40 [1]).

2. School and study in Cologne

I grew up in Cologne, which is known for possessing the largest Gothic cathedral in the world (two towers of 157 m each). Because of the extreme noise and vibration of the world largest swinging bell, one cannot enter the bell tower when the bell is in action. It is activated very seldom, for example on the night of New Year's eve. During my high school days I participated in a special course in hydrobiology. I was given the task of catching all small organisms in a pond and analysing them under the microscope. This helped in crystallizing my decision to study general biology at the University of Cologne after my Abitur (high school final examination).

During the study of general biology, which included zoology, developmental biology, pharmacology and electron microscopy, I did not have a definite idea of the field that I wished to choose for my research work. Until the war, scientists in many institutes in Germany were working in the field of developmental biology, named Entwicklungsmechanik or Entwicklungsgeschichte (today Entwicklungsbiologie and Entwicklungsgenetik). Quite a few had studied under Hans Spemann and went on to become heads of institutes or at least of a Department of Entwicklungsphysiologie. There was a separate institute of Entwicklugsphysiologie in Cologne even after the war. The head of the institute of zoology from 1945 to1950 was Professor Eckhard Rotmann, a former student of Hans Spemann, and he was working on eye development in newts. Several coworkers were engaged in classical developmental biology. As a graduate student I was looking for a thesis in my field of interest – embryology.

I did not have many choices. Only Professor Hans Engländer (figure 1a) had a place for experimental work at this time. What amounted to a random search for a 'Doktorvater' (thesis supervisor) turned out to be an excellent decision. He and his colleague Professor Anna Gisela Johnen had published several interesting papers about the role of the time factor in early embryonic induction

Keywords. Central nervous system; Embryonic induction; Growth factors; Spemann-Mangold-Organizer; Vegetalizing factor (activin)

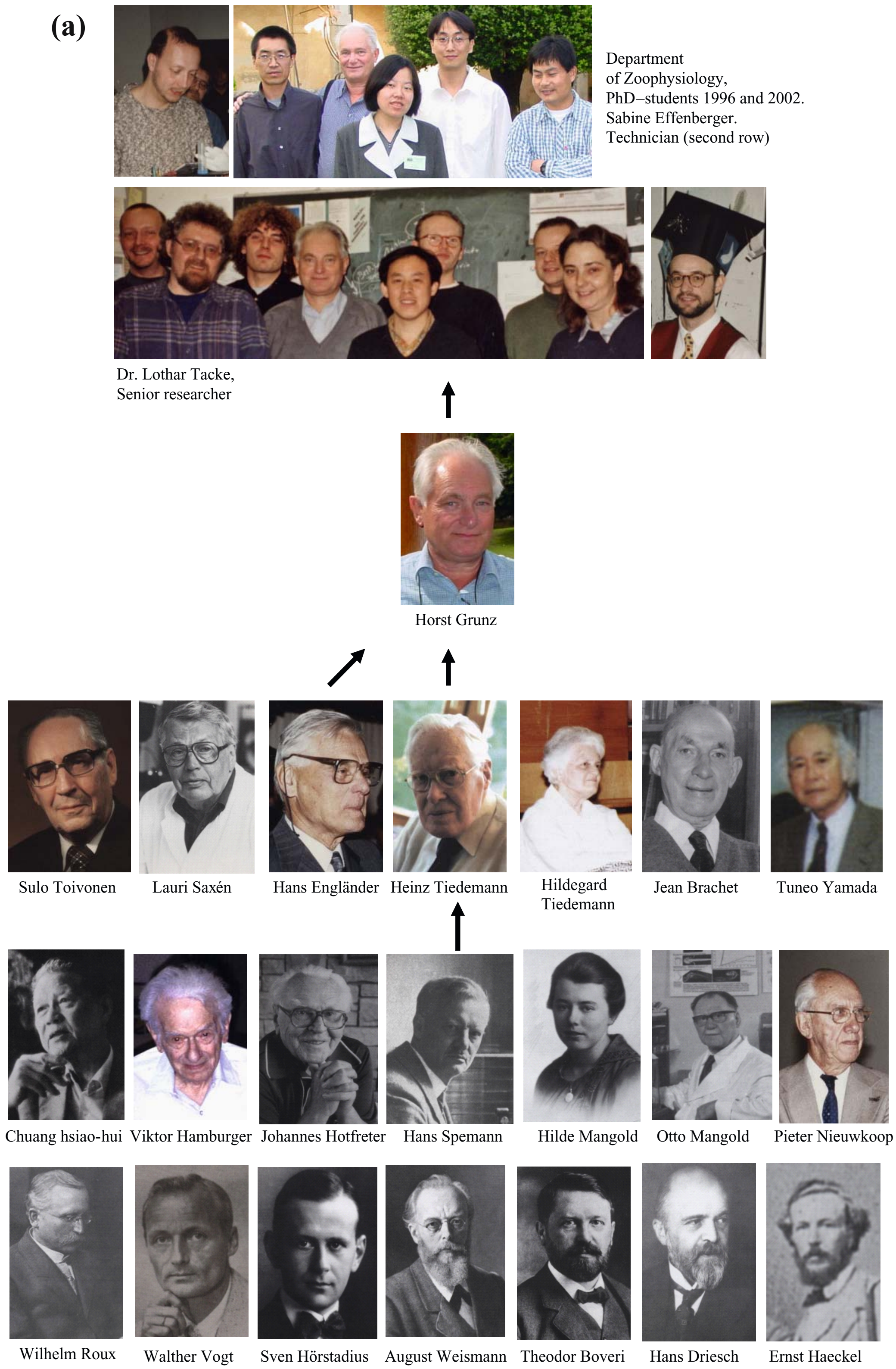

Figure 1a. For caption, *see* page No. 7.

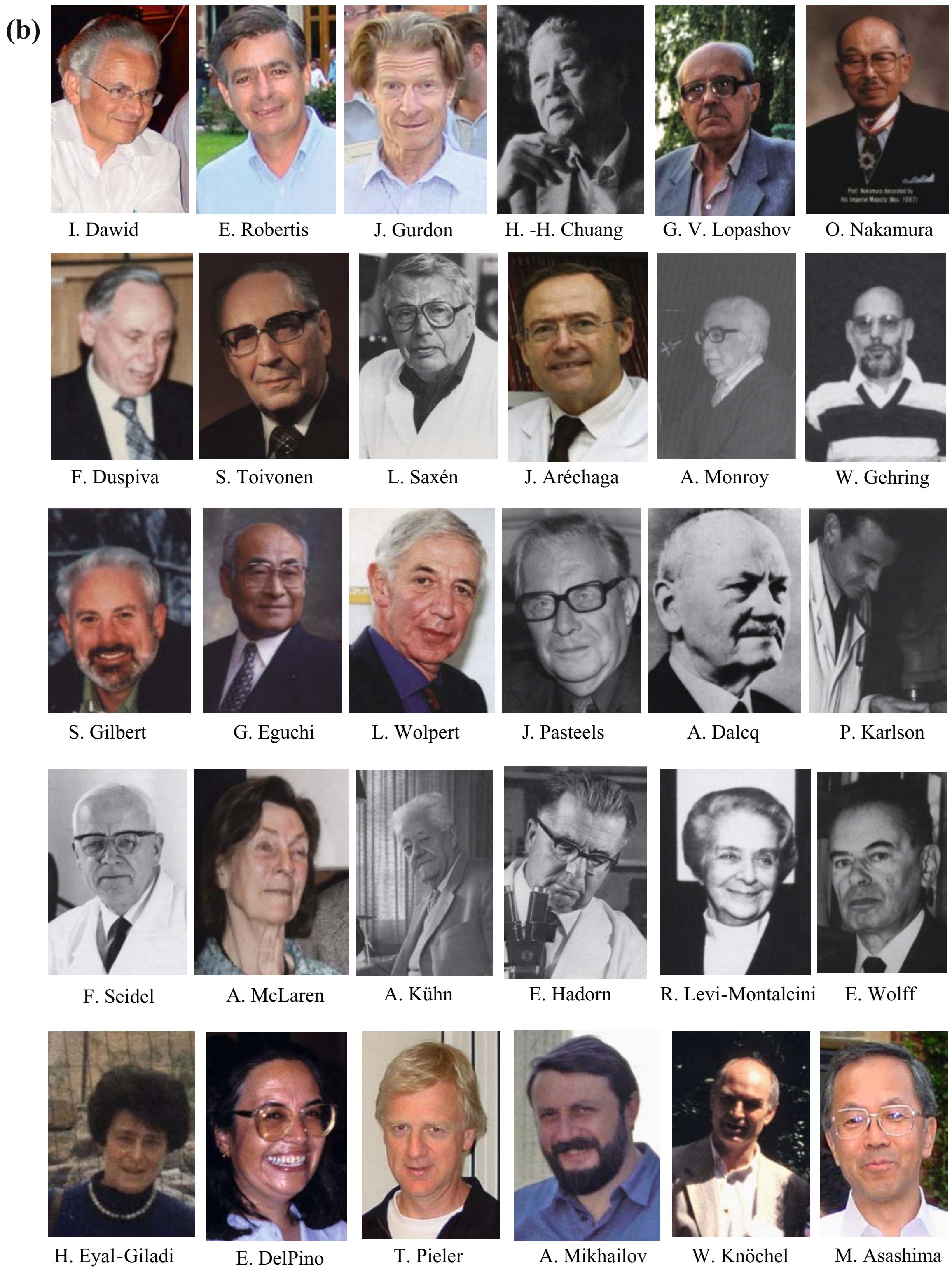

Figure 1. **(a)** Colleagues with immediate influence on my scientific work. Arrows show that I am the 'grandson' of Spemann. **(b)** Persons with important influence on my scientific work. *Last row:* Younger colleagues with whom I have collaborated and published.

and development. Johnen had spent some time with the distinguished Dutch embryologist Pieter Nieuwkoop in the Hubrecht Laboratory, Utrecht, prior to her habilitation. I received excellent training in the classical techniques on newt embryos (*Triturus vulgaris* and *Ambystoma mexicanum*). Most techniques were still reminiscent of the Spemann school, especially the internationally well-known fine glass needles (*see* the video clips entitled 'Spemann Glasnadeln' on *http://www.uni-due.de/zoophysiologie/*) and the equally fine, specially sharpened, watchmaker's forceps. With these instruments, it was possible to perform microsurgery on newt or frog embryos that were 1–1.5 mm diameter. My thesis dealt with early embryonic competence and was published in 1968 (cited in *IJDB*, **45** [1]). The questions raised there continue to attract attention today in stem cell research, because embryonic cells progressively lose their competence (or toti/pluripotency) to react to determination factors as further development occurs. These central questions in stem cell research and early embryonic development are expected to be solved by molecular genetics and developmental biology.

3. Tiedemann

Heinz Tiedemann together with his wife Hildegard (figure 1a) had embarked on a search for the chemical nature of inducing factors responsible for the determination of organ systems such as the brain, skeletal muscles, kidney and gut at the Heiligenberg institute near Lake Constance. He had obtained his Master's and PhD degrees under the Nobel prize winner Otto Warburg in Berlin. Interesting facts about Tiedemann and the scientific situation prior, during and after the war in Germany are to be found in a small booklet in German (Tiedemann 2002). One finds there valuable information about the life of an outstanding scientist of the twentieth century, of whom Jonathan Slack said in his book *Egg and ego* (Slack 1998) 'The readers may well be surprised that Tiedemann did not receive a Nobel prize.'

Tiedemann began his research in developmental biology in 1954 as a postdoctoral fellow at the Heiligenberg Institut. Mangold (figure 1a), a coworker of Hans Spemann, moved from Freiburg to Heiligenberg after the Zoological Institute in Freiburg was destroyed during the war. Mangold was the successor of Hans Spemann in Freiburg and was director of the Department of Developmental Biology in Heiligenberg. After his habilitation in Biochemistry in Freiburg, Tiedemann and his coworkers tried to isolate inducing factors from chicken embryos. He developed the phenol extraction method, which allowed the separation of ribonucleic acids and proteins. It turned out that, in contrast to proteins, ribonucleic acids had no inducing activity. Tuneo Yamada (figure 1a) in Nagoya performed experiments at this time with proteolytic enzymes, which could inactivate the biological inducing activity of embryonic tissue extracts. His approach too indicated that embryonic inducing factors must be protein in nature. Curiously, until 1985, most biologists did not accept the important role of proteins in early embryonic development. Even Jean Brachet (figure 1a), the famous Belgian embryologist, thought that ribonucleic acids represented the inducing principle. However, he himself showed that ribonucleases which appeared to destroy the biological activity of tissue extracts were contaminated with proteolytic enzymes. Tiedemann's group was the only one in Germany that tried to isolate inducing factors by biochemical methods.

As a young PhD student, I was impressed by the modern biochemical approaches of the Tiedemann group. Today, we can say that he was a pioneer in the isolation of embryonic inducing factors. Tiedemann had moved from Heiligenberg to Wilhelmshaven to head his own department. During the last year of my thesis, Tiedemann visited Engländer in Cologne and asked him if he could recommend a PhD student who would be interested to work in a biochemical research programme on embryonic induction in amphibians. Thanks to Nobel Laureat Adolf Butenandt's recommendation, Tiedemann offered me a postdoctoral position in his department of the Max Planck Institute in Wilhelmshaven (on the North Sea). In 1967, I was invited to Wilhelmshaven to have a look at the institute where I should begin as a postdoctoral fellow after receiving my PhD in Cologne. However, in 1967, the Free University of Berlin offered Tiedemann the position of Director of the Institute of Biochemistry and Molecular Biology. Hence, I never visited Wilhelmshaven again, but started as a postdoctoral fellow in Berlin immediately after receiving my PhD degree in Cologne in 1968.

4. In Berlin: from 1968 till 1980

One of my first projects in Berlin was the study of disaggregated early gastrula ectoderm cells and their reaggregation. It was shown that reaggregation could be prevented by inhibition of protein synthesis (Grunz 1969). At this time, the study of the role of the outer cell membrane (plasma membrane) and cell-to-cell interactions was a favourite topic in many laboratories. Gerald Edelman's early studies on the structure and diversity of antibodies fetched him the Nobel prize for Physiology or Medicine in 1972. He focused on cell–cell interactions in early embryonic development, and the formation and function of the nervous system. These studies led to the discovery of cell adhesion molecules (CAMs).

It turned out that the dissociation technique was a prerequisite for one of our main contributions many years later. Our findings on the formation of neural (brain) structures of gastrula ectoderm (Grunz 1989, 1990) were the basis for postulating the Neural Default Model of the ectoderm (*see below*).

I always worked on several topics at the same time, because only a few would turn out to be experimentally feasible and could be pushed to a stage where the results could be published in journals of international repute. Since Tiedemann had isolated the so-called vegetalizing factor in purified form, I tried to study its biological action. He named his inducer vegetalizing factor, because it was able to act on competent ectoderm derivatives located in the vegetal half of the early embryo, i.e. give rise to endodermal tissue such as gut, intestine, pancreas and liver, as well as mesodermal tissue such as heart, skeletal muscle, blood cells and kidney. From the mid-blastula to the mid-gastrula stages, ectoderm (presumptive epidermis and brain) is competent to react to inducing factors and can be induced to form neural, mesodermal and endodermal structures. At this time, I could already transform these pluripotent cells into a variety of tissues. In more systematic studies carried out later with *Xenopus* ectoderm, it was possible to show that the quality and quantity of induced tissue depended on the time of exposure and concentration of inducing factors (Grunz 1983 cited in *IJDB* **40** [1]). This research has recently assumed importance in the context of stem cell biology.

By 1970, we had demonstrated that it was possible to prolong the pluripotency of ectoderm by inhibiting protein synthesis. In this context, in 1969, I also combined animal caps with the vegetal half of the embryo. In several cases, notochord could be observed between the two entities. Since Heinz and Hildegard Tiedemann both thought that the result was doubtful on account of a contamination by mesoderm right from the start, they did not trust it. As a young postdoctoral fellow, I did not have the experience to insist that my observations were valid, that the ectoderm had indeed been induced by an interaction with endoderm, leading to mesodermal differentiation. The restoration of Spemann's organizer (a mesodermal structure) by interaction of endoderm and ectoderm was published by Nieuwkoop (figure 1a) in 1969, cited in *IJDB* **40** [1]). It was one of his main contributions. This is one of many examples where different laboratories observe similar phenomena at nearly the same time, but only one can be the first to report them. There is a well-known debate concerning the discoverer of the organizer phenomenon in amphibian embryo. Gavin de Beer (1927) suggested that W H Lewis (1904, 1907) had discovered the organizer effect well before Spemann and Mangold. Apparently, Lewis obtained neural tissue, but did not recognize it as such. Spemann reacted to the doubts of his claim to being the first discoverer by saying, 'You may well make a discovery without intending to do so, but not without noticing it.'

In the following years I used vegetalizing factor to study different mechanisms of cell differentiation. These included changes in cell affinity, the ultrastructural changes in induced cells by mesodermalization using scanning and transmission electron microscopy, and the importance of the initial cell mass for pattern formation. A small number of ectodermal cells differentiated into gut structures, while larger numbers differentiated into muscle and notochord (Grunz 1979, 1980). Using similar approaches, John Gurdon (figure 1b) found that in order to get mesodermal induction, a critical size of the ectodermal mass was critical; he named the phenomenon the 'community effect' (Gurdon 1988). Elongation of the mesodermalized ectoderm by vegetalizing factor results in an elongation of the tissue and causes a change in cell affinity properties. This is comparable with what happens during the involution of the upper blastopore lip of the early gastrula. A similar observation was made later by Jim Smith with the supernatant of XTC cells.

5. Tuneo Yamada

In 1969, Tuneo Yamada (figure 1a) stayed for several weeks in Tiedemann's laboratory. While in Nagoya, he had isolated a mesodermalizing factor from guinea pig bone marrow. However, he gave up this trial, since purification to homogeneity needed a large team of coworkers and sophisticated biochemical isolation procedures. Tiedemann's group was the only one in the world which had such infrastructure available (in Berlin-Dahlem, at the Free University). Meanwhile, Tuneo Yamada shifted both his research field and geographical location. On the recommendation of Johannes Holtfreter (figure 1a), then at Rochester, and Viktor Hamburger, former coworker of Spemann (see also an article by Viktor Hamburger in the special issue of IJDB, **40** [1]), he got a position as group leader at the Oak Ridge National Laboratory, Tennessee, USA. There his studies focused on another important field in developmental biology, namely Wolffian lens regeneration (transdifferentiation of iris epithelium cells into lens cells in newts; recovery of a fully functional lens after lensectomy from the upper iris rim). In Berlin, I showed him my dissociation techniques of early embryonic amphibian cells (Grunz 1969). My findings relating to cell affinity resulted in a return visit to Yamada's laboratory in Oak Ridge. The aim of our research was to show that the pigmented upper iris cells that transform into lens cells after lens removal in newts (metaplasia or transdifferentiation) also change their cell affinity. The iris cells lose their characteristic melanin granula and parts of the cytoplasm, reaching a transient state prior to their redifferentiation to transparent lens cells and ability to synthesize specific lens crystallin. This problem is of relevance to the current situation with stem cells, since scientists like to convert so-called adult stem cells (better termed as somatic stem cells) with restricted determination potential into pluripotent cells comparable with embryonic stem cells. In this context, I should mention that in 1978 G Lopashov (figure 1b) invited me to Moscow to convert anuran lens cells, derived from pigmented epithelium, into muscle cells with purified vegetalizing (mesodermalizing) factor (see below). However, this was not possible. To this day, it is difficult to convert differentiated cells into pluripotent derivatives.

The time in Oak Ridge with my wife and older daughter (then 2 years old) was very pleasant, both in the laboratory and outside. Tuneo was an excellent Head of the department and a modest scientist. Strikingly, even after many years in the USA, he never learned to speak English fluently. I remember a situation that was typical both of his reaction and diplomacy. On the first day of my stay he introduced me to the other coworkers in his laboratory. One of them complimented me on my English and added 'You have a strong German accent.' Yamada replied with a smile to the young American, 'Your English is too excellent.' After my return to West Berlin (Free University) I continued to work on the mechanism of vegetalizing factor. After his retirement, Tuneo Yamada got a small laboratory at the Swiss Institute for Experimental Cancer Research in Lausanne in the department of Sohan Modak, an Indian scientist, who had earlier been a postdoctoral fellow in his laboratory in Oak Ridge. Yamada passed away in 1996, and it was a pleasure to attend a Mahabaleshwar meeting organized in his honour by Modak and Goel (figure 4) in 1997. During my stay in the USA, I had the opportunity to meet Viktor Hamburger (figure 1a) at his home in St Louis. At this time, he was 96 years old and in excellent mental shape (Grunz 2001, cited in *IJDB* Vol. 45 [1]). He had to leave Germany in 1934 when the Nazis came to power. He received me in a very friendly manner and we discussed, in German, the recent scientific results of my laboratory.

Back to Tuneo Yamada. In 1975, Tuneo invited me to continue studies on cell affinity in Lausanne. Unfortunately for me, I started my stay in Switzerland in May. Tiedemann did not like my decision. One must know that in Berlin we used newt embryos (*Triturus alpestris*) for our studies. Since you get eggs in the breeding season only (May till the end of June), we had to catch the animals in ponds and keep them in large tanks of the size of a small swimming pool in the institute garden. It used to be said in Freiburg that Spemann got very nervous in April; all his coworkers had to participate in newt-catching expeditions. As successor of the Spemann–Mangold school, Tiedemann followed the same procedure in Berlin. Therefore, my absence for the month of May was a sacrilege. After my return in early June, I had a hard time in the institute. Tiedemann refused to see me for a whole half year. I mention this only to explain what happened in the next month (later, I had an excellent relationship again with Tiedemann). In the meanwhile I had published a fair number of papers in well-known international journals. This was a requirement to begin the procedure of habilitation, a compulsory step of scientific evaluation that one had to go through to receive the *venia legendi* as university docent which, in turn, was a prerequisite for applying for a position as professor. Tiedemann was supposed to act as mentor and supervisor in this procedure. Nearly half a year later I got an invitation from Professor G V Lopashov in Moscow, for a stay in the Soviet Union to perform experiments with the vegetalizing factor on anuran lens cells. As mentioned above, the idea was to transform the lens cells into muscle or other mesoderm-derived tissues by vegetalizing factor. A large number of students and many scientists could not follow my talk, which was in English. Dr Olga Hoperskaya, coworker of Lopashov, translated every sentence into Russian. We needed two hours for each talk. Lopashov told me later that he had announced several weeks prior to my visit that a scientist from West Berlin would visit Moscow and give several talks in his laboratory. I was said to be a coworker of Tiedemann who used techniques in the tradition of Spemann; in a manner of speaking, I was Spemann's grandson. Apparently, many people misunderstood his announcements and thought that the real grandson of Spemann would show up. This may have been the reason why the lecture hall was overcrowded – 300 people showed up on the first day. Lopashov subsequently visited my laboratory at the university in Essen. The invitation to me by the Soviet Academy of Sciences was considered sufficiently prestigious for the Dean of our faculty to persuade Tiedemann to forget his annoyance with my absence during a critical period and stay in Yamada's laboratory in Lausanne. The habilitation procedure could be started.

In 1978, Lopashov and his coworker Olga Hoperskaya were invited by Tiedemann to participate in the XIII International Embryological Conference and give a talk. However, neither of them managed to get the necessary permission. I received the manuscripts of their intended talks and presented them during the meeting in addition to my own talk. This shows how difficult it was until 1990 to have contacts with Russian colleagues, especially if one was in West Berlin. During my time in Berlin until 1980, there was again a second goldrush-like period among many laboratories to look for factors responsible for early embryonic determination and differentiation. In Germany, most scientists expressed reservations about the role of Tiedemann's vegetalizing factor. At that time, traditional biologists and embryologists had little knowledge of chemistry and biochemistry. The hunt for inducing factors was considered hopeless. As a postdoctoral fellow I participated in several meetings in Germany when Tiedemann presented his results. But in his absence, I heard many well-established German biologists smirking about Tiedemann's work. It was difficult for them to understand why the vegetalizing factor required to be isolated by phenol extraction (first step) from

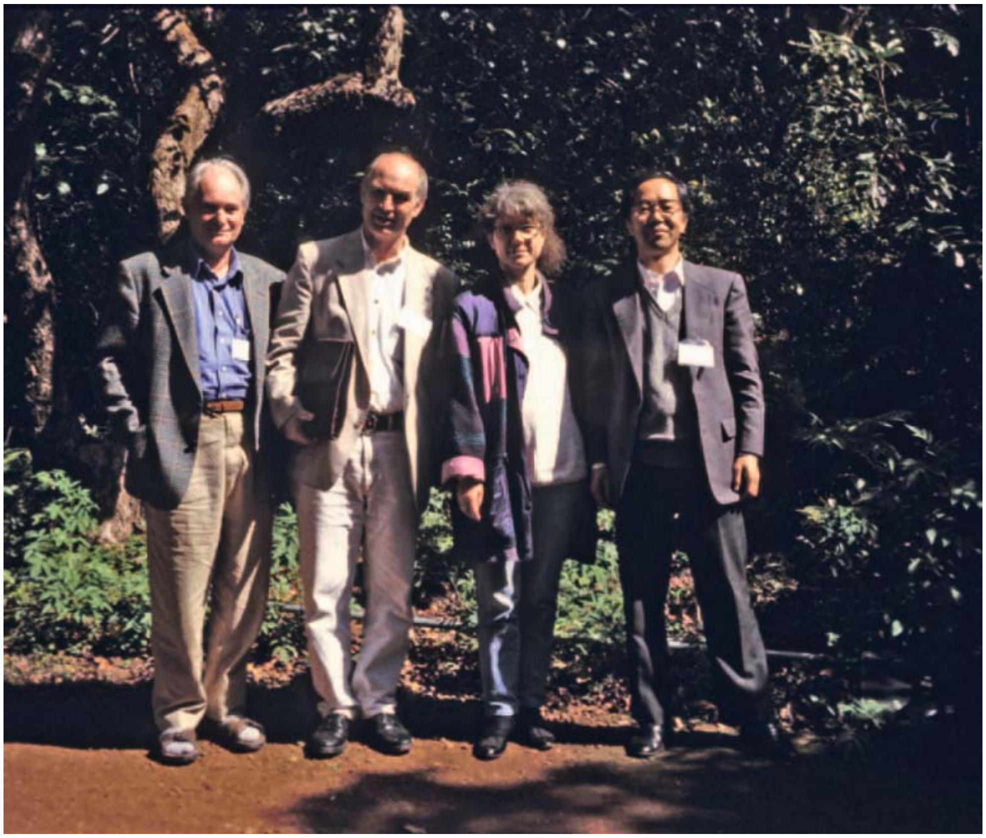

Figure 2. Horst Grunz with Walter Knöchel and his wife and Makoto Asashima in India.

12-day-old chicken embryos and then tested on amphibian embryos. Phenol was used to sterilize hospital rooms and instruments. Tiedemann as a biochemist introduced phenol extraction to separate proteins from nucleic acids, which came to be used by molecular biologists. Biologists assumed that the protein was denatured by this procedure; now we know that this was not the case. Furthermore, they could not understand how a factor isolated from chicken embryos could show biological activity in amphibian embryos. Isolation of factors from amphibians was not possible with the biochemical methods then available, because the amount of starting material was too small and was contaminated with a lot of intracellular yolk. Tiedemann can be considered as the pioneer of all further studies on the isolation of embryonic-inducing factors. It was proved later by many laboratories that embryonic factors are not species-specific and that vegetalizing factor, a homologue of activin, can be found even in human embryos. Ignoring the critics, Tiedemann's group continued to purify the factor to homogeneity with sophisticated methods. On the other hand, colleagues outside of Germany observed the activities of the Tiedemann laboratory with interest. Among them were Brachet (Belgium), Toivonen and Saxen (Finland), Slack, Smith, Gurdon (Great Britain), Igor Dawid and Eddy DeRobertis (USA), Hefzibah Eyal-Giladi (Israel), Alexander Mikhailov (Russia, now Spain) (figure 1a,b). A detailed description of the hunt for different embryonic factors (factor from XTC-cells, FGF and TGF*ß*) can be found in a review ('Developmental biology of amphibians after Hans Spemann in Germany', 2001, *IJDB* **45**; Tiedemann *et al* 2001). In 1989, Makoto Asashima (figures 2 and 3), reported during a stay in Berlin that he had discovered an erythroid factor. Together with Tiedemann's group, his group showed that the vegetalzing factor is a homologue of activin A. Finally, it was documented that vegetalizing factor was not a crazy idea and a dubious substance in the hands of Tiedemann's group (Tiedemann *et al* 1992, cited in *IJDB* **40** [1]).

6. Essen and visits to the US

After my habilitation in 1979, I got a position as Professor of Zoophysiology at the University of Essen. It was hard for me to find a position as a developmental biologist in Germany. There were several reasons for this. As a biologist at a biochemical institute (medical faculty) I had to give courses for medical students in biochemistry rather than

Figure 3. Horst Grunz and Makoto Asashima at the International Xenopus Meeting 2002 in Cambridge.

in biology. Zoological and biological faculties in Germany were looking for candidates with experience in teaching undergraduate zoological courses. Also, developmental biology was out of fashion. Since Karl von Frisch, Konrad Lorenz and Nikolaas Tinbergen received the Nobel prize in 1973, most new Chairs were being offered to candidates working on animal behaviour or neural physiology. The fact that I had published many papers describing the working of a 'crazy' vegetalizing factor (not yet known as an activin homologue) made applying even more difficult. Last, my habilitation in embryology and cell physiology was done in a medical faculty and not in a faculty of natural sciences. Developmental biology in Germany underwent a renaissance rather late; in fact, after the award of the Nobel prize to Nüsslein-Volhard in 1995. These days, positions in developmental biology and developmental genetics are being offered once again. Meanwhile, molecular genetic techniques have become well established in many laboratories. Even in Essen, two professorships for developmental biology were announced. As I explain later, only one could be filled.

I was lucky to get a position at a relatively new university even if the facilities in terms of basic equipment for my new department were poor. The faculty of biology offered me a position as a professor of zoophysiology. In fact, the faculty was not interested in developmental biology, but they needed a teacher for physiology (therefore the name zoophysiology). Colleagues urged me to give lectures in animal physiology (metabolic physiology) rather than developmental biology. The poor infrastructure (few coworkers, small laboratories and nearly no equipment) was the reason why I shifted from newts as a model system to *Xenopus*. The situation improved slowly because of grants from the Deutsche Forschungsgemeinschaft (DFG) and some financial support from the university recommended by colleagues (cell and molecular biologists) from the medical faculty. Colleagues from our faculty, working on environment protection and supported by the local government (the Social Democrats had to contend with the green movement represented by the Green Party), tried to devalue our internationally acknowledged research. The reason was the tough competition for financial support and additional equipment. Tiedemann's group in Berlin used newts (i.e. urodeles which, unlike anurans, do not go through full metamorphosis) because anuran embryos were not suitable for testing vegetalizing factor. Newt embryos needed several technicians to maintain them. Since we had only one technician in Essen for the entire group, we could not establish such an infrastructure. The shift to *Xenopus laevis*, which lays eggs on experimental hormone stimulation and can be kept in the laboratory during the whole year, was an excellent decision. Worldwide, many laboratories had introduced *Xenopus* as a vertebrate model system. Thus, results were not obscured by species differences. While in Berlin, together with Makoto Asashima (figure 3), I had already used *Xenopus* embryos to study the effect of inducers on the inner and outer layer of ectoderm. In contrast to anurans, newts have only one ectoderm layer (Asashima and Grunz 1983, cited in *IJDB* **40** [1]). In Essen I found that the differentiation pattern of *Xenopus* ectoderm depends on the incubation time and concentration of the vegetalzing factor (activin A) (Grunz 1983, cited in *IJDB* **40** [1]).

In addition to mesoderm/endoderm induction we were interested in neural induction (embryonic brain development) (Tacke and Grunz 1986; Grunz and Tacke 1986). Since Spemann's time, many researchers had tried to find neural inducers. Tiedemann's group too had started experiments to characterize the nature of the Spemann organizer (Janeczek *et al* 1984; Tiedemann and Tiedemann 1999, cited in *IJDB* **45** [1]).

I introduced methods of gene analysis based on my stay in Igor Dawid's laboratory (figure 1b) at the National Institutes of Health (NIH) in the US. Besides northern blotting, we had established the whole mount *in situ* hybridization technique in *Xenopus*. In this way, we could show the localization of a neural-specific gene (Oschwald *et al* 1990, cited in *IJDB* Vol. 40 [1]). I also continued earlier studies on *Xenopus*

Sitting : (*L to R*) G. Eguchi (Nagoya), M. I. Michael (Alexandria), L. S. Ramaswami (Bangalore), R. Bellairs (London), M. Tseng (Shanghai), S. C. Goel, *Convenor* (Pune), P. N. Srivastava, *Acting President*, Ind. Soc. Dev. Biol. (Delhi), T. S. Okada, *President*, Int. Soc. Dev. Biol. (Kyoto), G. T. Tonapi (Pune), R. O. Kelley (Albuquerque), H. Grunz (Essen), D. K. Magon (Nairobi), S. P. Modak (Pune), D. E. S. Truman (Edinburgh), B. I. Sunderaraj (Delhi).

Standing : (*L to R*) I Row F. Y. Al-Ani (Baghdad), D. M. Bania (Kathmandu), P. Thakkar (Bombay), M. Yoneda (Kyoto), M. S. Katdare (Pune), K. Singare (Bombay), R. C. Biswas (Bombay), S. Daosuko (Bangkok), A. R. Nerurkar (Pune), Y. Shih (Beijing), S. S. Navagiri (Nagpur), P. Mohanty-Hejmadi (Bhubaneshwar), S. M. Sirsat (Bombay), M. K. Khare (Shillong), I. A. Niazi (Jaipur), J. Jacob (Edinburgh), H. V. Ghate (Pune),

II Row : S. B. Prasad (Pune), P. K. Sarkar (Calcutta), S. Y. Paranjape (Pune), M. V. Joshi (Pune), R. V. Pandit (Nagpur), J. K. Mathur (Dehradun), S. A. Nevgi (Dharwad), A. Therwath (Paris), K. V. Rao (Delhi), S. K. Agarwal (Meerut), C. R. Bhaskar (Ujjain), J. K. Pal (Pune), D. R. Ranade (Pune), U. V. Wagh (Pune), S. L. Shinde, (Pune), S. R. R. Reddy (Pune), A. K. Indurkar (Pune), D. N. Deobagkar (Pune), N. Telang (New York), N. N. Godbole (Pune).

III Row : A. N. Latey (Pune), V. Patwardhan (Pune), A. Bhanot (Pune), S. Jawale (Pune), D. Gupta (Pune), J. Savla (Pune), A. Bhui (Pune), R. Narsimhan (Pune), R. Kalavagunta (Pune), S. Kamble (Pune), R. Kamakaka (Pune).

Figure 4. International Conference on "Teaching and Research in Developmental Biology in Afro-Asian Countries". Organized by the Indian Society of Developmental Biologists (University of Poona, 23 to 26 November 1982, Pune).

which we had started with *Triturus alpestris* to show that ectoderm cells react on inducing factors in a different way when they are disaggregated into single cells. We wanted to show that ectoderm cells after cultivation as single cells for several hours will differentiate into endoderm only, while immediately reagregated cells will differentiate also into mesoderm and neural derivatives. We concluded that for the formation of mesodermal and neural structures, the cells need mutual cell contact. We used untreated ectodermal cells as controls, which we kept for 4 h as single cells prior to reagregation. It turned out that just the controls brought about a breakthrough in the understanding of neural induction; these results were among our main scientific contributions. The cells differentiated into brain structures without an inducer. Therefore, it was postulated that the default status of ectoderm is not presumptive epidermis but neural tissue. We could show that the supernatant of disaggregated cells could prevent the neuralization of disaggregated cells (Grunz and Tacke 1990). The biological activity in this supernatant turned out to be BMP (Wilson and Hemmati-Brivanlou 1995, cited in *IJDB* **40** [1]). Neuralizing factors bind to BMP and inhibit it. This leads to neuralization of competent ectoderm.

Prior to my stay in Igor Dawid's laboratory, I had visited the USA in 1985 and participated in two meetings. One took place at Lace Placid. In my talk, I showed that Concanavalin A is able to induce neural structures in ectoderm. Today, we can explain this by its possible interaction with BMP-like substances at the surface of the ectoderm which results (as with neuralizing factors) in prevention of epidermal differentiation. At the 1985 First International Symposium on Cellular Endocrinology, Johannes Holtfreter (figure 1a) was an honorary guest on his eightieth birthday. I still remember his comments. When Tiedemann introduced me to him, his words were: 'Is this the guy with the Concanavalin A?' Apparently, he was not very impressed by my results. Every embryologist at that time thought that neural induction takes place by neuralizing factors that acted by entering the cell or by interacting with specific receptors on the cell membrane.

7. The German university system

Many politicians in Germany complain that outstanding scientists hardly want to return, especially from the USA, to accept a position at a German university. I have worked as a postdoctoral fellow and guest professor, on three occasions for periods longer than 7 months each at leading institutes in the United States – Oak Ridge National Laboratory, NIH and the University of California, Los Angeles. During my stays we obtained quite a few results. I did not derive any advantage from this in our faculty. On the contrary, coworkers of other groups greeted me after my return with the question, 'Did you have a nice holiday in the USA?' Every serious scientist who has worked in the USA knows that the host laboratory expects you to work very hard. Otherwise, a second invitation will not be sent. Foreign visitors always wondered why I could not improve my infrastructure at the University of Essen, although the results of our laboratory received international acceptance. Of course, such acceptance means that one will get financial support from grant-giving institutions outside the university; for example, in my case, the DFG and German–Israeli Foundation for Scientific Research and Development (GIF). Optimal utilization of support depends on your having a basic infrastructure in the department. However, it is hard to get postdoctoral positions in Germany. It is nearly impossible to get additional postdoctoral positions or technicians from the university. Although we had a large *Xenopus* colony, it was not possible to get a permanent position as an animal keeper. A leading official from the university administration (Personaldezernent) argued thus in response to my application for the position of an animal keeper: 'Why do you need an animal keeper, are your animals all ill?' He compared the situation with a hospital, where nurses care for (ill) patients. Since I said that our animals were in good shape, the application was rejected. By the way, this person was an intelligent man, a lawyer, who later became the chancellor of another university! Another shortcoming in Germany is that some professorship positions come with poor basic infrastructure while others come with much better backing. In our department, two colleagues were transferred as full professors (C4, now W3) from a college for primary schoolteachers, which was closed and integrated into the university. Although they had never completed a habilitation or held a record of good publications, they had several permanent coworker positions including an animal keeper, two postdoctoral positions and two technicians. If a C3 (now W2) Professor wants to improve his situation he must apply for a W3 at another university. I was fortunate; with the help of continuous financial support (including PhD positions) from the DFG from 1971 till my retirement I could compensate partially for these disadvantages.

Today, the political situation has changed. The support for environmental science by the local government is no longer the dominant factor that it was. Things have gone to the other extreme: the study of ecology has been abandoned in Essen. A new study area was created, called medical biology. Two professorships in developmental biology were considered essential for this area. After my retirement in 2003, one (W2) had to be filled with a successor; an additional one (W3) was created by the ministry and a university committee. I mention this in detail because this situation is not unique for a university in Germany. For both professorships the committee asked for a habilitation and outstanding publications. So, in principle, for both positions the same requirements were necessary. There were clear differences in resources. The W2 would get 1 postdoctoral position (research assistant) and 1 technician. The W3, on the other hand, would be assured of 3 postdoctoral positions, 2 technicians and 1 secretary. Only the W3 position could be filled. Till today, the W2 position has not been filled. This kind of situation explains why outstanding young scientists prefer positions in industry or try to prolong their stay in the USA. The acceptance of the W2 professorship mentioned above would result in unacceptable working conditions for an ambitious scientist. In addition, W3 professors feel superior to W2 ones and like to delegate a lot of teaching for undergraduate courses. Both the W2 and W3 have the same duties, and must spend a lot of time in administrative work.

The state of German universities has become very confusing since the 1968 student revolution. New (old) ideas do not make the situation better. In the 1970s, the universities introduced so-called assistant professor positions. They were cancelled after a while. Now junior professors (for 5 years) without a habilitation requirement have been created. The idea is that young scientists should perform research independent of a personal supervisor (traditionally a professor with a permanent position). Some people argue that the older professor has the tendency to suppress the younger one. This may be true in many cases in the past. However, in the field of natural sciences (especially in molecular biology and molecular genetics), a beginner does not possess the resources required by way of instruments and laboratory space. Maybe the new (old) idea for a junior professorship came from groups that had bad experiences. Or perhaps they were thought of in the humanities faculties (Geisteswissenschaften), where in most cases you need no sophisticated or expensive apparatus. The other new idea, to give certain universities the title ‘elite’ (where the universities get additional financial support after a special application procedure), must be examined critically. Elite universities such as Harvard, Stanford, MIT, Cambridge or Oxford, models for German politicians, received their international reputation by having outstanding scholars over long periods and not by being so defined. Even in elite universities, it is questionable if the best scientists will always receive financial support from their faculties. From my experience, I know that the distribution of money was not always transparent or correlated with the international reputation of the group.

The retirement of German professors is unique. You could be very active, and you could get financial support from outside the university (for example, from the DFG). However, you have to leave your laboratory and office at exactly 65 years of age. I still remember the day of my retirement. I came into the office of the rector of the university and received a paper with the words, ‘Congratulations, you have visited many places in the world. Here is your retirement document.’ Several reasons make it important for a retired scientist to leave his office and laboratory as soon as possible. Colleagues are waiting for free rooms and instruments. In particular, the successor is interested in a quick departure. The university and ministry (financial) are happy when a scientist retires, since his salary is reduced to 70%. As I know from colleagues in Japan, they do semi-scientific work as honorary directors or honorary presidents. Since they have long experience in science and administration, they can do valuable work for students and younger scientists. Because they know the university and laboratories from the inside and have many contacts with foreign colleagues and institutions, they can give valuable advice on communication and scientific cooperation.

Since high-speed internet is now available now at home, I could continue with some scientific work (evaluation of papers, etc.) by email. On the other hand, I have gone from molecular biology to my roots, zoology. I visited the Galapagos islands three times and prepared a film, which I have shown to larger audiences in the university. My interest is to make underwater films. To this end, in 2007 I passed an examination and received an international license (PADI) as Advanced Open Water Diver. May be this is late for a 68-year-old, but perhaps not too late. It became possible to use an underwater video camera off the Seychelles islands and Red Sea in cooperation with a marine biologist. New perspectives are available after retirement if you are willing to look for them.

Acknowledgements

The author thanks Prof. Dr Juan Aréchaga and the *International Journal of Developmental Biology* for permission to use portraits of colleagues from different issues of the journal.

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*e*Publication: 24 February 2009