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Herbert Steinbeisser: a life with the Xenopus embryo

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IN MEMORIAM

Fig. 1. Herbert Steinbeisser (1958-2014). *During a cruise on the river Mosel, on occasion of the 12th International* Xenopus *Conference in Leiwen, Germany. Photo by Christof Niehrs, (September 2008).*

Herbert Steinbeisser: A life with the *Xenopus* embryo

Herbert Steinbeisser was a developmental biologist completely immersed in science. Of a cheerful disposition and constant good humor, he was the best collaborator one could hope for. When such a nice, kind colleague is taken by cancer at age 56 it seems so unjust. Yet, his life is an illustration of how wonderful a life in science can be and we would like to relate it here.

Ketsch, Heidelberg and Ecuador

Herbert was born in Ketsch, a small village near the Rhine river where he grew up fishing for pike with his grandfather. He did his Ph.D. at the nearby University of Heidelberg with Michael Trendelenburg studying the transcription of protein coding genes in *Xenopus* oocytes (Steinbeisser *et al.*, 1989). After graduating he won an Alexander von Humboldt Foundation postdoctoral fellowship to work with Eugenia del Pino at the Pontificia Universidad Católica in Quito, Ecuador. There, in 1990, he worked with the giant oocytes of the marsupial frog *Gastrotheca*, which carries fertilized eggs on its back (del Pino *et al.*, 1986). Herbert loved Ecuador and maintained a long-standing exchange of students.

California

One day one of us (EDR) received a letter from Eugenia del Pino saying she had this German postdoc that wanted to set up Richard Harland's new whole-mount *in situ* hybridization method and could he come to Los Angeles to use our *Xenopus* embryos. He eventually stayed for four highly productive years. At that time Ken Cho and Bruce Blumberg had just cloned a homeobox gene called *goosecoid*. In one of his first *in situ* experiments Steinbeisser observed a region of staining on the dorsal side of a *Xenopus* gastrula. We all congregated over the dissection microscope as the color developed. It was a great moment in embryology, for it was the first time that Spemann's organizer had been directly visualized (Cho *et al.*, 1991). Previously, the existence of Spemann's organizer was deduced from the inductive activity of transplanted dorsal blastopore lips, but now we had a gene that marked this tissue.

One of Herbert's favorite experiments was to dorsalize an embryo by lithium chloride (LiCl) treatment (which mimicks the early canonical Wnt signal by inhibiting GSK3, leading to an embryo in which the entire mesoderm becomes organizer) or to ventralize it by irradiation with ultraviolet (UV) light; *goosecoid* behaved exactly as expected for a Spemann organizer gene (Cho *et al.*, 1991).

With an easy-going and optimistic personality Steinbeisser was always ready to collaborate even in the most technically difficult experiments. Martin Blum, who became a life-long fast friend of Herbert's, had cloned the mouse homologue of *goosecoid* and we had found that it was expressed at the anterior primitive streak in 6.75 day mouse embryos. I presented this at a meeting and it caused great consternation, for the Spemann organizer of the mouse was presumed to reside on the node, a structure that appears later. Fortunately, Azim Surani suggested that we could test the inductive potential of this region by transplantation into *Xenopus* embryos. Mice breed at night, and each female has embryos at a wide range of stages. We embarked on a huge experiment in which Herbert prepared *Xenopus* gastrula stage embryos, Martin Blum dissected pregnant mice to extract embryos at the exact stage, and EDR transplanted the anterior tip of the primitive streak or its posterior region into the blastula cavity of *Xenopus* embryos (by the Einsteck method). We were at it all day long, and by the time we had gone through all the mice it was midnight, and we went to enjoy a beer in camaraderie at a popular restaurant and bar near UCLA. It was one of those magical days that make scientific research so worthwhile. Two days later we were rewarded with some beautiful inductions of head structures, of *Xenopus* origin, induced specifically by the *goosecoid*-expressing region of the mouse gastrula (Blum *et al.*, 1992).

Steinbeisser's main project was to study the ventralizing effect of Bone Morphogenetic Protein 4 (BMP4), which had the opposing effect to that of *goosecoid* mRNA (Steinbeisser *et al.*, 1995). In loss-of-function studies he showed that antisense RNA for BMP4 could

Fig. 2. Herbert Steinbeisser and wife Sarah Cramton, with Ana and Eddy De Robertis in Pacific Palisades, California. *Photo by Marta Steinbeisser (August 2013).*

reverse the ventralizing effects of UV irradiation, and that *gsc* antisense could inhibit the dorsalizing effects of LiCl treatment. These two antisense RNAs worked very well, as had been seen before by Herbert Jäckle with *Drosophila Krüppel*. Unfortunately, studies in zebrafish claimed that other antisense RNAs had non-specific effects, and it became very difficult to publish any studies using antisense RNA loss-of-function. Thus, very valuable reagents were lost to *Xenopus* researchers due to scientific fashion. The observed results (Steinbeisser *et al.*, 1995) were probably the result of the formation of interfering RNAs, but RNAi had not been discovered at the time. Our studies on BMP4 were the result of a very fruitful collaboration between Herbert and a sabbatical visitor from Jerusalem, Abraham Fainsod, with whom he kept a long lasting friendship (Fainsod *et al.*, 1994). Because Steinbeisser was working on BMP4, he was in a good position to make rapid progress when the BMP antagonist *chordin* was cloned as a downstream target of *goosecoid* in the lab (Sasai *et al.*, 1994 and 1995).

One anecdote worth telling is how Herbert received the Mayor of Ketsch in Los Angeles. In California we sometimes have the most wonderful sunsets. One such gorgeous day the two authors looked at each other and decided they just had to watch this sunset from a bar at the Santa Monica Pier, about 5 miles from UCLA. We sat right by the water, and overheard German spoken behind us. There were Herbert Steinbeisser, the Mayor of Ketsch and a fellow citizen. We bought them a round, and they were so happy with the glittering on the Pacific Ocean and grateful for conversation in German that they invited us to fish for pike anytime at Ketsch village. Obtaining a permit to do this if you are not a local is probably next to impossible; this was a great honor although the offer was never taken up. Herbert was completely dedicated to science yet also a very social person who knew how to enjoy the simple pleasures of life. The most important event of this postdoctoral stay was meeting Sarah Cramton, a Ph.D. student in the lab right across the corridor at UCLA. They married upon his return to Germany, had two children, Karl and Marta. This was a happy marriage.

Tübingen

In 1995 Herbert became a Group Leader at the Max Planck Institute for Developmental Biology in the Department of Peter Hausen, a phage geneticist turned a strong leader of *Xenopus* research. His first paper there was a collaboration with Stephan Schneider, a graduate student of Hausen. They made the important discovery that endogenous β-catenin protein translocated inside nuclei in the dorsal side of the midblastula embryo, both in *Xenopus* and zebrafish (Schneider *et al.*, 1996). In this study we can see Steinbeisser's imprimatur for he used LiCl to show stabilization of β-catenin all around the embryo and UV irradiation to block nuclear translocation on the dorsal marginal cells. This paper was a landmark study in embryology. The dorsal localization of β-catenin is caused by a cortical rotation of the egg cytoplasm, a mechanism to which Herbert was to return (Medina *et al.*, 1997; Ding *et al.*, 1998).

Steinbeisser's interest turned to Frizzled seven-transmembrane proteins, which had been recently discovered by Roel Nusse and Jeremy Nathans as the long sought Wnt receptors. In a series of papers he explored the role and structure-functional aspects of Fz7 during *Xenopus* development (Swain *et al.*, 2001; Medina and Steinbeisser, 2000; Medina *et al.*, 2000). This culminated in the discovery, together with Rudi Winklbauer, that Fz7/PKC signaling regulates sorting out of early involuted anterior endomesoderm from ectoderm. This comes about by a switch in cell adhesion and is essential in *Xenopus* gastrulation (Winklbauer *et. al.*, 2001). The paper featured an elegant explant assay, where embryonic cells are placed on naïve ectoderm and either sink-in or stay put, the type of experiment that makes *Xenopus* such a unique system. This study set the stage for a series of follow-up papers on tissue separation, notably involving the then poorly understood protocadherins. These transmembrane proteins play an important role in cell adhesion and morphogenesis. In Tübingen, and later in Heidelberg, Herbert showed that paraxial protocadherin (PAPC) regulates tissue separation and that it is a signaling molecule, which activates Wnt/Planar Cell Polarity (PCP) signaling (Medina *et al.*, 2004; Wang *et al.*, 2008; Kraft *et al.*, 2012) and inhibits canonical Wnt signaling by sequestering casein kinase 2 beta (Kietzmann *et al.*, 2012). Steinbeisser's studies on tissue separation renewed interest in an extracellular matrix region of the *Xenopus* embryo that separates the endomesoderm from the ectoderm called Brachet's cleft (Gorny and Steinbeisser, 2012). This narrow region is now thought to provide a signaling highway during patterning of the gastrula.

Heidelberg and return to Ketsch

In 2002 Herbert was appointed professor by his *alma mater,* Heidelberg University, where he joined the Institute of Human Genetics. He was able to return to his roots, and entirely renovated the home of his grandparents in the tiny village of Ketsch. It was a beautiful house full not only with his own two children but also with kids from the neighbors that would wander in and out, as did their cats. This was a very happy home.

The human geneticists in Heidelberg realized that they needed a tractable animal model system to study the mechanisms of inborn human malformations and syndromes. In Steinbeisser they hit on the ideal colleague because a distinguishing feature was his strong collaborative nature, which manifested itself already in his postdoc days. Modest and optimistic by nature, he was always ready to contribute to studies with his *Xenopus* or Wnt expertise, even if it meant a less visible author position for himself. For example he coauthored a series of papers with Gudrun Rappold on the Leri-Weill and Turner syndrome homeobox gene SHOX (Hoffman *et al.*, 2013; Puskaric *et al.*, 2010; Blaschke *et al.*, 2003), and with Jochen Zschocke on the mitochondrial enzyme 2-methyl-3-hydroxybutyryl-CoA dehydrogenase (Rauschenberger *et al.*, 2010; Deutschmann *et al.*, 2014), congenital defects of which lead to childhood neurodegeneration.

Between 2008-2014 Steinbeisser was the leader of a Wnt research network (Wnt-FOR) funded by the German Research Foundation (DFG). The Wnt-FOR was a success story that is inextricably linked to Herbert's name, even if he was far too modest to ever articulate this. The Wnt-FOR brought together scientists from the Heidelberg, Karlsruhe and Mannheim area, who study different aspects of Wnt biology. The Heidelberg area is fortunate to have a critical mass of colleagues working in this field (including A. Aulehla, M. Boutros, G. Davidson, D. Gradl, S. Hardt, T. Holstein, A. Martin-Villalba, C. Niehrs, S. Özbek, S. Scholpp, H. Steinbeisser and D. Wedlich). Herbert not only made major contributions to Wnt signaling in early *Xenopus* development, but above all he had a special touch to integrate a heterogeneous group of scientists and balance varying interests. He had an open ear for everybody's concerns, particular needs, and interests, and everyone felt respected and uplifted by him.

Steinbeisser successfully steered the research group through the cliffs of the installation grant, coordinated its successful extension, and organized two very successful Wnt symposia in Heidelberg that gave international visibility to the Wnt-FOR. The Wnt-FOR trained more than a dozen young scientists, created a series of Wnt studies in development and beyond, and established the Rhine-Neckar area as an internationally visible Wnt research hub. The last few months, already in poor health, Herbert tirelessly worked to transform and expand the Wnt-FOR beyond the boundaries of Heidelberg to a DFG-Transregio initiative. Colleagues who witnessed his deteriorating health admired how stoically and persevering he pursued this goal, undeterred by his fate. Fortunately, he was able to complete and submit the concept paper to the funding agency before passing away.

The Heidelberg Wnt community will long benefit from his relentless commitment. A donation fund was established in Herbert Steinbeisser's name, which will support young scientists of the Pontificia Universidad Católica del Ecuador, where Herbert spent happy months doing research on *Gastrotheca.* Herbert will be missed by many scientific friends that collaborated with him throughout the years, but his scientific contributions and personal legacy will remain.

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