BIOGRAPHICAL MEMOIRS

Walter Jacob Gehring

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A Biographical Memoir by Edward M. De Robertis

Figure 1 Walter Gehring. *Photo by Eddy De Robertis, 2005.*

Walter Gehring was a pioneer of the fusion of molecular biology and Drosophila developmental genetics that revolutionized biology. His relentless pursuit of molecular explanations for developmental control genes led to the discovery of the homeobox, the enhancer trap method, and the common origin of all eyes in the animal kingdom. These findings were entirely unexpected at the time and made Gehring one of the most important biologists of the end of the twentieth century.

Education in Zürich

Walter Gehring was born on March 20, 1939, in Zürich, Switzerland, to Marcelle Rembann and Jakob Gehring, an engineer. As a child, he developed an interest in insects and birds and their migration patterns. He attended Realgymnasium Zürich (high school) and then entered the University of Zürich to study zoology. He was a passionate birdwatcher, and for his master's thesis, he used the Swissair radar at Zürich Airport to follow bird flock migration. His mentor, Ernst Hadorn, allowed him this amusement on the condition that he would do his thesis on Drosophila. Hadorn trained with Fritz Baltzer in Bern, who in turn had trained with Hans Spemann and Theodor Boveri in Würzburg. Much of European cell biology can be traced back to Boveri.[1]

Hadorn worked on the determination of Drosophila larval imaginal disc cells.[2] Using cell hair and bristle markers, he showed that imaginal cells could be transplanted serially for many generations inside the body cavity of recipient larvae, where they proliferated in the hemolymph-rich environment. Upon metamorphosis, the transplanted cell formed structures that correspond to the disc of origin, such as eye, antenna, and wing. During the year that Walter was chasing birds, Hadorn discovered transdetermination. When disc cells were cultured inside the abdomen of adult flies for a period of time and then returned to larvae and allowed to metamorphose, sometimes they differentiated into adult structures corresponding to a different disc. Gehring's thesis was titled "Transdetermination of the Antennal Discs in *Drosophila,*" and he found that this change was not clonal: cells would change fate as a group rather than as individuals. He also described a spontaneous mutant that he called Nasobemia, later found to be in the Antennapedia gene. Gehring also met Elsbeth Lott during this period, and the pair married in 1964. Gehring completed his Ph.D. the following year.

Postdoc at Yale

In 1965, Gehring became a postdoc in the lab of molecular biologist Alan Garen at Yale University in New Haven,

Connecticut. After completing the postdoc, he was hired as an assistant professor at Yale Medical School. With L. N. Chan he showed that anterior blastoderm cells, before any signs of differentiation, were already determined to form anterior structures after transplantation.[3] Walter's first graduate student, Eric Wieschaus, performed analyses of blastoderm clones induced by X-ray recombination that demonstrated that blastoderm cells were restricted to form particular segments of the fly before any morphological changes appear.[4]

In Switzerland during this period, University of Geneva professors Werner Arber and Edouard Kellenberger persuaded the city of Basel to build a new institute of molecular genetics, which was named the Biozentrum and became a premier European research center. This investment proved an invaluable human resource for the local Swiss pharmaceutical companies. One of their first recruitments was Walter Gehring, who joined the faculty in 1972 and became a full professor at the tender age of thirty-two.

Introducing Molecular Biology to Drosophila Genetics at the Biozentrum

From the very beginning, Gehring realized that there was much to gain from the application of nascent molecular biology techniques to Drosophila developmental mutants. He turned his Department of Cell Biology at the Biozentrum into the place to be. Drosophila—with its well-established mutants, linear genetic maps, polytene chromosomes, and translocation, inversion, and duplication strains—proved a uniquely advantageous organism with which to take advantage of the emergence of gene cloning. Many of the techniques were invented at Stanford University by Gehring's close friend David Hogness (who pioneered colony hybridization with radioactive probes, mapping of DNA clones to polytene chromosomes, and walking along the chromosome) and were rapidly implemented in Basel. With the help of Alfred Tissieres, from the University of Geneva, they established gene banks (called banks instead of libraries, as they were made in Switzerland) and cloned the heat-shock genes,[5] the 5S rRNA genes,[6] and the *white* eye gene.[7] David Hogness spent a sabbatical in Basel, and his office was designated the Hogness Box because it was where he discovered the promoter sequence now called the TATA box. Other visitors included Monte Westerfield from the University of Oregon and Frank Ruddle from Yale. One of Gehring's earliest postdocs was Christiane Nüsslein-Volhard, who analyzed maternal-effect mutants that affected larval cuticle.[8] She later teamed together with Eric Wieschaus, who had moved together with Gehring from Yale, for their Nobel Prize-winning screen of lethal zygotic mutations that affected larval cuticle carried out at European Molecular Biology Organization in Heidelberg, Germany.

The overarching theme of the Gehring laboratory was the quest for molecular evidence for Drosophila developmental mutations. I joined the department in 1980 as full professor but had a small laboratory. We had common weekly seminars, which were a joy. Walter would stand up in the middle of a talk, walk to the blackboard, and fill it up with drawings of genetic crosses or whatever the topic was. Some of his own postdocs did not welcome these enthusiastic interruptions, yet I am forever grateful that I completed my education in developmental biology with Walter Gehring, who was a wonderful scholar.

The Discovery of the Homeobox

In 1983, Richard Garber completed a chromosome walk of 250 kilobases using overlapping phage lambda clones, finally reaching the gene *Antennapedia*.[9] There was contemporaneous, independent research from Thomas Kaufman and his postdoc Matthew Scott in Bloomington, Indiana, that stimulated creative competition.[10] Garber obtained both genomic and cDNA clones with help of Atsushi Kuroiwa. The *Antp* gene was very large (100 Kb) and, in addition to its many exons, a nearby gene, later found by Kuroiwa to correspond to *ftz* (*fushi tarazu,* which means "not enough segments" in Japanese), also hybridized with the *Antp* cDNA probe. Garber then departed on a job search tour of many universities in the United States for several months. His timing was most unfortunate, for during this hiatus much happened with the *Antp* cDNA in the miraculous year of 1983–84 at the Biozentrum. I had the privilege of a front-row seat during these exciting times when the homeobox was discovered.

Ernst Hafen, a brilliant graduate student at the Biozentrum, had been trying to establish a method for in situ hybridization of mRNAs in Drosophila embryos for several years without success. Michael Akam had given a seminar in Basel after his return from Hogness's lab and had given Ernst a then-unpublished midgut probe that was expressed very abundantly and allowed him to get the mRNA in situ hybridization method with radioactive probes perfected for *Antp* cDNA.[11] Michael Levine arrived in Basel and proved a powerhouse. Teaming up with Bill McGinnis and Hafen, he tested Edward Lewis's proposal that homeotic genes, which change body segments into the likeness of another, were descended through duplication of an ancestral gene during evolution. They used low-stringency Southern blots by lowering temperatures and formamide concentration, a technique in which McGinnis had previous expertise. They found that a short fragment of *Antp* cross-hybridized only at low stringency (indicating they had mismatches) with at least seven other genomic Drosophila DNA fragments.[12] Walter requested lambda clones from the *Ultrabithorax* locus from

the Welcome Bender and Hogness Bithorax genomic walk, and it too contained the cross-hybridizing sequence. Walter's team screened duplicates of a Drosophila genomic library, provided by Tom Maniatis, for plaques that hybridized with both *Antp* and *ftz* probes and isolated two novel genes that mapped near the *Antennapedia* and *Bithorax* chromosomal regions.[13] Using similar methods, over time the Gehring laboratory cloned a plethora of developmentally crucial genes such as *deformed*, *sex combs reduced* (*scr*), *abdominal-a, abdominal-b*, *caudal,* and many others.[14,15] Gehring's *engrailed* probe was particularly useful for Peter Lawrence and others studying imaginal disc patterning.[16] The conserved sequence was called the homeobox and later proved to be a Rosetta Stone for developmental biology. The homeobox was independently found by Matt Scott in the United States in almost contemporaneous experiments.[17]

A crucial moment came when Ernst Hafen presented in our weekly seminars an in situ hybridization with *Antp* cDNA. In his initial image of a late embryo, *Antp* mRNA showed up in the ganglion neurons of the ventral nerve cord of the second thoracic segment and, more weakly, on the third.[18] This was tremendously exciting: homeotic genes were expressed in the region in which they affect the fly phenotype. I immediately jumped up and argued that since *Antp* was expressed in the central nervous system, the conserved sequence might encode a neuropeptide that is secreted to impart positional information to the ectoderm. Walter politely explained that this could not possibly be, because *Antp* was a cell-autonomous gene. However, not knowing at the time what cell-autonomous meant, I remained insistent, prompted by work in Hydra, in which Chica Schaller had reported that a head-inducing peptide had an amino acid sequence almost identical to that of a bovine brain peptide (sequencing of the Hydra genome later showed that this peptide did not exist in its genome, so probably it was just an artifact). Later results in Drosophila showed that *Antp* was expressed in epidermis at earlier stages; my idea was completely wrong, but at the time neuronal expression was all that had been detected.

After the seminar, I followed Walter into his office and argued that despite his reasoning, it would be very easy for us to test this crazy idea in a vertebrate because we had freshly plated *Xenopus laevis* frog genomic libraries, a gift grom Igor Dawid and Walter Wahli from which we had already cloned many genes. There was little to lose, and Gehring agreed. In my excitement, the next stop was to chase Bill McGinnis, who later reminded me (and the entire audience of the fortieth anniversary symposium celebrating the homeobox) that he was in the bathroom and asked me to please wait a minute. McGinnis had trimmed down short probes not only for *Antp* but also for *ftz* and *Ubx* homoboxes and had defined low stringency hybridization conditions. Back in my lab, my two most expert molecular biologists, Iain Mattaj and Rolf Zeller, who had been at the seminar, passed on the experiment. Fortunately, another postdoc, the late Andrés Carrasco from Argentina, took on the task, and on the first try he isolated a genomic *Xenopus* lambda clone that hybridized with *Antp* and in Southern blots with all three Drosophila homeotic probes. Carrasco's gene, now called *HoxC6*, contained a remarkably conserved sequence. Shortly before, McGinnis had obtained the sequences of the homeoboxes of *Antp*, *Ubx,* and *ftz*. The homeobox sequences of Drosophila and *Xenopus* encoded a sixty-amino-acid, DNA-binding protein domain that was designated the homeodomain.[19-21] When DNA sequencing revealed that frog *HoxC6* shared fifty-five out of sixty amino acids with the *Antennapedia* homeodomain, Walter believed we had a contamination with a Drosophila library. But this was not the case; we had just been very lucky that evolution had conserved the homeodomain to an extraordinary extent, probably more than any other DNA-binding domain. Even to this day, for example, we do not know why the *engrailed* homeodomain has the unusual property of higher conservation at the nucleotide level than at the amino acid level. These results were published in two back-to-back landmark papers in *Cell*.[22,23] The Carrasco, McGinnis, Gehring, and De Robertis paper explained in the discussion that "cross-hybridizing sequences, first found in the frog, have subsequently been found in other vertebrates." The abstract stated, "This gene could perhaps represent the first development-controlling gene identified in vertebrates," and so it was.[24] Gehring generously distributed the Drosophila homeobox probes to many laboratories, and an unexpected era in the analysis of vertebrate development commenced.[25]

The Enhancer Trap Method

The awesome power of Drosophila molecular genetics was used by Gehring to devise a powerful method to detect DNA enhancers. Cahir O'Kane, a postdoc from Ireland, fused the weak promoter of P-element transposase to *E. coli* β-galactosidase and made many transgenic lines in which the P-element transposon, initially developed by Gerald Rubin and Allan Spradling, integrated randomly into the genome. When stained for β-galactosidase enzyme activity, 70 percent of the transposons had integrated close to genomic sites able to drive expression in interesting embryonic patterns; the Drosophila genome was, unexpectedly, full of elements that regulate gene expression.[26] The enhancer trap method was subsequently perfected to mobilize the transposon by simple genetic crosses with fly strains expressing transposase.[27] When β-galactosidase was replaced with the yeast Gal4 transcription factor, using the UAS reporter method invented by Andrea Brandt and Norbert Perrimon, enhancers were able to drive the expression of proteins or interfering RNAs in

target tissues. The enhancer trap method became a widely used tool in molecular genetics of developmental genes that are redundant and not required for viability in Drosophila.

The Universal Evolutionary Origin of Eyes

During studies of proteins interacting with homeodomain DNA-binding sites, the Gehring lab isolated a cDNA clone that corresponded to the *eyeless* locus. Its sequence had strong homology to *Pax6*, a vertebrate gene then known to encode the mouse *small eye* and the human *aniridia* mutations.[28] Gehring immediately proposed that *Pax6* represented a master control gene for eye development in all living organisms. Previously, it was believed that eyes had evolved independently multiple times during animal evolution. For example, faceted insect compound eyes, with their many ommatidia, differ greatly om morphology from octopus or human eyes. This was a radical hypothesis.

To prove a common function, the Gehring group used their enhancer trap method to drive the expression of mouse *Pax6* in imaginal discs. From his Ph.D. transdetermination studies, Walter knew that eyes could sometimes transdifferentiate from transplanted antenna or leg discs. They generated enhancer trap lines driving overexpression of mouse *Pax6* in these imaginal discs using the Gal4-UAS method and were eventually able to induce ectopic eyes in antennae and legs.[29] Of course, the eyes were fly ones; the mouse only provided one gene and the fly the other 2,000 downstream genes required to form eyes. The Gehring group went on to study the role of gene networks that collaborated with *Pax6* in eye development. He also showed that the genomic *Pax6* DNA enhancer that drives lens crystallin formation in chicks is also functional in the fly ommatidium lens formation.[30] Walter defined *Pax6* as a master control gene of eye development.[31]

Epilogue

Walter Gehring was driven by an unquenchable curiosity and was completely immersed in science. He was a true scholar. He co-authored a general zoology textbook in German with Rudiger Wehner after its original author, his mentor Hadorn, died. He taught an annual marine biology course at the marine station of the Centre National de la Recherche Scientifique in Banyuls-sur-Mer in the south of France. When the plankton net came in, he delighted in teaching his Biozentrum tudents how to classify invertebrate larvae. He was a joyful man of science. A happy and jolly man, he cut a figure not unlike Santa Claus. He told jokes with a sparkle in his eyes. He would recount that he was probably the only Swiss professor who discharged his periodical military duty at the low rank of soldier. A gregarious man, he traveled regularly with friends to Italy to purchase Barolo wines. He never lost his passion for birdwatching, delighting in watching pelicans dive along the coast of California.

Figure 2 Walter and Elsbeth Gehring birdwatching in Pacific Palisades, California, 2014. *Photo by Eddy De Robertis.*

Gehring was a gifted speaker who gave fascinating lectures. For years, he delivered talks on the evolution of eyes. He had a collection of jokes he recycled, and the audience showed up in part to find out which ones he would use that day. During his final year, he had assembled a stimulating new lecture on the in vivo DNA binding of the *Sex Combs Reduced* (*Scr*) homeodomain protein, which is expressed endogenously in salivary glands, to polytene chromosomes. Although Walter had to close his lab at age seventy, according to Swiss law, two of his postdocs continued working on *Scr* in Sweden. DNA-binding proteins have both low- and high-affinity for their targets. High-resolution imaging revealed that instead of binding and sliding along chromatin until finding high-affinity sites, *Scr* comes on and off DNA until it meets its high-affinity targets in living salivary glands.[32,33] This was fascinating for those interested in how DNA binding proteins work.

Walter loved Greece, and he attended eighteen consecutive biannual European Drosophila meetings there. While on vacation there in 2014, he rented a car on the island of Lesbos. It was a rainy day, and on a slippery hillside a tractor-trailer truck jackknifed in front of him. A crash was unavoidable, and he was severely injured, although his wife, Elsbeth, was unharmed. He was flown back to Switzerland and underwent two operations, but he passed away shortly thereafter at the age of seventy-five.

Walter Gehring was an intellectual leader during a most exciting time for evolutionary developmental biology. His legacy carries on through his many trainees and their discoveries.

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