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A visit to the Hubrecht laboratory

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The seven month sabbatical that I spent in Holland from January until July 1978, had a profound effect on my future research career and was personally a thoroughly enjoyable experience. It was a lesson in cooperation and multidimensional investigation.

At that time I was at Princeton University and I had planned to spend part of my sabbatical, jointly with John Gerhart, who was at Berkeley, investigating some aspects of early development in *Xenopus* at the Hubrecht Laboratory in Utrecht. By 1978 some areas of biology had moved forward at great speed, spurred by molecular biology, but others like embryology still seemed to be mired in vague premolecular concepts, such as induction, competence or regulative development. The scene at the Hubrecht in those days was a curious one. It seemed to John and me that the Institution was dedicated to the most interesting unsolved problems in developmental biology and with its emphasis on *Xenopus* it employed one of the best systems to answer them. Yet compared to most laboratories, the Hubrecht was unusually focused in descriptive morphology and lacked the modern core of molecular biology and biochemistry found in most places. For us, the descriptive morphology was just what we were lacking. We particularly enjoyed talking to Pieter Nieuwkoop, who at that time seemed rather disconnected from the other work going on. John and I soon discovered that he had performed a wealth of fascinating experiments with Elze Boterenbrood on the mechanism of mesoderm and neural induction, experiments that have a continuing influence today. We would sit with him at lunch with our open faced ham and cheese sandwiches, imbibing the lore of induction and germ cell formation and trying to connect to a tradition of experimental embryology that was almost completely gone

John and I were identified as cell biologists and biochemists, and assumed to be neophytes in embryology, although in fact both have us had used *Xenopus* for several years. We were offered the chance to collaborate with Geert Ubbels, who was working on the histology of the *Xenopus* egg during the first cell division, when it was known that the dorsal ventral axis was established. Geert had a particular interest in the cytoskeleton, but for *Xenopus* before confocal microscopy this was still difficult. The importance of the cytoskeleton in axiation was a perceptive intuition of hers, though the significance would not be felt until several years later and would derive from experiments that we did at the Hubrecht. Our first weeks at the Hubrecht were both productive and frenetic. Since we wanted to examine the first cell cycle in the egg, we needed to fertilize eggs artificially. To our surprise, there was a virtual prohibition on *in vitro* fertilization, since that required sacrificing a male for every experiment. So the first two weeks were spent learning how to store sperm, which we finally could do for a period of weeks, and how to store eggs, which we did by modifying Ringers to make MMR (which John named for me-Marc's Modified Ringers, though I am not sure I made any real contribution to its formulation). During those first few weeks we learned two important things from Geert: the existence of a pigmented spot on the egg surface, where the sperm entered the sperm entry point, and the existence of an extraordinary library of

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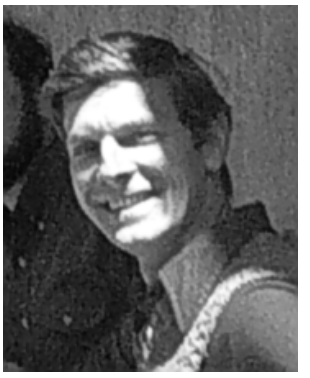


John Gerhart

Geert Ubbels

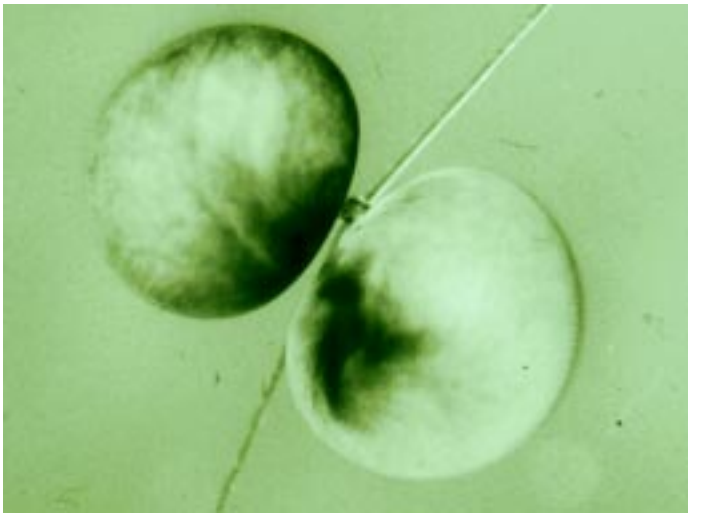
Koki Hara

old reprints at the Hubrecht. Each night John and I would take home several reprints, dating back almost 100 years and a trail of nearly forgotten experiments from Schulze to Ancel and Vitemberger pointed to rotation of the egg cortex as being involved in setting up the embryonic axes.

At this time the "egg rotation" literature was forgotten and unknown. There were several reasons. First, mechanical rotation of the cortex or physical displacement seemed distinctly medieval, as an explanation for something as tangible as the specification of embryonic tissues. Second, experiments by Adam Curtis had shown that the determinants for the dorsal axis lay in the cortex and were transplantable, though no one had repeated these difficult experiments. Third, the important cortical element in these transplantation experiments was supposed to reside in the grey crescent, which John and I liked to refer to by the Dutch name for the Holy Ghost, since the grey crescent was obviously of transcendental importance but seemed without substance. Fourth, the experimental protocols for rotating eggs were distinctly unimpressive, since the eggs had to be continually turned over as they rotated freely within the vitelline envelope. The latter problem, we solved by finding that large polymers like Ficoll would dehydrate and reduce the perivetelline space and hold the eggs in any position, an innovation that simplified many experiments with fertilized eggs. We carried out several experiments that showed that twinning and axis reversal was related to cytoplasmic displacements in highly reproducible ways, and this was the beginning of our modern understanding of axiation in *Xenopus*.

I was lucky enough to be able to come to Holland with my whole family and could stay 7 months, experiencing 7 months of seemingly constant Dutch weather. My wife loved Holland and made a successful attempt to learn the language. John, who came without his family left after 3 months. In my monolingual existence in the lab I unfortunately absorbed little of the surrounding culture. In the lab John and I met another pivotal figure for us, Koki Hara, who for years had dedicated himself to making time lapse movement of developing eggs. Koki was an artist at micromanipulation and at cinematography. His nervous quiver at 40X magnification was about equal to mine at 1X. This discrepancy was a challenge for me to design methods that would work for my modest abilities. Koki and I finally had a bit of a showdown over the ability to remove the vitelline membrane from newly fertilized eggs without puncturing them. Using Ficoll, I found that I could be as quick and as successful as he was. I think at that point Koki realized the foreboding power of biochemistry. Koki's movies changed my view of the world, for they were not just beautiful but they revealed processes unappreciated by anyone else. In one movie he showed me dissociated axolotl embryos with isolated blastomeres dividing in unison until suddenly, at the midblastula stage division became asynchronous and slower. That movie in itself became the inspiration for experiments I later did with John Newport on the midblastula transition. After John Gerhart returned to California, Koki and I continued to collaborate on experiments to see the effect of colchicine on early cortical events in the egg related to the axiation problem. I was stunned by the results. In one simple movie the entire autonomous oscillator of the cell cycle was revealed. The eggs of course did not cleave but instead showed rhythmical contractions timed with the cell cycle in cleaving eggs. When we enucleated the eggs by tying them in half with baby hair, the enucleated half also heaved up and down with the same periodicity as cleavage in the unperturbed eggs.

I wrote to John back in Berkeley, that a process governing the cell cycle must exist separately from the known events of the cell cycle, such as mitosis and DNA replication. The only known chemical phenomenon that could explain this was maturation promoting factor. Therefore, when I returned to the US to take up



Genesis of an enucleate fragment by constricting a *Xenopus* zygote with a baby hair.

my new job at UCSF, John and I began collaborating on experiments designed to relate MPF to the autonomous mitotic oscillator and my career in the cell cycle field had begun.

John and I were lucky to take our joint sabbatical in Utrecht. We had wonderful support from the scientists and staff to investigate anything of interest. Peter Nieuwkoop was still there anticipating a revival in experimental embryology based on his classical experiments. The reprints from the late 19th and early 20th century were there, straining our limited knowledge of German but exposing us to phenomena that could serve as a basis of many new investigations. Most important, there were no interruptions. Phone calls were a rarity, since when they did come they would be announced over a loudspeaker and we would have to run to one of a few telephone booths where the calls were directed. In many ways the laboratory was constructed on an old fashioned principle, where students of the great Professor work the rest of their lives on problems he laid out. But the problems were good ones, and older approaches have a way of shedding new light on problems overlooked by current fads of investigation. Even small things like the importance of time lapse cinematography and the existence of a morphological sperm entry point were key ingredients in future progress using more molecular methods. Our time was productive: four papers co-authored with Geert Ubbels and Koki Hara. Two of these might even be considered of special importance, but the real productivity was a collection of ideas that would fuel many more discoveries for years to come. Soon after we left, the Hubrecht evolved into something more conventional and more modern. However, for us 1978 was a period suspended in time when it seemed that simple experiments could still be performed on complex systems to yield important answers. In retrospect, our sabbatical fit into a 17th century Dutch painting: a tranquil landscape, an expansive but cloudy sky with industrious people in the corner of the painting using humble tools doing something worthwhile.