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BIO

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Today's generation of women graduate students, postdocs, and junior faculty worries about getting a good job (and often an additional one for a spouse within commuting distance), day-care, maternity leaves, and granting agencies expecting high productivity no matter what. My generation would have been happy to have had such problems. Ours were Will women be admitted into graduate school? Can I get any academic job as a woman? Can I have a job at all if I get married? The answers were often No. Born a few months before the United States entered World War II, I grew up in Durham, New Hampshire, USA, population 1500 and home to the University of New Hampshire (UNH). When I was 9, my parents took me to a public lecture at UNH given by biologists Lorus and Margery Milne. That lecture convinced me that if Margery Milne could be a scientist, I could too. She had a PhD from Harvard. In 1948 the Milnes both joined the UNH faculty. However, Margery was "forced to resign" in 1951 because of a new university rule—perhaps formulated specially for them—that barred married couples from both being faculty in the same department. She never had another job. Her second class status was reflected in the many books on natural history she cowrote with her husband—she was always second author. A notable example is "The lower animals; living invertebrates of the world, by Ralph Buchsbaum and Lorus J. Milne. In collaboration with Mildred Buchsbaum and Margery Milne." Her life was typical of highly intelligent, educated women who tried to combine marriage and an academic career in the mid-20th century. They were not allowed to.

About the time I attended the Milnes' lecture, I found some frog eggs in a neighbor's pond, brought them home, and raised them in a hubcap I'd placed in my sandbox. After school, I'd watch them for hours. I thought that the Scientists certainly knew just how it was the frogs made legs and resorbed their tails, etc. By age 12, I had decided to be a marine biologist—after all Durham is on Great Bay, and the boys would chase the girls with sea lampreys coming up the stream behind the grammar school. In my junior year in high school (1957), we watched Sputnik go overhead—a small moving light. I couldn't see the banner behind it announcing "Free Lunch for Scientists," but I am sure it was there. Sputnik presaged a perfect time for scientists.

That same year, my English teacher at Oyster River High told 3 of us out of a class of 20 to forget our last year of high school and go to college. I immediately wrote away for brochures from colleges in warm places that offered marine biology (I had shoveled enough snow). The cover of one showed some students playing volleyball. Not serious enough.

I chose Stanford, where advanced undergraduates could take Don Abbott's summer classes in marine biology. I planned accordingly, starting with the required classes, one of which was Clifford Grobstein's embryology course. Grobstein, who had just come from a research position, heard that Stanford was supposedly the Harvard of the west coast. His class was excellent, although most of us were miffed that he refused to grade on the curve and for a class of 110 gave 2 As, 15 Bs (one was mine), and the rest Cs, Ds, and Fs. Although I really liked developmental biology, because Grobstein was not approachable, I chose Art Giese for independent study on echinoderm reproductive physiology. After 5 min of instruction from a medical student in the lab on how to do Kjeldahl protein nitrogen assays, I began to learn from my mistakes. Today I tell my students that my only excuse for staying in science is that I generally don't make the same mistake twice.

During my senior year, to avoid expulsion from Stanford for returning to the woman's dorm 30 sec late (there was a three strikes and you are out policy that year), Nick Holland, who was a graduate student, and I found the circuit judge of Santa Clara County and were married. In one way, that was a good decision; we are still married. In another way, it was a bad one. Ten years after UNH fired Margery Milne for being married, I was going to see if I could do better. I hadn't considered that all my professors at Stanford except one German instructor, were men. The dearth of women faculty should not have been surprising. About 1900, the Stanford President David Starr Jordan, an ichthyologist, refused the application of Dr. Julia Platt, famous for Platt's vesicle in the shark, for a faculty position. She became mayor of Pacific Grove, California. Even so, I only realized how bad things were for women when I applied to Stanford for a master's degree. Stanford's policy was not to admit its own undergraduates as PhD students. I was admitted, but the chairman of the graduate admission committee, in whose graduate class in plant physiology I had done well, said to me "Of course we admit fewer women than men. Women have such a bad record finishing." I thought, "We'll see about that."

After finishing a master's on proteins of sea urchin coelomic fluid (I disproved the dogma that it contained no protein), Nick and I and our daughter moved to Naples, Italy, where he had a 2-year NSF fellowship to study at the Stazione Zoologica. Our two other children were born there. It took all my time to shop, pay the bills, and wash the diapers in a wringer washer. In 1966 Nick accepted an assistant professor position at Scripps Institution of Oceanography (SIO). Once again, I found that the situation for married women hadn't changed. All job applications required listing marital status, spouse's profession, and number of children. I could get no job. Finally, to retain my sanity and keep my hand in, I volunteered at SIO, doing an electron microscopic study of regeneration of sea urchin pedicellariae. Although SIO was relatively rich because of the cold war emphasis on antisubmarine warfare, the director, William Nierenberg, denied me US$20/week (the cost of daycare), claiming that if he paid me US$1/hour, he'd have to pay Mrs. Hubbs retroactively for all the years she'd been volunteering her time. She too had a master's degree from Stanford, and was her husband Carl Hubbs' unpaid assistant at SIO. Although her contributions were unofficially widely recognized, she was a co-author on only 3 of Carl's 79 articles.

The first of many breaks came for me in 1971 when a fellow graduate school mate from Stanford, Meredith Gould, obtained a postdoctoral position in Dan Lindsley's *Drosophila* genetics lab in the UCSD biology department and asked me to work for her. I was paid as a part-time temporary "laboratory assistant," a position usually held by undergraduate dish washers. However, no matter how lowly, a paid position gave me respectability. For Meredith, I was really a fellow postdoc. After trying to make temperature-sensitive *Drosophila* mutants and finding that bread mold grew much better than flies at 60°F, we began a side project on development of *Urechis caupo*, on which she had done her graduate work. Meredith volunteered to teach and was appointed a lecturer. Then, because the study of the electrical polyspermy block we did with Laurinda Jaffe was so exciting, Meredith obtained an NSF grant and was promoted in one step to associate professor with tenure. In turn, I was promoted to technician. It was the ideal job for me. However, Meredith, who wanted to help disadvantaged students, finally realized that most UCSD students only wanted to learn enough to pass the exam. Early in 1983, she resigned her position at UCSD and emigrated to Mexico. At the Universidad Autónoma de Baja California in Ensenada she found the motivated students she had been looking for. At least 50 attended her memorial service in 2006.

At this point, I began to liken myself to a mouse at the end of the age of dinosaurs. With three children in expensive colleges, or nearly so, we needed two incomes. Consequently, I asked two professors in the biology department for recommendations. One, Herb Stern, the department chair, said to me, "There aren't any jobs in exactly what you've been doing, so you're finished." The other said "You have risen above your station in life and must be prepared to come down." I was 41, and speechless, but I thought "We'll see about that." I looked at job advertisements to see what skills were in demand and on a Friday, put in an application at the nearby Scripps Research Institute. I claimed I was knowledgeable in protein chemistry, chromatography, electrophoresis, etc., with an excellent theoretical knowledge of immunology (I was a third of the way through an immunology book). On Monday I interviewed with Ted Zimmerman, who offered me a position in his human blood clotting lab. Later he admitted that as it was now illegal to ask job applicants about their families, he selected technicians in their early 40s because, if they were going to have children, they already would have done so. In 4 years there I really did become quite a good protein chemist and quite knowledgeable about antibodies. However, it was a real technician's job. In the beginning I was learning new techniques, but after 4 years I simply could not bear high-pressure liquid chromatography any longer.

Therefore, as the children were through college, I quit my job, Nick took a sabbatical, and in 1986, we went to the Station Zoologique in Villefranche-sur-mer, France. I wanted to return to developmental biology and study genes and development in sea urchins. Before we left for France, because Nick was out of town, I rang up an old friend, George Somero, who was on the SIO faculty, and invited him to a movie. I can't recall the film, but by its end, I had learned all about his new girl-friend (now his wife for 20 years) and convinced him to hire me 3/4 time to manage his fish biochemistry lab. That way I could have the other 3/4 time for my own research. At the Station Zoologique, I was told, surprise, that I couldn't do molecular biology as there was neither a room nor any money for it. However, at the suggestion of one of the developmental biologists, I went through the invisible iron curtain between the developmental and plankton biology groups and arranged with Gaby Gorsky, a physiologist, who had the appendicularian tunicate, *Oikopleura dioica*, in culture, to look at its sperm-egg interaction. Thanks to Gaby and Christian Sardet and the developmental biology group, I showed that, like other marine deuterostomes, appendicularians have sperm with an acrosome and a typical acrosome reaction, while the eggs of at least *O. dioica* have cortical granules that undergo exocytosis at fertilization. Therefore, contrary to widely accepted dogma, appendicularians were probably basal in the tunicates as they had retained these features, which ascidians and most thaliaceans had lost.

Near the end of that year, Nick suggested moving on to amphioxus. We went back to Naples, but in 2 h of dredging, we only got six. Returning to La Jolla, I was given another big break. Adelaide T.C. Carpenter, in the UCSD biology department, gave me free use of her electron microscope. Thanks to Adelaide, I expanded my study of gamete morphology and fertilization in tunicates and extended it to amphioxus.

The summer of 1988 was a milestone for amphioxus research. Because populations in Italy had declined and the cultural revolution in China had interrupted university research, studies of amphioxus development had languished. A friend from Stanford, John Lawrence, who was on the faculty of the University of South Florida in Tampa, Florida, alerted us to amphioxus in Old Tampa Bay. I brought all the chemicals known to induce meiotic maturation in marine invertebrates, and Nick brought an old neurophysiology stimulator for another project. None of the chemicals worked but on our last day in Tampa, Nick, remembering an old report that electrical stimulation caused sea urchins to spawn, shocked the last female. She jumped. Five minutes later I looked in the dish. It was full of eggs. We squeezed a drop of sperm from a male and fertilized the eggs. It was the first time that controlled fertilization of amphioxus had been achieved in the laboratory.

During the next 2 years, I focused on tunicates and on fertilization in amphioxus. I also began to look at gene expression in amphioxus, starting with antibodies raised against *engrailed* in other species. While working on fish enzymology, I learned molecular biology, which became very useful when a Scientific American article by Eddy De Robertis and colleagues. appeared in October 1991 with a figure showing the collinear expression of *Hox* genes in the mouse CNS and in *Drosophila*. Nick came into the lab waving it, saying "Let's look at *Hox* genes in amphioxus." Because the *Hox* genes have nested expression in the vertebrate hindbrain and spinal cord, we could use their expression to ask if amphioxus has a homolog of the vertebrate brain and if so, how much. However, all of our NSF proposals were turned down on the grounds that we had no published track record in molecular biology. Finally, in 1991, Thurston Lacalli from the University of Saskatchewan, who was mapping the neurons in the amphioxus central nervous system, told us about a young lecturer at Oxford, Peter Holland, who was cloning bits of *Hox* genes out of the European amphioxus but had no embryos. I sent Peter DNA of the Florida amphioxus and, thanks to a US$2,000 grant from the American Philosophical Society, went to his lab in March, 1992 with a box full of fixed amphioxus embryos. I was 50, Peter was 28, yet we were on the same wavelength. He had done the first in situ hybridization for the mouse, beating out the competition by 6 months. He tried tissue-section in situs with radioactive probes while I tried whole mount in situs. I first tried one recipe—the embryos were all purple. I tried another—they were all white, finally, after 3 weeks of mixing the best bits from recipes for flies and mouse, I obtained a weak signal for *Hox*3. Peter and I looked at each other. We said nothing. I repeated the labeling. The in situs were better. We smiled. Our careers were both made that day. Peter is now the Linacre Professor of Zoology at Oxford University, UK. He wrote the article and it was published in *Development* in November 1992. Not long after, Judy Plesset, the program manager at NSF, called Nick to tell him that our grant proposal was turned down again. We needed to do better in situs. To which Nick said, "If they are no good, why is one on the cover of *Development* for November" Judy Plesset said, "Hmmm. I am coming to San Diego in January. I'll stop by." She did. I had just gotten off a scientific cruise for tunicates and had developed Bell's palsy. Half my face was paralyzed. Almost the first thing she said to me was "If amphioxus is so exciting, the guys with the big labs will just mop the floor with you." I replied, "No, they won't. We have a corner on the market on the embryos." That was it. We were finally funded.

During this time, George Somero and his wife both took positions elsewhere. Times were changing. He kept me on to supervise his remaining students, which allowed me to keep a small lab and an office. I decided it was time to move upward. I asked the division chair for an appointment to specialist, a semi-independent, soft-money academic position that is nominally under a faculty member, but allows submitting grant proposals with permission. Initially, the chair was noncommittal, but a few weeks later at a party put his arm around my shoulders and said "I think it's time for that promotion." Letters were requested, but one faculty member shouted "Nepotism!!!" More letters were obtained. Two weeks before the appointment went through, I asked the department manager for my own email account. "You can't have email," she said. You're only a technician. Besides, we're going to throw you out of your office and lab very soon." "Oh," I said. "I'm about to have an academic position. Besides, where am I supposed to go." She replied "You can have a corner of your husband's lab." "Have you asked him?" I said. No reply. I thought "We'll see about that." When the billows of smoke cleared, I was appointed specialist, had my office, little lab, and email. That was the last slap in the face I experienced.

In 1998, having obtained a woman's grant (POWRE) from NSF and written some articles, I asked to move to the research series—an academic series that parallels the faculty series, for which a PhD or equivalent is required. It has no teaching requirement but is all soft money at the assistant level, 25% soft money at the associate level and 50% soft money at the full level. Nearly half the "faculty" of SIO are in the research series. The new division Chair, Bob Hessler, said, "I think that's a good idea, but don't expect any start-up funds. The level that is equal to your present salary is full-researcher step I." As I didn't have a PhD, I was prepared for trouble. There was none. Attitudes toward women had changed. The appointment came through at one level higher than requested. With a half salary from SIO and some grant money, for the first time since coming to SIO, I could earn a full salary. The next surprise was that the chair of the appointment committee decided I really should have a PhD. He went about seeing how many rules could be bent. At one point, the head of the UCSD graduate office called me to ask "Did you have at least a 3.0 as an undergraduate?" "I did," I said. "Where did you get your degree?" "Stanford," I said. It was one of the few times that a Stanford degree mattered. I paid 2 years of half-time tuition, turned four articles I wrote during those 2 years into a dissertation, and the colleagues on my PhD committee, all but one of whom was younger than I, enjoyed themselves giving me a hard time. "Well, your work doesn't seem to be falsifying hypotheses . . . ." I was 60 when I obtained a PhD. My first de facto graduate student had already obtained his degree. Ironically, I am now at the level I would have been at had I had a doctrinaire career and started as an assistant researcher in 1966.

The last 10 years have been especially exciting for amphioxus and evo-devo. Jeremy Gibson-Brown and I wrote the white paper to NIH for sequencing the amphioxus genome. Although NIH gave it only moderate priority, Dan Rokhsar at the Joint Genome Institute (JGI) in Walnut Creek, CA asked if he could resubmit the white paper to JGI. I also had an NSF grant to make BAC libraries, which I subcontracted to Pieter de Jong at Children's Hospital, Oakland, CA. Nori Satoh in Kyoto, Japan had money for a major EST project. I supplied the amphioxus RNA and a graduate student, J.-K. (Sky) Yu, to make the libraries. Yuji Kohara and Asao Fujiyama at the National Institute of Genetics in Mishima, Japan sequenced 160,000 EST clones and the ends of 20,000 BACS. JGI, thinking the amphioxus genome was bigger than it is, did 11-fold coverage of the genome and sequenced another 60,000 EST clones. With all these resources, Nick Putnam, a postdoc at JGI, was able to assemble both alleles separately. We published two genome papers. My lab developed techniques for gene knock-down and overexpression in amphioxus. All that is lacking is having amphioxus in continuous breeding culture, which would allow genetic experiments. This should be possible because the Florida amphioxus and other warm water species breed every 1–2 weeks in summer and can develop to sexual maturity in about 6 weeks. Several laboratories in the world are working toward this goal, and it seems likely it will be achieved in the next few years.

It has been very rewarding to help bring amphioxus from relative obscurity into the light. A search of the key words Branchiostoma and amphioxus in The Web of Science for 1986, lists four and six publications, respectively. For 2008, the numbers are 64 and 105, respectively. It is also heartening that today about 50% of the graduate students and 20–25% of the faculty and researchers at SIO are women. There is a ways to go, but women are getting there. When Rie Kusakabe, who is now a researcher at Kobe University in Japan, is married and has three children, told me when she was a postdoc that I was her role model, I was more than pleased to have survived in science and helped set some precedents.