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Interview

A conversation with Craig C Mello on the discovery of RNAi

CC Mello[1]

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While we would like to congratulate on behalf of CDD and our readers for the fantastic achievements that have revolutionized our field, a change in paradigm witnessed by the greatest scientific honour, thanks to the Nobel Foundation (Copyright© The Nobel Foundation 2006), we are pleased to share Dr. Mello's autobiographic thoughts.

I recall a sunny September morning in Virginia. I remember the sound of the school bus taking away the older kids, including my two siblings Jean and Frank. My mother, no doubt, was busy with my baby brother Roger. I was playing in the creek as I often did, turning over stones, looking for small animals. I remember a mourning dove cooing on the telephone wire, and the way the sunlight felt on my red sweatshirt and my rolled up hand-me-down blue jeans. I remember a sense of contentment with being alive, a feeling that infuses many of my early memories in a general, fuzzy, unfocused way. However, this memory is different. It was etched with stunning clarity in my mind by adrenalin and other sharper emotions. That morning, a box turtle decided to choose this peaceful moment to make its way across the street adjacent to the field where I was playing. My attention was drawn to the road by the sound of an oncoming car, and I remember my excitement with seeing the turtle changing to shock as I watched the car swerve with clear intention toward the turtle. I remember a smirking teenage boy driving off, leaving the turtle, his shell broken, still struggling to move to the edge of the road. The turtle died before my eyes, etching this scene deeply into my mind. Even though this might seem a sad memory, the fact is that I am grateful in a sense. That morning of my youth seems timeless now. I can see in my heart that the child playing in the creek is me, and that I haven't changed much really in the intervening years. I'm still turning over stones, hoping to find something new. I'm still struggling to understand what drives us humans to cruelty and hoping that knowledge of our place in the world can help us to achieve a higher purpose.

I was born in New Haven, CT, on 18 October 1960, the third child of a paleontologist father and an artist mother (James and Sally Mello). In 1962, my father completed his doctorate in paleontology at Yale University, and my family moved to Falls Church in Northern Virginia so that he could take a position with the US Geological Survey (USGS) in Washington, DC. My parents met while attending Brown University and were the first children in their respective families to attend college. My grandparents on both sides withdrew from school as teenagers to work for their families. My paternal grandfather, Frank Mello, was of Azorean descent although he was born in Warren, RI. He was an outstanding athlete nicknamed 'Bullet' Mello for his speed. He played semipro baseball and football. He worked a variety of jobs, including delivering grain for many years and operating trucks for the town. My grandmother, Elena (Primiano) Mello, was of Italian descent, but was also born in Warren, RI, and she worked in the local textile factories. They both worked for their families for close to ten years before they were able to marry and start their own household at the age of 24. On my mother's side I have English and Scottish roots dating to colonial times and including a distant link to Lyman Hall, who signed the declaration of

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independence. My maternal grandfather, William Cameron, ran a very successful plumbing business in Middletown, CT. My grandmother, Ida (Hall) Cameron, was a homemaker. I'm proud of my melting pot origins and of the accomplishments of my grandparents. They worked hard and sacrificed so that their children could go to college. They were wonderful, creative, thoughtful, and extremely loving people who gave me a refreshing perspective on what's important in life.

After a brief stay in Falls Church, we moved to Fairfax, VA, when my father switched from the USGS to a position as assistant director at the Smithsonian Museum of Natural History. Among my fondest early memories are field trips with my father and the whole family to Colorado and Wyoming and more frequent trips to the Blue Ridge Mountains in Virginia. I remember searching for fossils, hiking, exploring, and wonderful family discussions around the campfire.

My family had a very strong tradition of discussions around the dinner table. This experience was extremely important to me. I learned to argue, to listen, and to admit it (sometimes grudgingly) when I was wrong about something. These were often lively discussions, and my parents did a great job of allowing each of us to be heard. At a time when I was not performing so well in school, these daily discussions helped to build my confidence and self-esteem. I struggled during the first few years of grade school. I started first grade at the age of five in a local private school because I was too young to enter first grade in the public system. I don't know if I was a slow learner, or just not interested, but I did not do well in school until the seventh grade. In second grade, I remember faking that I could read and the embarrassment of being called on in class. I much preferred playing outdoors, in the woods and creeks, to time spent in the classroom. Meanwhile, my older siblings were model students, raising the teacher's expectations for me. If not for the family discussions, where I was respected and could hold my own in arguments, I might have been discouraged with my academic prospects.

During these early years, I remember having no doubt that I would be a scientist when I grew up. I was amazed that so few adults (including my teachers) understood basic concepts such as deep (geologic) time, the vastness of the universe, and the common evolutionary origins of life. In first grade, the private school I attended had a Bible session each day, and I remember being shocked that the teacher presented the story of Noah and his ark as fact. Similarly, I was exposed to religious instruction in Sunday school. My father had agreed to raise us as Catholics. My mother was a Methodist by birth but did not practice her religion and did not attend Catholic services with the family. I remember learning the argument of intelligent design in Sunday school, as a counterargument to evolution. Given my own exposure to my dad's museum, and our family discussions about evolution and the history of the earth, these exposures to religious dogma actually had the effect of intensifying my interest in science as a way of knowing about the world.

By the time I was in middle school, I had decided to reject religious dogma altogether. The 'absolute knowledge' offered was, in my view, inadequate to explain the world around me. Furthermore, it seemed wrong to claim knowledge based on one's culture or upbringing. I saw the leap of faith involved in religion as smothering dialogue, closing the door on non-believers and walling them out of one's society. In contrast, the scientific method with its focus on asking questions and admitting no absolutes, was and continues to be refreshing to me. Science is grounded on, and values, dialogue. It is a human enterprise that breaks down walls and challenges its practitioners to admit ignorance and to question all ideas. However, we must all arrive at and defend our moral choices of right and wrong. Science can't touch these issues and shouldn't try. I believe that there is no more spiritual and worthwhile undertaking than that of trying to understand the world around us, and our place in it. The world is a far more remarkable place than we can imagine. Its mysteries define the human condition: to exist without knowing why. My first exposure to academic science came in seventh grade, and during that year I can remember for the first time applying myself to my studies. I became an avid reader of science fiction, an amateur astronomer, and a serious student. I remember organizing my desk at home and doing homework, with music blasting, for at least a couple of hours every night. I attended Fairfax High School, where I took all of the science courses offered except advanced physics. My earth science, chemistry, and biology teachers were excellent. My biology teacher, Randy Scott, was also my wrestling, football, and track coach. He was a wonderful man, who had a large role in fostering my interest in biology. I reconnected with him and was able to thank him after the news of October, but tragically, he has since lost his battle with cancer.

In 1978, I learned about molecular biology from a newspaper article in the Washington Post. The article described the cloning of the human insulin gene in bacteria, and described how the bacterial cells were able to read the human genetic code and produce functional human insulin. I found this concept incredible and extremely exciting. Incredible because the bacterial cells were able to speak the same language as the human cells, reading out the genetic code to make functional, life-giving, human protein for diabetic patients. Prior to that time, diabetics used animal insulin. I found this extremely exciting because I could see the potential for understanding disease at the genetic level and for treating it with molecular medicines, like insulin, and with gene therapy.

At Brown University, I pursued biochemistry and molecular biology as my major and had inspiring teachers, including Frank Rothman, Ken Miller, Susan Gerbi, and Nelson Fausto. Brown provided a wonderful environment for learning, and had the added benefit of being close to my grandparents' home in Warren, RI, and to my small sailboat on the Warren River, a tributary to the upper Narragansett Bay. Sailing continues to be an important part of my life. It gives me a sense of place that settles and refreshes my mind. I sail in a wide range of conditions, for hours and hours (if possible). I don't prefer to race, but rather to explore. I gradually trained my parents and grandparents to accept the fact that if the wind died, then I might not get back until long after dark. Eventually, they even agreed to my taking camping gear and doing overnight trips ranging along the coast from Martha's Vineyard to half way up Long Island Sound.

After Brown, I went to Colorado for graduate school, where I enjoyed the mountains again and a really fantastic and inspiring course in molecular, cellular and developmental biology. The course consisted of a small group of 15 or so

students with outstanding instructors, including Drs. Dick McIntosh, Mike Yarus, Larry Gold, Bill Wood and others. At Boulder, I was introduced to *Caenorhabditis elegans* in the laboratory of Dr. David Hirsh. David's lab was fantastic – filled with people who would prove to be really important in my future training. These included Dan Stinchcomb, who introduced me to the practice of molecular biology; Mike Krause, Jim Kramer, and Ken Kemphues, with whom I collaborated; and Jim Priess with whom I did my postdoctoral work. When I joined David's lab in 1982, no one had succeeded in introducing DNA back into *C. elegans* (a method referred to as 'DNA transformation'). Work in yeast had identified functional DNA elements that direct replication and partitioning of chromosomes (replication origins and centromeres, respectively). Working with Dan Stinchcomb, my project was to identify such elements from the worm, with the goals of (1) understanding these essential functional chromosomal elements, and (2) using them to produce stable artificial chromosomes for worm molecular genetics. During my first year in Boulder, David Hirsh decided to take a position in industry, and so I chose to move to Harvard University where I could continue my research with Dan Stinchcomb, who was starting up an independent lab there.

I thoroughly enjoyed Harvard! Dan set up his lab at the Biolabs in Cambridge next to Victor Ambros, another brand new, junior faculty member at Harward working on *C. elegans*. Dan and Victor integrated their labs to make a single 'wormlab', and both served as advisors to me during my studies. I loved my project and worked long hours in the lab, never going home until I had a gel running or something incubating, so as to use the overnight hours. I took advantage of opportunities to attend lectures on a wide range of subjects. I obtained permission to use the large refracting telescope located atop the Science center, which was, surprisingly, available for individual use. I got to meet and teach with Stephen J Gould, whose essays on natural history and the philosophy of science had inspired me over the years. Gould's, '*The Freezing of Noah*,' is one of my favorites as it captures the essence of good science: admitting when your theory is wrong and developing a new theory.

I learned an important lesson in graduate school, that it's not enough to be persistent and to work hard, it's also important to attack the question you wish to address from every conceivable angle. By focusing on identifying worm centromere activities using yeast as a model system, I ended up learning about the yeast centromere, not the worm centromere. While this project was fulfilling and interesting to me, it was flawed. To study the yeast centromere, I should have been working with the yeast sequences directly. To study the worm centromere, I should have been injecting DNA into the worm. Only after I began to experiment directly with the worm did my project really take off.

Technology is what drives science, and yet, developing new technology is often a thankless task. Getting something to work that has never been done before can be exceedingly frustrating because you may never know how close you were to success, and failures quite often teach you nothing. Partly because of this, those working on technology development often tend to band together and share ideas more than would otherwise be common among scientists. This was certainly the case for Andrew Fire and me. We were both working on developing techniques for DNA transformation in worms. Andy had some early success and developed a number of clever methods. I followed up with some improvements. And together we made DNA transformation a routine procedure for the worm. In the course of these studies, we became frequent correspondents, spending hours on the phone (before email was invented). We developed the mutual trust and respect that ultimately led to our collaboration on RNAi.

After graduating from Harvard, I joined the lab of Jim Priess at the Fred Hutchinson Cancer Research Center in Seattle Washington. Jim is one of those rare scientists who has 'a feeling for the organism' as EF Keller put it when describing Barbara McClintock. Jim put me in touch with my own feelings for the worm. Through Jim, I was able to learn genetics, without which our later work on RNAi would have remained entirely descriptive. In Jim's lab, we identified genes that act as regulators of early development in *C. elegans*. It turns out that some of these genes are connected to RNAi-related mechanisms in ways that we are still trying to understand.

In Seattle, my daughter Melissa was born in 1992. I wish she could remember those first two years of her life in Seattle. We hiked and biked together regularly and had a wonderful time. However, her mother and I struggled to find enough time together as a family. Melissa's mother, Margaret Hunter, worked mornings and weekends as a chef at a Café in Seattle. Because my schedule often demanded late nights and weekends, we handed Melissa off from one to the other and rarely had enough time to be together as a family. Shortly after we moved to Massachusetts in 1994, we separated and divorced. Fortunately, we remain respectful and friendly to this day. I focused on my work and continued to have Melissa with me half of each week.

Shortly before I started my lab in 1994, I learned from Ken Kemphues and his student Sue Guo about an 'antisense' RNA injection technique that surprisingly well in *C. elegans* silenced target genes. I began using this method to study the genes we had identified during my genetic studies with Jim Priess. The genome-sequencing project for *C. elegans* had begun in earnest and had revealed dozens of genes in the sequence data base that were similar in DNA sequence to those that I had discovered in Jim's lab. These related genes (or homologs, as we call them) could have important developmental functions, and so I began using the RNA injection method described by Guo and Kemphues to silence them in order to identify those functions.

At that time, RNA injection was performed according to the same procedure that Andy and I had developed for DNA injection. A fine, sharp, glass needle was inserted with care through the cuticle of the worm and positioned inside the large shared cytoplasm of a gonad that contains hundreds of germline nuclei. After positioning the needle and injecting, the procedure was then carried out a second time on the other gonad arm, two injections per worm. The power of this gene-silencing approach accelerated our studies and we began to make rapid progress in understanding the developmental mechanisms that specify cell fate in the early embryo. However, we also became interested in the silencing phenomenon itself. The first observation that truly galvanized my interest occurred when, having injected RNA targeting

apx-1, a gene essential for embryogenesis, I observed by chance that some embryos hatched and matured to adulthood only to produce 100% *apx-1* dead embryos. The silencing phenomenon had skipped a generation and had been passed on *via* the germ line to the next generation! This was truly amazing and prompted further studies that demonstrated the transmission of silencing for multiple generations via both the sperm and the egg.

The first graduate student to work on RNAi in my lab, Sam Driver, discovered, in part by accident while learning to inject, that the RNA need not be delivered directly to the germ line. Injection anywhere in the body was sufficient to induce interference that spread into the germ line and was transmitted to progeny. These findings, along with the inheritance properties, and the lack of strand specificity (first noted by Guo and Kemphues) prompted us to recognize the silencing phenomenon as an active response in the organism to the RNA. To distinguish this mechanism from the earlier 'antisense' methodology, we decided to give it the simple name RNAi (for RNA interference). We envisioned a mechanism where either strand could template the production of the other strand and could somehow build up silencing RNA levels. The specificity of the silencing indicated that ultimately, after amplification, the antisense strand must unwind from its complement to find its target RNA and induce silencing.

Throughout this period, Andy and I continued to correspond and collaborate. It was Andy's suggestion that dsRNA contaminating our preparations could be the actual trigger molecule underlying RNAi. At the time, I was still thinking of dsRNA as an amplification intermediate, rather than trigger. It was not until after Andy sent me purified dsRNA to test in my own hands that I became a believer in this molecule as a potent trigger for gene silencing. We now know that dsRNA is both a trigger and intermediate in RNAi. The concept of dsRNA as a trigger for sequence-specific gene silencing only makes sense if one recognizes that the organism is actively responding by unwinding the RNA strands both for amplification and to generate single strands capable of base pairing with targets. This concept of an active response in the animal prompted Hiroaki Tabara in my lab to undertake his exciting genetic studies that identified cellular gene products that mediate silencing. As discussed in my lecture, dsRNA is not the only trigger for this silencing mechanism. However, importantly, dsRNA turned out to be a highly conserved trigger that rapidly led to the application of RNAi in diverse species including humans.

1998 was a truly outstanding year. In January of that year, Andy and I published our paper on RNAi. In August, I married Edit Kiss and became the stepfather of two wonderful kids, David and Sarah Apotheker. In the year 2000, our daughter Victoria was born. In an unfortunate twist of fate, Victoria developed type I diabetes in the fall of 2001. Suddenly, I had to learn how to inject into a human, my own daughter, for the first time. Ironically, human insulin, the same bacterially synthesized molecule that inspired me to pursue molecular biology, is now giving Victoria her very life. This experience has given me a new perspective on the importance of medical research. Edit, who is a wonderful nurse, is now taking care of Victoria, and serving as a diabetes counselor for newly diagnosed families.

With RNAi and the completion of the genome sequences for humans and numerous other organisms, we now have unprecedented opportunities to develop new, life-saving therapies and to advance the basic understanding of our biology. We humans have a potentially very bright future. The biological mechanisms at work inside our cells are truly ancient and remarkably stable, more stable even than the positions of continents and oceans on the face of the Earth. However, in my view, our thriving global economy has engendered serious problems. Climate change and other forces beyond our control could easily disrupt our economies causing widespread human suffering at unprecedented levels. We are fishing out oceans, depleting our topsoils, and exhausting our sources of fossil fuel and fresh water. Scientists and policy makers must begin to work together to foster the development of technologies that are sustainable and resilient. As humans, we must work with common purpose around the world to prepare for the challenges and opportunities ahead. I hope that I can further that cause.

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