Q & A

Xuemei Chen

Xuemei Chen grew up in the northeastern city of Harbin in China and received her BS degree in Biology from Peking University in Beijing. She came to the USA in 1989 to pursue her PhD at Cornell University. Under the supervision of David Stern at the Boyce Thompson Institute, she used molecular genetic approaches available at the time to study chloroplast gene expression in the unicellular green alga Chlamydomonas reinhardtii. *It was during this time that she began to appreciate the extensive, posttranscriptional mechanisms that impact chloroplast gene expression. After obtaining her PhD in 1995, she moved to the California Institute of Technology to study the molecular mechanisms of floral patterning in* Arabidopsis thaliana *in the lab of Elliot Meyerowitz. It was an exciting time, as key factors and pathways underlying floral cell fates were being discovered in the field. In 1999 she started her own research group at the Waksman Institute at Rutgers University. She continued to study floral patterning, and the genetic screens conducted in her lab found genes that act on RNA, and this then led to the discovery of microRNAs. She then turned her attention to microRNAs and other small RNAs in subsequent years. In 2005 she moved to the University of California, Riverside, where she is currently Distinguished Professor. She was elected to the National Academy of Sciences in 2013. Her lab is currently studying small RNAs and other posttranscriptional mechanisms.*

What turned you on to biology in the first place? I think that my upbringing led me to biology. I was born at the beginning of the ten-year Cultural Revolution, which thrusted everyone into social turmoil and sent my father — a professor — to labor camps. I, however, had a carefree childhood living with my grandmother in the countryside with lots of nature: eggplants, green peppers, and sunflowers in the front and back yards; huge gourds from our neighbor, the shells of which we used as kitchen utensils; herds of cattle that sometimes walked in front of our house, causing ground vibrations. I roamed the riverbeds and fields with other kids, catching butterflies and dragonflies. We dug to find earthworms, picked caterpillars off eggplants, and collected ladybugs. My favorite 'books' were my father's collection of glossy prints of a type of Chinese painting that meticulously depicts flowers and bugs. These prints were sent to the countryside for safe keeping, only to be destroyed by my scribbles, which my doting grandmother thought were creative! I returned to the city to go to school, in retrospect with an appreciation for nature, which eventually led to an interest in biology.

As to how I got into plant biology, I think that this also had to do with my fascination with plants as a child. Harbin's winters were long and harsh. After months of winter, signs of spring — tiny weed seedlings popping out of the ground and yellow buds emerging from willow branches — brought so much joy. I thought that plants were fascinating as they use sunlight as their energy source and wondered if I could make people photosynthesize if I studied plants when I grew up. An incident in high school gave me a bit of a nudge toward plant biology as well. I was the biology teacher's assistant and had to dissect salivary glands from *Drosophila* larvae to make slides for the class. The nauseating experience told me that I could not work with animals.

Do you have a scientific hero? Madame Curie was the scientific heroine of my childhood. I was deeply struck by her dedication to and perseverance in science. However, as I entered a scientific career myself, I looked up to people who were more 'accessible' to me for inspiration, guidance, and support along the way. My PhD advisor David Stern and my postdoc advisor Elliot Meyerowitz were most instrumental in the early stages of my career. They provided intellectually stimulating yet friendly laboratory environments and struck a balance between offering guidance and fostering independence. While at Cornell, the Yeast Genetics course taught by Tom Fox and the Chromatin (I forget the exact name) course taught by John Lis struck a chord with me and made me realize the powers of molecular genetics in understanding life processes. Another person who influenced me was Bik Tye at Cornell. She was one of the few female faculty members in the Biochemistry Department at the time and she was originally from Hong Kong. Being a shy graduate student, I did not interact with her much, but she unknowingly served as an inspiration and a role model, simply by being an Asian female faculty member. Twenty some years later, I was invited to give a talk at the Chinese University of Hong Kong and was totally and pleasantly surprised when Bik walked into the auditorium before the seminar! She

was then a visiting professor at the Hong Kong University of Science and Technology and had learned about my seminar. I finally got a chance to let her know that she had been an inspiration to me! Two other women scientists, Ruth Steward at Rutgers University and Natasha Raikhel at the University of California, Riverside, were great mentors to me during my assistant and associate professor years.

If I have to name only one person as a scientific hero in my life though, this person would be the late Ray Wu, who was a faculty member at Cornell University. Wu was a pioneer in DNA sequencing: he published his first paper on the topic in 1968 and devised the primer extension principle with which a sequence of eight nucleotides in lambda DNA was determined in 1970, a few years before the Nobel prize-winning DNA sequencing methods from Maxam and Gilbert and from Sanger were published. Wu later worked on improving agronomical traits of rice with the noble intention of bettering the lives of people in the developing world. I admire his scientific achievements and his foresight, courage, and determination to switch to working on rice at a time when this organism was not so amenable to molecular genetic studies. More importantly, I am grateful that Wu provided a path to science for me and other young and eager minds by organizing the CUSBEA (China US Biology Examination and Admission) program, through which hundreds of Chinese students came to the USA to pursue PhDs in biology in the 1980s. Imagine hundreds of these original trainees pushing the scientific frontier, generating cures for diseases, improving crops, and more importantly mentoring their trainees. What a legacy!

Do you have a favorite paper? Yes, two actually. One is the 1993 paper from Victor Ambros's lab showing that the *Caenorhabditis elegans lin-4* gene encodes a small RNA, a microRNA as it would be called eight years later (Cell (1993) *75*, 843–854). They started with *lin-4* mutants with defects in making developmental transitions and got to the gene through map-based cloning. They narrowed the gene down to a small region without any open reading frames. I thought that it was very clever of them to have compared the sequences of *Caenorhabditis briggsae* and *C. elegans* in this region and identified a short region of sequence identity. This, together with the positions of the mutations in the *lin-4* mutants, perhaps led to the hypothesis that the gene product was a small RNA, and this was confirmed by RNA gel blot analysis. I was impressed by the identification of the small RNA as well as the hairpin precursor, the discovery of sequence complementarity between the *lin-4* small RNA and the 3'UTR of its target gene, and the demonstration that target regulation occurred through the 3'UTR. Thus, major concepts of microRNA biogenesis and regulatory function were laid out in the study.

The other one is the 1999 paper by Hamilton and Baulcombe showing for the first time that small interfering RNAs (siRNAs) were associated with foreign sequences (transgenes and viruses) that underwent posttranscriptional gene silencing (PTGS) in plants (Science (1999) *286*, 950–952). Many PTGS phenomena had been described in plants during the past 10 years by then, but the underlying mechanism of the sequence-dependent gene silencing at the RNA level was unknown. In 1998 RNA interference was found to be triggered by long double-stranded RNA in *C. elegans* and it came to be realized that PTGS in plants and RNAi in *C. elegans* were related phenomena. The discovery of siRNAs reported in the Hamilton and Baulcombe paper ushered in this central player in PTGS and RNAi.

What's your favorite experiment? My favorite experiment was one that was designed to test a far-fetched hypothesis and proved it correct. When I set up my lab at Rutgers University in January 1999, we set out to perform a genetic screen in *Arabidopsis* to isolate floral homeotic mutations: ones that would change one floral organ type to another. These efforts were an extension of my postdoctoral studies and aimed at understanding the molecular mechanisms that govern cell fate specification in floral patterning. The genetic screen was performed with an *Arabidopsis* strain with two genes (*HUA1* and *HUA2*, which I had discovered during my postdoctoral work) mutated in order to circumvent genetic redundancy. Thus, the isolated mutations were called *hua enhancer* (*hen*) as they enhanced the phenotypes of the *hua1 hua2* mutant and caused the male reproductive organs in flowers to be replaced by petals. *HEN1* was the first gene that we mapped and cloned (in 2000). While *HEN1* was clearly important in floral patterning as reflected by the mutant phenotypes, its function at the molecular level was unclear.

Several clues led to the hypothesis that *HEN1* acts in microRNA biogenesis. microRNAs? Yeah, I know. They would not be discovered in plants until 2002. This is why the hypothesis was far-fetched in late 2000/early 2001. One clue was that the phenotypes of *hen1* mutants were strikingly similar to those of another mutant named *carpel factory* (*caf*) at the time. The *caf* mutant was studied by Steve Jacobsen when we were both postdocs in Elliot Meyerowitz's lab. Our benches were next to each other and I was very familiar with the phenotypes of the *caf* mutant. The second clue was that the *CAF* gene is a homolog of animal Dicer and was to be renamed *DICER-LIKE1* (*DCL1*) a few years later. The third clue was that *C. elegans* Dicer was shown to be required for the biogenesis of *lin-4* and *let-7* (the first two microRNAs to have been discovered) in the year 2000 and then microRNAs were found to be widespread in animals in 2001. These clues led to the aforementioned bold hypothesis, which we set out to test by first cloning microRNAs from plants and then examining their abundance in *hen1* and *dcl1* mutants. When my postdoc came to me with an X-ray film, still dripping water, we knew that the hypothesis was actually right. The RNA gel blot showed a strong microRNA signal in wild type and nearly no signals in the *hen1* or *dcl1* mutants.

That experiment marked a turning point in my career. My PhD experience had long cultivated an appreciation for posttranscriptional processes in gene expression regulation. The discovery of plant microRNAs and HEN1 — a protein of previously unknown function — as a microRNA biogenesis factor prompted us to move into the then new and exciting field of small RNAs. Of course, I could not have switched from studying flower development to investigating small RNAs all of a sudden, as students and postdocs were already well into

their studies of various other *HEN* genes. But, interestingly, most of the other *HEN* genes (*HEN2*, *HEN4*, *HEN5*, *HEN7*) turned out to be factors in RNA metabolism, and this reinforced the conviction that posttranscriptional processes impact many life processes. In addition, the first microRNA that we discovered, miR172, was found to have sequence complementarity to a master regulator of floral patterning, *APETALA2* (*AP2*). We went on to show that miR172-mediated regulation of *AP2* is crucial in floral patterning — while a wild-type flower has six stamens (the male reproductive organs), flowers expressing miR172-resistant *AP2* have a 'superman' phenotype with tens or even hundreds of stamens! During this transitional period we revealed the molecular function of HEN1. It is a methyltransferase that deposits a 2'-*O*-methyl group onto the 3'-most ribose in microRNAs and siRNAs.

Which historical scientist would you like to meet and what would you ask them? I wish that I could meet Elizabeth Betty Keller again. When I did a rotation in her lab in 1989 to 1990 she was perhaps in her late 70s or early 80s, and her research interests had shifted to transcriptional regulation by TATA and TATA-less promoters. I only vaguely knew at the time that she had studied protein synthesis previously. I recall that she once mentioned the days when the genetic code was being cracked and the excitement she had experienced at a meeting in Moscow when a talk on this was given. I was not bold enough to pester her for a historical account of the exciting years of investigation into protein synthesis. It was not until two decades later when my research on microRNAs had led me to read up on translation on the endoplasmic reticulum that I ran into her paper from 1954 on the role of microsomes in protein synthesis. She showed in this paper that, among different cellular fractions from the liver, microsomes gave the highest level of protein synthesis. In fact, in the mid-1950s she, M.B. Hoagland, and P.C. Zamecnik published a series of papers establishing an *in vitro* translation system and characterizing various aspects of translation. All this was prior to — and perhaps set the stage for — the discovery of tRNA and mRNA. Betty later went on to characterize the initiator $tRNA^{Met}$ using the wheat germ *in vitro* translation system. She also studied polyA polymerases in chloroplasts and tRNA modifications, among a broad swath of research topics on which she worked. I wish that I had asked her what it was like in the early days of studying protein synthesis and particularly how she navigated the overwhelmingly male research community.

Do you feel a push toward more applied science? In plant biology, as funding steers research toward crop species, it is increasingly difficult to study the model species *Arabidopsis thaliana*, with which most molecular frameworks underpinning plant life were established. Although many plant species are becoming increasingly amenable to genetic manipulations and thus easier to study, thanks to advances in technologies such as transformation and genome editing, it is still essential to have a few model species in which the plant community invests to accumulate a knowledge base and a resource collection, so that these species can be used to efficiently uncover universal principles and mechanisms. I wish that research funding were question-based rather than organism-based and a balance could be struck between funding efforts to understand various life processes and supporting those to solve imminent societal issues. It is clearly crucial that efforts are directed toward applied science to stop the COVID-19 pandemic, cure cancer, control the citrus greening disease, treat sudden oak death, and so on. But our ability to solve these issues relies on knowledge and technology, which are often gained through discoveries aimed at answering basic questions. RNAi and CRISPR-based genome editing, technologies that have far-reaching potentials in agriculture and medicine, are two examples of basic research that have led to technological leaps.

In my research group, our initial efforts to understand floral patterning could hardly be deemed important from a practical point of view. Yet, the initial efforts led us to discover microRNAs from plants, and plant microRNAs would turn out to be key regulators in so many biological processes from development, growth, and stress responses to immunity. Although my lab did not study microRNAs in crop species, others including my former students and postdocs have elucidated effects of various microRNAs in numerous agriculturally important species, such as rice, maize, cotton, soybean, and so on. I expect that plant microRNAs will be targets of manipulations for the engineering of better traits in agriculture. In terms of research in my group moving forward, we will continue working on key questions that we believe to be of fundamental importance in plant biology and that we are in a position to address because of our expertise in RNA biology, while being mindful about the potential applications of our discoveries.

If you had to choose a different field of biology, what would it be? My research interests have actually shifted quite a bit in the past 30 years, from gene expression in plastids, to mechanisms of floral patterning, to small RNA biogenesis and function. Moving forward, we have again adjusted our research directions. We are intrigued by the fact that key cellular metabolites, such as NAD^+, UDP-glucose, and UDP-GlcNAc, can serve as the cap of messenger RNAs. This implies to us that metabolism and RNA processes communicate and regulate each other. We plan to study how these metabolite caps influence gene expression. We are also 'revisiting' plastids, hoping to use the newest technologies to better understand these organelles that are essential to not only plant life but also nearly all life on Earth.

If I had the time and resources to choose a different field of biology, I might study heterokonts, particularly diatoms. I have always been fascinated by these marine autotrophs that exhibit mind-blowing diversity and exquisite patterns. They are important players in the Earth's carbon cycle. Their four-membraned plastids are thought to be the outcomes of two endosymbiotic events. Hidden in the immense diversity and curiosity of diatoms, both morphological and genetic, are probably many of life's secrets waiting to be uncovered.

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