Winged migration

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ABSTRACT **It is an honor to become a part of the talented group of cell biologists who have received this award before me. While running a research group certainly has its ups and downs, I love being a faculty member and am continuously excited by the prospect of scientific discoveries yet to be made. I have benefited from the support of many people over the years and hope to be able to do the same for others through my mentoring and teaching.**

SKYDIVING SWANS AND CENTROMERES

Born and raised in Italy, I worked as an undergraduate assistant in Milan, studying DNA replication in budding yeast. The lab was small and tight-knit, and the work of undergraduate students was valued and rewarded. Energized by this experience, I decided to move Edinburgh to pursue my dream: being a full-time researcher. I didn't know exactly what I wanted to do, everything sounded exciting, but understanding the organization and stability of genomes particularly intrigued me. An opportunity arose to work in a basic biology laboratory that studied chromatin, so, in 1998, I joined the group of Robin Allshire at the Medical Research Council Human Genetics Unit in Edinburgh, Scotland, for my doctoral work.

Photo by Amy Nocton

Barbara Mellone

Edinburgh was very different from any other city I had known. I'll never forget the time I was walking to the lab from my apartment in the lovely Comely Bank neighborhood on yet another rainy day. Out of the blue, I heard a strange crashing noise coming from the road, the sound of something large and soft hitting the cobblestones. When I stopped and turned around, I could not believe my eyes. A swan had attempted to land on the slick, shiny, wet surface of the road, mistaking it for water. My apartment was near a park with a swan pond, and this was the second time I had to call the local police and wait for them to come and rescue an injured bird, warning drivers to slow down and maneuver carefully around it. The police were accustomed to getting these calls from residents in the area, but when I called the lab to let people know I was running late, yet again because of a fallen swan, my lab mates wouldn't stop laughing.

It was a very exciting time for chromatin biology, as the importance of novel histone modifications was just beginning to emerge. My project focused on understanding the chromatin requirements for the silencing of pericentric heterochromatin in the fission yeast. Because of previous work in budding yeast, we had predicted that silencing mechanisms in other organisms would be mediated by the hypoacetylation of the histone H4 N-terminus. I had just embarked on mutating individual histone H4 lysines when Thomas Jenuwein's lab discovered that mouse Suvar39h had histone H3 lysine 9 methyltransferase activity (Rea *et al.*, 2000). Knowing that Suvar39h proteins are required for silencing in the fission yeast *Saccharomyces pombe* and flies, I quickly shifted my attention to histone H3 and showed that mutating lysine 9 completely abolished silencing, providing direct evidence that this residue is the key target of this family of methyltransferases and highlighting the remarkable resemblance between the heterochromatin of *S. pombe* and that of more complex organisms (Mellone *et al.*, 2003). Seeing how much we understand today about heterochromatin and silencing, I cherish having been part of the early work and marvel at how much progress the field has made

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Abbreviation used: CAL1, chromosome alignment 1; CENP-A, centromere protein A; CENP-C, centromere protein C; RNAi, RNA interference.

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since then. Working with Robin Allshire taught me to be critical of my data, to connect concepts in a deep and mechanistic manner, and to think about my experiments in the context of the bigger picture. Learning to write about my work in a different language felt overwhelming, but he painstakingly edited my thesis and papers, showing me the importance of editing and revisions. Working on a basic biology problem was exciting, and the broad implications of the potential findings motivated me. I also realized how much we didn't know about the basic principles of chromatin dynamics and their impact on chromosome inheritance.

Toward the end of my PhD work, I became interested in the centromere-specific histone H3, called CENP-A, which had been hypothesized to epigenetically "mark" the centromere location. I performed experiments that manipulated both canonical histone gene dosage and centromere size, and we determined that CENP-A chromatin was "plastic," that is, it had the ability to spread and shrink (Castillo, Mellone, *et al.*, 2007). My exposure to fluorescence microscopy in the Allshire lab made me realize how much I loved being able to visualize the structures that fascinated me most: chromosomes and mitotic spindles.

The idea that centromeres might be defined epigenetically through the presence of CENP-A, rather than by specific DNA sequences (Karpen and Allshire, 1997), got me completely hooked on centromeres. I became very interested, perhaps even obsessed, with understanding how CENP-A becomes specifically localized to centromeres and maintained there across cell generations. For my postdoc, I decided that I wanted to study this process using a genetic system that would also afford beautiful cytology. In 2003, I joined the laboratory of Gary Karpen, which uses *Drosophila* to study centromeres and heterochromatin, at the Lawrence Berkeley National Laboratory and the University of California, Berkeley. The novel high-throughput RNA interference (RNAi) technology that was being developed by Norbert Perrimon at the *Drosophila* RNAi Screening Center at Harvard seemed like an ideal entry point to identify the largely unexplored regulators of centromere chromatin assembly using a cell-imaging screen. This collaborative genome-wide RNAi screen (which involved Sylvia Erhardt along with myself in the Karpen lab and Craig Betts in Aaron Straight's lab at Stanford) identified critical factors of centromere function at a time when very little was known about the CENP-A deposition pathway. These factors included centromere-associated proteins and cell cycle regulators (Erhardt, Mellone, Betts, *et al.*, 2008), which I was able to later pursue in my own research group. Gary Karpen was a fantastic mentor. He gave me freedom and responsibility; supported me unconditionally through two maternity leaves; and worked closely with me on developing grants, papers, and research seminars. His relentless encouragement and faith in my abilities were instrumental in my decision to seek a faculty position.

BROCCOLI FIELDS AND NEW CHALLENGES

In 2009, my husband, two young daughters, and I uprooted ourselves to move to Connecticut, "the land of broccoli fields," as my good friend from Italy calls it, due to the appearance of its tree-covered landscape from an airplane. The hilarious photo on my University of Connecticut ID, taken on my first day as a faculty member, captured my initial emotions: "Is this really happening? What if I can't do this?"

The initial few months were challenging; the gorgeous old university campus was a maze of brick buildings and trees, and I kept getting lost. At UConn I was once again surrounded by large birds, but instead of swans, it was the plentiful Canada geese that pass through campus every year in the thousands. I missed lunches with my fellow postdoc friends from Berkeley, needing a good laugh or to commiserate about difficult experiments. Fortunately, it wasn't long before I mastered my new campus's geography and made new friends, both at UConn and outside work. While it seemed I had less time to socialize, I soon found colleagues and friends who supported me and laughed with me.

When I started my lab, our understanding of the mechanisms of centromeric chromatin assembly was very limited. As a first step in my independent research program, I followed up on experiments started in the Karpen lab and elucidated the functional cell cycle dynamics of CENP-A and its effectors from our RNAi screen—cyclin A, CAL1, and CENP-C (Mellone *et al.*, 2011). An inherent challenge of dissecting the centromere assembly pathway is that a mutation or knockdown of centromere components often results in centromere disruption and subsequent cellular lethality. To overcome this, we used genomic engineering in *Drosophila* S2 cells, implementing an inducible tethering system (LacI/lacO) to dissect the stepwise formation of CENP-A chromatin without compromising viability. This was critical in providing the first experimental evidence that CAL1 is a CENP-A assembly factor required for centromere establishment (Chen, Dechassa, *et al.*, 2014).

Our studies showing that centromeric chromatin can be established de novo in flies are consistent with the occurrence of neocentromeres (centromeres that form at new locations) in humans. But many fundamental questions remain: How are canonical nucleosomes reorganized to make room for CENP-A during its deposition? Are cells able to detect the presence of a spurious centromere and to inactivate it? How is centromere function maintained in spite of its rapid evolution? Recent graduate students in my lab have taken on some of these challenges, showing that CENP-A deposition is directly coupled to and requires transcription (Chen *et al.*, 2015) and that CENP-A evolves in concert with its chaperone (Rosin and Mellone, 2016), but much exciting work remains to be done not only to inform mechanistic models for centromere specification but also to explore the genomic landscape and evolution of these fascinating chromosomal structures.

FINAL THOUGHTS

As with most new science faculty, I have experienced the ups and downs of staffing a functional laboratory, obtaining funding, publishing, and teaching effectively. In fact, many times I felt like those swans in Edinburgh—confused, disappointed, and alone—but just like those swans, who ultimately made it safely back to their pond (in one case after some rehabilitation) I have always had somebody to turn to for help and support when things didn't go as planned (by the way, they rarely do). Despite the highs and lows, doing research for a living remains a highly rewarding and creative career that offers both great flexibility (especially helpful when also raising a family) and the fortunate opportunity to encounter new ideas, meet new people, and travel to new places along the way.

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