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14 \text{ days} \quad \text{acceptance to publication}

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The mismeasure of scientific research articles and why MBoC quickly embraced preprints

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In this issue, we publish essays from the 2016 recipients of awards bestowed by the American Society for Cell Biology (ASCB). All of the awardees are being honored, at least in part, for the excellence of their research. But how do we recognize the value of scientific research?

In his criticism of attempts throughout history to quantify human intelligence, Stephen Jay Gould highlighted two fallacies (Gould, 1981): the “fallacy of ranking,” which is the “propensity for ordering complex variation as a gradual ascending scale,” and the “fallacy of reification,” which is “our tendency to convert abstract concepts into entities.” These fallacies also apply to attempts to quantify the value of scientific research articles.

There are many nonquantifiable ways in which a scientific research article may have value—it may provide new information, a new concept, a technical advance, and so on. Often, the value of an article is in the eyes of the reader. The value is definitely not determined by where the article was published. While an article may be of little interest to one researcher, it may provide the key piece of missing information or the key technical advance that allows another researcher to make a significant advance. Moreover, the “value” of a research article sometimes is not appreciated until many years after its publication (Wang et al., 2016). This is why Molecular Biology of the Cell’s (MBoC’s) founding editor-in-chief, David Botstein, opted to “leave it to future generations to decide whether an article was significant.”

And that is why scientific award committees generally seek to look at, understand, and appreciate a candidate’s research rather than just counting citations and tallying up the journal impact factors, or JIFs, of the journals in which the work appeared.

WHY MBoC EMBRACES PREPRINTS

Posting unrefereed manuscripts on preprint servers has been common practice in the physical sciences for 25 years and is rapidly catching on in the life sciences (Berg et al., 2016). While some journals are hesitant to consider manuscripts posted on preprint servers, the ASCB’s research journal MBoC has no restrictions and allows citation of preprints in the reference sections.

Why would some journals be reluctant to consider manuscripts posted on preprint servers? After all, preprint servers, like poster presentations and research talks at conferences, are just an additional option for communication of results before publication. One reason is that preprint servers are a threat to journals that are slaves to the JIF. This is because certain articles, particularly those in trendy areas, are mostly cited only during a short period of time after publication. When a journal decides to consider an article for publication that was already posted on a preprint server, the journal is missing out on the window of time when that article is likely to get the most citations. In the physical sciences, it is not uncommon for preprints to receive more citations than the final, published, research article. If a journal dedicates itself to the JIF, rather than to serving science, preprints could be a disaster.

MBoC was started by the ASCB for the sole purpose of serving cell biologists. During the peer-review process, we ask only whether the science is of the highest quality and moves the field forward. Our mission is to serve science, not to jockey for the highest position possible in the journal pecking order. We welcome articles posted on preprint servers, because this practice reduces delays in the communication of scientific results caused by an unpredictable peer-review process. Preprint servers give scientists control over when their results are communicated.

WHY JOURNALS STILL MATTER

While preprints give authors control over when their research is seen by the public, journals still play an important role. For one thing, it is only during the peer-review process that two or three experts will spend several hours thoroughly evaluating the experiments,
interpretations, and clarity of the writing and data presentation. The monitoring editor—an expert in the field at scientist-edited journals—assesesses the author’s responses to the reviews, giving the work the final stamp of approval. The reviewers and the editorial board of a journal therefore play a large role in improving and validating the work. Once the article is accepted for publication, it is copyedited to further improve the grammar and clarity and to make sure that the style, language, and abbreviations conform to accepted norms. Many journals also have now adopted author checklists to improve reproducibility (Schwarzbauer et al., 2016). Best practices for how to list on an individual’s curriculum vitae articles that have appeared first as preprints and then as peer-reviewed publications must still be established and standardized to avoid double counting and to allow aggregation of total citations to the work.

REFERENCES
A microscopic view of the cell

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ABSTRACT Light microscopy has long been an indispensable tool for cell biology research. From biological problems to biological knowledge, there are two more critical links in the light microscopy approach: labeling and quantitative analysis. Therefore, an integrative approach is desirable in order to deal with practical challenges in biological light microscopy.

In 1665, the word “cell” was first used by Robert Hooke to describe the honeycomb structure of a thin piece of wood cork that he saw under his microscope. Ever since, light microscopy has been an indispensable tool for cell biology research. With its ability to resolve cellular and subcellular structures, analyze their molecular compositions, and follow their dynamics in live specimens, light microscopy has provided us vivid pictures about the inner life of cells. Over the years, the capabilities of light microscopy have continued to expand with many instrumental breakthroughs and innovations. For example, optical sectioning by confocal microscopes provides crisp three-dimensional images. Two-photon fluorescence microscopes allow us to peer deeply into thick tissues. Superresolution microscopes push the spatial resolution from the organelle level to the macromolecule level, turning light microscopy into a new method for architectural analysis of molecular complexes and thus helping to bridge structural biology and cell biology. These technical advancements have greatly expanded the scope of biological problems that can be visualized and investigated using microscopy.

Microscopy, however, requires more than just the microscope itself. From biological problems to biological knowledge, there are two more critical links: labeling and quantitative analysis (Figure 1). For any structures or activities that do not produce a natural contrast under a microscope, labeling is essential to make them visible. Fluorescent proteins, antibodies, and nucleic acid probes have been widely used to tag specific proteins, DNAs, and RNAs for fluorescence microscopy. Lipids and small-molecule metabolites can be labeled by special chemical bonds or isotopes for Raman detection. Functional probes such as calcium indicators and enzymatic activity reporters shed light on how cells do their everyday work. In all these cases, the information regarding the target is transferred into the positional and optical properties of the labeling reagent, which can be read out by the microscope. To the extreme, in expansion microscopy, the original cellular structure can be entirely removed after the fluorophores are anchored to a polymer matrix.

Although a picture is worth a thousand words, it takes more than just pretty pictures to answer a biological question definitively. Quantitative analysis of microscopic images is crucial in order to objectively extract information such as shape, abundance, colocalization, and movements. Some of these tasks can be accomplished with a few mouse clicks or several lines of scripts in software packages like ImageJ and CellProfiler; in more complicated cases, serious algorithm development and programming may be required. With microscopes becoming more automated and cameras becoming faster, the volume of data has nowadays emerged as yet another challenge as well as an opportunity. Advanced computation such as machine learning has now proven its value in plowing through gigabytes or even terabytes of imaging data.

Because instrumentation, labeling, and analysis are all essential components of microscopy, any challenge in applying microscopy to biological research could be tackled from multiple angles. Let us use a very common issue in fluorescence microscopy as an example.
When acquiring live confocal stacks of a low-abundance protein, we may find that the signal fades away too quickly because of photobleaching. What can we do then? On one hand, we could move to a light-sheet microscope that prevents out-of-focus regions from light exposure during a three-dimensional scan (Keller and Ahrens, 2015). We could also switch to brighter labels, using the HALO tag (Grimm et al., 2015), SunTag (Tanenbaum et al., 2014), or tandem FP11 tags (Kamiyama et al., 2016), so that the intensity of the excitation light can be lowered without sacrificing the intensity of fluorescence signals. Yet another route is to consider some of the de-noising/deconvolution algorithms (Carlton et al., 2010). Using redundant information among camera pixels and across time points and prior knowledge about the structure of interest can dramatically reduce the signal level required for robust statistical interpretation of images. In practice, any one of these approaches could become the optimal choice, depending on the specific biological system studied.

Even more powerful is when the three approaches are integrated. A perfect example is single-molecule switching-based superresolution microscopy, more commonly known by acronyms such as STORM and PALM (Huang et al., 2010). While the challenge is a physical one, that is, the diffraction of a light wave fundamentally limits the spatial resolution of light microscopes, the solution is not just to be found in the optical arena. In addition to a microscope that is sensitive enough to capture snapshots from a single fluorophore molecule, there are two more critical components. First, using fluorophores that can switch from a nonfluorescent to a fluorescent state, we can achieve a low density of active fluorophores in a densely labeled sample, thus making single-molecule recording possible. Second, by computationally determining fluorophore coordinates from their diffraction-limited snapshots, we can reconstruct a final image with much higher spatial resolution. In this way, the physical diffraction barrier is circumvented without violating the physical principles.

Seamless integration of these three approaches is not easy in practice, though. It is uncommon for a single lab to possess expertise in areas ranging from biology to bio-chemical labeling to optical instrumentation to computational analysis. Collaboration is extremely helpful here but can sometimes be difficult. People in different fields speak different languages and have different priorities. It can be frustrating when a biologist comes to a microscopy core facility, only to realize that he or she has chosen the “wrong” labeling, which will not match the instrument. It is also a disappointment if a huge pile of images sleeps in the hard drives, simply because the friend in the statistics department cannot be motivated without seeing “new” algorithms coming out of his/her effort. Solving these problems calls for people who have basic understanding of all three fields and can take the initiative to facilitate communication among scientists with diverse expertise. Our systems of funding and evaluation also need to better support and credit people who play “technical service” roles that are actually critical for biological discoveries. In the end, just as microscopy is not just about the microscope, the advancement of microscopy in biology is not only in the hands of microscopists but also in the hands of everyone involved.

REFERENCES
The thrill of scientific discovery and leadership with my group

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ABSTRACT My group and I feel tremendously honored to be recognized with the 2016 Early Career Life Scientist Award from the American Society for Cell Biology. In this essay I share the scientific questions that my lab has been excitedly pursuing since starting in August 2009 and the leadership behaviors we have adopted that enable our collective scientific productivity.

MY LAB’S SCIENTIFIC CONTRIBUTIONS AND VISION
My lab is fascinated by understanding how tissues renew themselves throughout an organism’s lifetime. Specifically, we are interested in understanding how cells orchestrate tissue growth and make cell-fate choices that result in balanced tissue regeneration. Tissue regeneration is often looked at as a process happening in a vacuum: each individual cell is an actor in a play, enacting its role according to an unchanging “script.” Yet continuous insults, such as tissue tear and somatic mutations, among others, can create continuous variations to the “script.” Thus, since these variations may call for improvisation, cells must adapt their roles to keep a tissue (play) functional (entertaining). As I started my lab, I felt that the biggest challenge in understanding mammalian tissue regeneration was that the field largely used static analyses that prevent the ability to capture cells in action in the context of an intact organism in which variations occur. During my doctoral thesis, I experienced firsthand how live imaging provided me not only a better understanding of tissue patterning but also allowed us to discover new biology that we had not anticipated, whereby epithelial cells secrete vesicles (called argosomes) that carry morphogens and disperse them throughout the tissue (Greco et al., 2001).

Thus, as I set up my lab, we studied tissue regeneration by investing in a high-risk/high-reward approach to establish skin stem cell imaging in live mice. After more than one year of troubleshooting and several discouraging roadblocks, we were finally able to visualize, track, and manipulate stem cells and their niches within the skin epithelium of an intact living mouse (Rompolas et al., 2012; Pineda, Park, et al., 2015). These novel approaches have allowed us to get a fresh look at processes that have been investigated for decades, leading to the capture of novel principles of stem cell biology and tissue regeneration. In retrospect, what I had accomplished was combining my passion for visualizing biological processes in vivo with my knowledge of stem cells gained during my postdoc (Greco, Chen, et al., 2009). The ability to directly observe a biological phenomenon is the reason why I fell in love with science, and I was able to bring this angle to bear on our research, which uniquely poised my lab to address previously inaccessible questions and distinguished my lab from the laboratories of my previous mentors (Park et al., 2016).

With these tools, my lab has contributed to the understanding of fundamental principles of the equilibrium of cell choices reached during tissue regeneration and has explored the edges of this equilibrium, which we describe in more detail below. Questions that...
fueled our science include, What are the rules that sustain robust daily tissue regeneration? How many ways are there to ensure tissue function? How does this equilibrium evolve when the normal tissue is in the presence of cancerous proliferative clones?

The niche’s requirement for stem cell fate

Tissue regeneration is achieved through a balance of cell production (growth) and elimination (regression). Yet we still fail to understand how stem cells and their environment balance tissue growth and regression during regeneration in a live mammal. To address these questions, we used the mouse hair follicle, which cycles between these phases while maintaining a pool of stem cells to sustain tissue regeneration. By visualizing stem cell behavior and manipulating stem cells’ niche during growth, we have shown that 1) stem cell fate depends on the position (surrounding niche) that the cells inhabit, and 2) while the niche is required for tissue regeneration, dedicated stem cells are dispensable. Specifically, we showed that cells can switch their fate to adopt new stem cell functions in the face of loss of a specific stem cell pool. These discoveries have provided a new understanding of the role of stem cell location and how fundamentally important the native/local niche is with respect to stem cell decisions and overall tissue regeneration, which could not have been observed without our ability to track the same cells over time in a live mammal. Our results also reveal a robust mechanism of compensation in which cells from other epithelial compartments can adopt new stem cell functions and fuel tissue regeneration (Rompolas et al., 2012, 2013). We have shown that hair follicle epithelial stem cells are eliminated during regression through a spatial gradient of apoptosis along the same axis utilized for growth. Furthermore, we have demonstrated that hair follicle stem cells collectively act as phagocytes to clear dying epithelial neighbors. Through cellular and genetic ablation, we have shown that epithelial cell death is extrinsically regulated by the local niche through transforming growth factor (TGF)-β activation. Strikingly, our data show that regression acts to reduce the stem cell pool, and the inhibition of the regression phase results in excess basal epithelial cells with regenerative abilities (Mesa et al., 2015). These findings are surprising, because the field previously thought of stem cells as having a finite lifetime/capacity of divisions that eventually leads to their elimination by exhaustion. Indeed, this work shifts the understanding to the niche environment, which, if altered, can lead to the aberrant coopting of the system toward deregulated growth. It also demonstrates that reinstalling a proper niche can correct stem cell–driven aberrancy. This principle is key to TGFβ-driven cancer models and, importantly, also elevates the significance of the niche in the broader study of mechanisms of cancer initiation.

Currently, we are addressing how these cellular interactions are regulated by surrounding niche populations such as mesenchymal cells and immune cells and structural elements such as the extracellular matrix. Together, these approaches will allow us to determine whether specific niche populations that are interspersed or adjacent to the epithelium serve as regional checkpoints to locally control the regeneration process. Additionally, this work has allowed us to branch out and begin to study how cells ensure rapid tissue repair after injury. We have been able to study how the interplay between repair behaviors, such as migration and proliferation, lead to effective reconstruction of the epithelial tissue and the extent to which homeostatic processes such as differentiation are affected during the repair process. We are particularly excited to study how these interconnected cellular behaviors contribute to the tissue-scale changes observed in the repair process.

Individual and group stem cell behaviors in normal and cancerous tissues

Tissue regeneration relies on a multitude of distinct cellular behaviors. Yet we lack an understanding of how these individual cellular behaviors are regulated and how mutations may influence them. To answer these questions, we have traced the entire lifetime of epidermal stem cells and interrogated their behaviors. We have demonstrated that stem cells do not appear to be intrinsically biased toward either self-renewal or differentiation but instead seem to be influenced by the behaviors of their neighboring sister cells. Additionally, as basal stem cells stochastically commit to differentiation, they reuse existing structural organizations (Rompolas, Mesa, et al., 2016; Xin et al., 2016). Recent studies have reported that morphologically normal skin often carries oncogenic mutations. We therefore began to interrogate the interface between normal tissue and cancerous clones by using the evolutionarily conserved pathway, Wnt/β-catenin. Our efforts uncovered a novel mechanism of action for β-catenin that acts non–cell autonomously within the hair follicle stem cells by recruiting wild-type cells to induce de novo hair growth that ultimately results in tumors. Additionally, we show that β-catenin–driven growth is triggered and expands independent of the local niche’s influence (Deschene, Myung, et al., 2014). This work changes our understanding of how cells that carry mutations can interact with neighboring cells, providing insights into how cancer initiation and progression may be fueled.

To understand how to counterbalance cancerous growth, we took advantage of two contrasting mouse cancer models: a unique, benign skin tumor that regresses spontaneously, keratoacanthoma; and a malignant skin tumor, squamous cell carcinoma (SCC). We demonstrated that self-regressing keratoacanthoma tumors counterbalance excessive proliferation by employing a homeostatic mechanism of terminal differentiation to regress. When this differentiation cue, retinoic acid, is used on SCC it could also induce the regression of these malignant tumors (Zito et al., 2014). Taken together, this body of work modifies prevailing views in the field regarding how cells that carry mutations can interact with neighboring cells, expanding our understanding of how tumor progression and regression is regulated.

Currently, we are interested in mutations associated with SCC, such as clones bearing Hras mutations in combination with a loss of TGFβ function. Thus, we are using live imaging to study the dynamic behaviors and interactions between mutant clones (double and single mutants) and wild-type neighboring tissues. Functional investigation of both oncogenic signaling pathways and different cellular interactions will help us elucidate the critical set of decisions that lead to cancer.

RESPONSIVE LEADERSHIP: ADAPTING TO MY GROUP TO GENERATE SCIENTIFIC DISCOVERIES

During my journey, I have often reflected on the “features” of a successful leader and will explain below the thought process that brought me to evolve a model of leadership that is based on the complementary strengths and weaknesses of all who work in the lab. In searching for my voice in a leadership role, I encountered a conundrum. The search for leadership “features” revolved around the principal investigator (PI) as the leader. While the PI is fundamental in the initial phases of a lab—the founder who needs to begin the lab with a great idea—as the group expands in size over time, the lab’s effectiveness increasingly depends on the vision of all of its constituents, not just the PI. Yet models of leadership I have been exposed to, from grant preparation to promotion to credit in papers, seemed to single out one element in this collective project,
tening to feedback and developing effective solutions to the prob-
to acquire at least two essential behaviors: first, knowing one’s own
successful behaviors that people in positions of leadership would
the group itself. I finally came to realize that, in my view, the most
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which we work. In fact, leadership is about the effective work of a
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behaviors not necessarily connected to the groups with
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which we work. In fact, leadership is about the effective work of a
collective group, and those behaviors seem to have little to do with
the group itself. I finally came to realize that, in my view, the most
successful behaviors that people in positions of leadership would
need to adopt would evolve around the demands of the group.

Thus, in my view, this requires people in positions of leadership
to acquire at least two essential behaviors: first, knowing one’s own
weaknesses, and second, effectively adapting to the group by lis-
tening to feedback and developing effective solutions to the prob-
lnesses, I also engage my lab members in compensating for them
(and complementing my strengths) by working together on tasks
and creating a better outcome on all fronts. Thus, my weaknesses
turn into a positive tool that empowers my lab members and allows
them to grow better and faster, preparing them for future roles in
positions of leadership while building a more cohesive group in
which everyone is valued.

Establishing a feedback model
I would not be where I am today were it not for the insightful and
invaluable contributions of each person in my lab (Figure 1). Para-
doxically, that also places me in a position of vulnerability. I have al-
ways felt that we use more reductionist approaches in viewing peo-
ple, ranking the first and seeing the rest as less valuable. I have
found that it takes courage, time, but most importantly, genuine
belief to view everyone we have hired within an organization, some
of whom fall “below” in a formal hierarchical structure, as significant
contributors. I have come to realize that this belief in the meaningful
contribution of all members of the lab is a fundamental component
of a successful group. Therefore, I work hard to maintain a culture in
my lab in which feedback from lab members is not only encouraged
but listened to carefully and very often leads to changes that deeply
affect the course of our lab decisions. While it has not always been
easy and requires a significant investment of time and a willingness
to accept that the PI’s ideas are not always welcomed with open
arms by the lab members, it has created an evolving entity that
empowers each individual within our small organization.

I believe an obstacle to establishing this model comes from the
fear we carry of not feeling adequate. I believe this feeling is shared
within any profession in which present and future performance is
what determines our own and our peers’ appreciation independent
of previous accomplishments. A mentee once asked me what hap-
pens if a lab member is “better” than the PI. This thought was
posed to me with the assumption that such a lab member is a po-
tential threat. I started to think that people feel a need to establish
a hierarchical scale to rank people from best to worst (similar to the
ranking of the traits I was discussing earlier). In a separate conversation with a colleague, I was told that I needed, once in a while, to establish the superiority of the PI over the lab members. My hypothesis is that both conversations were stemming from this fear we carry of becoming dispensable, which in turn may trigger a dominant, repressing behavior in us. Thus, this repression is not based on actual superiority but on fear. Regardless, these behaviors that our group will silently watch and learn from will inhibit a transparent critical dialogue, which in turn limits the power of the lab for discovery. If we instead foster a system in which 1) each individual is valued for his or her strengths and weaknesses (one person’s weaknesses leave room for the contributions of others) and 2) the PI is committed to soliciting and responding to lab members’ feedback on important lab decisions, I believe we create the opportunity for significant scientific achievement.

I believe that these reflections on leadership could be applied to any group setting and therefore to any person working in a position of leadership. Thus, our scientific community at large could profit from a similar model, wherein more engagement of our members, seeing them as accountable and driving forces of the group itself, may accelerate both individual and collective growth.

ACKNOWLEDGMENTS

I am very grateful to many people. My dear family and friends who support my passions, believing in me and investing on our relationships. My kids Lola and Gael, who inspire me to have the courage to pursue a life filled with experiences that help me grow. My colleagues and friends at Yale, who help me develop in a world that does not always favor the group I belong to. My many supportive collaborators Ann Habermann, Christine Ko, Jim Duncan, Zhongzhi Liu, Don Nguyen, Miriam Domowicz, Alion Klein, Kyogo Kawaguchi, Yohanns Bellaiche, Boris Guirao, Slobodan Beronja, and many others. They help my lab expand the expertise and intellectual contributions necessary to get the answers we seek. Most importantly, my lab members, each one of them providing excitement and dedication as we work together on our favorite scientific puzzle. Additionally, I am grateful to the courage of my group in working together to create a safe environment where challenges can be a positive stimulation and failures are turned into continued personal and scientific growth. Finally, I thank David Berg, Cristiano Pineda, Kai Mesa, Sangbum Park, Antonio Giraldes, Daniel Colón-Ramos, and Marc Hammarlund for their invaluable feedback on this essay.

REFERENCES

Boldface names denote co-first authors.


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.

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.
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.

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.

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 UC-Irvine 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 UC-Irvine 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 in 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 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.

**ACKNOWLEDGMENTS**

I am grateful to my family for their love and support. I am indebted to all the generous mentors and colleagues who have supported me, shared their experiences, helped improve my grants and papers, and discussed scientific ideas with me. I thank all of the dedicated people who have done the most critical work in my lab to date, especially Kate Grive, Sarion Bowers, Chin-Chi Chen, Leah Rosin, and Jason Palladino. I also thank Rachel O’Neill, Dave Daggett, and my daughter Livia for their suggestions for this essay. Research in the
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REFERENCES

Boldface names denote co–first authors.


Think differently

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ABSTRACT

Asked to reflect on my own research and career after being selected for the great honor of the Women in Cell Biology Mid-Career Award for Excellence in Research, I found myself contemplating not only how I approach my own science but also how this approach contributes to the larger scientific enterprise. Here I discuss my motivations and their impact on how I conduct my research as one example of the myriad ways to be a scientist. I invite you to consciously consider how, as scientists, we view one another’s unique approaches and argue for the importance of diversity of perspective in scientific progress.

I recently participated in a community art project that asks participants to answer the question “Why do you do what you do?” in a single sentence, one short enough to fit on 8 x 10 card stock. The whole point was to go beyond superficial reasons and to identify your true driving passions. My answer was “Because asking questions makes our world purposeful.”

While that’s my motivation, no one else in the room had the same answer. And that’s actually a great outcome. A diversity of passions makes our society rich.

In much the same way, diversity of perspectives is the pillar of scientific progress: advances in understanding only emerge when one of us connects observations in a new way. Yet I fear that we, as scientists, don’t consciously appreciate, protect, and promote this diversity. By not doing so, we risk losing it.

For example, I’m one type of scientist. I came to my profession via my own unique path. In graduate school, I was drawn to the study of gene expression regulation in Epstein-Barr virus. During this early training, I developed a deep appreciation for interesting biology, fostered by my advisor’s repeated question: “Why would the virus do that?” For me, this appreciation sparked a passion for understanding the awe-inspiring complexity underlying the biology in the greatest mechanistic detail possible.

My current area of research has shifted to a phenomenon that first sparked my interest when I was an undergraduate student: how a unique group of proteins known as prions can act as elements of inheritance and infectivity. I approach this question from the same perspective that drove my thesis research: my fascination with the biology and its underlying mechanisms. This perspective, more than any other, has defined how I choose to conduct my research today:

1. I treat all hypotheses as working ones and continually try to disprove them, because my goal is to figure out how things really work rather than to be prescient. Even when our observations are consistent with our ideas, I ask my students and postdocs, “What about this?” or “If we’re right, then what would we predict?” I offer other interpretations or extensions of our work and collaborate with my students and postdocs to think about how we might use these questions to gain additional experimental support. Because we are thinking so deeply about a problem, I believe that we have the responsibility to be our own harshest critics.

2. I view research as a scholarly pursuit that occurs equally at and away from the bench. My PhD advisor frequently paraphrased Westheimer’s discovery to remind us that “six months at the bench can save you half an hour in the library.” Even now, I spend a significant amount of my time rereading published papers to view them from the new perspective of our advancing knowledge. And I try to pay this gift forward by including a journal club in our lab meeting rotation, extensively reading with new members of my group, and starting all writing projects by asking my

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trainees to develop detailed outlines of their papers, proposals, and theses that place their work within the context of the pertinent literature.

3. I believe that every question answered creates many new ones, moving us toward increasing mechanistic detail. As our work has progressed, it seems as though whole new levels of the unknown are revealed, providing an endless source of motivation and inspiration for me. But sorting through this complexity takes time and focus. For me, this progression works best when one person is responsible for one project, following lines of investigation where they lead, learning whatever approaches are needed to answer the next question, and holding all of the observations in his or her mind until they can be connected.

Of course, my way is just one way. And that’s a good thing, because there are downsides to my approach to science, as there are for all approaches to science. In my case, we study prions in Saccharomyces cerevisiae, because it is amenable to answering the type of questions that drive my passion for details without losing sight of the biology, but this choice raises questions about the validity of generalizing our findings to mammals. My self-critical approach to hypothesis testing and my insistence on individual projects by no means brings us even close to perfection and admittedly makes for slow progress. My view that papers are opportunities to tell stories about how some aspect of biology works limits my publication list. But I am proud of the new knowledge that we have created and of the successes of my trainees. And I am humbled that I have been selected to receive the incredible honor of the Women in Cell Biology (WICB) Mid-Career Award for Excellence in Research, despite these limitations.

When I consider the previous recipients of the WICB awards and other colleagues whom I admire in the community of science, I’m struck by the different ways that we all go about the business of advancing scientific understanding. I’m inspired by those who are different from me: they think at higher levels to propose conceptual advances and translate basic findings into practical uses. I would never see these things, but I use their insights as the foundation to make my own unique contributions. From my perspective, this network of thinking is what makes the scientific enterprise successful; if we all operated at the same level, we would never move forward.

A senior colleague, whom I admire greatly, once told me that she weighs the uniqueness of a candidate’s perspective heavily in her review letters for tenure decisions, arguing that this characteristic, more than any other, predicts long-term success. Yet I don’t think that this enlightened opinion is our default. Instead, scientists tend to view and evaluate our discipline as a series of never-ending either/or characteristics that don’t acknowledge the importance of our unique perspectives: significant versus incremental, innovative versus standard, translational versus basic, impact versus productivity, discovery versus hypothesis driven. I have bought into these dichotomies myself in reviews of other people’s work and have been on the receiving end of them as well. When I propose to push our understanding of some biological phenomenon to a greater depth of detail, I have to admit, if I’m honest with myself, that the critiques questioning whether or not this line of investigation will lead to new insights are fair. I often don’t know if they will myself, but I do know that staying grounded in the biology has never led me astray.

I’ve seen the relative importance of these dichotomies in the scientific community shift during the course of my own career—study sections were not supportive of genetic screens when I wrote my first grant, but the age of ‘omics has led us to embrace discovery-based research. In the face of such a drastic change in perspective by the time that I’ve reached the middle of my career, we should ask ourselves, “How useful are these dichotomies in evaluating good science?” The priorities we identify now shape the future of the scientific enterprise, and in setting those priorities, we must ask ourselves, “Are these choices purposeful?” In our restricted funding environment, our decisions act as a form of selection to limit diversity in perspective, and this changing landscape will be passed on to the next generation, who depend on existing laboratories for training.

When I look back at the aspects of my work of which I’m most excited and proud, they are almost exclusively the insights that I never predicted when we began the projects that led to their discovery. If I can’t always see the endpoints in my own work, then it’s ridiculous for me to try to do so in the work of others. Rather, I think the best that we can do is to evaluate our work by the soundness of the ways we ask and interpret questions. The rest is up to the biology, which is exactly the point of what we’re all trying to do.

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The path from student to mentor and from chromosomes to replication to genomics

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ABSTRACT
The American Society for Cell Biology Women in Cell Biology Sandra K. Masur Senior Award recognizes leadership in scientific accomplishments and in mentoring, which are intertwined. My development as a scientist reflects important mentors in my life, including my father and Joe Gall, who is my “Doktor Vater.” In turn, as an established investigator, my scientific successes in researching 1) chromosomes, their replication and genomics, and 2) ribosomes, their structure, evolution, and biogenesis, reflects the hard work of my students and postdocs, for whom I act as a mentor, guiding them in their research and along their career paths.

It is a wonderful honor to receive the American Society for Cell Biology (ASCB) Women in Cell Biology (WICB) Senior Leadership Award that is named after my friend Sandra Masur, who has done so much for WICB. I attended the very first WICB meeting in 1971 and served as the chair of WICB in 1991. Subsequently, I led the action to make it a standing committee of ASCB, thus ensuring its longevity and its acceptance by the ASCB as a way to promote women in science. This is also the charge of the Rosalind Franklin Society, of which I am a founding member. In this short article, I will trace my training and key mentors who have impacted my career.

THE EARLY YEARS
It was natural that I would become a biologist. My father was a physician-scientist who grew up in Italy. After graduating from medical school in Milan, he emigrated to the United States during World War II, arriving by boat during the Great Hurricane of 1938, to pursue research with Harry Goldblatt, who had established the first animal model for renal hypertension. Soon thereafter, Mussolini’s Manifesto of Race stripped Jews of their Italian citizenship and professional positions. Unable to practice medicine in Italy, my father remained in the United States and joined the faculty of the College of Physicians and Surgeons (P&S) of Columbia University (serving as a faculty member from 1942 to 1979), where he continued his research on hypertension and saw patients. He wrote an exhaustive review of the field and proposed an explanation for renal hypertension (later proven correct by others), but since it was counter to a hypothesis espoused by his department chair, he was not allowed to publish the work. I vividly remember my father shelving his opus and stating that although he would terminate his research, his patients would be the beneficiaries of his knowledge of the area. At that moment I became determined to become a scientist and carry forward the name of Gerbi in biomedical research. Years later, a study presented at an ASCB WICB meeting showed that successful female biologists hold their fathers as role models. How true this was for me!

At Hunter College High School, I had marvelous teachers for ninth grade biology (Ruth Lilienthal) and for advanced placement biology (Lynn Pasztor). I wrote a term paper about J. Herbert Taylor’s discovery published just a few years earlier that chromosomal duplication is semiconservative (Taylor et al., 1957). In that classic...
paper, Taylor popularized the use of tritium for autoradiography and was able to follow the label in successive cell divisions. However, he could not imagine how DNA was organized into chromosomes and how DNA replication occurred. It was exciting times, and Taylor invited Matt Meselson to give a seminar about his demonstration that DNA replication in *Escherichia coli* was semiconservative (Meselson and Stahl, 1958), a study that had been published a year after Taylor’s findings of semiconservative duplication of chromosomes (for further discussion, see Gall, 2016). Taylor served as ASCB president in 1970.

As an entering Barnard undergraduate, with New York at my doorstep, I registered for a Brookhaven symposium where I was met at the train station by a chauffeur sent from Brookhaven to escort me to the meeting, never dreaming that his passenger was an undergraduate and not a professor! The impetus to attend this meeting was to learn more about giant chromosomes. This wish was fulfilled. Joe Gall spoke about his DNase studies on amphibian giant lampbrush chromosomes that supported a uniune model for chromosome structure (i.e., one DNA double helix per chromatin; Gall, 1963), thus settling the issue of DNA arrangement in chromosomes that had puzzled Taylor. At the same meeting, Cordowald Pavon spoke about the polytene chromosomes of *Rhyynchocista larval salivary glands*, whose DNA puffs underwent intense DNA synthesis (Ficq and Pavon, 1957). Although I did not introduce myself at the time, I already knew that I wanted to pursue a PhD under Gall’s mentorship. Moreover, I became hooked on sciarid DNA puffs, and we are still studying them in my lab.

Early on in my studies at Barnard, I was taught about the experimental basis for biological facts in a developmental biology course given by Lucena Barth. Subsequently, she and her husband moved to the Marine Biological Laboratory (MBL) to continue their research. With her introduction to the MBL, I came to appreciate this very special place, where research is an intense experience shared with colleagues who are incredibly excited by scientific discoveries. As a graduate student, I took the physiology (cell biology) course at MBL, and later, as a faculty member, I did some collaborative research at MBL, taught in an undergraduate January course, and served on several MBL review committees. Lucena Barth was my first role model of a female research scientist, helping me to choose research as a career path. My next female role model was the vivacious Reba McLeistock, and spent her career studying *Sciara* from Charles W. Metz at Johns Hopkins University, did her PhD research with Barbara McClintock, and spent her career studying *Sciara* (Gerbi, 2007). We now maintain the *Sciara* stock center and welcome new investigators who wish to work with *Sciara* in their labs to explore its many unique biological features (DNA amplification, chromosome imprinting, a monopolar spindle in meiosis, X dyad nondisjunction, chromosome elimination, etc.). Such studies are now made possible by our expanded toolbox of the genome sequence and transformation methodology for *Sciara* (brown.edu/go/sciara-stocks).

**GRADUATE SCHOOL AND BEYOND**

My PhD studies at Yale with Joe Gall were transformative in terms of my career. He is a biologist par excellence who chooses whatever biological system is best suited to answer the question at hand, including frogs, salamanders, fruit flies, beetles, and protozoa (Endow and Gerbi, 2003; Endow et al., 2013). Although I had wanted to study amphibian lampbrush chromosomes with him, he encouraged me to bring *Sciara* to his lab. I wanted to pursue DNA amplification at the polytene chromosome “DNA puffs,” but the molecular methodology was not yet available (cloning and sequencing had not yet been invented). Instead, we made use of *Sciara’s* gigantic polytene chromosomes (which undergo more rounds of endoduplication than *Drosophila* polytene chromosomes) as the first chromosomes to be used for in situ hybridization (Pardue et al., 1970). These were exciting times in the Gall lab, as he was developing this method with my classmates Mary Lou Pardue, using an *rRNA* probe against its amplified extrachromosomal genes in amphibian oocytes (Gall and Pardue, 1969; Pardue and Gall, 1969). The power of new methods to advance the field is a lesson I took with me to my own lab, where we have developed several new techniques, including replication initiation point (RIP) mapping, which allows the start site of DNA synthesis to be mapped to the nucleotide level (Bielsky and Gerbi, 1998, 1999). The basis for RIP mapping is λ-exonuclease, and we are refining its use to map replication origins genome-wide (Fouk et al., 2015).

Following graduate school I spent two years as a postdoctoral fellow at the Max Planck Institute for Biology in Tübingen, Germany, where Wolfgang Beermann had created a mecca for polytene chromosome researchers. Just as I had wished to work on lampbrush chromosomes for my graduate research but ended up working on *Sciara* polytene chromosomes, at the Max Planck, instead of working on chromosomes, I started my studies on *rRNA*. Having used *Xenopus* *rRNA* to probe polytene chromosomes at Yale, I wondered where the regions of evolutionary conservation resided. Beginning in my postdoc and continuing on in my own lab at Brown, I used the relatively new method of molecular hybridization and then the even newer methods of DNA sequencing and cloning to explore this question. My fascination with ribosomes began in college, when my father had taken me to hear a seminar at the New York Academy of Sciences given by George Palade about his isolation and electron microscopic visualization of ribosomes. The dogma of the time was that ribosomal proteins were the enzymes for ribosome function in protein synthesis, but I suspected that *rRNA* might play an important role, as is now well documented. We derived the first sequence for *rRNA* from a metazoan (*Xenopus*) and discovered highly conserved sequences of vital importance for ribosome function (peptidyl transferase center, etc.) and “expansion segments” (Gerbi, 1996), whose positions but not sequences are conserved in eukaryotes. Their eukaryotic-specific roles are currently emerging. Our recent bioinformatic study has mined the now extensive database of *rRNA* sequences from the three domains of life to define conserved nuclear elements (CNEs), some of which are universally conserved. Other CNEs are domain specific, including several that line the wall of the tunnel in the large ribosomal subunit in eukaryotes, suggesting a eukaryotic-specific function (Doris et al., 2015). At Brown we also delved into ribosome biogenesis and used *Xenopus* oocytes to demonstrate the function of U3 small nucleolar RNA (*snoRNA*) in 18S *rRNA* processing (Savino and Gerbi, 1990; Borovjagin and Gerbi, 2001) and discovered the conserved elements that guide U3 and other *snoRNAs* to the nucleolus (Lange et al., 1998, 1999).

Since joining the faculty of Brown in 1972, I have tried to mentor the next generation as payback for the mentoring I received. At the local level, besides the many wonderful undergraduate and graduate students and postdocs mentored in my lab, I served for more than three decades as the director, principal investigator (PI),
and then co-PI of our National Institutes of Health (NIH) graduate student training grant. As the founding chair of the Department of Molecular Biology, Cell Biology, and Biochemistry, I also mentored junior faculty. I have been active in graduate and postdoctoral training at the national level, serving as a founding member and chair of the Association of American Medical Colleges Graduate Research Education and Training Group and as chair of a Federation of American Societies for Experimental Biology conference on this subject, publishing several articles with Howard Garrison (deputy executive director for policy). As part of my activities in ASCB public policy, I testified before the House and Senate Subcommittees on Appropriations about the importance of NIH funding for graduate education. It was awesome to realize how many thousands of scientists would benefit from my three-minute testimony!

The ASCB plays an important role in nurturing the careers of cell biologists from their time as students to established investigators. As a beginning graduate student, I attended my first ASCB meeting in 1965 and later served as program chair (1986), member of the ASCB Council (1988–1990), chair of WICB (1991), and president (1993). It is noteworthy that my PhD advisor Joe Gall, who was ASCB president in 1968, trained three ASCB presidents (Mary Lou Pardue, Liz Blackburn, and me). Moreover, all three of us are women, and for his nurturing of women in science, Gall received the WICB Senior Award in 2006. It is a great honor to follow in his footsteps to receive this three-minute testimony!

ACKNOWLEDGMENTS
I thank the mentors discussed in this essay, my supportive husband James Mcllwain, and the many others not mentioned who have nurtured my career. I am indebted to my students and postdocs, as my career is based upon their successes at the bench. During my career, my research has been supported by the NIH (currently NIH R01 HG008160), the National Science Foundation (currently NSF MCB 1607411), and several other agencies.

REFERENCES


**The need to connect: on the cell biology of synapses, behaviors, and networks in science**

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**ABSTRACT** My laboratory is interested in the cell biology of the synapse. Synapses, which are points of cellular communication between neurons, were first described by Santiago Ramón y Cajal as “protoplasmic kisses that appear to constitute the final ecstasy of an epic love story.” Who would not want to work on that?! My lab examines the biological mechanisms neurons use to find and connect to each other. How are synapses formed during development, maintained during growth, and modified during learning? In this essay, I reflect about my scientific journey to the synapse, the cell biological one, but also a metaphorical synapse—my role as a point of contact between the production of knowledge and its dissemination. In particular, I discuss how the architecture of scientific networks propels knowledge production but can also exclude certain groups in science.

**NEUROGENESIS**

In second grade, I almost failed science class. I was not a bad student. I was a nerd and I loved science—but I was bored. The books we used were filled with concepts that I found foreign and irrelevant. There was a picture of a kid, with really straight hair, whose hair was standing on end after being rubbed with a balloon. It was used as an example for static electricity. It looked fun, so I tried it. It did not work.

I was born and raised in the archipelago of Puerto Rico. In the tropics, where there is ~80% humidity year-round, you do not need to rub a balloon for your hair to stick straight up. Your hair is always sticking up.

Most of the scientific examples I learned in school were similarly irrelevant to my reality growing up in Puerto Rico. The books we used were written for kids from Europe and North America. I had to memorize examples that seemed fictional to me. For seed dispersal, I learned about the helicopter-shaped maple tree seeds, something I had never seen, because maple trees do not grow in the Caribbean. There is nothing wrong with using examples from elsewhere to illustrate scientific concepts. But when only examples from elsewhere are used, one learns that science is a distant, irrelevant thing done elsewhere.

Outside the classroom, my experiences were different. I was stimulated by the richness of the biological diversity around me, and my mind was constantly churning out questions. If plants can’t feel, then why does the *morivivi* (*Mimosa pudica*) close when touched? How does a *tinglar* (*leatherback turtle, Dermochelys coriacea*), know to go to the ocean when it comes out of its egg? How does it remember, many years later, to come back to the same beach on which it was born to lay its eggs?

Scientific curiosity is a shared human instinct. Regardless of where we are born on planet Earth, we wonder and marvel about the world around us. While the knowledge produced by science is of universal importance, not everyone has equal access to scientific networks that produce knowledge. How we communicate and make scientific discoveries relevant to others—a strategy known in...
education as “contextualization”—contributes, by design or by accident, to who sees themselves as belonging in science.

**DIFFERENTIATION**
I did not fail science class in second grade. Instead, I became that annoying kid who is constantly interrupting and asking questions, many of them largely irrelevant to what is being taught in class. By the time I graduated from elementary school, I had earned the nickname “el estudiante de las mil preguntas” (the student with one thousand questions). I was fortunate that my parents and many of my teachers in Mater Salvatoris elementary school and, later, in Colegio San Ignacio high school had the patience and the disposition to answer my endless barrage of questions.

There is such a thing as a stupid question. I know, because I have asked many of them. But asking questions, even questions that might seem stupid, is a critically important skill, particularly for a scientist. I admit it can be daunting—a question can expose one’s ignorance. But in scientific research, as in any form of learning, what one knows is a starting point toward the unknown. A question is the first step in a journey seeking to connect our brains to a broader network of knowledge.

Some questions lack answers. In research, knowledge serves as a platform on which one stands and, through questioning, staves against and recognizes the dark boundaries where our collective human knowledge ends. Those boundaries are the special places where scientific discoveries become most impactful, extending new paths toward the unknown. But to find those boundaries that haven’t been mapped, one cannot be afraid to ask questions and stare at the precipice of one’s own ignorance.

**OUTGROWTH**
In high school, some of my questions turned into scientific projects. For example, after learning that plantain sap, considered largely useless in Puerto Rico, was used in the Dominican Republic for treating tuberculosis, I developed a project that demonstrated the antimicrobial properties of the plantain sap. The project, rooted in my experience and surroundings, felt relevant and helped me see science as a tool of discovery and learning. I was hooked.

By the time I went to college at Harvard University, I was convinced that I wanted to be a scientist. I majored in biology, attended scholarly lectures by world-class scientists, and received well-meaning mentorship. Yet, in college, science again felt foreign and distant, and I struggled to connect.

In the enormous lecture halls where the basic science courses were taught, I found little space for what I enjoyed most in science: asking questions. My questions became casualties to the fast-paced, meat-grinder courses structured as filters for pre-med students. I joined a lab to gain research experience but felt lost in the concepts and intimidated by the environment. By the end of my sophomore year, I had performed mediocrely in my science courses, gotten fired from my lab as a tech, and started seriously wondering whether science was really for me.

The search for my place in science was an intellectual journey that eventually took me thousands of miles away from Cambridge, into the jungles of Central America. Working in collaboration with the Smithsonian Tropical Research Institute, I traveled in dugout canoes to remote Tawahka villages in Honduras and Emberá communities in Panama. I lived among indigenous groups and documented their use of medicinal plants and the rain forest. These experiences allowed me once again the flexibility to develop and ask my own questions. In the remote villages, I met many individuals without formal scientific training who were asking critical questions about the world around them. Science felt relevant again. These were important experiences that greatly influenced my development as a scientist and as a person, and they resulted in my first publication (Godoy et al., 1998). There, far away from any lab, in villages that lay completely off the grid and went entirely dark after sunset, I started to clearly see my path toward becoming a scientist.

**SYNAPTogenesis**
In the rain forests of Central America, I had the flexibility to formulate my own questions but lacked the training to push them forward. Science is an apprenticeship, and I needed a mentor to teach me how to transition from being a consumer of knowledge to being a producer of knowledge.

I also needed a role model. At a time of much self-doubt, I needed to see that people like me could contribute to science. So I reached out to the only Puerto Rican scientist I knew, Mariano García-Blanco. As a postbaccalaureate student in his lab at Duke University, I studied the nuclear architecture of cells undergoing organelle regeneration. I established a collaboration with Robert Singer’s lab at Albert Einstein College of Medicine and developed a protocol for fluorescent in situ hybridization in the alga *Chlamydomonas reinhardtii* (UNIacke et al., 2011). I discovered changes in the nuclear architecture of *C. reinhardtii* corresponding to transcriptional changes occurring during flagellar regeneration (Colón-Ramos et al., 2003b). I also discovered my interest in cell biology and decided to go to graduate school.

I joined the University Program in Genetics at Duke University and the lab of Sally Kornbluth. In Sally’s lab, I worked on the molecular mechanisms of programmed cell death and identified a viral family of proteins similar to Drosophila Reaper that induce apoptosis (Holley et al., 2002; Colón-Ramos et al., 2003a; Olson et al., 2003). I also made the surprising discovery that the proapoptotic protein Reaper and the viral proteins I identified regulate translation by directly binding to ribosomes and modulating ribosomal subunit assembly (Colón-Ramos et al., 2006). To answer these questions, I had to leave the comfort zone of the techniques I used regularly and set up collaborations that allowed me to establish in vitro systems to study ribosomal profiles and protein synthesis. It was a journey that resulted in internships in labs in Oklahoma and California. A critical skill I learned during this period was how to seek and establish meaningful collaborations that open up new areas of discovery.

By the end of my PhD, I decided to switch fields and do a postdoc in developmental neuroscience. I became interested in how the complex but organized architecture of neural circuits emerges during development to regulate behavior. Neuroscience had never been part of my formal training, but science is less about what you know and more about what you are willing to learn. I reached out to the network of mentors and peers I built during graduate school and used them as sounding boards to refine my interests, identify potential postdoctoral mentors, and learn about the outstanding questions in the neuroscience field. I then systematically approached (some would say pestered) labs working in model organisms and using approaches that linked cell biology and genetics with circuit connectivity and behavior.

**MATURATION AND PRUNING**
In breezy summer nights in the archipelago of Puerto Rico, leatherback turtle hatchlings can be seen racing across the moonlit beaches of the island of Culebra toward the surf. These turtles are born with an ancestral memory: they instinctively know to go toward the ocean. This behavior is wired into the leatherback turtle’s nervous system and was selected for by evolution. Once born, the hatchling
forms a new memory, one that it will carry throughout its lifetime. It will remember the beach on which it was born, and many years later, after traveling the world’s oceans, it will return to that same beach to nest. How does the leatherback turtle know to go to the sea upon hatching? How does it remember where it was born?

These were questions that I asked myself as a child, and they are directly related to the fundamental questions in neuroscience that my lab examines today. I joined Kang Shen’s lab at Stanford University to establish a system in the nematode Caenorhabditis elegans to study these questions. “A worm is only a worm,” said Diderot. “But that only means that the marvelous complexity of its organization is hidden from us by its extreme smallness.” C. elegans forms memories. For example, C. elegans does not have an innate preferred temperature and can instead remember the temperature at which it is cultivated (Hedgecock and Russell, 1975). The neurons that control this behavior and their connectivity are known (White et al., 1986; Mori and Ohshima, 1995), but how synapses are established and modified to form these memories is not. During my postdoc, I adapted markers that allowed me to inspect the cell biology of synapses during development. Using these cellular markers, I discovered a role for glial cells in specifying synaptic connections in vivo through Netrin signaling (Colón-Ramos et al., 2007).

Now, in my own lab at Yale (Figure 1), we are interested in understanding how synapses are assembled and maintained to build the neuronal architecture underlying thermotactic behavior, and how they are modified to store memories. We established collaborations to develop and use new instrumentation and approaches for better visualization of the events leading to correct synaptogenesis (Rankin et al., 2011; Wu et al., 2011, 2013a,b; Kumar et al., 2014; Christensen et al., 2015; Santella et al., 2015). We also collaborate to visualize neuronal activity and thermotactic behavior in C. elegans (Ha et al., 2010; Luo et al., 2014a,b).

Using these tools, we identified conserved mechanisms of neurodevelopment and synaptic assembly (Christensen et al., 2011; Smith et al., 2012; Stavoe and Colón-Ramos, 2012; Stavoe et al., 2012; Nelson and Colón-Ramos, 2013; Zhang et al., 2014) and unexpected roles for glia in establishing and maintaining synaptic positions (Shao et al., 2013). We also discovered a role for autophagy in synapse formation (Stavoe et al., 2016) and documented a metabolic subcompartment that powers synaptic function and animal behavior (Jang et al., 2016). All of our projects started by asking simple, fundamental questions, understanding how to break them down into solvable experimental problems and pioneering new approaches, by forging collaborations, that provide tractable ways of addressing our questions.

CIRCUITS AND NETWORKS
My journey into science has been one of searching for connections. Now, as part of the scientific network, I see connections everywhere.

Scientific ideas do not sprout in isolation. Scientific knowledge results from a robust network of influences and cross-pollination of ideas. Scientists influence one another through their publications, research talks, collaborations, and, of course, through teaching, training, and mentoring. How these networks of knowledge are wired influences who is connected to the world of science. It also influences who is kept out.

When I trained with scientists like Mariano García-Blanco, Sally Kornbluth, and Kang Shen, I synapsed onto this larger network of knowledge, one that extends back through time and links me to a global community of scientists. These networks were key in my education and training as a scientist and in my ability to do science today.

The scientific community produces knowledge with the aspiration that it will be consequential and influence the way that we understand the world around us. For science to fulfill this aspiration, the ideas and knowledge produced by scientists need to be accessible. Yet our networks are not necessarily representative of our aspirations, as they remain inaccessible to most. They are instead representative of the history of science, one that until recently, exclusively served a very narrow demographic. It is a history that affected E. E. Just as an African-American scientist (Manning, 1983). It is also a heavy-handed legacy that today influences who belongs in science, who benefits from the scientific enterprise, and who does not.

I frequently reflect on my role as a scientist in these networks of knowledge. These interests led me to found an organization called Ciencia Puerto Rico (CienciaPR; www.cienciapr.org). It is an online network of more than 7500 scientists, students, and educators who are geographically dispersed across 50 countries, but who are connected in their commitment to promoting scientific research and education in Puerto Rico (Guerrero-Medina et al., 2013). Wonderful things happen when you connect people’s minds. By crowd-sourcing essays that contextualize science for kids growing up in the tropical Caribbean, the CienciaPR community was able to produce a book that communicates to children in Puerto Rico that science is relevant to them (González-Espada et al., 2011). The book discusses the discovery of giant fossilized sharks in the Puerto Rican karst country. It talks about how parts of the Puerto Rican archipelago were “born” in the Pacific Ocean and shifted to the Caribbean basin through tectonic plate movements. It describes the resilient microbial communities that paint many colors in the salt flats of the Puerto Rican southwestern coast. The book is now being used in elementary schools in Puerto Rico.

FIGURE 1: Colón-Ramos lab in New Haven, CT.
My hope is that networks like CienciaPR sprout elsewhere. When developed carefully and deliberately, networks have the potential of extending the impact of our knowledge and discoveries. In doing so, they bring down structural and geographical barriers that have historically limited access to science. They also help scientists like me fulfill our responsibilities as points of contact between the production of knowledge and its dissemination, not to a privileged few, but to all.


ACKNOWLEDGMENTS

I am aware that I am able to follow my passion thanks to scientists like E. E. Just who contributed to extending the scientific networks of knowledge. To them, I am profoundly grateful. I acknowledge the support of my parents, my family, my teachers, and my friends (too many to name here) for always encouraging my passion. I acknowledge the Yale University Program in Multidisciplinary Research, the National Institute of Neurological Disorders and Stroke, and the Department for Cell Biology, led by de Camilli and Steve Strittmatter, for giving me the opportunity to work, please see www.ibiology.org/ibiomagazine/issue-10/daniel-colon-ramos-making-science-relevant.html.

REFERENCES


2016 ASCB Award Essays
MIG-10/Lamellipodin isoform and ABI-1 downstream from Netrin. Genes Dev 26, 2206–2221.
Dear microtubule, I see you

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ABSTRACT This essay summarizes my personal journey toward the atomic visualization of microtubules and a mechanistic understanding of how these amazing polymers work. During this journey, I have been witness and partaker in the blooming of a technique I love—cryo-electron microscopy.

FROM PHYSICS TO BIOLOGY AND FROM X-RAYS TO ELECTRONS
I was trained in physics (solid state) in Madrid after I decided that medicine was not for me. I had amazing science and math teachers in high school, all very powerful and inspiring women! When I was getting ready to graduate from college and thinking about my PhD, the hot topic in Spain was synchrotron radiation. With third-generation synchrotron sources sprouting all over the globe, it was the time of x-rays! The European Synchrotron Radiation Source was being built in Grenoble, and the Spanish government wanted to train Spanish scientists in this area, so it was relatively easy to get a fellowship to carry out my thesis work at the British synchrotron. Quite by accident, I ended up deciding to study biological macromolecules and tried a number of interesting self-assembly systems (the acrosomal bundle of the horseshoe crab sperm; clathrin cages and coated vesicles) before settling on tubulin.

My studies used time-resolved small-angle x-ray scattering (SAXS) to follow the assembly of tubulin in the presence of vinblastine, an antimitotic, anticancer agent, and the effect that temperature had on the aberrant, spiral-like polymers that vinblastine induced (remember that, in the absence of drugs, the in vitro assembly of tubulin into microtubules can be controlled by temperature). At the time, computational analysis of SAXS scattering curves was limited, and part of our interpretation of the temperature changes needed help from a more direct visualization of the structures. This was why I first used cryo-electron microscopy (cryo-EM), helped by Dick Wade, who was visiting Daresbury to work with the computational group on his model of microtubule lattice accommodation (of all things!) (Wade et al., 1990). At the time (ca. 1990), cryo-EM was in its infancy. A practical method for vitrification of a protein solution had just been developed a few years before by Jacques Dubochet (Lepault et al., 1983). And 1990 was the year that Richard Henderson published the atomic model of bacteriorhodopsin using electron crystallography (Henderson et al., 1990). But the idea that atomic structures would one day be obtained by cryo-EM analysis of frozen-hydrated samples in solution (i.e., single-particle studies) was then remote.

FROM AN ABERRANT POLYMER TO THE STRUCTURE OF TUBULIN
The biggest stroke of luck in my scientific career was to meet Ken Downing at the Lawrence Berkeley National Laboratory and to join his lab for my postdoctoral studies. Ken had been a player in the electron crystallographic studies of bacteriorhodopsin and was starting to use this methodology to study yet another aberrant polymer of tubulin. In the presence of zinc, tubulin assembles into straight protofilaments resembling those in microtubules. But while the natural polymer is formed by the parallel association of ~13 protofilaments
to form a hollow tube, zinc makes protofilaments associate in an antiparallel way to form sheets. For all extents and purposes, these sheets can be considered small two-dimensional crystals, and therefore perfect samples for electron crystallography. Having worked with Taxol as a microtubule stabilizer during my PhD, I added this drug to the zinc-induced sheets and found that it also had a stabilizing effect. After about four years of data collection and analysis, my postdoc colleague Sharon Wolff and I obtained the first structure of tubulin in an assembled form and bound to one of the most broadly used anti-cancer agents (Nogales et al., 1998b).

The structure showed that tubulin is not a classical GTPase (Nogales et al., 1998a), provided the Taxol-binding site, explained the different nucleotide exchange properties of unassembled αβ-tubulin dimers and microtubules, described the longitudinal interaction between tubulin subunits along a protofilament, and explained the coupling of assembly and GTP hydrolysis central to the dynamic behavior of tubulin (discussed later) (Nogales et al., 1998b; Lowe et al., 2001). However, it told us nothing about how protofilaments came together to form the cylindrical microtubule. For that, we needed the structure of the bona fide microtubule.

At the time, the groups of Linda Amos, Ron Milligan, and Dick Wade were making significant progress in the cryo-EM study of different kinesins bound to microtubules (Arnal et al., 1996; Hirose et al., 1996; Sosa et al., 1997). (The x-ray structure of the kinesin motor domain was then fresh from the oven [Kull et al., 1996].) The resolution of those cryo-EM structures was typically 25 Å, but the use of hybrid methods to place crystal structures of components into the EM map was very powerful to define interfaces and interpret relative motions. We teamed up with Milligan and used his 20 Å map of the microtubule (Figure 1A) to “dock” the electron crystallographic structure of the protofilament into it. As a result, we were able to produce a model of the microtubule that positioned the different structural elements in the tubulin molecule with respect to the outside and the lumen of the microtubule, and we identified potential structural elements involved in lateral interfaces (Nogales et al., 1999).

This work landed me a job as assistant professor at University of California, Berkeley (1998), so I did not have to move very far! As an independent investigator, I continued to use EM. By then, single-particle cryo-EM, pioneered by Joachim Frank, was gaining momentum, and I started using it to study GDP-bound tubulin rings, an assembly form that mimics the rams’ horns of bent protofilaments seen at the end of depolymerizing microtubules. This methodology also allowed me to start the second line of research in my lab, the study of the eukaryotic transcription initiation machinery. But that is another story.

**FROM BLOBS TO ATOMS IN THE VISUALIZATION OF MICROTUBULES**

The regime of 20–30 Å resolution in cryo-EM is familiarly known as “blobology.” Improving the resolution of cryo-EM maps of microtubules beyond blobs required the use of high-end instruments and the merging of large data sets. A major breakthrough came with Ken Downing and Huilin Li’s visualization of the structure at ~10 Å resolution in 2002 (Li et al., 2002). Ten years later, many cryo-EM studies of microtubules, alone or decorated with a number of motors or other associated factors, had provided a richness of biological information, but none of these microtubule structures had broken the 8 Å resolution barrier (Sindelar and Downing, 2010; Sui and Downing, 2010; Alushin et al., 2012; Maurer et al., 2012; Redwine et al., 2012).

It was at this point that two brave souls in my lab, postdoc Gabe Lander and graduate student Greg Alushin, decided to go all out in a joint effort to break this resolution curse. They developed and implemented a reconstruction scheme that used helical/single-particle hybrid reconstruction methodology developed by Ed Egelman and that took advantage of the pseudohelical symmetry of the microtubule, while still accounting for the presence of the so-called seam (discussed later). As a result, we produced better than 5 Å resolution structures, a new record at the time (Alushin et al., 2014). These studies used data collected on film, the best detection media for high-resolution cryo-EM studies available to us at the time. And then the revolution happened.

In cryo-EM images, resolution is limited by poor signal due to the intrinsic low contrast of proteins on a water background and the need to use low doses to minimize radiation damage. Historically, the problem was made significantly worse by charge-coupled device (CCD) detectors, which introduced noise and lowered the signal across the whole resolution spectrum. New direct electron-detection technology has recently dramatically improved the contrast

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**FIGURE 1:** Microtubule and tubulin structures then and now. (A) A 20 Å cryo-EM density map (gray mesh) shown for three protofilaments, with the alpha trace of the docked electron crystallographic structure of the protofilament shown in green, except for one tubulin dimer highlighted in blue (modified from Nogales et al., 1999). (B) A 3.5 Å cryo-EM map of one αβ-tubulin dimer segmented from the map of full microtubules shown in C. α- and β-tubulin are shown in lighter and darker blue, respectively (modified from Zhang et al., 2015).
in cryo-EM data, resulting in an ever increasing number of atomic structures for molecules or assemblies that were considered structurally unreachable or highly challenging.

In our most recent studies, postdoc Rui Zhang has used a direct electron detector and improved data-processing strategies to obtain cryo-EM reconstructions of microtubules at 3.5 Å or better resolution (Figure 1B; Zhang et al., 2015). This journey has taken us from blobs to atomic models for the full microtubule. Among other things, we can now see, for the first time, the details of the lateral contacts between protofilaments, the stitches that hold together the microtubule lattice (Figure 1C).

FROM THE BASIS OF DYNAMIC INSTABILITY TO UNDERSTANDING ITS REGULATION

Essential to most microtubule functions is the phenomenon of dynamic instability, a property first described by Mitchison and Kirschner in 1984. Microtubules switch stochastically between phases of slow growth and rapid shrinkage, a metastable behavior powered by the energy of GTP hydrolysis. A mechanistic understanding of this process requires a detailed description of the structural changes that accompany GTP hydrolysis in the microtubule. The present cryo-EM methodology has allowed us to obtain atomic models for microtubules bound to GMPCPP (a slowly hydrolyzable GTP analogue), to GDP (after GTP hydrolysis has taken place), and to GTPγS. Comparison of these states shows that hydrolysis results in a conformational change in α-tubulin and a compression of the dimer–dimer longitudinal interface along protofilaments that generates tension in the lattice (Zhang et al., 2015). Interestingly, hydrolysis has a negligible effect on lateral interfaces, with the notable exception of the “seam.” The seam is the special lateral contact between protofilaments in the microtubule that involves heterotypic contacts (α–β and β–α), instead of α–α and β–β, and is thought to be involved in microtubule closure. By taking advantage of the higher contrast afforded by the direct electron detector, we have determined that the position of the two protofilaments involved in seam contacts deviates from the cylindrical shape of the rest of the tube (Zhang et al., 2015) and that this deviation is larger after GTP hydrolysis.

Cryo-EM is also now providing us with the atomic details of the interactions microtubules establish with their associated cellular factors. Microtubule-associated proteins (MAPs) can regulate microtubule dynamics and organization and thus are critical to their essential cellular functions. We have visualized in atomic detail the interaction with microtubules of the +TIP protein EB3. EB proteins track growing microtubule ends by recognizing a particular microtubule structure, and also directly regulate microtubule dynamics. Our structures provide a mechanistic understanding of how EB3 recognizes an intermediate state following hydrolysis, and how EB itself promotes GTP hydrolysis within the microtubule (Zhang et al., 2015). With the myriad of MAPs that interact with and regulate microtubule function, our bucket list of studies is a long one.

THE JOURNEY SO FAR AND THE JOURNEY AHEAD

It is a rare thing in science to witness how your field of research undergoes a revolution of mind-blowing proportions. This is where I feel I am in my scientific career. I have been using cryo-EM since my PhD, and I have seen it grow and evolve at a consistent pace for more than two decades while I pursued the structural characterization of the microtubule. Cryo-EM practitioners steadily pushed the limits of resolution and applicability and along the way generated beautiful structures, landmarks of technical achievement, and new biological insights. But while all of us in this field knew that our technique was only going to get better, the explosion of results we have witnessed in the last few years and, even more, the potential that now has become all too apparent, has caught most of us by surprise. Our heads are spinning and the possibilities seem almost limitless. Keith Porter, an accomplished and pioneering electron microscopist, would have uniquely appreciated the exceptional moment we are experiencing in the visualization of macromolecular structures. He would be particularly excited about the potential to bring molecular resolution to the realm of cell biology, which we are expecting will be the next landmark of cryo-EM. It is a wondrous and exciting time, and there is little doubt in my mind that the cryo-EM revolution will lead to a bright new vision of how the cellular machinery works. You will see.

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REFERENCES


Thinking in three dimensions: discovering reciprocal signaling between the extracellular matrix and nucleus and the wisdom of microenvironment and tissue architecture

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ABSTRACT I thought long and hard whether I could avoid talking about family and personal life, and just share the excitement of being a scientist and how science continues to sustain us all. But so many people, especially younger scientists, want to know—and always ask—How did you do it? A woman from Iran, a Middle Eastern country and essentially Muslim, now considered backwards and misguided if not downright scary, traveling very young and alone to the United States, finishing college and graduate school together with having children, first-year graduate school and second-year post doc—years ago, going against a number of entrenched dogmas, and yet succeeding against many odds and obstacles, and all the while on soft money? Below is my personal narrative answering some of these questions.

If there is one generalization that can be made from all tissue and cell culture studies with regards to the differentiated state, it is this: Since most, if not all, functions are changed in culture, qualitatively and/or quantitatively, there is no constitutive gene expression in higher organisms; i.e. the differentiated state is unstable and the (micro)environment regulates gene expression.

Mina J. Bissell
International Review of Cytology, 1981

That was then….After more than 35 years of probing, I know this to still be true.

But now I feel a huge sense of responsibility and awe: the honor of winning the E. B. Wilson Medal is truly humbling. There are scores of deserving individuals who have spent their lives to discover the secrets of the cell and to enlighten and educate with generosity and kindness. My heartfelt thanks to all who provided moral support and funding, especially in early days of my career, and to those who kept an open mind and considered my laboratory’s contributions seriously. To my family, who may have made peace with my constant grant writing and work ethic, and still seem to like me! To my many collaborators, especially to my fellows and students for their hard work, passion, and original ideas, and also for putting up with my excessive mentoring and in the process teaching me many things themselves. This singular honor is possible because of you. For students and fellows who are willing to be mentored still: If you are reading this, I hope you will go back and read many more essays by Wilson medalists since each of us is unique; we choose to advise and mentor in our own ways and this is the way it should be.

For all of you, and also for myself, I will continue to teach that we must be bold and speak without fear, exposing mediocrity, injustice, and greed, and questioning conclusions and mindless authority. I will continue reminding myself and others that scientific results are not written in stone: well-designed experiments and unexpected data that lead to new paradigms, and maybe even beget medals and prizes, sooner or later will have to be reexamined as we become wise enough to admit how much more remains to be discovered. I believe deeply that the pull and the beauty of science is its humbling complexity, which leaves no room for arrogance, and that looking at new data with unbiased eyes and awe is the sacred duty of science and scientists.

WHAT ARE THE QUESTIONS TO ANSWER AND WHY?
I thought long and hard whether I could avoid talking about family and personal life and just share the excitement of being a scientist

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Abbreviations used: AFME, American Friends of the Middle East; ECM, extracellular matrix; STEM, science, technology, engineering, and mathematics; TME, tumor microenvironment; UCSF, University of California–San Francisco.
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and how science continues to sustain us all.

But so many people, especially younger scientists, want to know, and always ask, “How did you do it? A woman from Iran, a Middle Eastern country and essentially Muslim, now considered backward and misguided, if not downright scary, traveling very young and alone to the United States, finishing college and graduate school together with having children in your first year of graduate school and during your second postdoc year, going against a number of entrenched dogmas, and yet succeeding against many odds and obstacles, and all the while on soft money?” First, a few facts: Iran used to be called Persia, which confuses many in the United States, who also don’t know that Iranians are not Arabs and before the seventh century were Zoroastrians—one of the first monotheistic religions before Christ—and the name has been Iran for thousands of years—it means “the land of Aryans.” It was Alexander the Great who brought his army to the capital of Iran in the province of Pars (hence Persia) and burned the Persepolis, the palace of the kings of Iran.

So, when people ask, “How?!” the answer I give is: “Badly!” I am not being coy when I say that. Almost no one does it perfectly or even well, but the trick is to choose what you love to do and persist! I persisted. But I believe it is precisely because of my background in Iran that I could persist in the era of many odds and obstacles, and all the while on soft money?!”

COULD THERE BE ONE OR MORE LESSONS HERE FOR ALL OF US, ESPECIALLY OUR POLITICIANS?!

My father believed all religions are sources of much exploitation, wars, and misery. He held either we were reasoning humans, under- stood our responsibilities to our fellow humans, including our families and ourselves, or we weren’t! He also believed that, whereas some clergy all over the world do “good” (as did his father, and now best exemplified by the magnificent current pope), most did more harm than good. He would debate my sister and me across the lunch table and would tell us we could join any profession we wanted as long as we could maintain our integrity. He was a fantastic orator and was known to defend people from all levels of society if they were victims of injustice or victims of the regime in charge at that moment! This meant helping both the Shah’s enemies and the Shah’s supporters, even if they were on opposite sides! In retrospect, my father was one of my early heroes and the reason I have lived my life the way I have. One of my mentoring points to mothers of sons and young men: if fathers believe in their daughters and have high expectations for both sexes, many of their daughters will succeed in having productive and satisfying lives.

On the maternal side, there was less interest in scholarly pursuits and no interest in politics, but one aunt was a U.S.-educated medical doctor and the chair of immunology at Tehran University, and the other was in public health; one uncle was in government, and the other was a professor of mathematics in the United States. The only member of this extended family who had not finished college was my mother. Her father was one of my early heroes and the reason I have lived my life the way I have. One of my mentoring points to mothers of sons and young men: if fathers believe in their daughters and have high expectations for both sexes, many of their daughters will succeed in having productive and satisfying lives.
higher education and become independent no matter what. I think, even without reading Virginia Woolf’s *A Room of One’s Own* (1921) (a book to read by both sexes, even though it was written in the 1920s), she had arrived at the same conclusion: “For a woman to succeed, she must have a room of her own and 500 pounds a year” (Bissell, 1981). Hence, one of my other key mentoring refrains is: there is huge dignity in work and earning your own living.

**CHILDHOOD**

I had a happy childhood despite one very traumatic experience. I was born with what could be called a photographic memory for written and spoken words, a trait that surprised some grown-ups, who kept testing me for their amusement. This made me uncomfortable and separated me from my many cousins and friends in school. However, I lost quite a bit of this “gift” at age 8, being a bit of a tomboy, climbing trees and engaging in sports and other unseemly behavior for girls in those days. While jumping over a high bar set between two columns with nothing but hard ground underneath (a contrivance of one of my boy cousins who was 15 years old), I fell hard and was bedridden for months but survived and recovered against all odds. The result was essentially complete loss of hearing in my left ear and loss of most of my photographic memory. Despite the injury, I felt better integrated after recovery! Persians have a saying that I often still repeat: Enemy can bring “good,” if goodness is willing!

My mother, who I adored, never seemed to be interested in books and politics but understood quite early that I would be able to amount to something when I grew up; she also understood how easily I would get hurt and how easily I cried when there was any kind of perceived injustice or unkindness, and she felt I needed some protection. (I used to be teased mercilessly by my sister, who looked up to and loved and who is still my best friend, and cousins alike, who would make me cry by making fun of my crying!) I liked nothing more than hiding and reading books, and she tried to help me become more integrated socially, including sending me to a ballet class (although I was a bit too old at 10). I loved it—even if I never could do the split no matter how hard I tried! Nevertheless, it actually allowed me to become more at ease, and made me more open, socially and otherwise. When talking about the significance of form and function in my studies, I often show slides of dancers and quote Yeats’s “Among School Children,” as I did in a TED Talk: “How can you tell the dancer from the dance?” If you decide to have kids, try harder with them if they are loners, and don’t be a loner yourself; there is wisdom in group activities, in scientific collaboration and team sports: we are born to be social creatures, and almost all of us have inherited the capacity for empathy from our ancestors and fellow creatures (de Waal, 2013).

**SCHOOL YEARS AND MAKING A FAMILY**

I loved school; it was effortless, and I was a top student in my primary and secondary schools for 12 years. In the last year, in the countrywide exams, I became the top student in the country. I was not particularly “special”—everyone is special—but I was lucky in the (micro)environment of my childhood. There are scores of children all over the world who could and will do many things better than I did in all areas if they have the opportunity. I received a medal and award from the Shah of Iran (there are a couple of pictures of me with the Shah, with me getting the medal and a book), and my fellowship for going abroad was not from a college in the United States but was earned by taking an exam for a special award given to five students in science, technology, engineering, and mathematics (STEM) fields in that year in Iran. My father felt the United States was too young a country to educate women, but I wanted to go to the United States, and as I said earlier, my grandfather—the Ayatollah Haeri Mazandarani—explained to my father, “She has earned it, she is good, and she deserves to go wherever she wants!” My father consented. I applied to a few universities in the United States by sending applications to the American Friends of the Middle East (AFME)—a branch of the American government—where we had to send my application for a U.S. visa. When the head of AFME saw my application, he called my father (who spoke reasonable English and, of course, French) and asked for me to come see him. He suggested I should go to Bryn Mawr, one of the Seven Sister colleges in the United States (analogous to the seven Ivy League schools, which then accepted only male students), since to his mind—and later to my mind—Bryn Mawr was and remains one of the best colleges in the United States. I knew nothing about the place, and luckily I was never asked to take the college entrance exam! At this point, my English was quite elementary, but they let me in!

After a short stay in New York City to take a perfunctory English course, arranged with the help of my uncle, the math professor, I spent an enchanting two years at Bryn Mawr. Math and chemistry were easy for me, due to my high school classes. But I did not enjoy English and Arabic at all in high school, partly because, apparently along with the hearing loss in my left ear, I had also damaged the “nomic gland” behind the left frontal lobe and had difficulty even then remembering names, places, or anything to do with the left side of the brain that I had to memorize. But I liked literature in Iran, and fell in love with English literature, because I love well-written words. I had an amazing and brilliant freshman English professor, Ann Berthoff. To this day, she is my favorite teacher and at age 93 is one of my most beloved friends. I debated between majoring in English literature or chemistry, and the latter won out, mainly for practical reasons. I will always remain grateful to the head of the AFME, the gentleman who cared enough and took the time to meet up with my father and me, and showed me the way. I never learned his name, since he died shortly after I had left for the United States, and I also found out much later from American newspapers that the AFME was a front for the CIA and behind a coup that toppled Mosadegh, then the most popular, beloved, and democratically elected prime minister Iran had ever had. For the young: politics matter in a democratic society. Get involved if you want to keep democracy alive—please vote! If you want to understand the times we live in now, please read and understand the history of different regions more deeply, particularly that of the Middle East.

In my sophomore year in college, in short order, I met a graduate student from Harvard University who was Iranian and was getting a PhD in political science and economics; he proposed and I said yes. I declared chemistry as my major, moved as a junior to Radcliffe/ Harvard University, won the Medal from the American Institute of Chemists as a junior, married in my senior year, and ended up being a vanishing “Cliffe,” since I received my bachelor’s degree in chemistry as one of the inaugural members of the “integrated” Harvard class along with people like David Botstein. The degree was written in English. I had hoped for a classy Latin!

My husband was still a graduate student, so I opted to stay in the area to get a PhD in bacterial genetics from Harvard Medical School. Do not ask why—I am not sure I know myself! No sooner had I started graduate school than I got pregnant. This was eons ago, and at that time, there were 200 men and three women in the Harvard Medical School class that year, and the entire place had one woman faculty member. In the entering class in bacteriology, there were three men and three women (the latter all Clifffes!). Despite this early balance, after the first year, all three men left—they found HMS to be too ingrown, too arrogant toward “outsiders,” and too wanting in
ADVENTURES IN VIROLOGY AND CELL AND CANCER BIOLOGY: DISCOVERING THAT HALF THE SECRET OF THE CELL IS OUTSIDE THE CELL!

Based on the existing literature, a model is presented that postulates a “Dynamic Reciprocity” between the extracellular matrix (ECM) on the one hand and the cytoskeleton and the nuclear matrix on the other. The ECM is postulated to exert physical and chemical influences on the geometry and the biochemistry of the cell via transmembrane receptors so as to alter the pattern of gene expression by changing the association of the cytoskeleton with the mRNA and the interaction of the chromatin with the nuclear matrix. This, in turn, would affect the ECM, which would affect the cell, which …

Bissell et al., Journal of Theoretical Biology, 1982

When I wrote the above article, even my close colleagues who were my friends asked what I was smoking! I had no intention of just postulating; my students and fellows (and often many undergraduates) and I worked long and hard for more than three decades, essentially on one organ, the mammary gland, to unravel how the extracellular matrix (ECM) may talk to the nucleus and vice versa. The majority of the critics have come around, and the number of papers in the area of tumor microenvironment (TME) has increased logarithmically. The model I proposed—not the name, but the concept of ECM regulating gene expression at many levels and the roles of basement membrane and tissue architecture—is here to stay. Many are working in these areas all over the world. Dozens of our own papers have shown the significance and mechanism of interaction between the ECM and cytoskeleton and the nuclear matrix and chromatin. We showed in the early 1990s that the promoters of milk protein genes contained ECM-response elements and that loss of interaction of laminin 111 with integrins leads to loss of milk function and induction of apoptosis. In the presence of laminin-rich gels (a 20% pure laminin 111 and rat-tail collagen gel can substitute for Matrigel) and certain oncogenic pathways, inhibitors can “revert” the malignant cells to a normal phenotype, despite the tumorigenic genome. Together with a number of outstanding collaborators, including Zena Web (UCSF), Ole William Petersen (Copenhagen), Cathy Park (UCSF), and more recently David Lyden (Weil Cornell Medical College), we have explored many aspects of mammary gland morphogenesis, function, and homeostasis and breast cancer and exosomes, and have dared challenge other paradigms.

My apologies for short-changing the science I so love. But I have used up my print space here. There are, however, more than 30 interviews and write-ups listed on my website (www2.lbl.gov/LBL-Programs/lifesciences/BissellLab/main.html), a few of which I note in the reference list (Vaughan, 2002; Abbott, 2003; Flintoft, 2003; Friedrich, 2003; Graebner, 2003; Bissell and Devine, 2005; Bonetta, 2005; Novak, 2005; Pon, 2005; Cohen, 2006; Klein, 2006; Mason, 2006; Schuldt, 2006; Ary, 2007; Blow, 2007; Mervis, 2007; Platoni, 2007, 2008; Shekhar, 2007; Wong, 2007; Beashon, 2008; Marx, 2008; Fleischman, 2009; Kolata, 2009; Lako and Daher, 2009; Short, 2009; Yann, 2009; Hayden, 2010; Zagorski, 2010; Claiborn, 2011; Bissell, 2015). There are also scores of write-ups on our publications and press releases in the Lawrence Berkeley National Laboratory archives (Today at Berkeley Lab, https://today.lbl.gov), and then there are more than 400 publications from our laboratory (also on the website).

I have been asked a few times: “Do you have any regrets?”

But of course! Even though I look at the bright side more often, and I am blessed with much, it takes extreme indifference and arrogance not to have regrets. Indeed, only idiots have no regrets! So I would like to leave you with a sentiment that has resonated with me deeply. It is in a tiny, tiny book, may be even still on the Web. It is the text of a convocation address to a graduating class at Syracuse University given by George Saunders. It brought tears to my eyes, and I envied him for putting it so well. I quote: “Here’s something I know to be true, although it’s a little corny, and I don’t know what to do with it: What I regret most in my life are failures of kindness” (Saunders, 2014).

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Science, who not only was one of a very few scientists in granting agencies who was open to unorthodox ideas, but was modest, intuitive, and kind—in short a hero to many! He was willing to take chances. He also understood concepts and the significance of what we were trying to do. He overruled a number of scientists in his division and allowed our grant to be reviewed in the Post Genome Committee. The grant received the top score and was funded until the program was discontinued. It took 20 years to receive similar scores and acceptance as well as a Merit Award from the NCI. The Merit Award never materialized, because the program was abolished altogether at the NCI, even those few of us who had already been granted the Merit Award by the council were denied.

REFERENCES
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