The microenvironment matters

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ABSTRACT **The physical and biochemical properties of the microenvironment regulate cell behavior and modulate tissue development and homeostasis. Likewise, the physical and interpersonal cues a trainee receives profoundly influence his or her scientific development, research perspective, and future success. My cell biology career has been greatly impacted by the flavor of the scientific environments I have trained within and the diverse research mentoring I have received. Interactions with physical and life scientists and trainees and exposure to a diverse assortment of interdisciplinary environments have and continue to shape my research vision, guide my experimental trajectory, and contribute to my scientific success and personal happiness.**

NURTURING NATURE

I am honored to receive the Women in Cell Biology Sustained Excellence in Research Award. I am delighted to be part of a vibrant and supportive cell biology community. I recognize that I am the fortunate recipient of this prestigious award because of the mentoring and encouragement I have enjoyed throughout my career and the group of superb trainees with whom I have had the pleasure to work with.

My career trajectory has not always been straightforward. I grew up as part of an extended, working-class family in northern Ontario, Canada, where the only educational expectation placed on a young woman from my background was to acquire practical skills to secure a well-paying job that could supplement the family income if required. However, as fate dictated, I was born with an insatiable curiosity and an inquiring nature that both shocked and perplexed my parents. In hindsight, the mad disassembly of dolls, melting of cosmetics, and dragging home of various skeletons and insects hinted at the beginnings of a scientist. Fortunately, this "research" potential was recognized by a series of teachers and colleagues who encouraged me to attend university and to pursue graduate studies.

Valerie Marie Weaver

DISCOVERING PASSION: SEED AND SOIL

Graduate school was a revelation to me. For the first time, not only was I able to indulge my desire to learn and appease my curiosity, but at last I had discovered an environment in which I could express my creativity and challenge my intellect. My doctoral studies in biochemistry, made possible by two graduate scholarships, were completed at the University of Ottawa, where I studied vitamin D metabolism and the pathophysiology of vitamin D deficiency with J. E. Welsh. During my thesis studies, I was immersed in a community involved in a wide range of research, including work on brown fat metabolism, developmental apoptosis, enzymology, lipid biochemistry, and protein crystallography. Strong ties between the Departments of Biochemistry and Cell Biology ensured that I was also exposed to an array of cell biology research. This diverse scientific portfolio instilled in me an

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Abbreviations used: 3D, three-dimensional; DOD BCRP, Department of Defense Breast Cancer Research Program; ECM, extracellular matrix; IME, Institute for Medicine and Engineering; MEC, mammary epithelial cells; NIH NCI, National Institutes of Health–National Cancer Institute; NRC, National Research Council; rBM, reconstituted basement membrane.

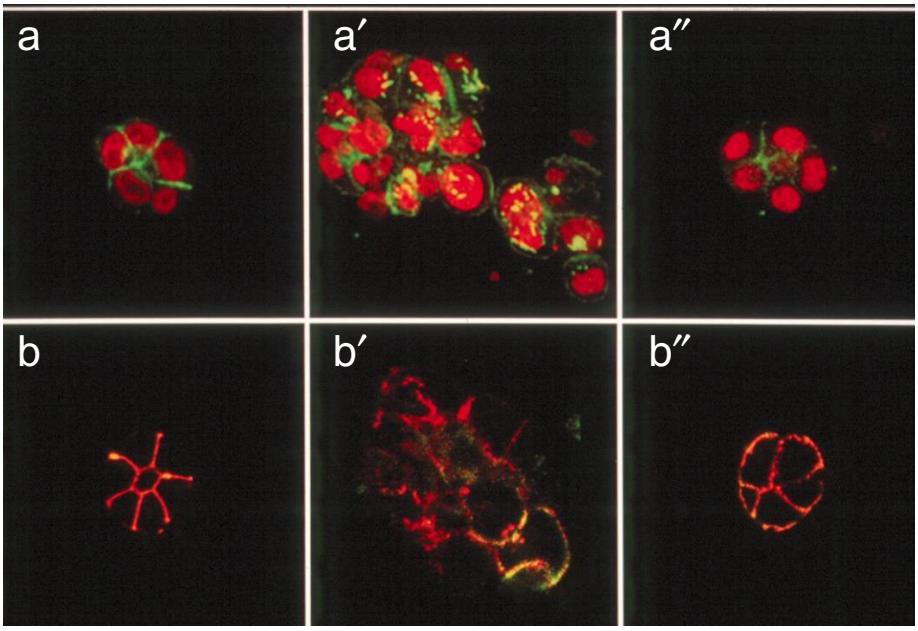

FIGURE 1: Phenotype dominates over tumor genotype. β1-inhibitory antibody treatment of tumor cells leads to the formation of reverted acini. (a–a″) Confocal fluorescence microscopy images of F-actin: both the nonmalignant HMT-3522 S-1 (a) and its malignant cell derivative T4-β1 reverted acini (a″), showed basally localized nuclei (propidium iodide), and organized filamentous F-actin (fluorescein isothiocyanate), while the tumorigenic HMT-3522 T4-2 mock-treated colonies (T4-2 immunoglobulin G) formed disorganized, hatched bundles of actin and pleiomorphic nuclei (a′). (b–b″) Confocal immunofluorescence microscopy images of E-cadherin (FITC) and β-catenin (Texas Red): in S-1 (b) and T4-β1 reverted acini (b″), E-cadherin and β-catenins were colocalized and superimposed at the cell–cell junctions. (©Weaver VM *et al.*, 1997.Originally published in JCB. doi:10.1083/jcb.137.1.231. Reproduced with permission from Weaver *et al.*, 1997.)

appreciation for the sheer range of biological questions being asked and the various perspectives and approaches available to test them. Equally important during my training were my interactions with a variety of successful female scientists, which helped me to visualize myself as an independent academic investigator.

Toward the end of my graduate studies, I attended the first apoptosis workshop held at the Federation of European Biochemical Societies meeting in Budapest, Hungary, where I met several prominent investigators studying apoptosis and programmed cell death. Apoptosis research was in its infancy, and the ideas discussed at this meeting sufficiently impressed me that I decided to join the laboratory of Roy Walker and Marianna Sikorska at the Canadian National Research Council (NRC) to study links between higher-order chromatin structure and apoptosis regulation. My work at the NRC convinced me that a key regulator of apoptotic decisions in cells was its interaction with the extracellular matrix (ECM). It was during this time that I heard Mina Bissell present at the Canadian Federation of Cell Biology in Windsor, Ontario, on the importance of the ECM in mammary tissue behavior. Fortunately, when I inquired about the possibility of joining Mina's group, she looked at me intently and immediately agreed. Had I realized that she had just turned down several applicants, I may not have been so confident.

LIFE IS WHAT HAPPENS WHILE YOU ARE BUSY MAKING OTHER PLANS

Within the first few months of my starting graduate school, my father passed away from a terminal brain tumor. Midway through my graduate studies, after having successfully passed my qualification exam, I embarked on a short "celebratory" skiing holiday with friends in northern Vermont. While traveling to the ski hill one day, I was involved in a horrible car accident that resulted in a broken back and broken legs and hands and ribs, which generally left me pretty bashed up. Needless to say, these two events had a big impact on my life. However, while both traumas certainly, at least temporarily, impeded my thesis research work, they also instilled in me an appreciation for the personal advantages that I enjoyed and gave me a strong resolve to take full advantage of the opportunities provided to me and to live life to the fullest. Therefore, much to the dismay of my senior colleagues, as soon as my settlement funds arrived, I bought a ticket to West Africa with a return from India. Before relocating to Berkeley to train with Mina, I spent six months traveling and meeting people across Africa and Asia. However, despite what could be interpreted as a lackadaisical attitude to science, I have absolutely no regrets about my decision to take a break and explore the world. Not only was that traveling adventure enlightening and one I shall never forget, but the experience broadened my perspective and put my own life experiences into better perspective, and importantly, they renewed my desire to pursue a research career.

EXPANDING HORIZONS

Joining the Bissell laboratory was a turning point and another major life-changing event. In Mina's group, I was quite literally surrounded by an enthusiastic group of intelligent postdoctoral fellows and students who were completely engaged in their research and, indeed, in the world in general. The atmosphere in the Bissell laboratory was highly energized and one in which Mina encouraged everyone to think unconventionally and expand their scientific perspective(s). Not only did I learn about the mammary gland and the ECM, but I grew to think more critically and outside the conventional box. Ideas were bandied about freely, and laboratory meetings were lively events during which discussions served to expand my research vision and foster my love of science and amazement at the beauty and elegance of cell biology. My research with Mina followed up on an article she had recently published with Zena Werb and Nancy Boudreau, in which they showed that, in the absence of integrin engagement by the ECM, normal mammary epithelial cells (MECs) underwent apoptosis (Boudreau *et al.*, 1995). I was greatly intrigued by these findings and wanted to use my prior apoptosis experience to expand upon this work as well as on studies by Tony Howlett showing that transformed breast cells resist apoptosis even in the absence of ECM cues (Howlett *et al.*, 1995). In collaboration with Ole Petersen in Copenhagen, I established a human breast tumor progression series and set about clarifying why tumors no longer died in the absence of ECM ligation (Weaver *et al.*, 1995, 1996). What I observed, quite unexpectedly, was that not only did the malignant derivatives in this tumor series not die when I blocked the activity of the major ECM receptor β1 integrin, but the tumor cells

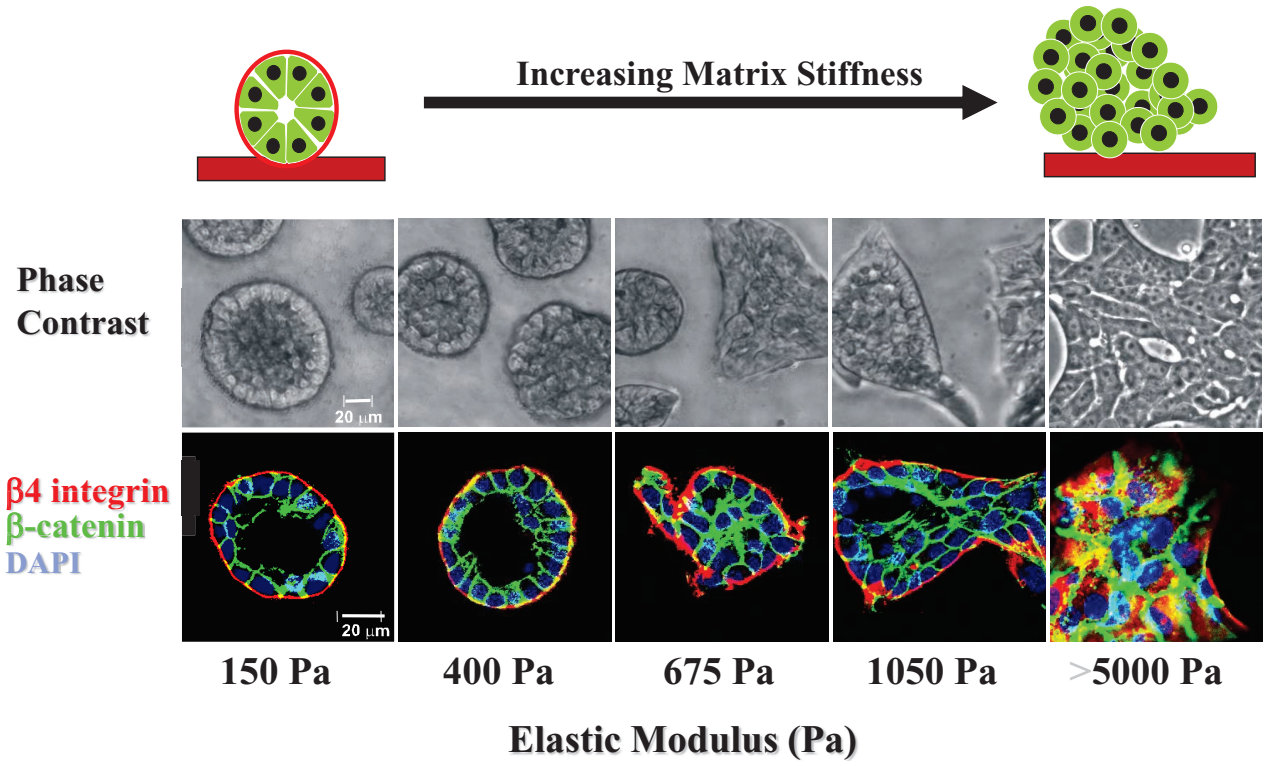

FIGURE 2: The importance of tissue context: ECM stiffness modulates mammary tissue morphogenesis. MEC growth and morphogenesis are regulated by matrix stiffness. Phase-contrast microscopy and confocal immunofluorescence images of nonmalignant MECs grown for 20 d on top of polyacrylamide gels of increasing stiffness (140–5000 Pa) conjugated with reconstituted basement membrane (rBM) and overlaid with rBM to generate a 3D rBM ECM microenvironment. Findings showed that increasing ECM stiffness enhanced MEC growth, as revealed by an increase in colony size and disrupted tissue organization indicated by aberrant tissue margins and invasive structures (phase-contrast images: top panels). ECM stiffness also progressively disrupted tissue morphology, as indicated by disrupted cell–cell localized β-catenin (green) and loss of basally localized (α6)β4 integrin (red) with nuclei costained with 4′,6-diamidino-2-phenylindole (DAPI; blue) (confocal images: lower panels). (Reproduced with modification and proper permission obtained from Elsevier as published in Paszek *et al.*, 2005.)

phenotypically reverted, ceased to grow and invade, and instead assembled a three-dimensional (3D) differentiated tissue structure or "acini" (Figure 1; Weaver *et al.*, 1997). They were also no longer tumorigenic when injected in vivo (Weaver *et al.*, 1997). This was the first of many "humbling" experiences I have experienced throughout my professional career regarding the importance of context and the impact of tissue structure on cell phenotype. I can honestly say that I haven't looked back since that first experience. In the years following my first observation, I was involved in a series of collaborative studies in which I worked with colleagues in the Bissell group to study the impact of 3D and tissue organization on receptor signaling, nuclear architecture, and apoptosis (Lelievre *et al.*, 1998; Wang *et al.*, 1998; Weaver and Bissell, 1999; Weaver *et al.*, 2002; Rizki *et al.*, 2008).

AN INTERDISCIPLINARY ENVIRONMENT

Bolstered by my success in Berkeley, and consistent with the interdisciplinary ethos fostered during my sojourn at Lawrence Berkeley National Laboratory, I secured a faculty position in the Pathology Department and gained membership in the new Institute for Medicine and Engineering (IME) at the University of Pennsylvania. After arriving at IME, I set about trying to understand how the 3D organization of a tissue could so dramatically modify cell behavior. I initially chose to focus on apoptosis regulation, because, during my last year with Mina, I had made the rather startling observation that MECs incorporated into a 3D polarized "tissue-like structure" resist apoptosis induction by extrinsic stimuli (Weaver *et al.*, 2002). My journey of discovery was unexpectedly bolstered by the unique environment at the IME, where I was physically surrounded by engineers and biophysicists who routinely discussed concepts such as viscoelasticity, emergent properties, and compression or flow, and who used a grab bag of approaches familiar to physical scientists but quite new to a biochemist/cell biologist. Luckily, my curiosity got the better of me, and it was just a matter of time before I began to apply some of the physical science concepts and methods to my own research. My aha moment came when I realized that ECM topography and compliance were major regulators of tissue behavior and that these ECM features might explain at least some of the different phenotypes in MECs when they grow in the context of a 3D reconstituted basement membrane or in the soft mammary gland in vivo or in the stiffened fibrotic microenvironment of a breast tumor (Figure 2; Paszek and Weaver, 2004; Paszek *et al.*, 2005). I also became enamored with assorted methods for deconstructing, manipulating, and testing how these biophysical cues modify cell and tissue behavior. Over the past several years, I have been converted to the wisdom of working with colleagues across disciplines and applying physical science concepts and approaches to understand cell and tissue biology. I have since relocated my laboratory to the University of California, San Francisco, and expanded my group's studies to include the development of novel in vivo mechano-regulated

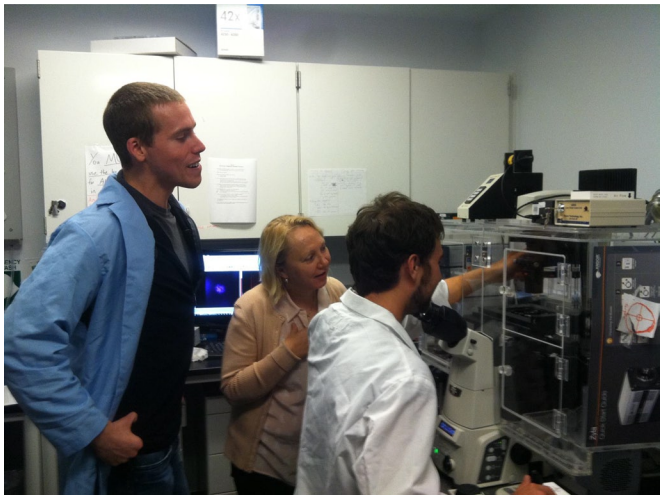

FIGURE 3: Scanning angle interference microscopy reveals impact of tissue mechanics on integrin adhesion organization. Joint University of California, San Francisco/Berkeley Bioengineering graduate students Luke Cassereau (left) and Matthew Rubashkin (right) and Valerie Weaver conduct supraresolution imaging studies using scanning angle interference microscopy to explore the interplay between integrin adhesions and tissue mechanics in metastatic breast cancer cells.

models and exploration of the role of force in stem cell fate and the impact of force not only on breast cancer but also on brain and pancreatic cancer (Butcher *et al.*, 2009; Levental *et al.*, 2009; Dufort *et al.*, 2012; Paszek *et al.*, 2012, 2014; Mouw *et al.*, 2014; Rubashkin *et al.*, 2014). Regardless, the vision and the passion with which I approach my research remain constant, so while the initial work from my group may have been met with some skepticism, persistence and hard work has paid off, and we are in good company these days. Thus, while years ago my engineering students may have felt isolated when they attended the American Society for Cell Biology conference, nowadays the cell biology community has incorporated interdisciplinary approaches into virtually every aspect of cell biology, and I genuinely look forward to seeing and becoming involved in many of the new and exciting discoveries being made at these interfaces.

PAYING IT FORWARD

Mentoring is one of the privileges and pleasures of being an academic researcher. The joy that I have experienced when one of my students has passed a qualification exam or obtained his or her PhD or when one of my postdoctoral fellows has secured a permanent job and established his or her independence is wonderful. The fun I have interacting with my trainees sustains and nurtures me in multiple ways, and I am constantly learning and being challenged by them (Figure 3). I view the laboratory community I have created as a microcosm of an ideal world in which scientists of all genders, races, and backgrounds and from different disciplines work together to solve key biological questions (Figure 4). Of course, mentoring scientists from different disciplines and team building are not without their challenges, as one struggles with different sensibilities, scientific languages, and perspectives. However, the rewards are many, and I believe that we are united by common goals, including a love of knowledge and an appreciation for the beauty of cell biology and the precision of engineering and the elegance and logic of physics that continue to challenge and motivate us toward the next discovery and the next new concept.

FIGURE 4: Fostering interdisciplinary science. It's not all work and no play. A day out, a bit of sunshine, and liquid refreshments go a long way to nurturing interdisciplinary research. Members of the Center for Bioengineering and Tissue Regeneration on the yearly wine tour. Clockwise from top: Suraj Kachgal (bioengineering postdoc, Boudreau Laboratory), Ori Maller (cell biology postdoc), Jon Lakins (biochemistry lab manager), Matthew Rubashkin (bioengineering graduate student), Janna Mouw (mechanical engineering senior scientist), Matthew Barnes (cell biology postdoc), Christopher Dufort (chemistry postdoc), Jason Tung (bioengineering postdoc), Russell Bainer (genetics postdoc), Laralynne Przybyla (cell biology postdoc), Amanda Wijekoon (cell biology laboratory specialist), Balimkiz Senman (premed student trainee), Laura Damaino (cell biology postdoc), Valerie Weaver (biochemistry principal investigator), and Irene Acerbi (bioengineering postdoc).

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more recently by a DOD BCRP Scholar expansion award and the NIH NCI Physical Sciences and Oncology program, and my group's pancreatic and glioblastoma work is currently supported by grants from the NIH NCI Tumor Microenvironment program, with additional support from the American Association for Cancer Research Pancreatic Action Network and the Susan G. Komen Foundation, and the stem cell work is being supported by the California Institute for Regenerative Medicine.

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