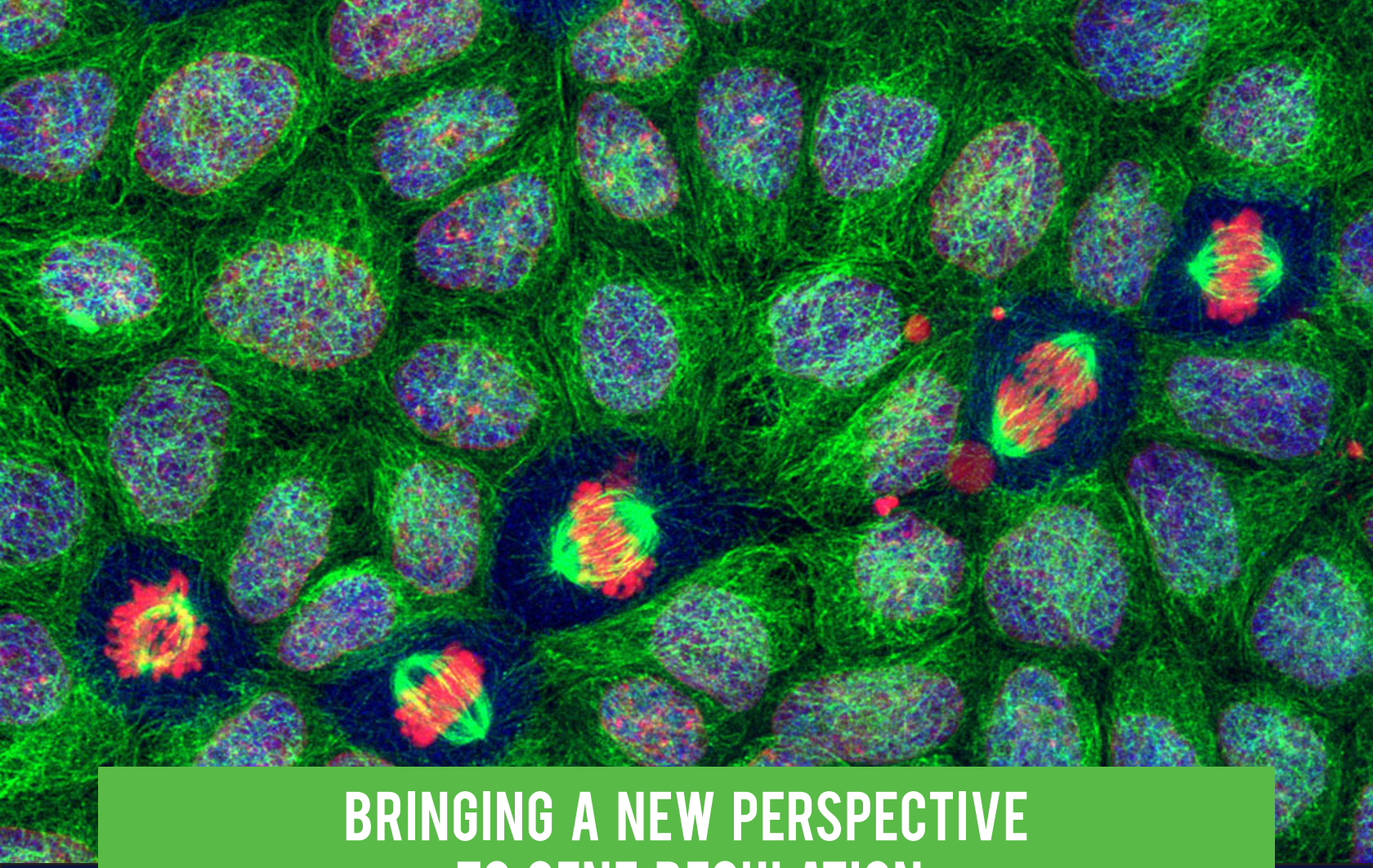

BRINGING A NEW PERSPECTIVE TO GENE REGULATION

Interview with Professor Elçin Ünal

BY MATTHEW COLBERT, EMILY HARARI, MICHELLE LEE, ELETTRA PREOSTI, SAUMI SHOKRAEE, AND NIKHIL CHARI

***Dr. Elçin Ünal** is an Assistant Professor of Genetics, Genomics, and Development at UC Berkeley. After growing up in Turkey, Ünal came to the United States for graduate school where she began her research career. Her lab currently studies meiosis in the context of gene regulation and cellular quality control. We spoke to Professor Ünal about her work on the processes of kinetochore inactivation prior to meiosis and mitochondrial segregation during cell differentiation.*

BSJ: How did your experiences growing up in Turkey make you interested in biology, and what led you to continue that type of research in the United States?

EU: I had a very engaging biology teacher in high school. Normally, you think of biology as memorization-based, but it wasn't taught that way in his class. In Turkey, the college admissions process is a little bit different than in the U.S.; you need to take a national exam, which is basically the only determinant of whether or not you make it into college. It's very competitive, and you announce your major before you actually get there. My teacher informed me about a new department, Molecular Biology and Genetics, that just opened a few years ago. The year I applied to college was the year Dolly the sheep was cloned, so there was a lot of excitement around molecular biology. Every year, my department would only get about 15 students. The training was great, but doing research was difficult for economic reasons. So I followed the footsteps of the previous years' classes, and went for an internship abroad. I actually went to graduate school at the same place abroad the next year.

BSJ: Where was that?

EU: Johns Hopkins.

BSJ: Much of your current research is in kinetochore inactivation. Could you elaborate on what a kinetochore is and why the activation and inactivation of kinetochores is important for meiosis?[1]

EU: A kinetochore is a large macromolecular structure that connects the chromosomes, which contain a cell's DNA, to the division machinery, namely the microtubules and the cytoskeleton. Kinetochores are essential for genome segregation during both mitosis and meiosis. What's interesting about meiotic cell divisions is that, prior to the divisions themselves, there are a lot of changes that occur to the chromosomes. The DNA is first shattered into pieces by programmed double-strand breaks, which then allow chromosomes to undergo recombination. Meiosis is unique because the genome size is reduced by half; chromosomes are replicated, and then segregated twice. To accommodate that, the kinetochore also undergoes changes, and this is observed in any model that has been studied so far, from yeast to mice. It has been found that the kinetochore loses its ability to interact with microtubules during prophase, while chromosomes are being repaired and undergoing recombination. Then prior to chromosome divisions it gains back that activity.

In my postdoctoral work, we asked why it matters for the cell to go through this sort of inactivation and re-activation, and we found that of more than 40 kinetochore subunits, there was one protein called Ndc80 that dramatically changed in abundance between the two stages of kinetochore activity. We overexpressed this protein in a cell during a time when it's normally not present, and we observed the cells revert from a meiotic to mitotic phase. That told us that kinetochore inactivation is probably important for establishing a meiosis-specific chromosome architecture during prophase. Since one lynchpin subunit dictated when this entire complex was functional during meiosis, we really wanted to understand how this gene is regulated at all steps, and that's how we got into this LUTI mRNA business.

BSJ: In addition to the main open reading frame (ORF) which codes for a specific protein, long undecoded transcript isoform mRNAs, or LUTI mRNAs, contain several upstream open reading frames (uORFs). What's the difference between the ORF that codes for the Ndc80 protein and the uORFs we find in LUTI mRNAs?[2]

EU: The LUTI mRNA is basically a longer version of the canonical mRNA. It has an entire ORF for a given gene—in this case, the Ndc80 ORF. In addition to this, due to its extended 5' region, it has uORFs. These are short translation units that have their own start and stop codons. How the translational machinery normally works is that the 40S ribosome subunit engages with the mRNA and starts scanning from the 5' end; when it finds an AUG start codon the ribosome is fully assembled and starts translating. Then, when the ribosome encounters a stop codon, it is released from the mRNA. In LUTIs, the uORFs compete with the main ORF, so the ribosome abortively engages with the short translation units but never gets to properly translate the actual ORF (Fig. 1). This molecule looks like an mRNA that would normally be decoded into protein, but in the case of LUTI, it does the opposite. The message itself is not translated, but when the LUTI is transcribed, the production of the canonical mRNA is repressed. In this way, by coupling both transcriptional and translational repression, mRNAs can effectively shut down protein synthesis. Basically, the cell can use the same components that it employs to activate gene expression to repress gene expression.

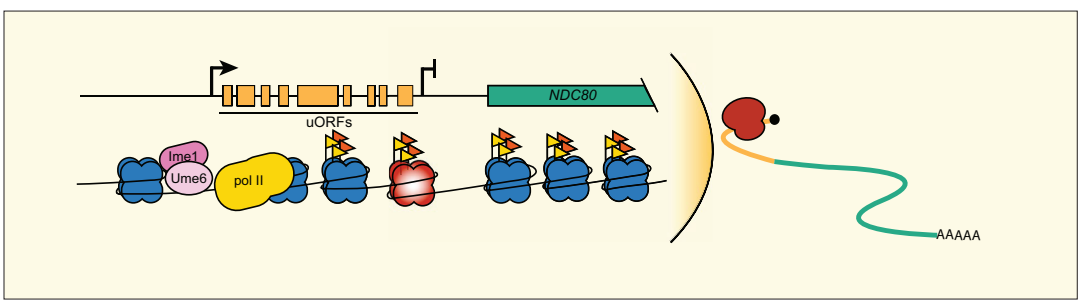

Figure 1: Translation diagram. *Upstream ORF prevents the ribosome from translating the downstream NDC80 ORF.*[2]

BSJ: Why can we call LUTI a mRNA if it doesn't encode a protein?

EU: It has all the information we normally find in mRNAs. By all criteria, an mRNA should have a cap, a poly-A tail, and an open reading frame, and it should be transcribed by RNA polymerase II. LUTIs fit all that criteria. In an mRNA-seq experiment, these non-coding LUTIs will be clustered together with all the other mRNAs because they contain all the other necessary signatures. They still have a coding potential, but because of the uORFs they become non-coding. We classify it as a LUTI because even though all the essential components of mRNA are there, it's not making protein.

BSJ: Could you elaborate on the transcriptional and translational mechanisms affected by LUTI-mRNA gene-regulation, specifically in kinetochore inactivation?[2]

EU: For meiosis in yeast, there are two major transcription factors that drive meiotic progression. One turns on early in response to both intrinsic and extrinsic cues. This first transcription factor activates genes involved in double-strand break formation, recombination, and DNA replication, but it also turns on about a hundred of these LUTIs, one of which is this NDC80LUTI. The transcription factor binds approximately 600 base pairs upstream of the ORF. When that happens, a message is transcribed that the mRNA is starting from an alternative upstream promoter. That message's transcription actually inhibits transcription from the canonical promoter. That's how the transcriptional repression works. Then you make a longer version of an mRNA—a LUTI mRNA—which is different from the canonical mRNA due to an approximately 500-nucleotide upstream extension. That extension contains uORFs which repress translation.

The second key transcription factor is activated just prior to the meiotic divisions, as the first one's activity goes down. It has a binding site in the canonical promoter, which codes for the regular NDC80 mRNA. This leads to a switch in production between two different NDC80 isoforms, leading to re-activation of the kinetochores. So, a cell can turn on either an ORF-directed or uORF-directed transcription factor, depending on whether it wants to activate or inactivate kinetochores.

BSJ: Besides kinetochore inactivation, are there any other events in meiosis or elsewhere that may be regulated by LUTI expression?

EU: I have a team lab with another professor, Gloria Brar. We're collaborators and basically best friends. Her group does a lot of global measurements of mRNA translation and quantitative protein measurements. When you look at meiotic prophase, NDC80 mRNA is expressed very highly, but because it's a LUTI it actually has a negative impact on protein production. Following our lab's work on NDC80, she used these global datasets taken over time during meiotic progression to see if there are other mRNAs that anti-correlate with their protein levels. Her lab found that 389 genes, about eight percent of the yeast genome, are expressed at different times through these luti-mRNAs, recognized by their signature uORFs. We're specifically looking at the genes regulated by this first meiotic transcription factor to understand the genome-wide rules for both transcriptional and translational repression. Beyond that, we looked at individual transcripts in human and fly cells, to see whether this kind of combinatorial integrated mechanism exists in other species. It seems to be conserved, and any kind of transcription factor-dependent gene expression program (which is pretty much every process in the cell) can, in theory, be regulated this way. It's just a matter of looking with a different lens and not making the assumption that mRNAs needs to make proteins. If you drop that simple assumption, then you start finding these mechanisms.

> "By coupling both transcriptional and translational repression, mRNAs can effectively shut down protein synthesis."

BSJ: How do you think the LUTI mRNA mechanism is evolutionarily advantageous?

EU: I think there is a nice parsimony in the system, in that the same trans-acting machinery is used to coordinate up-regulation and down-regulation of genes at the same time. Normally, when we think about anything in the cell that is dependent upon transcription factor regulation, like differentiation or sensing environmental stresses, we mostly think about how the cell activates a group of genes. But there are also genes that need to be inactivated. Cells can evolve a transcription factor and repressor separately to turn on and off genes at the same time. In this case, however, you evolve a single transcription factor, and the cis binding sites on the DNA sequence are what determine gene expression. If the transcription factor binds an upstream promoter, a LUTI mRNA can be produced; if it activates a downstream promoter, a normal protein-producing mRNA will be produced.

The other trans-acting factor is the ribosome. Ordinarily, we assume the ribosome always makes proteins. However, in this case, the ribosome associates with cis elements within the mRNA, which then prevent productive translation. Thus, the body is relying on the same toolkit to do both jobs, which is evolutionary advantageous.

BSJ: Like a transcriptional Swiss Army knife?

EU: Exactly!

BSJ: The meiotic mechanisms you study are critical to ensuring healthy cell progeny in yeast. Kinetochores are not the only cellular structures subject to this regulation, and your research also focuses on mitochondrial inheritance. Cells allocate half of their mitochondria to pass down to their daughter cells. Where are mitochondria normally found in the cell?[3]

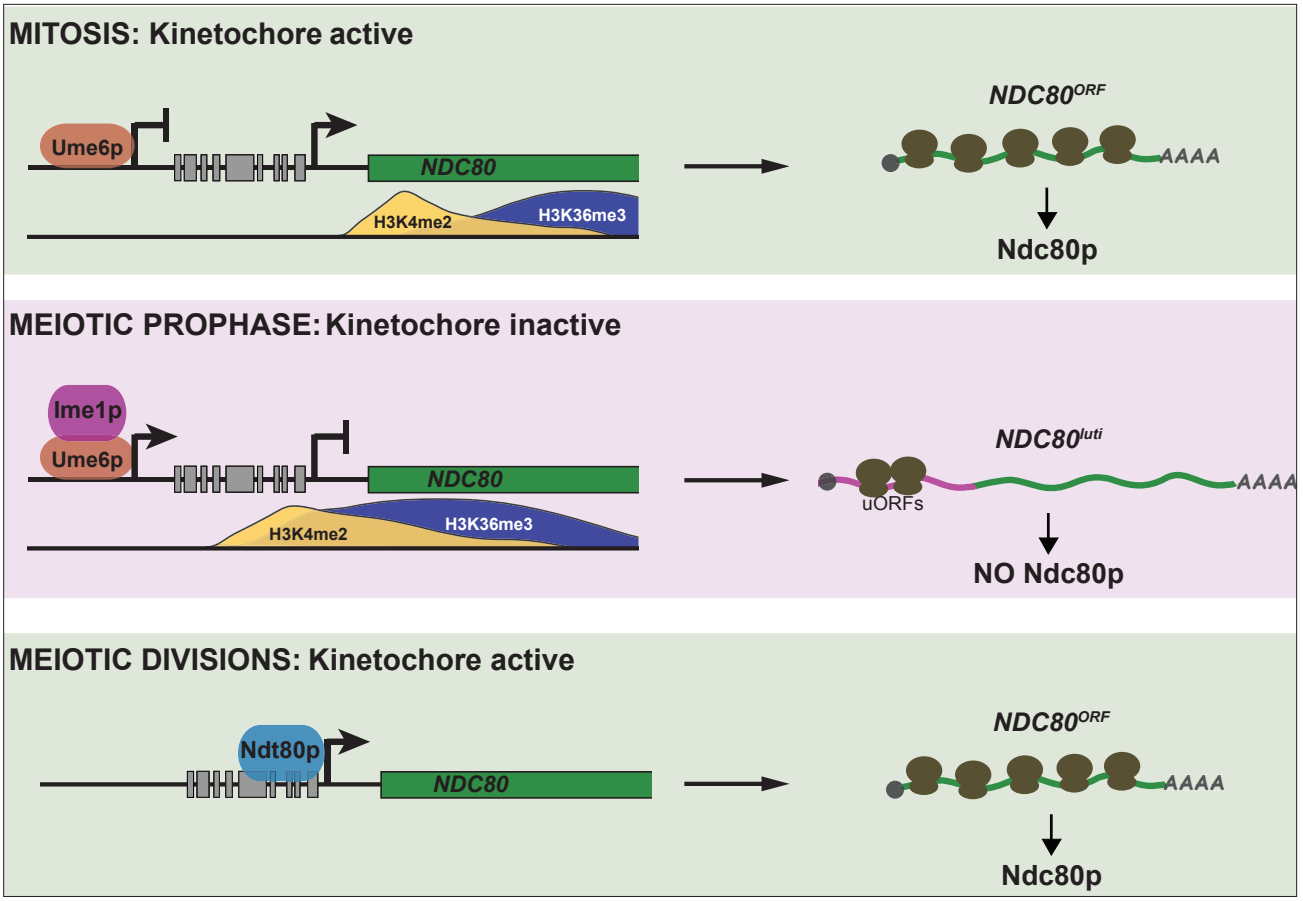

Figure 2: Formation of luti-mRNA during meiotic prophase. *It inactivates kinetochores. During meiosis, Ndt80 transcription factor prevents transcription of uORFs.*[1]

EU: Mitochondria normally reside in the cytoplasm of cells, but unlike the textbook picture of individual organelles floating around in the cell, the mitochondria form an interconnected network. When the cell undergoes division, it needs to inherit mitochondria. Besides being the major ATP production centers in the cell, mitochondria are also involved in many other metabolic regulation processes. The mitochondrial genome co-evolves with the nuclear genome, which contains most of the mitochondrial proteins that are imported into the organelle. So there is a symbiotic-type relationship. In organisms that have sex-specific gametes, the oocyte brings all of its mitochondria into the embryo at the end of fertilization, whereas single-celled organisms like yeast act like oocytes by inheriting a group of mitochondria, but also act like sperm by eliminating 50% of their genome, which won't be inherited. Ultimately, we are interested in whether there are differences between what is segregated into the gametes and what is left behind. We wanted to start by defining distinct stages where there are stereotypical morphological changes in mitochondria towards the segregation.

One of my students discovered that the first step of regulated mitochondrial detachment occurs at the molecular level. Mitochondria are connected to the cell cortex in the plasma membrane by a molecular organelle tether called the mitochondria-ER-cortex anchor (MECA) that binds to the mitochondrial outer membrane on one hand and the plasma membrane on the other. We knew that these contact sites are destroyed during meiosis because we see a very timed change in mitochondrial morphology when the cell is in transition during meiosis. After this, the mitochondria start associating with the nuclear membrane. The nuclear genome needs to get inherited somehow, so the mitochondrial genome "hitchhikes" with the nuclear genome to make it into the gametes. For this reason, we believe that the subset of the mitochondria that interacts with the nucleus is the part that makes it into the gametes, and the subset that does not interact with the nucleus is left behind and degraded.

BSJ: How does phosphorylation of protein kinase Ime2 promote the degradation of MECA and allow proper segregation of mitochondria during meiosis?[3]

EU: In mitotic cells, the MECA complex is very stable—it locks the mitochondria to the plasma membrane without dynamically changing. However, during meiosis, mitochondrial detachment occurs by modulating the activity of this tether. The kinase Ime2 phosphorylates the MECA subunits, changing the structure of these tethers and making them more amenable to proteasome degradation. Because phosphorylation is required for the degradation, we know that degradation occurs at the post-translational level. One

reason this probably happens is because a functional MECA is needed prior to meiotic division. Because it is a stable structure, shutting down its synthesis would not be enough for mitochondrial morphogenesis to be activated.

BSJ: Mitochondrial functions decline with age but are renewed during gametogenesis. How might this be possible?[3]

EU: Even in old cells undergoing meiosis, we see the cellular rejuvenation that occurs as part of meiosis. These progenitor cells are "replicatively aged" since they have undergone prior cell divisions to produce daughter cells through mitosis. They display some defects at the cellular level, but they are still partially functional. This is because aging is a progressive phenomenon.

Before meiosis, we anticipate that some mitochondria are more functional than others. Our question right now is whether the more functional ones are somehow selected over the others at the level of meiotic segregation. We can look at the quality control aspect of this by making basic fusions between young and aged cells or by damaging a subset of mitochondria. We want to find out whether there is selection in these mixed populations. Probably there is, but we don't know for sure.

BSJ: Many of your studies use a budding yeast model. What characteristics of budding yeast that make it an ideal organism for studying meiosis?[1,2,3]

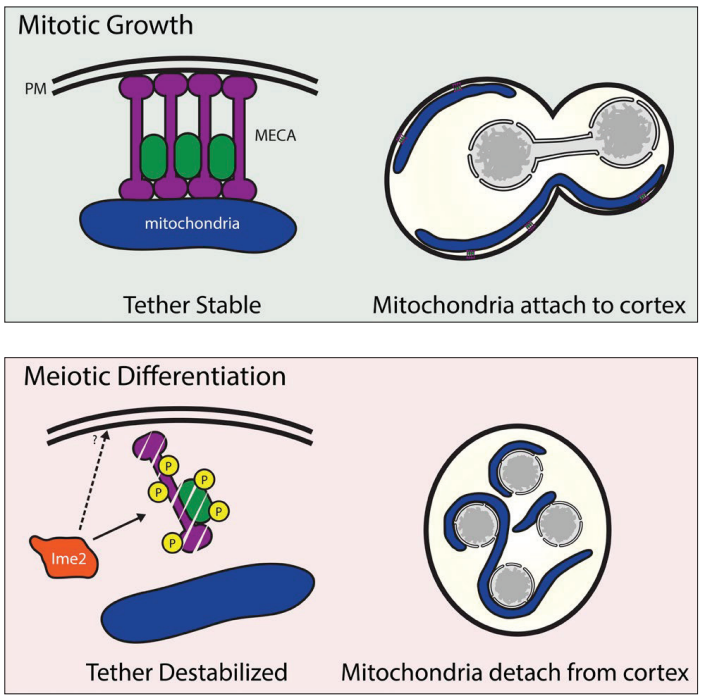

Figure 3: Mitotic growth and meiotic differentiation. *MECA tether is destabilized with phosphorylation by Ime2 during meiosis, untethering mitochondria from cell cortex.*[3]

EU: When it comes to meiosis, most of what we know comes from a very chromosome-centric perspective. But meiosis is a full cell differentiation program. Meiosis is an evolutionarily conserved process, which involves a lot of changes happening to the cell. If you want to understand those changes, you want to look at the most genetically tractable organism. When it comes to yeast, one big advantage is that we can induce meiosis. That's very important for looking at population-based studies of gene expression. If cells are performing a differentiation or some other change synchronously, the population behavior is more reflective of individual cell behavior. We can also live image meiosis in budding yeast cells from the very beginning to the very end of meiosis—it takes about 24 hours. Whereas in other cells like oocytes, we can't observe that refined framework or synchronous manner. Because there are so many unanswered questions, we want to start with the most simple and tractable organism—from there, we can begin to look for conservation and divergence in other systems.

BSJ: What motivates you to challenge canonical perspectives of gene regulation and cell differentiation in your research?

EU: In my mind, it's more a sense of curiosity than a desire to challenge. If I make an odd observation, I think, "How does this happen?" In our lab, we have the ability to observe cellular process in great detail, and I also have incredible graduate students. It all comes together because of their hard work, dedication, curiosity, and ability to make unique observations. When you do research, it mostly doesn't work. But when a discovery comes, for example, you check something in the scope and you're the first person to ever see it, you go back to childhood excitement. When that happens, you get the science high, which is better than any drug.

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