LRBGE connect

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Letter from the Editor

It's been another busy summer in the NCI Laboratory of Receptor Biology and Gene Expression! As well as attending conferences, publishing papers, and enjoying some well-deserved vacations, 80 LRBGE staff got together in the National Library of Medicine to take our latest branch photo (below). This included seven group leaders, two facility heads, eight staff scientists, and, of course, dozens of research biologists, postdoctoral fellows, postbaccalaureate trainees, graduate students, and visiting scientists.

In this issue of *LRBGE Connect*, we feature research from the labs of Tom Misteli, Dan Larson, and Gordon Hager. Our new "where are they now?" section profiles several former fellows and their successful post-LRBGE careers. We have a look at Dan Larson's career to-date and include an interview with Tineke Lenstra, one of his former fellows who is establishing her own research program. Finally, don't forget to check out our latest news updates, including awards, new faculty positions, and more!

On behalf of the newsletter team, I hope you all have a wonderful and productive fall. Look out for our next issue in December 2018!

► lain Sawyer Managing Editor, LRBGE Connect



Thumbs up! LRBGE branch photo, April 2018. Photo Credit: NCI.

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Banner Image (top): SVV840 human adenocarcinoma cells displaying chromosomal fusions. Centromeres are marked in red. Image credit: Arun Kumar Ganesan & Yamini Dalal.

LRBGE for →ard

Recent research highlights

Automated chromosome mapping

A recent paper published by Tom Misteli's Cell Biology of Genomes Group in *Methods* has outlined a new automated microscopy technique to study how DNA is organized into chromosomes inside human cells.

The 3D spaces occupied by each chromosome inside the nucleus are known as chromosome territories (CTs) and their relative cellular positions are responsive to both external and internal pressures. The current gold-standard method to visualize genetic regions by microscopy is fluorescence *in situ* hybridization (FISH) using specific DNA probes. These probes can paint genes and entire chromosomes allowing for target chromatin position or CT size to be studied by confocal microscopy.

However, previous single-cell studies showed that CT positions are stochastically distributed but exhibit some cell-, tissue-, and disease-specific positioning. With little known about the distribution of CTs, researchers must be able to image many cells to draw strong conclusions.

The introduction of high-throughput microscopy techniques has allowed researchers to image thousands of cells in minutes rather than hours or even days. Large datasets—often thousands or even millions of images—produce high statistical power and address user biases. The Misteli lab has previously shown that the position of individual genes can be recorded using highthroughput microscopy. However, it was unknown if this could be performed on larger genomic regions due to the size and complexity of CT-FISH analyses.

Postbaccalaureate student Ziad Jowhar developed a method called "HiCTMap", which automates CT-FISH for high-throughput microscopes, such as those in the CCR High Throughput Imaging Facility (HiTIF), housed in LRBGE. Cells are stained with specific chromosome paints, imaged, and automatically segregated by a custom machine learning algorithm. This pipeline performs image analysis and data compilation according to user-defined settings. The distance between the center of the nucleus and the chromosomes of interest is then calculated, as well as the distances between each chromosome.



Seeing chromosomes clearly. HiCTmap allows investigators to measure chromosome duplications and repositioning. Image Credit: Ziad Jowhar and Sigal Shachar.

As a proof of concept, the investigators studied the size and position of chromosomes X, Y and 18 in primary normal male and female skin fibroblasts. They showed that karyotype (XX or XY) can influence the size of chromosome X. They also demonstrated that chromosome X has a more peripheral position than chromosomes Y and 18, and this analysis is unaffected by measurement in 2D or 3D. By combining RNA FISH with chromosome paints, they confirmed previous observations that describe the active chromosome X (Xa) as larger and more centrally positioned than the inactive X (Xi) in normal XX cells.

This study demonstrated that CT architecture can be successfully analyzed using high-throughput imaging methods to rapidly map chromosome architecture in 3D. ► **Gregory Fettweis**

Learn more about this study:

Jowhar *et al.* (2018) "HiCTMap: Detection and analysis of chromosome territory structure and position by high-throughput imaging" *Methods* 142:30-38. DOI: 10.1016/j.ymeth.2018.01.013

LRBGE for →ard

LRBGE scientists have been busy publishing reviews and collaborating with other groups on several exciting projects.

Chromatin and enhancer deregulation in breast cancer

Regulatory mechanisms affecting chromatin dynamics and transcriptional activity are important prerequisites to understand gene regulatory networks in hormone-responsive breast tumors. A review from the Hager laboratory published in *Endocrine-Related Cancer* summarizes the complex network of regulatory events affecting chromatin landscape and transcription. The authors describe some of the recent technological advances in chromatin biology and single cell imaging which enable us to ask challenging questions in transcriptional regulation. They further discuss various models of enhancer-transcription factor interactions and stress on enhancer reprogramming in breast cancers by steroid receptors. Understanding the intricacies of transcription factor-enhancer biology will provide further insights into breast cancer progression and growth.

DOI: <u>10.1530/ERC-18-0033</u>

Developing microscopy tools to understand transcription in single cells

Single cell analyses are one the most powerful tools for studying highly dynamic and heterogeneous cellular processes. In two recent reviews published in Current Opinion in Systems Biology and Genome Biology, Dan Larson has discussed the merits and limitations of various methods to analyze gene expression and RNA splicing, including single cell RNA sequencing, single molecule FISH, and live cell imaging. Often, natural heterogeneity in cells poses both experimental and theoretical roadblocks to analyzing and interpreting single cell and molecule data, and, therefore, the authors discuss various understand approaches the functional to implications of cellular heterogeneity. These

reviews discuss how data from single cell systems could be used to simplify and model complex biological processes and networks that would represent a universal biological phenomenon.

The Larson lab has elegantly used the single molecule RNA FISH technique described in these reviews to characterize a muscle enhancer RNA that controls cohesin recruitment, thereby regulating transcription. This collaborative project with Vittorio Sartorelli (NIAMS) identified a distal regulatory region enhancer RNA (^{DRR}eRNA), located near the MyoD locus on chromosome 7, that acts in trans at the myoglobin locus on chromosome 1. This RNA directs cohesin loading and its maintenance at the trans locus to regulate muscle gene expression. Thus, this study presents a novel chromatin remodeling role for enhancer RNAs and cohesin.

DOIs: <u>10.1016/j.coisb.2017.11.011</u>, <u>10.1186/s13059-018-1467-4</u> & <u>10.1016/j.molcel.2018.06.008</u>

Ikaros: A Janus-faced myeloid cell transcription factor

Collaborative work between the Hager laboratory, former LRBGE scientist Dr. Mia Sung (who now runs her own research program at the NIA), and Dr. Iain Fraser (NIAID) has identified dual roles for the transcription factor Ikaros, published in the Journal of Immunology. Although well studied in the context of lymphoid progenitors, the role of Ikaros in mveloid cells and macrophages remains underappreciated. The authors therefore investigated Ikaros's chromatin binding profile and its effect on gene expression in macrophages stimulated using LPS, mimicking an innate immune response. This revealed both suppressor and activator roles for Ikaros, and further experiments pointed to a dose dependent control of the activation function of this transcription factor. Overall, this study presents an unexpected role of Ikaros in changing the transcriptional landscape in macrophages following infection.

DOI: 10.4049/jimmunol.1800158

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Robin Sebastian
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LRBGE alumni

Where are they now?

In this new section, we highlight past members of LRBGE and learn of their current vocations.

Dr. Marc Bailly (picture below) joined LRBGE upon Shalini Oberdoerffer's move from Frederick to Bethesda in October 2013. Marc was a postdoctoral fellow in Shalini's lab for about two years, followed by a three-month stint in Yamini Dalal's lab to help with protein purification. In February 2015, Marc was hired at Merck Pharmaceuticals and is now Associate Principal Scientist at the Merck Research Laboratory in Palo Alto, CA. He leads and supervises the Protein Purification and Characterization group. His work involves purification and physiochemical characterization of antibodies and/or antibody-like molecule selection for immunotherapy-related programs. Molecules are ranked and selected for development into potential drug therapies. Marc's time at LRBGE proved very important for advancing in his current career. Many aspects of the programs he is running at Merck involve T cell development and/or T cell-related activities, which he relates to the work he performed in Shalini's lab. His time in LRBGE and under Shalini's mentorship prepared him to quickly grasp the assays and analytics that are currently used at Merck Biologics.





Dr. Ido Goldstein (above, left) was a member of LRBGE from January 2013 to November 2017, as a postdoctoral visiting fellow in Gordon Hager's lab. Ido recently joined the faculty at Hebrew University in Jerusalem as an Assistant Professor. According to Ido, "Being a Principal Investigator is a long-sought dream come true!" He is looking forward to establishing his new lab and training students, as he enjoys the beautiful campus in Israel. He finds that starting from scratch and learning the ways of a large university do not come without challenges. He jokes that he often wishes he could fly back to his LRBGE bench to get work done with no hassles. At LRBGE, Ido appreciates having had the opportunity to learn from some of the greatest scientists and concentrate on science with few interruptions. He claims he "metamorphosed as a scientist" during his tenure at LRBGE.

Dr. Maayan Salton (above, right) joined LRBGE in 2010 and was a member of LRBGE for five years in Tom Misteli's lab as a postdoctoral visiting fellow. She is currently a principal investigator at the Hebrew University, in Jerusalem. Maayan enjoys leading a group of scientists and having the freedom to decide which directions to explore. She also appreciates the power of her position within the university which allows her to push forward on certain issues dear to her, such as gender equality. On the other hand, Maayan mentions, "being at the helm is not without its worries about funding and reviewers. I very often miss knocking on Tom's door and getting his perspective on the big as well as the

small things." During her tenure at LRBGE, Maayan developed as a scientist, learned the details of everyday work and the soft skills that are currently helping her navigate her way as a junior faculty member. Her time in LRBGE continues to inspire how she runs her own lab, to be as hard-working and supportive as the one she grew from.



Dr. Tina Miranda (above) joined LRBGE in 2009 and stayed as a research fellow in Gordon Hager's lab for five years. She enjoyed her NCI experience so much that she decided to stay and is currently a Scientific Health Analyst working in the Knowledge Management and Special Projects Branch, within the Center for Strategic Scientific Initiatives. Her job requires her to maintain a working understanding of NCI scientific research programs, policies and practices. She enjoys constantly learning about the great research that NCI funds. Her scientific expertise is not just focused in one specific area; thus, she can learn and disseminate a breadth of knowledge. Tina finds her job very rewarding and appreciates the work/life balance she is able to maintain. One reason Tina was initially hired for this position was her expertise in genomics and epigenetics. The time she spent as a research fellow in LRBGE allowed her to develop proficiency in those areas of research. She recalls back when she was a research fellow in Gordon's lab, "I was also very fortunate that Dr. Hager was supportive and allowed me to do a detail with my current office." The detail provided her with the scientific administrative experience she needed to be competitive for this position.

Dr. Huimin Chen (below) joined LRBGE in 2013 and stayed as a postdoctoral fellow in Dan Larson's lab for about four years. Current members may recall Huimin through her successful leadership of our LRBGE socials. Currently, she is working as a technical specialist at the intellectual property law firm Finnegan, Henderson, Farabow, Garrett & Dunner LLP. Within her recent transition into this career, she has gained experience on patent prosecution and opinion work. In patent prosecution, she writes and files patent applications and shepherds them through the Patent Office. The opinion work and due diligence involves helping companies determine if their new products or directions will infringe on other's patents. She loves that she gets to read, learn, write, think and listen to cutting-edge science 24/7, and is surrounded by smart and driven people. Some downsides she has encountered include long and arduous hours. She mentions, "In the job, every minute is spent thinking and reading, unlike in lab where the time spent pipetting or doing an experiment can be sometimes mindless." Huimin recalls her time at the NIH and within LRBGE as great exposure to the breadth of research topics and knowledge. Also, while she was here, she gained experience at the NCI Technology Transfer Office. These experiences have proven very useful and have helped guide her towards her current path. Mariana Mandler



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Finding the signal in transcriptional noise

In a 2011 paper published in *Science*, Dan Larson and colleagues wrote: "To gain a functional understanding of transcription networks, it is necessary to observe the output of transcription, namely RNA production." Dr. Larson LRBGE research program has been built around this approach: directly visualizing nascent RNA in live cells and studying the fluctuations of signal intensity at transcription sites in order to understand the mechanics and regulation of transcription.

The 2011 paper leveraged a tagging technique that had been used since the late 1990s to label mRNAs, in which a series of binding sites for bacteriophage coat proteins (PP7 or MS2) are inserted into an RNA and visualized with a fluorescent fusion protein. In this study, Larson and colleagues used PP7 stem loops to label an endogenous gene in yeast and tracked its expression in live cells by measuring the intensity fluctuations at the site of transcription. They observed little periodicity in these fluctuations and similar rates of transcription no matter where in the gene they placed their labels. The similarities in rates of transcription demonstrated that elongation and termination were deterministic, in particular that almost every initiation event resulted in a fulllength transcript. However, the lack of periodicity demonstrated that gene expression itself was stochastic, and thus knowing when one mRNA was transcribed would not give you much information about when the next one would be transcribed. Furthermore, the rates of initiation at this locus were similar to the search times for the relevant trans-activating factor, suggesting that in fact almost every time a trans-activating factor bound the promoter of this gene, the transcription machinery was loaded and a transcript was made. These were important fundamental insights as to how RNA regulation networks could function and

opened the door to two more papers studying the biophysics of RNA in live cells.

Transcription is only the beginning of an mRNA, or even its regulation. Most metazoan genes are regulated via control of mRNA splicing as well as control of transcription, meaning understanding splicing kinetics is crucial to understanding transcription. To detect splicing events, however, it would be necessary to label an intron and the growing transcript. Using orthogonal MS2 and PP7 loops to label both an intron and the 3' UTR of a single gene, Coulon et al. were able to separate initiation, splicing, and release of mRNAs in individual live cells. The results, described in a paper published in *eLife* in 2014, showed stochasticity at multiple independent steps. By cross-correlating the intensity from the intronic and 3' UTR regions of the same transcript, Coulon *et al.* determined that splicing can occur either before or after mRNA release. They further validated that the two events are independent with spliceostatin A, which prevents splicing but does not alter mRNA release, or campothecin, which slows elongation and thus mRNA release but does not alter splicing dynamics. However, under normal conditions, splicing occurred very shortly after release. Thus, it was the relative rates of two independently regulated but largely random processes —release and splicing—that determined when and where a transcript would be spliced.

The fact that the environment of a transcription site can be, in effect, regulatory in nature (i.e. could slow down splicing events) brings to mind regulation via non-coding transcription. In particular, it had long been postulated that the presence of the transcription machinery on the antisense strand was inimical to transcription of sense products. Studying this regulation had been difficult since strand-specific labelling and strandspecific repression were not yet well developed. In a paper published in *Molecular Cell* in 2015, Lenstra et al. leveraged the strand-specificity of the PP7/MS2 system (in which stem loops will only be formed in the sense orientation) and the strandspecificity of dCAS9-mediated silencing to study the ncRNA regulation at yeast GAL1 and GAL10. They found that ncRNA expression at this region is not strictly mutually exclusive with sense transcription, but that the two are anti-correlated. Antisense transcription functioned to prevent leakv transcription before activation but was insufficient to silence the region in the presence of strong activating signals. In the absence of ncRNA expression, spurious transcription of GAL10 would over-sensitize cells to the presence of galactose and cause a fitness defect. In effect, the ncRNA functioned to control the transcriptional noise in the silenced state, which would otherwise cause random spurious transcription of *GAL* genes.

In each case, the Larson lab had observed widespread stochasticity in transcription: the fundamental observation that transcriptional initiation complementary is stochastic. the observation of randomness in splicing and the observation that transcriptional noise, in the wrong place, had a fitness cost. These studies contributed to a model of transcriptional control in which stochasticity is the rule rather than the exception. The underlying randomness of the transcription machinery means that RNA transcription is highly heterogeneous over time and between cells, and upor down- regulation is mediated by local

concentration to shift kinetics. This stochasticity may even have implications for cancer progression.

Dan and his team continue to study how mRNA is controlled despite-or via-its production stochasticity, at multiple genes and in response to induction. This work has come full circle, as Dan new collaborates with two of his former fellows, Tineke Lenstra and Matt Ferguson, to simultaneously visualize both a transcription factor and its transcribing gene. A view is emerging that controlling this noise, rather than simply controlling gene expression, may be profoundly important for cells and organisms and that on the molecular level, as well as the organismal one, variability may be the stuff of life. ► Elizabeth Finn

Learn more about Dan's work:

Systems Biology of Gene Expression website: Link Larson *et al.* (2011) "Real-time observation of transcription initiation and elongation on an endogenous yeast gene" *Science* 332(6028):475-478. DOI: 10.1126/science.1202142

Coulon *et al.* (2014) "Kinetic competition during the transcription cycle results in stochastic RNA processing" *eLife* 3:e03939.

DOI: 10.7554/eLife.03939

Lenstra *et al.* (2015) "Single-Molecule Imaging Reveals a Switch between Spurious and Functional ncRNA Transcription" *Molecular Cell* 60(4):597-610. <u>DOI: 10.1016/j.molcel.2015.09.028</u>



Systems Biology of Gene Expression. Left to right: Abdalla Akef, Murali Palangat, Dan Larson, Yihan Wan, Simona Patange, Joseph Rodriguez. Not pictured: Ulrike Boehm, Gloria Garcia. Photo Credit: NCI.

LRBGE alumni

Tineke Lenstra, PhD

Junior Group Leader, Netherlands Cancer Institute https://www.nki.nl/people/lenstra-tineke/

Dr. Tineke Lenstra was an LRBGE postdoc from 2012-2016. After receiving her PhD in 2012 from the University Medical Centre Utrecht in the Netherlands, she joined Dan Larson's lab for her postdoctoral research. In 2016, Tineke accepted a prestigious group leader position at the Netherlands Cancer Institute (NKI) to establish her own independent research program. LRBGE Connect interviewed Tineke on her postdoc experience at LRBGE and the biological questions that motivate her career.



Tineke (lower row, center) and her new lab. Photo credit: Tineke Lenstra.

How did you decide on Dan Larson's lab and LRBGE as your research home for your postdoctoral fellowship?

I applied to Dan's lab after reading his paper in Science on visualizing transcription in live yeast cells. The possibility of measuring single molecules in single cells in real- time really appealed to me. In contrast to the genomics approaches of my PhD, these single-molecule experiments don't just describe the 'average cell' but provide information on the cell-to-cell variation, spatial organization and the dynamics of transcription. In addition, I was impressed by the collaborative and open environment at LRBGE during my interview, which is why I decided to join Dan's lab.

What were the most valuable aspects of working in LRBGE that helped your research during your time here?

I really enjoyed the critical discussions and the brainstorming sessions at LRBGE. It was really remarkable to work in an environment with such motivated scientists, all driven by the question to understand gene expression. I believe science gets better when done in teams, and in LRBGE I never felt like I was doing science on my own, even though I was the only person in Dan's lab working on yeast.

You are now a principal investigator (PI) at the Netherlands Cancer Institute (NKI). How did your work at LRBGE influence the research questions you now pursue?

I am intrigued by the question how cells decide which proteins to make. To have the chance to pursue this question together with a team of other scientists sounded very exciting. My exposure to all the excellent science at LRBGE convinced me that we need to understand the dynamics of biological processes to understand their mechanism. This dynamic aspect dominates my research questions, not only during my postdoc, but also as a PI.

You are not the only former member of LRBGE in your research group! Tell us how you met your graduate student, Heta, and how she decided to join your lab.

I met Heta Patel as a summer student in Dan's lab and I had never supervised someone so determined and motivated. We often joked that perhaps she could join me as a graduate student if I would start my own lab! Little did we know that two years later, after her postbac period fellowship in Mia Sung's lab (another LRBGE alumnus), Heta was applying for PhD positions at the same time that I was starting my lab. Since I knew that Heta was from California, I was of course a bit worried that the Dutch weather would scare her during her visit to Amsterdam (which was in February). Luckily for us, she was excited by our science, and even though she also received several offers from prestigious schools in the U.S., I was very happy when she decided to join our group.

Interviewed by Simona Patange

LRBGE news

Achievements at home and abroad

► Ruth Ashery-Padan, PhD (Hormone Action and Oncogenesis (HAO), 2017-2018) recently completed her sabbatical year with LRBGE. Dr. Ashery-Padan will return to Tel Aviv University, Israel where her team studies transcription factor function in the mouse eye. She will continue to collaborate with the Hager group.

► Congratulations to **Daniel Arango**, **PhD** (RNA Processing and Cellular Development, 2015-Present) who received the annual Fellows Award for Research Excellence (FARE). This NIH-wide competition recognizes outstanding research by junior intramural researchers.

► Michael Cordingley, PhD (HAO, 1983-1988) recently published a book entitled <u>"Viruses: Agents</u> of Evolutionary Invention", (Harvard University Press). Dr. Cordingley is President of Revolution Pharma Consulting and Senior Virology Advisor at Antiva Biosciences.

► Yamini Dalal, PhD (Group leader, Chromatin Structure and Epigenetic Mechanisms (CSEM), 2008-Present) recently celebrated her 10th anniversary of working at LRBGE. Dr. Dalal started her research program on chromosome centromere regulation on September 2, 2008.

► Marina Feric, PhD (Cell Biology of Genomes, 2016-Present) received an NIH PRAT (Postdoctoral Research Associate Training) fellowship. Dr. Feric investigates phase separation of mitochondrial DNA-bound proteins.

► Ido Goldstein, PhD (HAO, 2013-2017) recently joined the faculty at Hebrew University of Jerusalem, Israel. Dr. Goldstein's research program characterizes how transcription factors regulate metabolic pathways in the liver. For more insight on his journey, read his <u>interview on page 4</u> and his Twitter profile (@goldstein lab).

► Mary Hawkins (HAO, 2012-2018) recently retired. Ms. Hawkins joined the NIH in 1986 to investigate HIV. In 2012 she joined LRBGE and used

her biochemistry experience to develop *in vitro* systems for the study of chromatin biology. We wish Mary a happy retirement!

► Ville Paakinaho, PhD (HAO, 2015-2018) received the Academy of Finland Award. Dr. Paakinaho currently works as a postdoctoral fellow at the University of Eastern Finland where he studies steroid receptor function.

► Lorenzo Rinaldi, PhD (HAO, 2018-Present) was selected to receive an Advanced Long-Term Fellowship by the European Molecular Biology Organization. This will support Dr. Rinaldi's research into how transcription factors shape genome organization.

► Joseph Rodriguez, PhD (Systems Biology of Gene Expression, 2012-2018) has started a tenuretrack investigator position at the NIEHS Research Triangle Park in North Carolina. Dr. Rodriguez's research group will investigate single-cell transcriptional regulation.

▶ NIH Chromatin-DECODE, currently chaired by **Erin Swinstead, PhD** (HAO, 2013-Present) has just started its new seminar cycle. The series was founded in 2011 by LRBGE fellows and continues to attract large audiences for talks by NIH early career researchers. Please email <u>erin.swinstead@nih.gov</u> if you're interested in joining the committee or presenting your work. The next Chromatin-DECODE seminar will be held at noon on Tuesday, November 13 in Building 50, room 1227/1233.

► Haiqing Zhao, PhD (CSEM, 2013-2018), a doctoral student jointly advised by Dr. Yamini Dalal and Dr. Garegin Papoian (University of Maryland), successfully defended his thesis and received a PhD in Biophysics. Dr. Zhao was a member of the NCI-UMD Graduate Partnership Program for Integrative Cancer Research.

Do you have something exciting to share with the LRBGE community? Contact <u>rachael.stitely@nih.gov</u>.

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