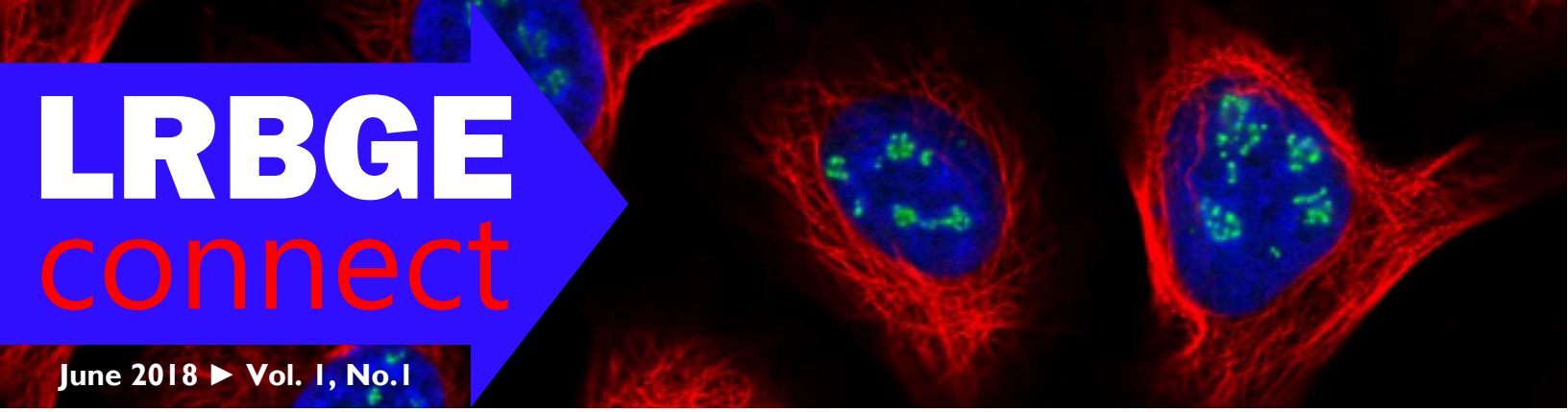

LRBGE connect

June 2018 ► Vol. 1, No.1

Welcome to your new LRBGE newsletter!

The Laboratory of Receptor Biology and Gene Expression was established during the reorganization of the National Cancer Institute by Harold Varmus and Rick Klausner (1994-1997) to take advantage of emerging developments in live cell biology, chromatin structure and function, and nuclear architecture. Early investigators included Jim McNally, John Brady, Tom Misteli, Cathy Smith, and Kevin Gardner. Beginning in 2008, we initiated a growth period with tenure track recruitments for Yamini Dalal, Shalini and Philipp Oberdoerffer, and more recently Dan Larson and Efsun Arda.

The lab currently includes seven independent groups and nearly 80 staff members. Over the history of the lab, approximately 450 fellows, students, sabbatical visitors, interns, and collaborators have passed through the groups. These colleagues represent an amazing family of gifted investigators, some young, some not so young, who continually form and support a rich intellectual environment. Specialties have covered a broad range of skill sets, from physics through biochemistry and cell biology to clinical practice. Throughout the life of the lab, the focus has always been on excellence in research accomplishment and keeping at the cutting edge of current biological questions, while maintaining a friendly, open environment. Our goal has been to foster the best possible culture for the development of young scientists in the early phases of their careers, and our track record is excellent. Dozens of our students and fellows have moved to the most competitive research institutes and flourished on their own. Indeed, many have risen to levels of major responsibility and made important contributions to the field of cell biology.

It has been a great pleasure to watch the growth of the lab, the emergence of new careers, and the wonderful friendships that have developed. I'm sure *LRBGE connect* will help all of us keep in touch and follow the future paths for our friends and colleagues. ► **Gordon L. Hager, Ph.D.** *Chief, LRBGE.*

IN THIS ISSUE

A blast from the past! LRBGE 1998. Photo credit: Gordon Hager.

PUBLISHER
GORDON L. HAGER - *CHIEF, LRBGE*
MANAGING EDITOR
IAIN SAWYER
WRITERS/EDITORS
GREGORY FETTWEIS ○ MARIANA MANDLER
KYSTER NANAN ○ JONATHAN NYE
SIMONA PATANGE ○ ROBIN SEBASTIAN
ERIN SWINSTEAD
ADMINISTRATIVE SUPPORT
ALLY BURKE ○ RACHAEL STITELY
EMAIL: RACHAEL.STITELY@NIH.GOV

LRBGE forward

Recent research highlights

Modeling the centromere

A defining and essential feature of all chromosomes is the centromere. This region is bound by microtubules and the kinetochore complex to ensure faithful chromosome segregation during mitosis. Despite its unique role in genome function, it shares several characteristics with other genomic regions, including the presence of nucleosomes that harbor post-translational histone modifications. However, these nucleosomes contain a unique histone variant called CENP-A, which replaces histone H3, that is essential for successful cell division. Several amino acids in CENP-A are known to be acetylated but the consequence of these events is unknown. The Dalal lab previously reported that centromeric chromatin becomes more accessible between G1 and S-phase, which they hypothesized may be a product of CENP-A modification.

To test this proposal, the team used all-atom molecular dynamics modeling to computationally reproduce the centromere. This method studies the conformational rearrangements made by a molecular structure of interest within a defined timeframe and across a range of environments. Bui, Pitman and colleagues revealed that acetylation of CENP-A lysine-124 and histone H4 lysine-79 increases DNA accessibility and strengthens the centromere protein core. However, a change in CENP-A conformation led to decreased binding of a partner protein essential for kinetochore assembly, CENP-C, which was confirmed in live cells by mutagenesis. Mass spectrometry analysis revealed a change in centromeric nucleosomes during different cell cycle stages, as CENP-A lysine-124 acetylation was present during G1/S transition but only methylated at early-to-mid S-phase. Dissection of the histone acetyltransferase pathways indicated this process might be specifically dependent on the p300 acetyltransferase.

The team took these results a step further and investigated the implications of this CENP-A conformational change for cell cycle progression. Cells that contained CENP-A lysine-124 mutants displayed both increased mitotic defects and aberrant replication timing, confirming the importance of correctly-timed and well-regulated CENP-A modification for cell division.

This highly multi-disciplinary project led by Dr. Dalal has major implications for centromere biology, mitosis, and beyond, and highlighted how epigenetic regulation of a single amino acid can influence essential cellular processes. The team has applied these interdisciplinary approaches to dissecting kinetochore protein binding to CENP-A nucleosomes, and to block cancer-specific chaperone-CENP-A interactions that drive new centromere formation. Further investigation into how variant histone modifications regulate nucleosome structure and function will be intriguing, as well as dissection of the structural features that make the centromere an exciting research target. ► **Iain Sawyer**

The home of LRBGE. National Institutes of Health Building 41, Spring 2018. Photo credit: Iain Sawyer.

Uniting DNA methylation and splicing

Non-protein encoding RNA regions called introns are removed from mRNAs by a large complex known as the spliceosome. This forms the basis of modular gene regulation and RNA diversity in higher eukaryotes. This process largely occurs co-transcriptionally and, thus, the link between splicing and epigenetics is a highly-researched topic. Often, the spliced-in exons are marked from the rest of the gene body by DNA 5-methylcytosine (5mC) and histone methylation (H3K36me3), but the cause-effect relationship between DNA methylation and splicing-associated genic histone

methylation remains unknown. A new system developed by Kyster Nanan and colleagues in the laboratory of Shalini Oberdoerffer, provides a tool to decipher the link between DNA and histone methylation on gene splicing.

Their minigene system involves single or two exon genes with variable splicing efficiency integrated into human 293T cells under the control of an inducible promoter. As a result, a researcher can quantitatively monitor the gene products from these cassettes in various genetic backgrounds and assess the factors that influence splicing outcomes.

Using publicly-available ChIP-seq data, the authors first looked at the global distribution of the transcription-associated histone mark H3K36me3 and the DNA methylation mark 5mC, expressed in single and multi-exon genes. These markers remained uniquely enriched at genes that underwent splicing. Reflecting a global pattern, the H3K36me3 mark was also enriched in multi-exon genes in the minigene system in a splicing- and transcription-dependent manner. However, genic DNA methylation status was not altered by transcription or splicing, even when the *de novo* DNA methyltransferases DNMT3A or DNMT3B were overexpressed.

Following this, the authors analyzed the influence of splicing on intragenic epigenome maintenance using *in vitro* methylated minigene constructs residing in a CpG-free vector, which was stably integrated into the 293T genome. Monitoring the methylation status over a period of 77 days revealed no difference in the maintenance of genic methylation in relation to splicing efficiency or H3K36me3 status.

Overall, Dr. Oberdoerffer's group showed a fascinating mechanistic uncoupling of DNA and genic chromatin methylation as it relates to splicing. The authors propose that, while the histone methyl transferase SETD2 can enhance H3K36me3 deposition in multi-exon genes in a splicing dependent manner, both *de novo* and maintenance DNA methylation could be transcription- and splicing-independent. The isogenic minigene tool developed and described in their work could be used to gain further insights into epigenetic modulators of transcription and splicing.

► **Robin Sebastian**

CTCF & transcriptional variation

Cell-to-cell variation is a trait inherent to all multicellular organisms. A barrage of single-cell analyses has recently revealed the extent to which this variability occurs within populations of ostensibly homogeneous cells in both tissue and *in vitro* contexts. The prevalence of this biological noise is somewhat at odds with the requirement of precise spatiotemporal control of gene regulation for normal cell development. Observations that cell-to-cell variation is both universal and heritable support the contention that there may be biological underpinnings that contribute to this trait, the details of which warrant further exploration.

To this end, Daniel Larson's team, in collaboration with Keji Zhao's group, investigated whether and how long-range chromosomal interactions contribute to cell-to-cell gene expression variability. CTCF is a key mediator of long-range enhancer-promoter loop formation and, by extension, the formation of larger topologically associating domains (TADs). By generating chromatin interaction maps for mouse Th2 cells, the authors observed a positive correlation between TAD interactivity and CTCF binding. More intriguing, however, was their observation that 80% of CTCF sites were positioned within active enhancers in Th2 cells. This prompted an investigation into the involvement of CTCF in regulating gene expression downstream of enhancer-promoter loop formation. In bulk populations, CTCF knockdown resulted in large-scale, bidirectional changes in gene expression. However, single-cell analyses revealed that depletion of CTCF, or gene-specific abrogation of CTCF-mediated promoter-enhancer interactions, increased cell-to-cell gene expression variations.

The authors further combined single-cell and single-molecule RNA-FISH approaches to show that the cell-to-cell variations in gene expression are related to the precise number of CTCF mRNA molecules per cell. More specifically, a higher

cellular concentration of CTCF mRNA resulted in decreased variability in cell-to-cell gene expression. Taken together, these findings indicate that the stabilization of CTCF-mediated promoter-enhancer loops is critical for reducing gene expression variability among cells.

The fact that complex organisms arise from a single cell has perplexed and intrigued scientists for centuries. Equipped with novel sample preparation and computational methodologies, we are better positioned to address this matter. A more comprehensive understanding of the biological source of cell-to-cell variability, such as the insight provided in this collaborative project, will improve our understanding of developmental and disease processes. ► **Kyster Nanan**

The double helix. Photo credit: Pixabay.com

Bookmarking fragile sites

DNA damage can occur through a variety of different mechanisms. Surprisingly, even normal cellular processes, like DNA replication can result in damage to the genetic code. For example, DNA polymerase progression can be impeded by transcription machinery leading to replication stress and double strand breaks. If not properly repaired, this type of damage can lead to genomic instability and potentially tumorigenesis.

Some genomic sites are naturally more difficult to replicate and, therefore, are prone to DNA damage associated with replication stress. These sites fall into two main categories including common and early replicating fragile sites (CFS and ERFS). Most of the time, damage resulting from stalled replication forks is efficiently repaired. However, fragile sites that exhibit chronic DNA damage because of replication stress increase the probability that errors in DNA repair will occur. These sites are frequently associated with translocations and amplification events, which are known drivers of disease.

In recent work published in *Molecular Cell*, Jeongkyu Kim, a postdoctoral researcher in the laboratory of Philipp Oberdoerffer, uncovered a pathway in which chronic DNA damage resulting from replicative stress leads to changes in the epigenetic landscape at fragile sites. Furthermore, they observe that these epigenetic changes can function to "bookmark" damage-prone sites to allow them to remain poised for quick and efficient DNA repair upon replication stress in subsequent cell cycles.

In their report, Kim *et al.* induce replication stress using the DNA polymerase inhibitor aphidicolin and observe the accumulation of the H2A histone variant macroH2A1.2 at sites of DNA damage. Moreover, they find that the DNA damage response machinery promotes incorporation of macroH2A1.2 via a replication fork-associated histone chaperone complex called FACT. In addition, they show these replication stress-induced changes to the chromatin landscape safeguard the genome by serving as a platform to recruit DNA damage repair machinery. Interestingly, accumulation of macroH2A1.2 occurs naturally after repeated rounds of replication. Indeed, macroH2A1.2 becomes enriched at fragile sites in late passage primary human fibroblasts and is necessary to prevent replication stress-induced senescence. Therefore, it seems that our cells have evolved a pathway to mark sites that are prone to DNA damage, setting up a chromatin environment that is primed for efficient repair.

These findings are particularly interesting in light of the observation that macroH2A1.2 is frequently overexpressed in cancer. The possibility that some tumors may rely on this important histone variant to resolve replication stress and prevent senescence redefines macroH2A1.2 as a potential novel therapeutic target against cancer.

► **Jonathan Nye**

LRBGE rewind

Protein-genome dynamics in 3D

At the turn of the 21st century, Tom Misteli started his *LRBGE* career strongly, publishing two back-to-back papers in *Nature* on the dynamics of nuclear proteins. Prior to these findings, the accepted dogma in the field was that unlike the cytoplasm, which is highly compartmentalized with membranous organelles, the nucleus lacked such organization. However, in the late 1990s, several groups reported the presence of specific nuclear compartments using high-resolution microscopy. Very little was known regarding these structures, the movement of nuclear proteins, and their functional impact on gene expression.

Due to the mere nature of these unknowns, protein movement, and the lack of membranous organelles, biochemical fractionation of the nucleus was met with little success. As a result, *in vivo* microscopy of fluorescently-tagged molecules was adopted to visualize and identify nuclear compartments. Protein dynamics were assessed with fluorescence recovery after photobleaching (FRAP), a method of photobleaching specific spots within a cell, which measures recovery of a fluorescent protein in that spot over time. Additionally, fluorescence loss in photobleaching (FLIP) was measured by repeatedly bleaching one spot in a cell with a high-powered laser and quantifying the loss of fluorescence in the area surrounding that spot.

FRAP and FLIP were key to determining live protein movement within a cell nucleus, permitting an April 2000 publication entitled, "High mobility of proteins in the mammalian cell nucleus." The authors discovered that proteins can move in an ATP- and temperature- independent manner, suggestive of passive diffusion of nuclear proteins. Nuclear compartments could also assemble and disassemble quickly. Proteins from three distinct nuclear processes were investigated: the nucleosome binding protein HMG-17, the pre-mRNA splicing factor SF2/ASF, and the rRNA processing protein fibrillarin. Though, FRAP recovery of each protein occurred within 30 seconds, residence times, kinetics, and compartmentalization were distinct, which likely depend on distinct functions within the nucleus. For example, blocking RNA polymerase I resulted in increased mobility of fibrillarin and diminished nucleolar integrity, but blocking RNA polymerase II resulted in quicker and larger formations of splicing factor compartments.

Setting a good (???) example! Tom Misteli, 2002.
Photo credit: Mirek Dundr.

The techniques and knowledge acquired from the April 2000 manuscript paved the way for another manuscript published in December's issue of *Nature* entitled, "Dynamic binding of histone H1 to chromatin in living cells." This article focused on understanding the dynamics of the linker histone H1. Here, the authors found that histone H1 is almost always associated with chromatin, displaying a slow exchange rate of several minutes. Interestingly, the residence of histone H1 to chromatin was sensitive to hyperacetylation of core histones, suggesting increased movement upon chromatin remodeling. Understanding the kinetics of histone H1 was fundamental to determining the mechanism underlying it repressive qualities.

This work was reviewed the following year in *Science* by Dr. Misteli, summarizing his landmark advancements in understanding the biophysical attributes of nuclear proteins. The findings from these groundbreaking papers incited a field of interest to further determine whether and how functional links exist between nuclear architecture, protein dynamics and the regulation of gene expression. ► **Mariana Mandler**

LRBGE rewind

A rapid exchange!

A core principle of LRBGE is to bring the stationary drawings of gene regulation in biology textbooks to life. From 1996 to 2004, Gordon Hager has led his group in several landmark studies to achieve a real-time view of the gene regulatory behavior of the glucocorticoid receptor (GR).

A major goal of the Hager lab was to observe real-time nuclear translocation and movement of GR in living cells. To do this, Han Htun and colleagues utilized the recently isolated green fluorescent protein (GFP), which had already begun to show its exceptional potential as a genetically-encoded marker of cellular proteins. Htun *et al.* created a chimera of GFP fused to rat GR, called GFP-GR. They expressed GFP-GR in a cell line capable of mouse mammary tumor virus (MMTV)-driven luciferase expression, which served as a readout of gene activation. Using time-lapse microscopy, they documented the first reported observations of a steroid receptor translocating to the nucleus in response to a hormone. The authors discovered that the GR response was a multi-step process consisting of hormone binding, nuclear translocation, and binding to gene targets. They also observed that different hormone ligands affected the timescales and efficiency of these independent steps. Their results highlighted fluorescent transgenes and time-lapse fluorescence microscopy as powerful tools to quantify the dynamic steps of receptor nuclear translocation.

But how does GR behave when it binds target gene to initiate transcription? The classic view was that receptors bound almost indefinitely to the chromatin template as long as the activating hormone ligand was present. To assess this issue McNally, Mueller and Hager took an *in vivo* approach. They expressed GFP-GR in a mouse neoplastic cell line containing a large tandem array of the MMTV long terminal repeat with several hundred binding sites for GR. Using fluorescence microscopy, the authors could visualize GFP-GR coalescing at the MMTV array upon hormone induction. They used this assay in combination with the imaging tools FLIP and FRAP, and discovered that GFP-GR exchange at the MMTV array was extremely rapid. McNally and colleagues advanced a new hypothesis, called "hit-and-run," which proposed that chromatin interactions of receptors and other transcription factors were short-lived, and provided only a transient scaffold for the assembly of other complexes at the regulatory site. This led to a re-interpretation for *in vitro* observations on footprints and hypersensitivity. They suggested that a population-averaged measurement, such as a footprint, did not necessarily indicate continuous binding by transcriptional regulators, but rather an equilibrium of dynamic binding that happened to favor the occupied state at a given time.

The Hager lab continued to pursue the idea of dynamic GR gene regulation. Akhilesh Nagaich and colleagues identified the cellular factors and timescales involved in GR-mediated chromatin remodeling. To achieve this, they incubated MMTV chromatin template reconstituted in *Drosophila* embryo extract with various combinations of GR, ATP, and SWI/SNF remodeling complexes. They removed a portion of each reaction for UV crosslinking and immunoprecipitation at 30 second intervals to construct a view of how GR and SWI/SNF associated with the chromatin template in real time. Nagaich's findings were consistent with McNally's *in vivo* observations in that GR-mediated chromatin remodeling was a dynamic process, and that GR itself rapidly exchanged at binding sites. The UV crosslinking study showed that both GR and SWI/SNF exhibited alternating periodic peaks of binding at five-minute intervals. The cyclical binding and dissociation by both GR and SWI/SNF were ATP dependent, and GR rapidly exchanged at the chromatin template.

These studies were highly novel in their use of *in vivo* imaging tools and *in vitro* biochemistry to construct a dynamic view of GR activity in the cell, and it resulted in a paradigm shift for the field.

► **Simona Patange**

LRBGE staff

H. Efsun Arda, Ph.D.

Head, Developmental Genomics Group

In the first issue of LRBGE Connect, there is no better candidate than our latest tenure track recruit to feature in our Q&A section. Dr. Efsun Arda was offered a Stadtman Investigator position in 2017 and has since made building 41 her new scientific home.

Can you describe your academic history and how you ended up at the NIH?

After finishing my undergraduate studies in Bogazici University, Istanbul, Turkey, I was accepted into the Ph.D. program at The University of Massachusetts Medical School in Worcester, MA. I was drawn to the principles of systems biology and joined the laboratory of Dr. Marian Walhout. Here we used *C. elegans* and yeast to delineate the gene regulatory networks governing metabolism. After I obtained my Ph.D. in 2011, I got the opportunity to work with Dr. Seung Kim for my postdoctoral training at Stanford University and moved to the West Coast. It was a great experience, surrounded by fantastic scientists, we pushed the boundaries of human pancreas research. The weather was a bonus, too! Towards the end of my training, with the goal of establishing my own research group, I spent a year as a guest researcher in Dr. Francis Collins' group at NHGRI, NIH. Through this opportunity, I became interested in the Stadtman program and decided to apply.

Can you give a summary of your future research program?

My research focuses on understanding the genomic information that governs human pancreas cell identity and function. Too many people are inflicted with the disorders of the pancreas, including diabetes and cancer. My research goal is to delineate the genetic, genomic, and molecular mechanisms that lead to the differentiation and development of the human pancreas. The premise is that studying the natural process of "making a pancreas" will provide us clues about the root causes of pancreatic diseases as well as help develop regenerative therapies.

Welcome aboard! Photo credit: H. Efsun Arda.

What has surprised you most about working at the NIH?

The abundance of resources that are available to the investigators as well as the trainees. We have many scientific, technical, and administrative support staff whose job is to help you achieve your goals. You don't feel like you have to do it all alone, and you can always find someone willing to assist.

Why do you think LRBGE is the right place for you to achieve your research goals?

During my interviews at different institutes and branches, LRBGE was the place I most felt at home. I remember the joy I experienced after my first conversation with Dr. Gordon Hager. It was as if I was wandering around in a foreign land and finally found someone who spoke my language! I think LRBGE has an exceptional group of investigators who are genuinely curious about the fundamental aspects of chromatin biology and how gene expression works. To me, being surrounded by such expertise is a great strength for the success of my research program.

► **Interviewed by Erin Swinstead**

LRBGE news

Achievements at home and abroad

► **Yamini Dalal, Ph.D.** (Group Director, Chromatin Structure and Epigenetic Mechanisms) was granted tenure at the NIH and promoted to Senior Investigator.

► **Lars Grøntved, Ph.D.** (HAO, 2008-2014) was granted tenure at the University of Southern Denmark. Dr. Grøntved's laboratory currently investigates the impact of the environment on transcription and chromatin structure using the mouse liver as a model system.

► **Tineke Lenstra, Ph.D.** (SBGE, 2013-17) was recently awarded the NVBMB prize by the Netherlands Society for Biochemistry and Molecular Biology. Dr. Lenstra currently works as a group leader at the Netherlands Cancer Institute, where she investigates the mechanisms of transcription regulation.

► **Diego Presman, Ph.D.** (HAO, 2012-18) recently started a tenure-track faculty research program at the University of Buenos Aires, Argentina. Dr. Presman will study transcription factor function using single-molecule techniques.

► **Tatini Rakshit, Ph.D.** (CSEM, 2017-) received an INSPIRE faculty award by the Indian government to start an independent research program.

Do you have something exciting to share with the LRBGE community? Please send details to rachael.stitely@nih.gov.

Acknowledgements

The *LRBGE connect* newsletter is published quarterly for and by LRBGE scientists.

Cover Image

Fixed U2OS human osteosarcoma cells stained with antibodies that detect NOLC1 (green, a nucleolar fibrillar center component) and microtubules (red). Cells co-stained using DAPI to visualize nuclei (blue). Image credit: *The Human Protein Atlas* (www.proteinatlas.org) - Thul PJ *et al*. (2017) "A subcellular map of the human proteome" *Science* 356(6340):pii:eaal3321.
DOI: 10.1126/science.aal3321. (link)

Citations

LRBGE forward

Bui, Pitman *et al.* (2017) "Internal modifications in the CENP-A nucleosome modulate centromeric dynamics" *Epigenetics & Chromatin* 10:17.
DOI: 10.1186/s13072-017-0124-6. (link)

Nanan *et al.* (2017) "Independence between pre-mRNA splicing and DNA methylation in an isogenic minigene resource" *Nucleic Acids Research* 45(22): 12780-1279.
DOI: 10.1093/nar/gkx900. (link)

Ren *et al.* (2017) "CTCF-mediated enhancer-promoter interaction is a critical regulator of cell-to-cell variation of gene expression" *Molecular Cell* 67(6):1049-1058.
DOI: 10.1016/j.molcel.2017.08.026. (link)

Kim *et al.* (2018) "Replication stress shapes a protective chromatin environment across fragile genomic regions" *Molecular Cell* 69(1):36-47.
DOI: 10.1016/j.molcel.2017.11.021. (link)

LRBGE rewind

Phair & Misteli (2000) "High mobility of proteins in the mammalian cell nucleus" *Nature* 404(6778):604-9; DOI: 10.1038/35007077. (link)

Misteli *et al.* (2000) "Dynamic binding of histone H1 to chromatin in living cells" Nature 408(6814):877-81. DOI: 10.1038/35048610. (link)

Misteli (2001) "Protein dynamics: implications for nuclear architecture and gene expression" *Science* 291(5505):843-7.
DOI: 10.1126/science.291.5505.843. (link)

Htun *et al.* (1996) "Visualization of glucocorticoid receptor translocation and intranuclear organization in living cells with a green fluorescent protein chimera" *PNAS* 93(10):4845-50.
DOI: 10.1073/pnas.93.10.4845. (link)

McNally *et al.* (2000) "The glucocorticoid receptor: rapid exchange with regulatory sites in living cells" *Science* 287(5456):1262-5.
DOI: 10.1126/science.287.5456.1262. (link)

Nagaich *et al.* (2004) "Rapid periodic binding and displacement of the glucocorticoid receptor during chromatin remodeling" *Molecular Cell* 14(2):163-74
DOI: 10.1016/S1097-2765(04)00178-9. (link)