
BIOGRAPHICAL SKETCH

NAME: L. Stirling Churchman, PhD

eRA COMMONS USER NAME (credential, e.g., agency login): CHURCHMANL

POSITION TITLE: Assistant Professor of Genetics, Harvard Medical School, Boston, MA

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Cornell University, Ithaca, NY	B.A.	05/1999	Physics
Stanford University, Stanford, CA	Ph.D.	01/2008	Physics
University of California, San Francisco, San Francisco, CA	Postdoctoral Fellow	07/2011	Cellular and Molecular Pharmacology

A. Personal Statement

My role in the proposed project is that of PI. Over the past five years in my first independent position as an assistant professor at Harvard Medical School, I have lead my group through projects that seek to quantitatively visualize gene expression processes at higher levels of resolution (e.g. Mayer *et al. Cell*, 2015 and Couvillion *et al. Nature*, 2016).

My interest in quantitative, high resolution measurements stems from my time as a physics graduate student in the laboratory of Dr. James Spudich (Stanford University). There I developed super-resolution single-molecule fluorescence microscopy techniques to elucidate the mechanism by which myosin V and myosin VI achieve directional and processive movement along actin filaments (Okten, Churchman *et al. NSMB*, 2004 and Churchman *et al. PNAS* 2005). The development of these techniques required novel analytical tools for the analysis of point-spread functions, which motivated a successful collaboration with Dr. Henrik Flyvbjerg, a theoretical physicist at the Technical University of Denmark (Mortensen*, Churchman* *et al. Nature Methods*, 2010, *equal authorship).

As a postdoctoral fellow with Dr. Jonathan Weissman (UCSF/HHMI), I developed native elongating transcript sequencing (NET-seq) in budding yeast, *Saccharomyces cerevisiae* (Churchman and Weissman, *Nature*, 2011). Yeast NET-seq data revealed frequent pausing during transcription and a general nucleosomal barrier for elongating RNA polymerase. In my independent career, I lead my group in re-engineering NET-seq to human cells (Mayer *et al.*, *Cell*, 2015). Directly relevant to this proposal, my lab has recently investigated the coordination of mitochondrial and nuclear gene expression processes that required adapting ribosome profiling to interrogate yeast mitochondrial ribosomes (Couvillion *et al. Nature*, 2016).

B. Positions and Honors

Positions and Employment

1997-1999 Undergraduate researcher in the lab of Watt W. Webb, Department of Applied Physics, Cornell University; Imaging intracellular oxidative stress using confocal and two-photon microscopy.

2000-2002 Graduate student in the labs of Steven Chu and Daniel Herschlag, Departments of Physics and Biochemistry, Stanford University; Single-molecule FRET studies of the folding of the *Tetrahymena* ribozyme.

- 2002-2008 Graduate student in the lab of James Spudich, Department of Biochemistry, Stanford University; Mechanisms of myosin processivity observed by super-resolution single molecule fluorescence techniques.
- 2008-2011 Postdoctoral Fellow in the lab of Jonathan Weissman, Department of Cellular and Molecular Pharmacology, University of California, San Francisco; Global analysis of transcription at nucleotide resolution.
- 2011-present Assistant Professor of Genetics, Harvard Medical School, Boston, MA
- 2011-present Associate Member of the Broad Institute of Harvard and MIT

Honors and Awards

- 2002 Kirkpatrick Teaching Award - Stanford Department of Physics
- 2008-2011 Merck Postdoctoral Fellow of the Damon Runyon Cancer Research Foundation
- 2011 Dale F. Frey Award for Breakthrough Scientists from the Damon Runyon Cancer Research Foundation
- 2011 Career Award at the Scientific Interface from the Burroughs Wellcome Fund
- 2013 New Scholar in Aging Award from the Ellison Medical Foundation
- 2015 Scialog Fellow of the Research Corporation and the Gordon and Betty Moore Foundation

C. Contributions to Science:

1) Regulatory control of transcription elongation and co-transcriptional processes

To deeply investigate the regulation of transcription elongation and coupled co-transcriptional processes, we need high-resolution approaches to localize RNA polymerase genome-wide. As a postdoctoral fellow, I spearheaded the development of native elongating transcript sequencing (NET-seq) that directly monitors Pol II at single nucleotide resolution across the *S. cerevisiae* genome (ref. 1). In my independent position, my group re-engineered NET-seq to enable its straightforward application to higher organisms, resulting in a transformative research tool that is empowering new research directions into gene expression regulation and genome biology (ref. 2). By resulting in a non-perturbative measure of transcription initiation, elongation and termination, NET-seq allows for the in-depth investigation of transcriptional complexities and provides insight into the *in vivo* dynamics of Pol II that are directly comparable to *in vitro* biophysical studies. We have discovered that transcription is interrupted by pausing events at nucleosomes, sites of transcription factor occupancy and the boundaries of exons to be retained in the mature mRNA (ref. 1&2). The NET-seq approach has been applied by many researchers to shed light on the roles of transcription, chromatin and termination factors. The large reach of the NET-seq methodology is enabled in part through publishing in-depth protocols (ref. 3). Most recently we applied NET-seq and quantitative proteomics to identify RNA polymerase II interactomes that revealed unappreciated connections between RNA polymerase II and splicing regulation (ref. 4). Together, NET-seq exposes the topography and regulatory complexity of human gene expression.

1. **Churchman, L. S.**, and Weissman, J. S. (2011). Nascent transcript sequencing visualizes transcription at nucleotide resolution. *Nature* **461**, 186-192. PMID: PMC3880149
2. Mayer, A., di Iulio, J., Maleri, S., Eser U., Vierstra, J., Reynolds, A., Sandstrom R., Stamatoyannopoulos J.A., **Churchman, L.S.** (2015). Native elongating transcript sequencing reveals human transcriptional activity at nucleotide resolution. *Cell* **161**, 541-554. PMID: PMC4528962
3. Mayer, A., and **Churchman, L.S.** (2016). Genome-wide profiling of RNA Polymerase transcription at nucleotide resolution in human cells with native elongating transcript sequencing, *Nature Protocols*, **11**, 813–33. PMID: 27010758
4. Harlen, K.M., Trotta, K.L., Smith, E.E., Mosaheb, M.M., Fuchs, S.M. and **Churchman, L.S.** (2016). Comprehensive RNA Polymerase II interactomes reveal distinct and varied roles for each phospho-CTD residue, *Cell Reports*, *in press*.

2) Co-regulation of mitochondrial and nuclear gene expression programs

Oxidative phosphorylation (OXPHOS) complexes pose a unique challenge for cells because their subunits are encoded on both the nuclear and the mitochondrial genome. Genomic approaches designed to study nuclear/cytosolic and bacterial gene expression have not been broadly applied to mitochondria, so the co-regulation of OXPHOS genes remains largely unexplored. We have developed approaches to monitor mitochondrial gene expression with the same accuracy and resolution afforded by nuclear genomic methodologies. We used these approaches to monitor mitochondrial and nuclear gene expression in *Saccharomyces cerevisiae* during mitochondrial biogenesis, when OXPHOS complexes are synthesized. We observed that nuclear- and mitochondrial-encoded OXPHOS transcript levels do not increase concordantly. Instead, mitochondrial and cytosolic translation are rapidly, dynamically and synchronously regulated. Furthermore, cytosolic translation processes control mitochondrial translation unidirectionally. Thus, the nuclear genome coordinates mitochondrial and cytosolic translation to orchestrate the timely synthesis of OXPHOS complexes, representing an unappreciated regulatory layer shaping the mitochondrial proteome. Our whole-cell genomic profiling approach establishes a foundation for studies of global gene regulation in mitochondria.

1. Couvillion, M.T, Soto, I.C., Shipkovenska, G. and **Churchman, L.S.** (2016). Synchronized mitochondrial and cytosolic translation programs, *Nature*, **533**, 499-503.

3) Molecular mechanism of myosin motor processivity

Myosin-based motility is responsible for trafficking molecular cargo across the actin cytoskeleton at the cell periphery. When I began my graduate work in the laboratory of Dr. James Spudich, the mechanism of processive myosin motors was still under debate. To directly observe myosin motility, I developed a novel super-resolution microscopy method that revealed that the two catalytic heads of the myosin V homodimer move alternatively along actin filaments using a 'hand-over-hand' mechanism, 36-nm at a time (ref 2). We observed a similar mechanism for myosin VI motility (ref 1). The mechanisms that power processive myosin motion have been used as models in subsequent work to determine how other cytoskeletal motors transport their cargo across the cell. Furthermore, this mechanism has critically influenced our thinking on how multiple molecular motors function and the role of accessory factors in living cells.

1. Okten, Z., **Churchman, L. S.**, Rock, R. S., and Spudich, J. A. (2004). Myosin VI walks hand-over-hand along actin. *Nat Struct Mol Biol* **11**, 884-887. PMID: 15286724

2. **Churchman, L. S.**, Okten, Z., Rock, R. S., Dawson, J. F., and Spudich, J. A. (2005). Single molecule high-resolution colocalization of Cy3 and Cy5 attached to macromolecules measures intramolecular distances through time. *PNAS* **102**, 1419-1423. PMCID: PMC545495

4) Optimization of super-resolution light microscopy

The resolution of conventional microscopy is limited to ~250 nm by light diffraction, mathematically described by the point-spread function (PSF). In contrast, the resolution of super-resolution localization microscopy is limited by the number of collected photons, the signal-to-noise ratio and the computational analysis method. We developed two-color super-resolution microscopy, single molecule high resolution colocalization (SHREC), to co-localize two fluorescent probes to within 10 nm (ref 1; also see description of our biological application in section 2 above). In another study, we derived and verified an accurate physical model for light microscopy images of point sources, enabling the highest precision possible by information statistics for super-resolution localization microscopy, such as PALM and STORM (ref 2). Using wave optics applied to a dipole emitter, we derived the PSF of the light microscope and showed that it precisely describes images of isolated fluorophores. We found that Maximum Likelihood estimation using the true PSF improved the localization precision by a factor of two, yielding the highest precision possible by information statistics (Cramér–Rao lower bound). Finally, we theoretically derived and confirmed experimentally the accurate localization precision formula that is now the standard in the field. In other work, we developed ways to increase signal-to-noise using zero-mode waveguides (ref 3) and by deriving optimal models for determining distances from noisy measurements (ref 4).

1. **Churchman, L. S.**, Okten, Z., Rock, R. S., Dawson, J. F., and Spudich, J. A. (2005). Single molecule high-resolution colocalization of Cy3 and Cy5 attached to macromolecules measures intramolecular distances through time. *PNAS* **102**, 1419-1423. PMID: PMC545495
2. Mortensen, K. I.*, **Churchman, L. S.***, Spudich, J. A., and Flyvbjerg, H. (2010). Optimized localization analysis for single-molecule tracking and super-resolution microscopy. *Nat Methods* **7**, 377-381. PMID: PMC3127582 *equal authorship
3. Elting, M.W., Leslie, S.R., **Churchman, L.S.**, Korlach, J., McFaul, C.M., Leith, J.S., Levene, M.J., Cohen, A.E., Spudich, J.A. (2013). Single-molecule fluorescence imaging of processive myosin with enhanced background suppression using linear zero-mode waveguides (ZMWs) and convex lens induced confinement (CLIC). *Opt Express* **21**(1):1189-1202. PMID: PMC3632498
4. **Churchman, L. S.**, Flyvbjerg, H., and Spudich, J. A. (2006). A non-Gaussian distribution quantifies distances measured with fluorescence localization techniques. *Biophys J* **90**, 668-671. PMID: PMC1367071

Complete List of Published Work:

<http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/45025847/?sort=date&direction=ascending>

D. Research Support

Ongoing Research Support

1010244.01 Churchman 9/1/11 – 6/30/16
 Burroughs Wellcome Fund- Career Award at the Scientific Interface
Regulation of the RNA polymerase motor mechanism in vivo
 The goal of this grant is to determine the biophysical properties of RNA polymerase II that allow it to be dynamically regulated.

Junior Faculty Grant Churchman 3/18/13 – 6/30/16
 Giovanni Armenise-Harvard Foundation
Visualizing the regulation of mitochondrial gene expression with nucleotide resolution
 The major goal of this grant is to develop methodologies to follow yeast mitochondrial gene expression with nucleotide resolution.

R01 HG007173 Churchman 4/1/13 – 3/31/18
 NIH / NHGRI
Mechanisms of transcriptional control revealed by nascent transcript sequencing
 The major goals of this project are to adapt native elongating transcript sequencing to human cells and to determine mechanisms of transcriptional elongation regulation.

AG-NS-1068-13 Churchman 12/1/13 – 11/30/18
 Ellison Medical Foundation – New Scholar in Aging Award
Mitochondrial gene expression landscape of S. cerevisiae during aging and life span extension
 The major goal of this grant is to determine how mitochondrial gene expression is altered during yeast aging.

Research Support Completed During the Last Three Years

Frey Award Churchman 9/1/2011 – 6/30/13
Damon Runyon Cancer Research Foundation
Visualizing global transcription in vivo at nucleotide resolution
 The goal of this grant is to measure the high resolution RNA polymerase on the mitochondrial genome.

Charles E.W. Grinnell Medical Research Award Churchman 3/3/15 – 2/28/16

Charles E.W. Grinnell Trust

High-resolution Analysis of Estrogen-Induced Transcription in Rare Human Mammary Cells using Native Elongating Transcript Sequencing

In this project we will adapt our NET-seq approach to require fewer cells in order to analyze rare cell populations.