BIOGRAPHICAL SKETCH

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NAME: Bryce Nickels

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

POSITION TITLE: Professor of Genetics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Miami University	B.S.	05/1995	Chemistry
Harvard University	Ph.D	07/2002	Microbiology
Harvard University	Postdoctoral	08/2007	Microbiology

A. Personal Statement

My lab's research focus is the mechanism and regulation of transcription by bacterial RNA polymerase (RNAP). Our work has provided fundamental insight into the mechanism of transcription initiation, transcription elongation, and transcriptional regulation, particularly in the areas of transcription start site selection, primer-dependent initiation, use of metabolites as non-canonical initiating nucleotides, and transcription pausing. To perform our studies, we use an approach that combines conventional methods (e.g. molecular biology, genetics, and biochemistry) with cutting-edge high-throughput sequencing (HTS) methods (e.g. massively-parallel transcriptomics and massively-parallel protein-DNA photo-crosslinking). Use of these HTS-based methods has enabled us to probe mechanistic aspects of transcription and transcriptional regulation, both *in vitro* and *in vivo*, that would be otherwise inaccessible. In addition, the development and use of specialized HTS-based methods for mechanistic studies of RNAP distinguishes work in my lab from that of others in the field.

Ongoing and Recent Support:

Transcription: Mechanism and Regulation
NIH-NIGMS, R35 GM118059; 6/1/21-5/31/26

Citations:

1. Goldman SR, Ebright RH, and **Nickels BE**. Direct detection of abortive RNA transcripts *in vivo*. *Science* (2009) 324, 927-928. PMC2718712
2. Vvedenskaya IO, Vahedian-Movahed H, Bird JG, Knoblauch JG, Goldman SR, Zhang Y, Ebright RH, and **Nickels BE**. Interactions between RNA polymerase and the "core recognition element" counteract pausing. *Science* (2014) 344, 1285-1289. PMC4277259
3. Bird JG, Zhang Y, Tian Y, Panova N, Barvík I, Greene L, Liu M, Buckley B, Krásný L, Lee JK, Kaplan CD, Ebright RH, and **Nickels BE**. The mechanism of RNA 5′ capping with NAD^+, NADH, and desphospho-CoA. *Nature* (2016) 535, 444-447. PMC4961592
4. Winkelman JT, Vvedenskaya IO, Zhang Y, Zhang Y, Bird JG, Taylor DM, Gourse RL, Ebright RH, and **Nickels BE**. Multiplexed protein-DNA crosslinking: scrunching in transcription start site selection. *Science* (2016) 351, 1090-1093. PMC4797950

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2017-present	Professor, Waksman Institute of Microbiology and Department of Genetics Rutgers University, Piscataway, NJ
2016-2021	NIH Prokaryotic Cell and Molecular Biology (PCMB) study section (member)
2013	NIH Prokaryotic Cell and Molecular Biology (PCMB) study section (*ad hoc*)
2012-2017	Associate Professor, Waksman Institute of Microbiology and Department of Genetics Rutgers University, Piscataway, NJ
2007-2012	Assistant Professor, Waksman Institute of Microbiology and Department of Genetics Rutgers University, Piscataway, NJ
2002-2007	Postdoctoral Research Fellow with Dr. Ann Hochschild, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA
1995-1996	Research Chemist, Monsanto Co., St. Louis, MO

Honors

2018	Fellow, American Academy of Microbiology (AAM)
2015	Chancellor's Scholar, Rutgers University
2010	NIH Director's Transformative Research Award, NIH
2008	Pew Biomedical Scholar, The Pew Charitable Trust
2003	Bernard N. Fields Prize in Microbiology and Molecular Genetics, Harvard Medical School
2002	Nestlé Award, Journal of Bacteriology
2002	Molecular Microbiology Poster Prize, Phage Meeting. Cold Spring Harbor, NY
1995	Phi Beta Kappa

C. Contributions to Science

1. *Synthesis and functional roles of short RNA products in vivo***:** My lab opened a new research area in RNA biology involving the synthesis and function of extremely short RNA products in living cells. We showed that abortive transcription initiation (which produces 2- to ~15 nt RNA products), occurs *in vivo*, thus answering a longstanding question in the field. In addition, we showed that 2- to 4-nt RNA products ("nanoRNAs") function as primers for transcription initiation in bacteria. We further showed that primer-dependent initiation by nanoRNAs is growth-phase dependent (with high prevalence in stationary phase and low prevalence in exponential phase) and that use of nanoRNAs as primers for transcription initiation modulates biofilm formation. Accordingly, our studies identified nanoRNAs as a previously unknown class of regulatory RNA that function by a previously undocumented mechanism of action, i.e. direct incorporation into RNA transcripts. In recent work, we have comprehensively defined the promoter sequence determinants for primer-dependent initiation with nanoRNAs in *E. coli.*

a. Goldman SR, Ebright RH, and **Nickels BE**. Direct detection of abortive RNA transcripts *in vivo*. *Science* (2009) 324, 927-928. PMC2718712
b. Goldman SR, Sharp JS, Vvedenskaya IO, Livny J., Dove SL, and **Nickels BE**. NanoRNAs prime transcription initiation *in vivo*. *Molecular Cell* (2011) 42, 817-825. PMC3130991
c. Druzhinin SY, Tran NT, Skalenko KS, Goldman SR, Knoblauch JG, Dove SL, and **Nickels BE**. A conserved pattern of primer-dependent transcription initiation in *Escherichia coli* and *Vibrio cholerae* revealed by 5′ RNA-seq. *PLOS Genetics* (2015) 11(7):e1005348. PMC4488433
d. Promoter-sequence determinants and structural basis of primer-dependent transcription initiation in *Escherichia coli*. Skalenko KS, Li L, Zhang Y, Vvedenskaya IO, Winkelman JT, Cope A, Taylor DM, Shah P, Ebright RH, Kinney JB, Zhang Y and **Nickels BE**. *Proc. Natl. Acad. Sci. U.S.A.* (2021) 118(27):e2106388118. PMC8271711

2. *Identification of an "ab initio" mechanism of RNA 5′-end capping: nucleoside-containing metabolites serve as non-canonical initiating nucleotides (NCINs)***:** Work from my lab identified a previously unknown "*ab initio*" mechanism of RNA 5′-end capping that occurs in both eukaryotes and bacteria. In this form of RNA 5′-end capping, nucleoside-containing metabolites such as nicotinamide adenine dinucleotide (NAD) are added to the RNA 5′ end during the first nucleotide addition step in transcription by serving as non-canonical initiating nucleotides (NCINs). My lab has (i) established NCIN-mediated capping occurs with bacterial RNAP, eukaryotic RNAP II and mitochondrial RNAP, (ii) established NCIN caps influence RNA stability, (iii) developed a high-throughput-sequencing method to detect and quantify NCIN-capped RNA ("CapZyme-Seq") and, (iv) defined, comprehensively, the promoter-sequence determinants for NCIN capping with NAD *in vitro* and *in vivo*, in *E. coli*. NCIN-mediated capping provides a direct regulatory connection between metabolism and transcription in which RNAP serves as both a sensor and an actuator in coupling changes in cellular metabolism (NCIN levels) to changes in transcriptional outputs (NCIN-mediated capping).

a. Bird JG, Zhang Y, Tian Y, Panova N, Barvík I, Greene L, Liu M, Buckley B, Krásný L, Lee JK, Kaplan CD, Ebright RH, and **Nickels BE**. The mechanism of RNA 5′ capping with NAD^+, NADH, and desphospho-CoA. *Nature* (2016) 535, 444-447. PMC4961592
b. Jiao X, Doamekpor S, Bird JG, **Nickels BE**, Tong L, Hart RP, and Kiledjian M. 5′-end Nicotinamide Adenine Dinucleotide cap in human cells promotes RNA decay through DXO-mediated deNADding. *Cell* (2017) 168, 1015-1027. PMC5371429
c. Vvedenskaya IO, Bird JG, Zhang Y, Zhang Y, Jiao X, Barvík I, Krásný L, Kiledjian M, Taylor DM, Ebright RH and **Nickels BE**. "CapZyme-Seq" comprehensively defines promoter-sequence determinants for RNA 5' capping with NAD^+. *Molecular Cell* (2018) 70, 553-564. PMC5935523
d. Bird JG, Basu U, Kuster D, Ramachandran A, Grudzien-Nogalska E, Towheed A, Wallace DC, Kiledjian M, Temiakov D, Patel SS, Ebright RH, and **Nickels BE.** Highly efficient 5' capping of mitochondrial RNA with NAD^+ and NADH by yeast and human mitochondrial RNA polymerase. *eLife* (2018) 7, pii: e42179. PMC6298784

3*. Transcription start site selection: determinants and mechanism***:** During transcription initiation, there is significant variability in the position of the transcription start site (TSS) relative to core promoter elements. To define the sequence determinants and mechanistic basis for variability in TSS selection by *E. coli* RNAP, we developed methods for massively-parallel transcriptomics and massively-parallel-protein-DNA photo-crosslinking. Using these methods, we monitored TSS selection for a library of 4^{10} (~1,000,000) promoter sequences. The results defined, comprehensively, the key promoter-sequence determinants for TSS selection and showed that variability in TSS selection occurs through transcription-bubble expansion ("DNA scrunching") and contraction ("DNA anti-scrunching") in the RNAP promoter open complex. In subsequent work, performed in collaboration with Richard Ebright's lab, we confirmed variability in TSS selection occurs through scrunching and anti-scrunching by directly measuring changes in the size of the transcription bubble during TSS selection using single-molecule nanomanipulation. We have also collaborated with Craig Kaplan's lab to identify determinants of TSS selection by *Saccharomyces cerevisiae* RNAP II.

a. Vvedenskaya IO, Zhang Y, Goldman SR, Valenti A, Visone V, Taylor DM, Ebright RH, and **Nickels BE**. Massively systematic transcript end readout, MASTER: transcription start site selection, transcriptional slippage, and transcript yields. *Molecular Cell* (2015) 60, 953-965. PMC4688149
b. Winkelman JT, Vvedenskaya IO, Zhang Y, Zhang Y, Bird JG, Taylor DM, Gourse RL, Ebright RH, and **Nickels BE**. Multiplexed protein-DNA crosslinking: scrunching in transcription start site selection. *Science* (2016) 351, 1090-1093. PMC4797950
c. Yu L, Winkelman JT, Pukhrambam C, Strick TR, **Nickels BE**, and Ebright RH. The mechanism of variability in transcription start site selection. *eLife* (2017) 6, pii: e32038. PMC5730371
d. Ssl2/TFIIH function in Transcription Start Site Scanning by RNA Polymerase II in *Saccharomyces cerevisiae*. Zhao T, Vvedenskaya IO, Lai W, Basu S, Pugh BF, **Nickels BE**, and Kaplan CD. *eLife* (2021) 10:e71013. PMC8589449

4. *Determinants of transcription elongation pausing, initial transcription pausing, and σ-dependent pausing:* My lab has defined the sequence determinants of transcription-elongation pausing and initial-transcription pausing. To define determinants for transcription-elongation pausing, my lab performed a genome-wide analysis of pausing in *E. coli* using native elongating transcript sequencing, "NET-seq." The results identified a consensus

sequence for elongation pausing: $G_{-10}N_{-9}N_{-8}N_{-7}N_{-6}N_{-5}N_{-4}N_{-3}N_{-2}Y_{-1}G_{+1}$, where Y is a pyrimidine and Y_{-1} is the position of the RNA 3' end. We further showed that sequence-specific interactions between the RNAP core enzyme and a core recognition element (CRE) facilitate pause read-through by stabilizing RNAP in a post-translocated register. To define determinants for initial-transcription pausing my lab developed a crosslinking-plus-sequencing-based method termed "XACT-seq" (crosslink-between-active-center-and-template sequencing), which provides a direct, single-nucleotide-resolution readout of the RNAP-active-center position relative to DNA. We applied XACT-seq to detect and quantify pausing in initial transcription at 4^{11} (~4,000,000) promoter sequences *in vivo*, in *E. coli*. The results identified a consensus sequence for initial transcription pausing: $T_{A-2}N_{A-1}\underline{Y_A}G_{A+1}$, where $\underline{Y_A}$ is the RNAP-active-center position. Thus, pausing in initial transcription and pausing in elongation are hard-coded by similar sequence elements, indicating that they share mechanistic commonalities. We have also used XACT-seq to define a consensus sequence for formation of a stable scrunched σ-dependent paused complex that is identical to the consensus sequence for initial transcription pausing.

a. Vvedenskaya IO, Vahedian-Movahed H, Bird JG, Knoblauch JG, Goldman SR, Zhang Y, Ebright RH, and **Nickels BE**. Interactions between RNA polymerase and the "core recognition element" counteract pausing. *Science* (2014) 344, 1285-1289. PMC4277259
b. Winkelman JT, Pukhrambam C, Zhang Y, Shah P, Taylor DM, Ebright RH, and **Nickels BE**. XACT-seq comprehensively defines the promoter-position and promoter-sequence determinants for initial-transcription pausing. *Molecular Cell* (2020) 79, 797-811. PMC7484426
c. XACT-seq: A photocrosslinking-based technique for detection of the RNA polymerase active-center position relative to DNA in Escherichia coli. Pukhrambam C, Vvedenskaya IO and **Nickels BE**. *STAR Protocols* (2021) 2(4):100858. PMC8517213
e. Structural and mechanistic basis of σ-dependent transcriptional pausing. Pukhrambam C, Molodtsov V, Kooshbaghi M, Tareen A, Vu H, Skalenko KS, Su M, Zhou Y, Winkelman JT, Kinney JB, Ebright RH, **Nickels BE**. *Proc. Natl. Acad. Sci. U.S.A.* (2022) 119(23):e2201301119. PMC9191641

5. *Development of HTS-based methods for studies of nucleic-acid biology***:** As mentioned above, my lab has developed methods for massively-parallel transcriptomics and massively-parallel protein DNA photo-crosslinking. These methods enable us to probe mechanistic aspects of transcription, both *in vitro* and *in vivo*, that were previously inaccessible, thereby distinguishing our work from that of others in the field. In published work, we have used these methods to analyze TSS selection, NCIN-mediated capping, and initial-transcription pausing. In collaborative work, we have developed HTS-based methods for analysis of the cleavage specificities of endoribonucleolytic toxins from bacterial pathogens **(**collaboration with Nancy Woychik's lab) and HTS-based methods to detect spacer precursors that accumulate in cells during primed CRISPR adaptation (collaboration with Konstantin Severinov's lab).

a. Schifano JM, Vvedenskaya IO, Knoblauch JG, Ouyang M, **Nickels BE** and Woychik NA. An RNA-seq method for defining endoribonuclease cleavage specificity identifies dual rRNA substrates for toxin MazF-mt3. *Nature Communications* (2014) 5, 3538. PMC4090939
b. Vvedenskaya IO, Goldman SR, and **Nickels BE**. Analysis of bacterial transcription by "massively systematic transcript end readout," MASTER. *Methods in Enzymology* (2018) 612, 269-302. PMC6352903
c. Shiriaeva AA, Savitskaya E, Datsenko KA, Vvedenskaya IO, Fedorova I, Morozova N, Metlitskaya A, Sabantsev A, **Nickels BE**, Severinov K, and Semenova E. Detection of Spacer Precursors Formed *In Vivo* During Primed CRISPR Adaptation. *Nature Communications* (2019) 10(1):4603. PMC6787059
d. Vvedenskaya IO and **Nickels BE**. CapZyme-Seq: a 5'-RNA-seq method for differential detection and quantitation of NAD-capped and uncapped, 5'-triphosphate RNA. *STAR Protocols* (2020) 1, 100002. PMC7384699

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/bryce.nickels.1/bibliography/42351139/public/?sort=date&direction=descending