BIOGRAPHICAL SKETCH

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NAME: Ebright, Richard H.

eRA COMMONS USER NAME (credential, e.g., agency login): EBRIGHT1

POSITION TITLE: Professor

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
Harvard University, Cambridge MA	A.B.	05/1981	Biology (*summa cum laude*)
Harvard University, Cambridge MA (with Jon Beckwith)	Ph.D.	01/1987	Microbiology and Molecular Genetics

A. Personal Statement

Ebright's research focusses on the structure, mechanism, and regulation of bacterial transcription complexes, and on the development of inhibitors of bacterial transcription as antibacterial therapeutic agents. His research employs tools of structural biology, biophysical chemistry, and drug discovery. His research contributions include defining the structural organization of transcription initiation complexes, defining the "scrunching" mechanism of initial transcription, defining the "recruitment" mechanism of transcription activation, defining novel antibacterial targets in the bacterial transcription machinery, and identifying novel antibacterial agents that exhibit activity against drug-resistant bacterial pathogens. He directs a laboratory of approximately fifteen postdoctoral associates, graduate students, and technicians and serves as project leader on two NIH grants.

Ebright serve as PI on the project.

Previous cryo-EM publications:

Lin, W., Das, K., Degen, D., Mazumder, A., Duchi, D., Wang, D., Ebright, Y., Ebright, R.Y., Sineva, E., Gigliotti, M., Mandal, S., Jiang, Y., Liu, Y., Yin, R., Zhang, Z., Eng, E., Thomas, D., Donadio, S., Zhang, C., Kapanidis, A., and Ebright, R. (2018) Structural basis of transcription inhibition by fidaxomicin. *Mol. Cell* **70**, 60-71.

Yin, Z., Kaelber, J., and Ebright, R.H. (2019) Structural basis of Q-dependent antitermination. *Proc. Natl. Acad. Sci. USA* **116**, 18384-18390.

Wang, C., Molodtsov, V., Firlar, E., Kaelber, J., Blaha, G., Su4, M., and Ebright, R.H. (2020) Structural basis of transcription-translation coupling. *Science* (in press; https://science.sciencemag.org/content/early/2020/08/19/science.abb5317.long).

B. Positions and Honors

Positions and Employment

1984-1987 Junior Fellow, Society of Fellows, Harvard University, Cambridge, MA
1987- Laboratory Director, Waksman Institute of Microbiology, Piscataway NJ
1987-1992 Assistant Professor, Department of Chemistry, Rutgers University, New Brunswick, NJ
1992-1995 Associate Professor, Department of Chemistry, Rutgers University, New Brunswick, NJ
1995-2013 Professor, Department of Chemistry, Rutgers University, New Brunswick, NJ
1997-2013 Investigator, Howard Hughes Medical Institute
2013- Board of Governors Professor, Department of Chemistry, Rutgers University, New Brunswick, NJ

Honors

1980 Phi Beta Kappa
1989 Searle Scholar Award
1990 Johnson and Johnson Discovery Research Fellowship

1995 American Society for Biochemistry and Molecular Biology Schering-Plough Award
1996 Fellow, American Academy of Microbiology
1998 Rutgers University Board of Trustees Research Excellence Award
2004 Fellow, American Association for the Advancement of Science
2011 Fellow, Infectious Diseases Society of America
2012 Theobold Smith Society Waksman Award
2013 National Institutes of Health MERIT Award
2016 Member, American Academy of Arts and Sciences

C. Contributions to Science

1. Sequence-Specific Protein-DNA Interaction

Ebright helped define the basis of sequence-specific protein-DNA interaction and developed artificial sequence-specific DNA cleaving agents.

Using genetic approaches and photocrosslinking approaches, Ebright established that sequence-specific DNA binding proteins recognize DNA sequences through direct contacts between amino acids and DNA bases. By conjugating a DNA cleaving agent to a sequence-specific DNA binding protein in a manner that permitted activity in specific complexes but not in nonspecific complexes, Ebright constructed a high-specificity DNA cleaving agent able to cleave megabase DNA substrates at single sites.

Ebright, R., Cossart, P., Gicquel-Sanzey, B., and Beckwith, J. (1984) Mutations that alter the DNA sequence specificity of the catabolite gene activator protein of *E. coli*. *Nature* **311**, 232-235.

Ebright, R. (1986) Evidence for a contact between glutamine-18 of lac repressor and base pair 7 of *lac* operator. *Proc. Natl. Acad. Sci. USA* **83**, 303-307.

Blatter, E., Ebright, Y., and Ebright, R. (1992) Identification of an amino acid-base contact in the GCN4-DNA complex by bromouracil-mediated photocrosslinking. *Nature* **359**, 650-652.

Pendergrast, P.S., Ebright, Y., and Ebright, R. (1994) High-specificity DNA cleavage agent: design and application to kilobase and megabase DNA substrates. *Science* **265**, 959-961.

2. Transcription: Transcriptional Activation

Ebright provided the first mechanistic and structural description of transcription activation.

Ebright analyzed transcription activation by *Escherichia coli* catabolite activator protein (CAP) at the *lac* promoter. He showed that transcription activation requires a small patch of the activator ("activating region") and a small patch of a flexibly tethered module of RNA polymerase ("activation target"), showed that transcription activation involves direct interaction between activating region and activation target, determined a crystal structure of the complex between activating region and activation target, and, most recently, determined an EM structure of the intact transcription-activation complex. His results establish that transcription activation by CAP at *lac* proceeds by a "recruitment" mechanism, in which interactions between CAP and RNA polymerase facilitate binding of RNA polymerase to DNA.

Zhou, Y., Busby, S., and Ebright, R. (1993) Identification of the functional subunit of a dimeric transcription activator protein by use of "oriented heterodimers." *Cell* **73**, 375-379.

Chen, Y., Ebright, Y., and Ebright, R. (1994) Identification of the target of a transcription activator protein by protein-protein photocrosslinking. *Science* **265**, 90-92.

Blatter, E., Ross, W., Tang, H., Gourse, R., and Ebright, R. (1994) Domain organization of RNA polymerase α subunit: C-terminal 85 amino acids constitute a domain capable of dimerization and DNA binding. *Cell* **78**, 889-896.

Benoff, B., Yang, H., Lawson, C., Parkinson, G., Liu, J., Blatter, E., Ebright, Y., Berman, H., and Ebright, R. (2002) Structural basis of transcription activation: structure of the CAP-αCTD-DNA complex. *Science* **297**, 1562-1566.

3. Transcription: Structures of Transcription Initiation Complexes

Ebright defined the structural organization of the nucleoprotein complexes that perform transcription initiation.

Using distance restraints from systematic photocrosslinking and systematic fluorescence resonance energy transfer (FRET), Ebright constructed the first structural models of bacterial, archaeal, and eukaryotic transcription-initiation complexes. Using x-ray crystallography, Ebright determined the first atomic structure of a promoter-dependent, initiation-factor-dependent, functional transcription initiation complex. More recently, using x-ray crystallography, Ebright determined atomic structures of transcription initiation complexes engaged in *de novo* initiation and initial transcription, providing comprehensive structural descriptions of the protein-DNA interactions involved in promoter recognition, promoter unwinding, *de novo* initiation, and initial transcription. Most recently, using x-ray crystallography, Ebright determined the first atomic structures of a gene-specific transcription activation complex and of Mycobacterial transcription initiation complexes.

Naryshkin, N., Revyakin, A., Kim, Y., Mekler, V., and Ebright, R. (2000) Structural organization of the RNA polymerase-promoter open complex. *Cell* **101**, 601-611.

Mekler, V., Kortkhonjia, E., Mukhopadhyay, J., Knight, J., Revyakin, A., Kapanidis, A., Niu, W., Ebright, Y., Levy, R., and Ebright, R. (2002) Structural organization of RNA polymerase holoenzyme and the RNA polymerase-promoter open complex. *Cell* **108**, 599-614.

Zhang, Y., Feng, Y., Chatterjee, S., Tuske, S., Ho, M., Arnold, E., and Ebright, R. (2012) Structural basis of transcription initiation. *Science* **338**, 1076-1080. PMCID: PMC359305.

Feng, Y., Zhang, Y., and Ebright, R. (2016) Structural basis of transcription activation. *Science* **352**, 1330-1333. PMCID: PMC4905602.

4. Transcription: Mechanisms of Transcription Initiation and Transcription Elongation

Ebright elucidated the mechanisms of initial transcription and promoter escape in transcription initiation and defined sequence determinants and mechanisms for transcriptional pausing in transcription elongation.

Using ensemble and single-molecule FRET, Ebright showed that the transcription initiation factor σ is not obligatorily released in promoter escape but, instead, can remain bound to RNA polymerase, translocate with RNA polymerase, and recognize regulatory DNA sequence elements during transcription elongation. Using single-molecule FRET and single-molecule nanomanipulation, Ebright showed that initial transcription involves a "scrunching" mechanism, in which RNA polymerase remains stationary on promoter DNA and reels in downstream DNA, and that promoter escape involves the accumulation of stress through scrunching, followed by the use of accumulated stress to break RNA polymerase-promoter interactions. More recently, using high-throughput sequencing approaches, Ebright and collaborators showed that transcription start-site selection also involves scrunching, defined, genome-wide, the DNA sequence determinants for pausing during transcription elongation, and demonstrated roles of a newly identified DNA sequence element recognized by RNA polymerase--the "core recognition element"--in transcription initiation, transcription elongation, and transcriptional pausing.

Revyakin, A., Liu, C., Ebright, R. & Strick, T. (2006) Abortive initiation and productive initiation by RNA polymerase involve DNA scrunching. *Science* **314**, 1139-1143. PMCID: PMC2754787.

Kapanidis, A., Margeat, E., Ho, S.O., Kortkhonjia, E., Weiss, S. & Ebright, R. (2006) Initial transcription by RNA polymerase proceeds through a DNA-scrunching mechanism. *Science* **314**, 1144-1147. PMCID: PMC2754788.

Chakraborty, A., Wang, D., Ebright, Y., Korlann, Y., Kortkhonjia, E., Kim, T., Chowdhury, S., Wigneshweraraj, S., Irschik, H., Jansen, R., Nixon, B.T., Knight, J., Weiss, S., and Ebright, R. (2012) Opening and closing of the bacterial RNA polymerase clamp. *Science* **337**, 591-595. PMCID: PMC3626110.

Vvedenskaya, I., Vahedian-Movahed, H., Bird, J., Knoblauch, J., Goldman, S., Zhang, Y., Ebright, R., and Nickels, B. (2014) Interactions between RNA polymerase and the "core recognition element" counteract pausing. *Science* **344**, 1285-1289. PMCID: PMC4277259.

5. Transcription: Transcription Inhibitors, Antibacterial Drug Discovery Targeting Transcription

Ebright is elucidating binding sites and mechanisms of antibacterial agents that function by inhibiting bacterial transcription and is developing small-molecule inhibitors of bacterial transcription as antituberculosis drugs and broad-spectrum antibacterial drugs.

Ebright defined the binding sites and mechanisms of the antibiotics microcin J25, streptolydigin, myxopyronin, corallopyronin, ripostatin, GE23077, salinamide, pseudouridimycin, and lipiarmycin, and of synthetic antibacterial agents of the phloroglucinol and aroyl-aryl-phenylalaninamide classes.

Ebright validated myxopyronins, phloroglucinols, and pseudouridimycins as advanced leads for broad-spectrum antibacterial therapy (with potent activities against priority pathogens in culture and in animals) and validated aroyl-aryl-phenylalaninamides as leads for antituberculosis therapy (with potent activities against *Mycobacterium tuberculosis* in culture). Guided by crystal structures of bacterial RNA polymerase in complex with these leads, Ebright and colleagues are designing, synthesizing, and evaluating analogs of these leads, seeking novel compounds with improved antibacterial activities and improved pharmacological properties.

Prompted by crystal structures indicating that rifamycins--a class of RNA polymerase inhibitors currently used as antibacterial drugs--and GE23077 interact with adjacent binding sites on RNA polymerase and can bind simultaneously to RNA polymerase, Ebright and colleagues linked a rifamycin to GE23077 and showed that the resulting "bipartite inhibitor" had exceptional potency and exceptional ability to overcome target-dependent resistance. Guided by crystal structures, Ebright and colleagues are designing, synthesizing, and evaluating additional novel bipartite inhibitors comprising rifamycin-site ligands linked to GE23077-site ligands.

Mukhopadhyay, J., Das, K., Ismail, S., Koppstein, D., Jang, M., Hudson, B., Sarafianos, S., Tuske, S., Patel, J., Jansen, R., Irschik, H., Arnold, E., and Ebright, R. (2008) The RNA polymerase "switch region" is a target of inhibitors *Cell* **135**, 295-307. PMCID: PMC2580802.

Zhang, Y., Degen, D., Ho, M., Sineva, E., Ebright, K., Ebright, Y., Mekler, V., Vahedian-Movahed, H., Feng, Y., Yin, R., Tuske, S., Irschik, H., Jansen, R., Maffioli, S., Donadio, S., Arnold, E., and Ebright, R. (2014) GE23077 binds to the RNA polymerase "i" and "i+1" sites and prevents the binding of initiating nucleotides. *eLife*, **3**, e02450. PMCID: PMC3994528.

Maffioli, S., Zhang, Y., Degen, D., Carzaniga, T., Del Gatto, G., Serina, S., Monciardini, P., Mazzetti, C., Guglierame, P., Candiani, G., Chiriac, A.I., Facchetti, G., Kaltofen, P., Sahl, H.-G., Dehò, G., Donadio, S., and Ebright, R. (2017) Antibacterial nucleoside-analog inhibitor of bacterial RNA polymerase. *Cell* **169**, 1240-1248. PMCID: PMC5542026.

Lin, W., Das, K., Degen, D., Mazumder, A., Duchi, D., Wang, D., Ebright, Y., Ebright, R.Y., Sineva, E., Gigliotti, M., Mandal, S., Jiang, Y., Liu, Y., Yin, R., Zhang, Z., Eng, E., Thomas, D., Donadio, S., Zhang, C., Kapanidis, A., and Ebright, R. (2018) Structural basis of transcription inhibition by fidaxomicin (lipiarmycin A3). *Mol. Cell* **70**, 60-71. PMCID: PMC6205224.

6. Complete List of Published Work in MyBibliography

https://www.ncbi.nlm.nih.gov/myncbi/richard.ebright.1/bibliography/public/

D. Research Support

1. Ongoing Research Support

Bacterial Transcription Complexes
NIH-NIGMS, R37-GM041376 (Ebright)
02/01/13-01/31/23
The major goal of this project is analysis of the structural and mechanistic basis of bacterial transcription.

Center to Develop Therapeutic Countermeasures to High-Threat Bacterial Agents
NIH-NIAID, U19-AI142731 (Perlin)
05/01/19-04/30/24
The major goal of this project is operation of a Center of Excellence for Translational Research focussed on development of compounds effective against drug-resistant bacterial pathogens. The major goal of the Ebright component of the project is synthesis and efficacy testing of novel arylmyxopyronins and arylalkylcarboxamido phloroglucinols effective against drug-resistant bacterial pathogens.

New Antibacterials: Library Screening
Janssen Pharmaceuticals (Ebright)
10/29/18-10/28/20
The major goal of this project is library screening for new broad-spectrum antibacterial agents and new antituberculosis agents.

2. Completed Research Support

Center to Develop Therapeutic Countermeasures to High-Threat Bacterial Agents
NIH-NIAID, U19-AI109713 (Perlin)
04/25/14-04/30/19
The major goal of this project was establishment of a Center of Excellence for Translational Research focussed on development of compounds effective against drug-resistant bacterial pathogens. The major goal of the Ebright component of the project was synthesis and efficacy testing of novel phloroglucinols and aroyl-aryl-phenylalaninamides effective against drug-resistant bacterial pathogens.

Therapeutics for Drug-Resistant Bacteria: Pseudouridimycins
NIH-NIAID, R01-AI090837 (Ebright)
01/15/13-12/31/18
The major goal of this project is structure-based design, synthesis, and efficacy testing of novel pseudouridimycin analogs effective against drug-resistant bacterial pathogens.

New Antibiotics: Microbial Extract Screening and Mutational De-Replication
Rutgers TechAdvance Fund (Ebright)
04/25/18-04/24/19
The major goal of this project is validation of a novel antibiotics-discovery platform involving microbial extract screening and mutational de-replication.