Chemistry 01:160:480, Chemistry 16:160:580, Quantitative Biomedicine 16:118:617:05
Structural Biology, Structural Biophysics, and Chemical Biology of Transcription

Instructor

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Location and Time

remote instruction (Zoom), Tuesday/Thursday, 7:00-8:30 PM

Course Description

Transcription is the synthesis of RNA using a DNA template. Transcription is the first step in gene expression, is the primary regulated step in gene expression, and is the target of two classes of currently used antibacterial therapeutic agents. Transcription is carried out by megadalton-scale, nanometer-scale molecular machines: RNA polymerase, complexes of RNA polymerase with one or more initiation factor, and complexes of RNA polymerase with one or more elongation factor. Transcription involves three stages: initiation, in which RNA polymerase binds to DNA and begins synthesis of an RNA molecule; elongation, in which RNA polymerase translocates along DNA as a molecular motor and extends the RNA molecule; and termination, in which RNA polymerase stops moving, stops RNA synthesis, releases the DNA molecule, and dissociates from DNA. Transcriptional regulation results in differences in gene expression in different cell types, developmental states, and environmental conditions. Transcriptional regulation occurs at each of these three stages of transcription: initiation, elongation, and termination. The course will present the identities, structures, mechanisms of the molecular machines that carry out transcription and then will present the molecular components and molecular strategies by which transcriptional regulation occurs.

The course will focus primarily on transcription and transcriptional regulation in bacteria, since transcription and transcriptional regulation in bacteria are better understood than--and are paradigmatic of--transcription and transcriptional regulation in higher organisms. Relationships between processes in bacteria and processes in higher organisms will be highlighted.

The course will focus primarily on structure and mechanism. Concepts of structural biology, structural biophysics, and chemical biology will be emphasized.

Protein-DNA interactions and protein-protein interactions will be major themes. The use of thermal energy, binding energy, and chemical energy to generate force, drive conformational changes, and power movement will be additional major themes.

The first half of the course will consist of lectures on transcription and transcriptional regulation. The second half of the course will consist of seminar presentations on research papers ("case studies") that provide the foundation for our understanding of transcription and transcriptional regulation and that exemplify use of x-ray crystallography, electron microscopy, chemical crosslinking, fluorescence resonance energy transfer, single-molecule fluorescence resonance energy transfer, magnetic tweezers, and optical-tweezers approaches to analyze structures and define structural transitions.

Course Description for Catalog

Chemistry 01:160:480, Chemistry 16:160:580
Structural Biology, Structural Biophysics, and Chemical Biology of Transcription (3)
Transcription and transcriptional regulation. Structures and mechanisms of RNA polymerase, initiation factors, elongation factors, activators, repressors, promoters, and terminators. Protein-DNA interactions, protein-protein interactions, and use of energy to drive conformational changes and translocation. Emphasis on RNA polymerase as a molecular machine. Primary focus on bacterial RNA polymerase. Lectures in first half of course. Seminars on primary research papers ("case studies") in second half of course.

Course Objectives

The course will provide a comprehensive understanding of transcription and transcriptional regulation.

The course will provide a thorough introduction to protein-DNA interaction and protein-protein interaction.

Through the intensive study of a representative, paradigmatic molecular machine, the course will provide an introduction to the mechanisms by which molecular machines use thermal energy, binding energy, and chemical energy to generate force, drive conformational changes, and power movement.

The course will provide exposure to modern research tools of structural biology, structural biophysics, and chemical biology, including x-ray crystallography, electron microscopy, chemical crosslinking, fluorescence resonance energy transfer, single-molecule fluorescence resonance energy transfer, single-molecule nanomanipulation with magnetic tweezers, single-molecule nanomanipulation with optical-tweezers, site-specific labelling of proteins, site-specific labelling of nucleic acids, analysis of protein-DNA interactions, and analysis of protein-protein interactions.

The second half of the course will provide training in reading, critically assessing,, and presenting and discussing the primary scientific literature.

Course Pre-Requisites/Co-Requisites

Special permission only

Required: 01:160:159-160, 01:160:161-162, 01:160:163-164, or equivalent coursework in general chemistry.

Required: 01:160:305-306, 01:160:307-308, 01:160:315-316, or equivalent coursework in organic chemistry.

Recommended: 01:160:323-324, 01:160:327-328, 01:160:342, 11:115:409, 16:160:537, or equivalent coursework in physical chemistry, physical biochemistry, or molecular biophysics.

Recommended: 01:694:407-408, 11:115:403-404,16:115:501-502, 16:115:511-512, or equivalent coursework in biochemistry and molecular biology.

Course Requirements

Exam (tentatively 25%).

Seminar presentation(s) on research paper(s) (tentatively 50%).

Attendance and participation (tentatively 25%).

Students enrolled in the course will be subject to the Rutgers University Academic Integrity Policy (http://academicintegrity.rutgers.edu/files/documents/AI_Policy_2013.pdf).

Course Schedule

session 1, 9/1: transcription and transcriptional regulation: overview; protein-nucleic-acid interactions

session 2, 9/3: transcription: RNA polymerase and initiation factors

session 3, 9/10: transcription: promoters

session 4, 9/15: transcription: transcription initiation, part 1, from R to RPo

session 5. 9/27: transcription: transcription initiation, part 2, from RPo to RDe

session 6, 9/22: transcription: transcription elongation, part 1, mechanism of elongation

session 7, 9/24: transcription: transcription elongation, part 2, editing, pausing, arrest

session 8, 9/29: transcription: transcription termination

session 9, 10/1: transcription: transcription antitermination and transcription-translation coupling

session 10, 10/6: transcription: small-molecule inhibitors of transcription

session 11, 10/8: transcriptional regulation: lactose promoter; bacteriophage lambda lysis-vs.-lysogeny

session 12, 10/13: case study 1: structural organization of RPo: chemical crosslinking

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.

session 13, 10/15: case study 2: structural organization of RPo: FRET

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 bacterial RNA polymerase holoenzyme and the RNA polymerase-promoter open complex. *Cell* **108**, 599-614.

session 14, 10/20: case study 3: structural organization of RPo: x-ray crystallography

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.

session 15, 10/22: case study 4: RNA polymerase clamp conformation: single-molecule FRET

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.

session 16, 10/27: case study 5: mechanism of initial transcription: single-molecule FRET

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

session 17, 10/29: case study 6: mechanism of initial transcription: magnetic tweezers

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

session 18, 11/3: case study 7: mechanism of initial transcription: chemical crosslinking

Winkelman, J., Winkelman, B., Boyce, J., Maloney, M., Chen, A., Ross, W., Gourse, R. (2015) Crosslink mapping at amino acid-base resolution reveals the path of scrunched DNA in initial transcribing complexes. *Mol. Cell* **59**, 768-780.

session 19, 11/5: case study 8: mechanism of initial transcription: HT-sequencing, chemical crosslinking

Vvedenskaya, I., Zhang, Y., Goldman, S., Valenti, A., Visone, V., Taylor, D., Ebright, R., and Nickels, B. (2015) Massively systematic transcript end readout (MASTER): transcription start site selection and transcriptional slippage. *Mol. Cell* **60**, 953-965.

Winkelman, J, Vvedenskaya, I., Zhang, Y., Zhang, Y., Bird, J., Taylor, D., Gourse, R., Ebright, R., and Nickels, B. (2016) Multiplexed protein-DNA crosslinking: scrunching in transcription start site selection. *Science* **351**, 1090-1093.

session 20, 11/10: case study 9: initiation-factor release: FRET

Mukhopadhyay, J., Kapanidis, A., Mekler, V., Kortkhonjia, E., Ebright, Y., and Ebright, R. (2001) Translocation of σ^{70} with RNA polymerase during transcription: fluorescence resonance energy transfer assay for movement relative to DNA. *Cell* **106**, 453-463.

session 21, 11/12: case study 10: mechanism of elongation: optical tweezers

Abbondanzieri, E., Greenleaf, W., Shaevitz, J., Landick, R., Block, S. (2005) Direct observation of base-pair stepping by RNA polymerase. *Nature* **438**, 460-465.

session 22, 11/17: case study 11: mechanisms of pausing: optical tweezers, high-throughput sequencing

Herbert, K., La Porta, A., Wong, B., Mooney, R., Neuman, K., Landick, R., Block, S. (2006) Sequence-resolved detection of pausing by single RNA polymerase molecules. *Cell* **125,** 1083-1094.

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.

session 23, 11/19: case study 12: mechanisms of termination: biochemistry, optical tweezers

Santangelo, T., Roberts J. (2004) Forward translocation is the natural pathway of RNA release at an intrinsic terminator. *Mol Cell*. **14,** 117-26.

Larson, M., Greenleaf, W., Landick, R., Block, S. (2008) Applied force reveals mechanistic and energetic details of transcription termination. *Cell* **132,** 971-982.

session 24, 11/24: case study 13: switch-region inhibitors: biochemistry, crystallography, and med-chem

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.

session 25, 12/1: case study 14: active-center inhibitors: biochemistry, crystallography, and med-chem

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.H. (2017) Antibacterial nucleoside-analog inhibitor of bacterial RNA polymerase. *Cell* 169, 1240-1248.

session 26, 12/3: case study 15: antitermination and transcription-translation coupling: cryo-EM

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://www.biorxiv.org/content/10.1101/2020.03.01.972380v2).

optional session 27, 12/8: case study 16: transcription activation at *lac*, CAP-RNAP interaction

Zhou, Y., Zhang, X., and Ebright, R. (1993) Identification of the activating region of CAP: isolation and characterization of mutants of CAP specifically defective in transcription activation. *Proc. Natl. Acad. Sci. USA* **90**, 6081-6085.

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.

session 28, 12/10: no class (exam preparation)

session 29, 12/15: exam