BIOCHEMISTRY (BCHM) 674 (NUCLEIC ACIDS) — FALL, 2005
TuTh, 8:00-9:15 a.m., Chemistry 0127

Assoc. Prof. Jason D. Kahn, Dept. of Chemistry and Biochemistry, UMCP

Office: Chemistry 2500A (Biochemistry, Wing 5 of the Chemistry complex)
Office hours: Weds. 1-2 p.m., Thurs. 1-2 p.m., Chemistry 2500A; there is no TA for the course
Contacting me: jdkahn@umd.edu much preferred to 301-405-0058. Please do not drop in to my office or lab, but I will be happy to set up appointments outside of office hours if necessary.
Web and email: http://www.biochem.umd.edu/biochem/kahn/bchm674; there is also an e-mail reflector.

Course Description

This course concerns the structure and function of nucleic acids and the mechanisms of nucleic acid transactions: a biochemical approach to molecular genetics. We will generally cover both prokaryotic and eukaryotic systems, emphasizing common logic and mechanisms. Topics are as follows:

- **Chemistry and structure of DNA and RNA, from nucleotides to chromosomes and genomes, and some methods for studying, synthesizing, sequencing and manipulating nucleic acids. Rudimentary bioinformatics and systems biology.**
- **Interactions between nucleic acids and ligands such as cations, drugs, and especially proteins.**
- **DNA Biology: Selected aspects of the biochemistry and regulation of DNA replication, transcription, recombination, and repair, and how these processes interact with each other.**
- **RNA Biology: Regulation of gene expression by RNA, RNA processing, RNA catalysis, translation.**

Procedures and Grading

This course is primarily lecture-based. Required papers from the literature will also be assigned for some lectures, as indicated on the Course Outline below. The papers can be read after the lecture, and study questions on the papers may be provided. All papers will be on reserve at the White Memorial Chemistry Library or otherwise provided to you. I may also provide occasional handouts with extra figures and additional entries into the literature.

There will be two 75-minute exams (100 pts each), a short (7-10 pp.) paper (100 pts), and a two hour final examination (150 pts). Exams will emphasize lecture material, with coverage of key concepts from the reading. You may be asked to design and interpret experiments as well as to discuss assigned material. Review sessions will be held and past exams will be available. The paper will be an examination of the historical development of our understanding of a particular topic, with emphasis on the critical experiments. I encourage questions and discussion in class, but class participation does not affect grading. The exams are quite difficult, but in the past I have had few complaints about final grades. Your course grade will be based on exam and paper performance relative to a curve and to my expectations. I anticipate roughly 50:50 A's:B's. The curve does not require C's and D's but I will give them without hesitation if necessary. Plus/minus final grades will be given.

If you absolutely must miss an hour exam, you must call me in advance or within 24 hours after the exam, and you must also present a valid University excuse (please secure a note from the Health Services if possible). You will then be assigned a grade based on the remaining exams and the paper. If you miss the final or both hour exams, you will receive a failing grade. I expect and enforce adherence to the

University's Code of Academic Integrity, found at http://www.studenthonorcouncil.umd.edu/code.html. I expect you to write out and sign the University honor pledge on each exam: "**I pledge on my honor that I have not given or received any unauthorized assistance on this assignment/examination."** Also, you are hereby notified that "plagiarism" will be interpreted in its broadest sense: ideas from others must be referenced; words from others must be in quotation marks and referenced. Paraphrasing without referencing will be considered plagiarism. Extensive paraphrasing from a single source is unacceptable, referenced or not. As a condition of remaining in the class, you are specifically directed to read my own web page on this: http://www.biochem.umd.edu/biochem/kahn/plagiarism.html.

Please try to be on time for class. Please make sure that your cell phone does not ring during class. Cell phones must be stored out of reach and sight during exams.

Textbooks

Required: Weaver, R. F. (2002). *Molecular Biology*. 3rd ed., McGraw-Hill, Boston. Excellent source for historical and modern experiments. Also see http://www.mhhe.com/weaver3.

Blake, R.D. (2004). *Informational Biopolymers of Genes and Gene Expression.* University Science Books, Sausalito, CA. The chemistry of molecular biology.

Recommended:

Bates, A. D. and Maxwell, A. (1993). *DNA Topology*. Oxford: IRL Press at Oxford University Press. 114 pp. Excellent short monograph on this difficult topic.

Bloomfield, V.A., Crothers, D.M., and Tinoco, I., Jr. (2000). *Nucleic Acids: Structure, Properties and Functions*. University Science Books, Sausalito CA. Biophysical chemistry.

Ptashne, M. and Gann, A. (2002). *Genes & Signals*. Cold Spring Harbor Laboratory Press. 192 pp. Heuristics of gene regulation.

Wolffe, A. (1999). Chromatin: Structure and Function. 3rd ed. San Diego: Academic Press, Inc. 400 pp. Covers from structure to biology.

Required Papers: a balance between classic papers and current research.

Cosma, M. P., Tanaka, T. and Nasmyth, K. (1999). "Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter." *Cell* **97**(3), 299-311.

Moser, H. E. and Dervan, P. B. (1987). "Sequence-Specific Cleavage of Double Helical DNA by Triple Helix Formation." *Science* **238**, 645-650.

Naktinis, V., Turner, J. and O'Donnell, M. (1996). "A Molecular Switch in a Replication Machine Defined by an Internal Competition for Protein Rings." *Cell* **84**, 137-145.

Ren, B., Robert, F., Wyrick, J. J., Aparicio, O., Jennings, E. G., Simon, I., Zeitlinger, J., Schreiber, J., Hannett, N., Kanin, E., Volkert, T. L., Wilson, C. J., Bell, S. P. and Young, R. A. (2000). "Genome-Wide Location and Function of DNA Binding Proteins." *Science* **290**, 2306-2309.

Seeman, N. C., Rosenberg, J. M., and Rich, A. (1976). "Sequence-specific recognition of double helical nucleic acids by proteins." *Proc. Natl. Acad. Sci. USA* **73**, 804-808.

Watson, J. D. and Crick, F. H. C. (1953). "Molecular Structure of Nucleic Acids: A structure for deoxyribose nucleic acid." *Nature* **171**, 737-738.

Westover, K. D., Bushnell, D. A., and Kornberg, R. D. (2004). "Structural basis of transcription: Separation of RNA from DNA by RNA polymerase II." *Science* **303**, 1014-1016.

Zaug, A. J. & Cech, T. R. (1986). "The Intervening Sequence RNA of Tetrahymena Is an Enzyme." *Science* **231**, 470-475.

Lecture Outline

Chapters to be read for background or amplification are specified for each lecture (W3 = Weaver, Chapter 3, etc. B = Blake). You are not responsible on exams for material covered only in the book unless this is specifically announced. You are, however, responsible for the answers to any study questions handed out in class.

I. Nucleic Acid Sequence, Structure, and Chemistry (10 lectures)

1. Introduction and nucleic acid building blocks; W1, 2, 3; B1, 4 — 9/1/05
   Introduction, central dogma, nucleotide structure, primary structure, chemical stability, nomenclature
2. Structures of double helices; W2; B6 — 9/6/05
   A, B, and Z form helices, base pairing and hydrogen bonding
   Watson and Crick, 1953
3. DNA and RNA hybridization and thermodynamics; W4, 5; B6 — 9/8/05
   Base-pair stability rules, melting, hybridization, hypochromism, microarrays
4. RNA structure and triple helices; W19; B5 — 9/13/05
   Tertiary structure and tRNA, prediction of RNA folding, antisense
   Moser and Dervan, 1987
5. Enzymatic manipulation of nucleic acids; W4, 5 — 9/15/05
   Restriction enzymes, nucleases, radiolabeling, basic genetic engineering, polymerases, PCR
6. Sequencing and synthesis of DNA and RNA; W5, 24; B7 — 9/20/05
   DNA sequencing, chemical and enzymatic synthesis, genomics, bioinformatics
7. Drug and cation binding; B8 — 9/22/05
   Intercalation, groove-binding, ion atmosphere, altered backbone chemistries
8. DNA bending, flexibility, and cyclization; B6 — 9/27/05
   Bending and twisting flexibility, sequence-directed bending, methods for detection and quantitation
9. Topology, supercoiling, topoisomerases; W20, B6 — 9/29/05
   Linking number, superhelix structure, topoisomerase reaction mechanisms, knots and catenanes
10. Review/catch-up day — 10/4/05

→ EXAM I ← Covers through Section I. — **10/6/05**

II. General Features of Protein-Nucleic Acid Interaction (4 lectures)

11. Biochemical methods for studying complexes; W5, 9; B9 — 10/11/05
    Binding curves, gel mobility shift, footprinting/interference, crosslinking, filter binding, ChIP
    Ren et al., 2000.
12. Protein structural motifs for nucleic acid binding; W9, 12; B9 — 10/13/05
    Helix-turn-helix, zinc fingers, bZIP proteins, TBP, hnRNP, etc.
13. Sequence- and structure-specific recognition of nucleic acids; W9; B9 — 10/18/05
    Major groove vs. minor groove, hydrogen bonding, direct vs. indirect readout, deformability, RNA recognition
    Seeman *et al.,* 1976
14. Chromosome structure; W13 — 10/20/05
    Nucleosomes, chromatin, higher-order structure, telomeres

III. DNA Transactions (9 lectures)

15. DNA replication: fundamental mechanisms; W20, 21; B10 — 10/25/05
    Polymerization reaction mechanisms, fidelity, structure

16. Genome replication; W20,21 — 10/27/05
    Origin recognition and polymerase holoenzymes in *E. coli*; the cell cycle.
    Naktinis *et al*., 1996
17. Transcription: fundamental mechanisms; W6, W10, W11; B5 — 11/1/05
    RNA polymerases, transcription cycle, transcription bubble, supercoiling
    Westover *et al*., 2004
18. Regulation in prokaryotes; repression, activation, looping; W7, 8 — 11/3/05
    Paradigms: lac operon, araC, ntrC. Searching mechanisms.
19. Transcription in eukaryotes: chromatin and complexity; W10, 11, 12, 13 — 11/8/05
    Assembly, activators, enhancers, chromatin remodeling, histone code, recruitment
    Cosma *et al.,* 1999

→ Paper Topics Due ← — **11/8/05**

20. Regulation of transcription by RNA; W14,W16 — 11/10/05
    RNAi, siRNA, microRNA, riboswitches
21. Recombination; W22, 23 — 11/15/05
    Holliday junctions, homologous recombination by RecABCD

→ EXAM II ← Covers through Lecture 20. — **11/17/05**

22. DNA repair; W20; B12 — 11/22/05
    BER, NER, mismatch repair, cancer

→ Thanksgiving← No lecture — **11/24/05**

23. "Interprocess Communication"W15.3, 20.3 — 11/29/05
    Review of regulatory and biochemical connections among replication, transcription, repair

IV. RNA Transactions (4 lectures)

24. Catalytic RNA; W14; B13 — 12/1/05
    Self-splicing RNA, ribozymes, origin of life
    Zaug and Cech, 1986.
25. RNA splicing, processing, and degradation; W14, 15, 16 — 12/6/05
    Splicing mechanisms, control of mRNA lifetime, nonsense-mediated decay
26. Translation; W17, 18, 19; B5 — 12/8/05
    Chemistry of protein biosynthesis, ribosome structure, the translation cycle

→ Paper Due ← — **12/8/05**

27. Review and/or catch up day. — 12/13/05

→ FINAL EXAM← Emphasizes 21-27 Tues., 12/20/05, 10:30-12:30 a.m., Chem. 0127