BIOCHEMISTRY 674 (NUCLEIC ACIDS) — FALL, 2001
TuTh, 8:00-9:15 a.m., Chemistry 0127

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

Office: Chemistry 2505 (Biochemistry, Wing 5 of the Chemistry complex)

Office hours: Weds. 2-3 p.m., Thurs. 1-2 p.m., Chemistry 2505; there is no TA for the course

Contacting me: kahn@adnadn.umd.edu much preferred to 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.

Class web site: 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 some methods for studying, synthesizing, sequencing and manipulating nucleic acids. Bioinformatics.
- Interactions between nucleic acids and ligands such as cations, drugs, and especially proteins.
- Selected aspects of the biochemistry and regulation of DNA replication, transcription, recombination, and repair, and how these processes interact with each other.
- RNA splicing, RNA catalysis, translation, and selection-amplification methods.

Required papers from the literature will be assigned for some lectures (may be read after the lecture), as indicated on the Course Outline below. All papers will be on reserve at the White Memorial Chemistry Library. In addition, two textbooks are strongly recommended for the course, for background and for reference in your research careers. I may also provide occasional handouts with some figures, hints on what you should take away from the assigned reading, and additional entries into the literature. Some of these additional sources, especially books, are on reserve.

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 some coverage of key concepts from the reading. You will be asked to design and interpret experiments as well as to recapitulate assigned material. Review sessions will be held and past exams will be on reserve. The paper will be an examination of the historical development of our understanding of a particular topic, with emphasis on critical experiments and how they caused reinterpretation of earlier work. I encourage questions and discussion in class, but class participation does not affect grading. Plus/minus final grades will be given.

If you absolutely must miss an exam, you must call me in advance or within 24 hours after the exam, and you must also present a valid University excuse, in order to be eligible for the assignment of a grade based on the remaining two exams and the paper. If you miss the final or both hour exams, you will receive a failing grade. 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 expect and enforce adherence to the University's Code of Academic Integrity, found at http://www.inform.umd.edu/CampusInfo/Departments/JPO/code_acinteg.html. Specifically, "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.

Course Outline

Recommended texts, available at the University Book Center. Chapters to be read for background or amplification are specified for each lecture (W3 = Weaver, Chapter 3, etc.). You are not responsible for material covered only in the book unless this is specifically announced.

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

Weaver, R. F. (2001). *Molecular Biology*. 2nd ed., WCB/McGraw-Hill, Boston. Excellent source for historical and modern experiments.

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

1. Nucleic acid building blocks; BCT2, W1, 2, 3 — 8/30/01
   Central dogma, nucleotide structure, primary structure, chemical stability, nomenclature
2. Structures of double helices; BCT4, W2 — 9/4/01
   A, B, and Z form helices, base pairing and hydrogen bonding
   Watson and Crick, 1953; Dickerson, 1983
3. DNA and RNA hybridization and thermodynamics; BCT8, W5 — 9/6/01
   Base-pair stability rules, melting, hybridization, hypochromism, gene chips
   Holstege *et al.*, 1999
4. RNA structure and triple helices; BCT8, W19 — 9/11/01
   Tertiary structure and tRNA, prediction of RNA folding, antisense
   Moser and Dervan, 1987
5. Sequencing and synthesis of DNA and RNA; BCT3, W5, 24 — 9/13/01
   Maxam-Gilbert and Sanger sequencing, chemical and enzymatic synthesis, genomics and bioinformatics
   Fleischmann *et al.*, 1995
6. Drug and cation binding, chemical probing methods; BCT11, 12 — 9/18/01
   Intercalation, groove-binding, ion atmosphere, reactivity of nucleotides, altered backbone chemistries
7. DNA bending, flexibility, and cyclization; BCT9 — 9/20/01
   Bending and twisting flexibility, sequence-directed bending, methods for detection and quantitation
   Zinkel and Crothers, 1987
8. Topology, supercoiling, topoisomerases; BCT10, W20 — 9/25/01
   Linking number, superhelix structure, topoisomerase reaction mechanisms, knots and catenanes
   Bauer *et al.*, 1980
9. Enzymatic manipulation of nucleic acids; BCT3, W4, 5 — 9/27/01
   Restriction enzymes, nucleases, radiolabeling, basic genetic engineering, polymerases, PCR
   Arnheim and Levenson, 1990
10. Catch-up day — 10/2/01

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

11. Biochemical methods for studying complexes; BCT13, W5 — 10/4/01
    Binding curves, gel mobility shift, footprinting/interference, crosslinking, filter binding, ChIP
12. Protein structural motifs for nucleic acid binding; BCT13, W9, 12 — 10/9/01
    Helix-turn-helix, zinc fingers, bZIP proteins, TBP, hnRNP, etc.
    Harrison, 1991; Nikolov *et al.*, 1995

→ EXAM I ← Covers through Section I. — **10/11/01**

13. Sequence- and structure-specific recognition of nucleic acids; BCT13, W9 — 10/16/01
    Major groove vs. minor groove, hydrogen bonding, direct vs. indirect readout, deformability, RNA recognition
    Seeman *et al.*, 1976

14. Chromosome structure; BCT14, W13 — 10/18/01
    Nucleosomes, chromatin, higher-order structure, telomeres
    Luger *et al.*, 1997

III. DNA Transactions (8 lectures)

15. DNA replication: fundamental mechanisms; W20, 21 — 10/23/01
    Polymerization reaction mechanisms, fidelity, structure
    Brutlag and Kornberg, 1972
16. Genome replication; W20,21 — 10/25/01
    Origin recognition and polymerase holoenzymes in *E. coli*; the cell cycle.
    Naktinis *et al.*, 1996
17. Transcription: fundamental mechanisms; W6 — 10/30/01
    RNA polymerases, transcription cycle, transcription bubble, supercoiling
    Nudler *et al.*, 1997; Liu and Wang, 1987
18. Regulation in prokaryotes; repression, activation, looping; W7, 8 — 11/1/01
    Paradigms: lac operon, araC, ntrC. Searching mechanisms.
19. Transcription in eukaryotes: chromatin and complexity; W10, 11, 12, 13 — 11/6/01
    Holoenzyme vs. initiation complex assembly, activators, enhancers, chromatin, recruitment
    Struhl, 1999; Cosma *et al.*, 1999
20. Recombination; W22, 23 — 11/8/01
    Holliday junctions, recABCD, λ phage integration and excision

→ Paper Topics Due ← — **11/8/01**

21. DNA repair; W20 — 11/13/01
    BER, NER, mismatch repair, cancer

→ EXAM II ← Covers through Lecture 20. — **11/15/01**

22. "Interprocess Communication" — 11/20/01
    Review of regulatory and biochemical connections among replication, transcription, repair

→ Thanksgiving← No lecture — **11/22/01**

IV. RNA Transactions (5 lectures)

23. Catalytic RNA; W14 — 11/27/01
    Self-splicing RNA, ribozymes, origin of life
24. RNA splicing and degradation; W14, 16 — 11/29/01
    Splicing mechanisms, control of mRNA lifetime
    Nilsen, 1994
25. Translation; W18, 19 — 12/4/01
    Chemistry of protein biosynthesis, ribosome structure, the translation cycle
    Nissen *et al.*, 2000

→ Paper Due ← — **12/4/01**

26. Selection-amplification methods for nucleic acids — 12/6/01
    Selection of optimal DNA and RNA ligands or catalysts, in vitro evolution
    Ellington and Szostak, 1990
27. Review and/or catch up day. — 12/11/01

→ FINAL EXAM← Emphasizes 21-27 **Sat., 12/15/01, 8:00-10:00 a.m., Chem. 0127**

Reading List

This list may change as the semester progresses. In the required papers, I have tried to strike a balance among review articles, classic papers, and current research. Please let me know if there are difficulties with the amount or depth of the reading.

General texts for further reading and background:

Kornberg, A. and Baker, T. A. (1992). *DNA Replication*. 2nd ed. New York: W.H. Freeman and Co. Focuses on classical biochemistry experiments.

Lewin, B. (1999). *Genes VII*. Oxford: Oxford University Press. Deeply flawed but relatively up-to-date.

Schleif, R. (1993). *Genetics and Molecular Biology*. 2nd ed. Baltimore: The Johns Hopkins University Press. Eclectic, emphasizing experiments leading to conclusions.

Monographs for more in-depth discussion of particular topics:

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.

Ptashne, M. (1992). *A Genetic Switch: Phage λ and Higher Organisms*. 2nd ed. Cambridge, MA: Cell Press and Blackwell Scientific. 192 pp. Heuristics of gene regulation.

Saenger, W. (1984). *Principles of Nucleic Acid Structure*. New York: Springer–Verlag. 556 pp. Very technical and detailed.

Steitz, T. A. (1993). *Structural Studies of Protein-Nucleic Acid Interaction: The sources of sequence-specific binding*. Cambridge, England: Cambridge University Press. 79 pp. Good pictures.

Travers, A. (1993). *DNA-Protein Interactions*. London: Chapman & Hall. 180 pp. And DNA structure.

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

Required Papers:

Arnheim, N. and Levenson, C. H. (1990). "Polymerase Chain Reaction." *Chem. & Eng. News* (October 1), 38-47.

Bauer, W. R., Crick, F. H. C., and White, J. H. (1980). "Supercoiled DNA." *Scientific American* **243**(1), 118-133.

Brutlag, D. and Kornberg, A. (1972). "Enzymatic Synthesis of Deoxyribonucleic Acid: XXXVI. A proofreading function for the 3′→ 5′ exonuclease activity in deoxyribonucleic acid polymerases." *J. Biol. Chem*. **247**, 241-248.

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.

Dickerson, R. E. (1983). "The DNA Helix and How It Is Read." *Sci. Am.* (December), 94-111.

Ellington, A. D. and Szostak, J. W. (1990). "In vitro selection of RNA molecules that bind specific ligands." *Nature* **346**, 818-822.

Fleischmann, R. D., Adams, M. D., White, O., many others and Venter, J. C. (1995). "Whole-Genome Random Sequencing and Assembly of *Haemophilus influenzae* Rd." *Science* **269**, 496-512.

Harrison, S. C. (1991). "A structural taxonomy of DNA-binding domains." *Nature* **353**, 715-719.

Holstege, F. C. P., Jennings, E. G., Wyrick, J. J., Lee, T. I., Hengartner, C. J., Green, M. R., Golub, T. R., Lander, E. S. and Young, R. A. (1999). "Dissecting the Regulatory Circuitry of a Eukaryotic Genome." *Cell* **95**, 717-728.

Liu, L. F. and Wang, J. C. (1987). "Supercoiling of the DNA Template During Transcription." *Proc. Nat. Acad. Sci. USA* **84**, 7024-7027.

Luger, K., Mäder, A. W., Richmond, R. K., Sargent, D. F. and Richmond, T. J. (1997). “Crystal structure of the nucleosome core particle at 2.8 Å resolution.” *Nature* **389**, 251-260.

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.

Nikolov, D. B., Chen, H., Halay, E. D., Usheva, A. A., Hisatake, K., Lee, D. K., Roeder, R. G. and Burley, S. K. (1995). “Crystal structure of a TFIIB-TBP-TATA element ternary complex.” *Nature* **377**, 119-128.

Nilsen, T. W. (1994). “RNA-RNA Interactions in the Spliceosome: Unraveling the Ties That Bind.” *Cell* **78**, 1-4.

Nissen, P., Hansen, J., Ban, N., Moore, P. B. and Steitz, T. A. (2000). “The Structural Basis of Ribosome Activity in Peptide Bond Synthesis.” *Science* **289**, 920-930.

Nudler, E., Mustaev, A., Lukhtanov, E., and Goldfarb, A. (1997). “The RNA-DNA Hybrid Maintains the Register of Transcription by Preventing Backtracking of RNA Polymerase.” *Cell* **89**, 33-41.

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.

Struhl, K. (1999). “Fundamentally Different Logic of Gene Regulation in Eukaryotes and Prokaryotes.” *Cell* **98**, 1-4.

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

Zinkel, S. S. and Crothers, D. M. (1987). “DNA bend direction by phase sensitive detection.” *Nature* **328**, 178-181.