C2006/F2402 '14 OUTLINE OF LECTURE #10

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Handouts: 10A -- Heterochromatin vs Euchromatin; Basic Nucleosome Structure
10B -- Details of Nucleosome & chromatin structure (in CWhandouts folder). Similar to Becker figs. 18-21 & 18-22 (18-20 & 18-21) or Sadava fig. 11.9.
For color versions of nucleosome pictures on 10B click here.
10C -- Typical Eukaryotic Gene; Cis & Trans

Additional Reading: The Hidden Codes that Shape Protein Evolution: https://www.sciencemag.org/content/342/6164/1325.full (Weatheritt & Babu, Science Magazine, 342, 13 December 2013, p. 1325) -- Not handed out in class.

For the Nucleosome/Histone movie, go to http://www.hhmi.org/biointeractive/dna/DNAi_packaging_vo2.html

Some handouts are posted in the CWhandouts folder (accessible to registered students only) because the handouts contain copyrighted material. Extra copies of all paper handouts are in boxes on 7th floor of Fairchild (after the corresponding lecture).

I. General Chromatin Structure -- Euchromatin & Heterochromatin

A. What is Chromatin? DNA in eukaryotes is not "naked" as it is in bacteria. It is in the form of chromatin = DNA complexed with histone proteins. So how do polymerases and TFs get to the DNA and how do they manage to carry out transcription with all those proteins stuck to the DNA? What actually turns transcription on and off? To answer these questions, we need to look closely at chromatin structure.

B. Composition of chromatin = DNA + associated proteins

1. Associated proteins are mostly histones (small, basic; more details below)

2. Associated proteins includes some (nonhistone) regulatory proteins

C. States of chromatin

1. Always includes proteins. In all states, DNA has proteins attached

2. Differences are usually due to different states of folding -- different folding with histones, not total removal of histones (there are some exceptions which will be discussed later).

3. Two Types of Chromatin -- Two basic states of chromatin are visible in the light microscope

a. Heterochromatin or heterochromatic chromatin/DNA

(1). What is it? Chromatin that is darkly stained, & relatively condensed (tighter),

(2). Heterochromatin is genetically inactive (not transcribed)

(3). Two kinds of (interphase) heterochromatin

(a). Constitutive heterochromatin

- Always heterochromatic in interphase in all cells

- Example: chromatin at centromeres, telomeres
- Usually repeating in sequence, non coding.

(b). Facultative heterochromatin

- Heterochromatic in interphase in some cells, but not others
- Example: inactive X. Same X is not inactive in all cells.
- Whichever X is inactive in any particular cell forms a Barr body (heterochromatin) in that cell.
- Same X will be euchromatic in one cell and heterochromatic in another.

(4). Is all inactive chromatin heterochromatic? No. ***Most*** of the DNA that is inactive during interphase (in a particular cell type) is euchromatic, **NOT** heterochromatic.

(5). Mitotic chromatin -- All chromatin is heterochromatic (tightly condensed) during mitosis & meiosis.

(6). Replication -- All chromatin, both heterochromatin & euchromatin is replicated in S. Heterochromatic DNA is not genetically active (not transcribed), but it ***is*** replicated.

b. Euchromatin or euchromatic chromatin/DNA

(1). What is it? Chromatin that stains more lightly, is less condensed (looser).

(2). Important Features of Euchromatin

- Capable of genetic activity (transcription) as vs. heterochromatin.
- Normal state of most DNA during interphase.
- Transcribable, ***but not necessarily being transcribed now***. DNA must be euchromatic to be active, but not all euchromatin is active.

(3). Reminder: Most interphase DNA is euchromatic, whether it is transcribed or not.

(4). Not all euchromatin is folded equally tightly

(a). Tighter vs looser. Euchromatin is often divided into several distinct states of folding, although tightness of folding is probably really continuous from relatively loose ↔ relatively tight.

(b). Different states of (eu)chromatin look about the same in the light microscope. Therefore indirect methods (such as DNase treatment) are necessary to test state of folding.

(c). Correlation between folding and function. In general, more active (transcribed) chromatin is looser.

II. Structure of Chromatin -- Histones & Nucleosomes

A. How are proteins and DNA arranged? How does all that DNA fit into a tiny cell? The evidence.

1. Appearance of chromatin in EM -- -- low salt ("beads on a string" appearance) vs physiological salt (30 nm fiber). See Becker fig. 18-18 (18-17) or Alberts (Or go to the PubMed Bookshelf at http://www.ncbi.nlm.nih.gov/books/ and use the search term box.) Shows DNA and protein arranged in repeating structure of 'beads on a string'.

2. Treatment with DNase

a. Specificity of DNase. Enzyme used here cuts DNA in areas that are most exposed or least protected. Where it cuts depends on local chromatin structure, not on the base sequence of the DNA. (Restriction enzymes are specific for certain sequences; this enzyme is not sequence specific.)

b. Procedure: Treat chromatin (not DNA, but DNA with associated proteins!) with DNase; then remove protein and isolate the DNA, then run DNA on a gel.

c. Results:

(1). If you use a little nuclease: A little digestion with nuclease → 200 Base Pair (BP) "ladder" = sequences of ***multiples*** of 200 BP on gel. Implies repeating structure of DNA/protein complex; DNA exposed (so easily cut) about once per 200 BP. (See Becker fig. 18-19 & 18-20 [18-18 & 18-19].)

(2). If you use a lot of nuclease: More treatment with nuclease → resistant core of around 145 BP. Implies that repeating structure of complex includes a core of 145 bases that is relatively protected and a stretch between cores that is more exposed.

d. Conclusions/model:

(1). DNA is in Nucleosomes:

- Chromatin = DNA-protein complex with a repeating structure.
- Repeating unit = One nucleosome = 200 BP + associated proteins.
- "bead" seen in EM = Nucleosome

(2). Nucleosome core: About 145 BP of 200 BP repeat is relatively protected in/on core of "bead" -- rest is a 'linker' that goes between beads and is more exposed.

(3). Linker: Linker DNA has one site every 200 BP that is relatively unprotected and readily cut by DNase.

3. Histones

a. 5 types: H2A, H2B (slightly lys rich), H3, H4 (arg rich) & H1 (lys rich). All relatively small, basic proteins.

b. All histones are highly conserved evolutionarily (this implies histones carry out a critical function that depends on a particular structure -- can't change structure much without ruining

function).

b. How much per 200 BP of DNA? 2 molecules each of H2A, H2B, H3, H4 plus one molecule of H1.

c. Low salt (or digestion of linker DNA) removes H1. H1 is on outside of bead; more easily removed.)

B. Model for Chromatin (Beads on string level) -- nucleosomes (See handout 10A and Becker fig. 18-21 [18-20] or Sadava fig. 11.9. Where is the *protein* relative to the DNA?

1. Octamer of 2 each of H2A, H2B, H3, H4 (+ some DNA) = one bead.

2. DNA wound 2X around (on outside of) each octamer -- protects core. For more pictures and info on nucleosomes, click here.

3. Linker DNA between cores = 50-60 BP; most exposed & sensitive to some nucleases (relative to DNA in core).

4. H1 is on outside of DNA/octamer (See handout 10A or click here for model)

5. Nucleosome = repeating unit = 200 BP DNA + octamer (H1 optional).

6. Chromatin structure is not completely fixed. It can be changed by:

a. Modification of Tails -- histones have 'tails' that stick out of 'bead.'

(1). Regulatory Function: Modifications of histone tails affect folding of chromatin and binding to regulatory proteins; consequently they affect the activity of genes. (More on this below and/or in next lectures.)

(2). Nomenclature: The peptide chains that stick out from the compact nucleosome structure are called 'tails'. The name does not imply that 'tails' are at the carboxyl ends (or tail ends) of the histones -- could be at either end of peptide.

b. Nucleosome sliding -- Proteins called remodelers allow RNA and DNA polmerase to progress along the DNA by sliding one or a few nucleosomes at a time along the DNA..

C. Nucleosomes & Higher Levels of Structure (requires H1) -- how does chromatin fold up? See 10B or books for detailed pictures. See Becker fig. 18-22 [18-21] or Sadava fig. 11.9 or click here & scroll down. Stages of folding are as follows (details in table below):

1. Nucleosomes. DNA + histones form a chain of nucleosomes; about 1/7 original length of DNA (see table below). Also called a "10 nm fiber."

2. 30 nm fiber. Chain of nucleosomes folds back on itself (supercoils) forming 30 nm fiber (sometimes called solenoid). Exact structure unclear. Fiber has about 6 beads/turn = 1/42 length of DNA. Need tails (of histones) and H1 to form 30nm fiber and higher orders of folding.

3. Loops. 30 nm fiber forms loops about 300nm in diameter (1/750 orig. length). Different sections may be tighter or looser. Individual loops are stretched out (probably to beads-on-a-string stage) when actually transcribed. Loops may be units of transcription or potential transcription.

4. Higher Orders of Folding. Looped structure folds further → → heterochromatin (not transcribable)

a. Chromatids: Folds back on self to form structures/fibers about 700 nm across (per chromatid)

b. At metaphase = tightest = 1/15,000-1/20,000 orig. length (Chromosome is 4-5 microns long but each chromatid contains about 75 mm of double helical DNA)

D. Summary of States of Folding (for reference) -- compare to handout 10B.

Structure	Compaction relative to previous	Packing Ratio*	Diameter	H1 Needed?
DNA	none	1	2 nm	--
Nucleosomes -- beads on a string	7X	7 (1/7th length of DNA)	10-11 nm	no
30 nm fiber	6X	42	30 nm	yes
loops	15-20X	750	300 nm	yes
heterochromatic chromatid (metaphase)	20X	15-20,000	700 nm (per chromatid)	yes

* Packing ratio = length of DNA or DNA/protein complex relative to original length of DNA. As packing ratio increases, chromatin fibers get shorter and wider.

E. Modifications of histones -- helps tighten up or loosen chromatin

1. Histones have 'tails' that stick out (see handout 10B or click here)

a. States of tails affect higher orders of structure.

b. Many different modifications of amino acids in tail are possible -- modifications affect transcription. For example:

(1) Phosphorylation -- decreases transcription -- e.g. at mitosis (see below)

(2). Acetylation -- increases transcription

(3). Methylation -- can increase (at H3K4) or decrease (at H3K27) transcription

2. An Example of a modification: Phosphorylation of H1.

a. When? Phosphorylation occurs at start of M & is reversed at end of M

b. How? Changes in kinase and phosphatase activity occur during the cell cycle.

c. What Effect? Alters state of histones and folding of chromatin in parallel with changes in lamins.

d. Question to think about: Should kinases help chromatin get looser or tighter? (Note that modifications of histone tails affect protein-protein interactions, not protein-DNA interactions.)

To review nucleosome structure, try problems 4-1, 4-3 A, 4-6 C, 4-9 A, 4-7 B, & 4-10 A.

III. Does Folding of Chromatin Correlate with Genetic Activity?

A. Basic Issue/Question: In interphase, almost all chromatin is euchromatic, but not all euchromatin is transcribed in any one cell. Is all euchromatin folded to the same degree or not? Does looseness or tightness of folding correlate with level of genetic activity?

B. How do you test state of euchromatin?

1. Why use DNase?

a. All (eu)chromatin looks about the same in the light microscope. Therefore indirect methods (such as DNase treatment) are necessary to test state of folding.

b. Use of DNases. States of folding of (eu)chromatin are often distinguished by effects of treatment of chromatin (not DNA) with various types of DNase.

(1). Rationale: DNA that is in tighter areas of chromatin will be more protected from degradation. (Some examples of this are discussed below and are in problem sets.)

(2). Results: State of (eu)chromatin can be deduced from relative sensitivity of DNA (while still in chromatin) to degradation by various DNases.

2. Basic Method -- see Becker fig. 23-14 (23-17) or handout 10A. Want to examine chromatin region containing a particular gene, say globin genes, from dif. tissues.

a. Issue -- This region is euchromatic in interphase in all these tissues, even though it is not expressed in all of them. No gross difference is visible, but is there a difference in folding?

b. What you *want* to do: Fish out section of chromatin containing globin genes (from cells that make globin and cells that don't) and test state of chromatin folding.

c. What you *have* to do: Need to test for folding indirectly, so you need to do experiment in reverse. You distinguish states of folding of chromatin first (indirectly) and then find which state contained the genes of interest, say, globin genes.

3. Actual Experimental Procedure

a. Overall:

(1). Treat chromatin with DNase, differentially degrading DNA in active and inactive chromatin.

(2). Remove protein to leave the DNA.

(3). Examine DNA -- Use probe to test DNA for state of genes of interest and see if the genes were degraded or not.

b. Details of DNase treatment

(1). Treat chromatin -- not DNA -- with DNase. *Then* isolate DNA and see what state it's in.

(2). Specificity of DNase used:

(b) Action of DNase: Cutting by DNase is related to how much the DNA is protected, which in this case correlates with how tightly nucleosomes are folded in on themselves.

(c). Specificity: This DNase is not sequence-specific, unlike restriction enzymes. This enzyme cuts relatively exposed DNA, whatever its sequence happens to be.

(3). Can vary conditions -- amount of enzyme, time, etc. to distinguish various degrees of sensitivity to the enzyme.

(4). Can test chromatin from many different tissues, say erythrocytes (in chickens -- still have nuclei) vs. brain

(5). Can test state of many different genes using diff. probes (see below).

c. Expected result:

(1). If euchromatin is relatively loose, DNA will be unprotected and readily degraded by DNase. There will be nothing left to hybridize to the probe for, say, globin.

(***2). If euchromatin is relatively tight***, DNA in that region will NOT be easily degraded, and there will be DNA left to hybridize to probe.

d. How to measure state of DNA (after treatment of chromatin with DNase)? Cut purified DNA with restriction enzyme and do a Southern blot with a probe to the gene of interest. Details below.

Details will not be covered in class, but they are included here, as you may want to refer to them for RP #5. Handout will be provided in recitation and/or posted later.

(1). Prepare DNA.

Step 2A on handout: Extract DNA (remove histones) -- get naked DNA.

Step 2B: Treat naked DNA with restriction enzymes. Why do you need to 'restrict' the DNA? To get pieces of reasonable size

- Will give discrete pieces IF DNA was in relatively tight region of chromatin -- therefore protected from DNase.
- Will not give discrete pieces if DNA was in loose region of chromatin -- in that case, DNA will not have been protected, and will already have been degraded by DNase.

(2). Find regions corresponding to known genes. Do Southern -- steps 2B to 3B.

Step 3A. Run restriction fragments on gel, blot to solid support.

Step 3B. Hybridize to labeled probe to identify DNA from regions of interest (say, globin genes).

Result: Only DNA from areas with relatively tight chromatin will give clear, undegraded (labeled) bands on the gel. (See figures on handout.)

Once you have gone over this in recitation or study group, try problem 4-9.

3. Summary of results of treatment of euchromatin with DNase.

a. Almost all eukaryotic DNA is associated with some protein. How do we know? -- Almost all DNA is much more resistant to digestion by DNase than naked DNA

b. Almost all eukaryotic DNA is in nucleosomes. All DNA but the very loosest euchromatin (see d) is in nucleosomes, and gives a ladder when treated appropriately with DNase.

c. Not all euchromatin is equally loose (equally sensitive to DNase), but all euchromatin is packed less tightly than heterochromatin. See table on handout 10A.

d. Actually transcribed (coding) DNA is more sensitive to DNase than ordinary euchromatic DNA.

> ***(1). Transcribed chromatin is 'looser' but it is still in nucleosomes*** -- it is much more resistant to DNase than naked DNA.
>
> ***(2). Nucleosomes are <u>not</u> stripped off during genetic activity.*** One or two nucleosomes, at the most, are probably moved aside as the polymerase moves relative to DNA during transcription or DNA replication.
>
> ***(3). 'Looser' euchromatin*** may correspond to stretched out loops that have been unfolded to the 10nm fiber or beads-on-a-string stage. Includes genes that are being actively transcribed, or were transcribed recently, or are next to transcribed regions, etc.

e. Hypersensitive sites exist -- these are the only sections of euchromatin that are not in regular nucleosomes.* See Becker fig. 23-15 (23-18). These are the 'loosest' regions of euchromatin.

> ***(1). Some hypersensitive sites found*** = very sensitive regions -- 10X more sensitive to degradation by DNase than average euchromatin. Degraded by very low amounts of DNase.
>
> ***(2). Hypersensitive sites correspond to regulatory, not coding, regions*** (in areas of active transcription).
>
> ***(3). Hypersensitive sites are different in different tissues*** -- different genes are 'turned on' (transcribed) in each cell type. DNA is the same in (almost) all cells, but only the genes that are 'turned on' (in that cell type) will have hypersensitive sites.
>
> ***(3). Hypersensitive sites correspond to sites without nucleosomes*** (or to regions with very few, loose, nucleosomes -- see 5b & note at * below).
>
> ***(4). DNA in hypersensitive sites is not naked*** -- it is bound to regulatory proteins.
>
> ***(5). Why are hypersensitive sites so sensitive to DNase? ****
>
> > *a. The traditional view:* Transcription factors (regulatory proteins) have replaced histones and the other proteins don't protect the DNA as well as histones do. See Becker fig. 23.15 (23-18).
> >
> > *b. An alternative view:* Histones are still present, at least in some hypersensitive sites, in addition to regulatory proteins. The histones are much more loosely attached to DNA than in normal nucleosomes, so the DNA is not as well protected as in ordinary nucleosomes.

* When doing the problems, assume that hypersensitive sites have no nucleosomes, unless the data in a particular experiment indicates otherwise.

To review the differences in states of chromatin, uses of DNase, etc., try the rest of 4-3, 4-9 & 4-10.

IV. How Do you turn a Eukaryotic Gene On?

A. The Problem: Need to unfold/loosen chromatin before transcription is possible. Can't just add RNA polymerase (& basal TFs) to DNA and start transcription. DNA is in chromatin and must be made accessible.

B. So how can transcription occur? ***If time, we will start this topic today; if not, this topic will be addressed next time. Regulation of transcription will be covered in detail at the start of the notes for lecture #11. (If you want to read ahead, see notes from last year's Lecture #11.)***