BICH/GENE 431 KNOWLEDGE OBJECTIVES

Chapter 7 – Genome Structure and Chromatin

Know relative genome sizes and gene densities between model organisms

Why is gene density less for "higher" organisms?

- introns
- more intergenic DNA: regulatory sequences, repetitive DNAs

Different types of repetitive DNA

- microsatellite repeats: very short, tandemly repeated
- genome-wide repeats: moderate size (100-1000bp) and interspersed

Essential features of eukaryotic chromosomes

- origins of replication: many, typically 30-40 kbp apart
- centromeres: only one per chromosome to direct proper segregation
- telomeres: protein/DNA complexes; one at each end; contain short repeats that are characteristic of organism; 3' overhanging ends fold back to make T-loops

Eukaryotic cell cycle

- know four phases
- ~24 hours long in mammals
- significance of cell cycle checkpoints and cell cycle arrest

Interphase chromatin vs. chromosome condensation in M phase

Events at S phase – cohesin rings hold sister chromatids together

Events at M phase – formation of kinetochore complex; attachment to microtubules and to microtubule organizing center to form mitotic spindle; cohesins are proteolyzed to allow migration of sister chromatids to opposite poles

SMC proteins (structural maintenance of chromosomes) – cohesins and condensins

- form rings with other proteins to hold chromosomes together
- condensins mediate chromosome condensation at M phase

You should review steps in mitosis and meiosis (Figs. 7-15 and 7-16) on your own.

Massive DNA compaction necessary to fit into nucleus – 2 meters of DNA into 10-15 micron size of nucleus

Nucleosome structure

nucleosome core particle: 147 bp DNA + octamer of core histones; 1.65 negative superhelical turns

linker DNA: size varies between organisms – core + linker for humans ~200 bp

basic properties of core histones

H3/H4 forms tetramer; H2A/H2B form two dimers

Histone fold motifs contain alpha helices and mediate histone-histone interactions

N-terminal tails stick out: are susceptible to protease treatment; sites of extensive modification to control functions

Dyad axis of core particle

Only approx. 6-fold compaction of DNA

Subunit structure of chromatin discovered by Roger Kornberg (and others)

Micrococcal nuclease assay to detect DNA repeat

Supercoiling caused by nucleosomes – understand supercoiling assay to detect nucleosomes (Box 7-2)

Histone-DNA interactions are very strong

- mostly via extensive hydrogen bonds to oxygens of phosphates on DNA
- involve minor groove interactions
- do not have DNA sequence specificity
- positive charges on histones help to shield neg. charge on DNA to enable bending of DNA around histone core
- requires high salt concentrations to break histone-DNA interactions

Higher-order chromatin structure

- involves binding of histone H1 to linker DNA
- formation of 30 nm fiber
- two models for 30 nm fiber: solenoid vs. zigzag structures
- need histone tails for 30 nm fiber
- approx. 40-fold DNA compaction with formation of 30 nm fiber (still a long way to go)

Nuclear scaffold – large loops of DNA attached; contains topoisomerase II and SMC proteins

Nucleosome remodeling – why needed?

- sliding vs. transfer
- nucleosome remodeling complexes – know some names, esp. SWI/SNF
- requires energy of ATP hydrolysis

Nucleosome positioning – some nucleosomes are positioned at precise locations on genomic DNA, especially near promoters

- mechanisms that contribute to positioning: protein binding; DNA sequences that favor or disfavor DNA bending

Histone acetylation

- on lysines, usually on histone tails, know structure, removes pos. charge
- comes from acetyl-CoA
- correlates with transcriptional activation, or needed for deposition of new histones, or for DNA repair
- remember some locations: H3K9, H4K8, H4K16

Histone methylation

- on lysines or arginines, usually on histone tails, know structure on lysines
- can be mono-, di-, or tri-methylated
- comes from SAM (S-adenosyl methionine)
- correlates with either transcriptional activation or repression, depending on site
- remember some locations: H3K4 (activating), H3K9 (repressive), H3K27 (repressive)

Histone phosphorylation

- on serines or threonines, know structure, adds a negative charge
- comes from ATP
- correlates with mitosis, apoptosis, or activation/repression of transcription
- remember some locations: H3S10 (activating), other H3 sites correlate with mitosis

histone code hypothesis
proteins containing specific domains bind to various histone modifications
- bromodomains bind to acetylated histone tails
- chromodomains bind to methylated histone tails
- SANT domains interact preferentially with unmodified histones

Histone modifications are reversible
Enzymes that catalyze histone modifications
- HATs (histone acetyl transferases); add acetyl groups; correlate with activation of transcription; nuclear HATs vs cytoplasmic HATs
- HDACs (histone deacetylases); remove acetyl groups; correlate with repression of transcription
- Histone methyltransferases; add methyl groups; generally more specific for a given site of modification; SET domain proteins
- Histone demethylases; remove methyl groups
- Kinases add phosphoryl groups
- Phosphatases catalyze hydrolysis of phosphoryl groups

An example of how histone modification is coordinated with nucleosome remodeling to control access to DNA in chromatin (Fig. 7-41)
Nucleosome assembly after DNA replication
- old H3/H4 tetramers stay together
- old H2A/H2B dimers stay together
- nucleosomes reassemble randomly on two new daughter molecules
- histone code is propagated because HATs and HMTs contain bromodomains or chromodomains to recognize old modification – then modify new histones in that region (see Fig. 7-43)

Histone chaperones are needed to assemble nucleosomes on DNA
- CAF-1 binds to H3/H4 tetramers
- NAP-1 binds to H2A/H2B dimers
- There are other chaperones too.
- CAF-1 interacts with PCNA in order to direct H3/H4 tetramers to newly replicated DNA (PCNA is proliferating cell nuclear antigen, a sliding clamp protein that binds to DNA polymerase at the replication fork)