CBIO 3400 – Week 12 Study Questions

1. Chromosome segregation is an important event during mitosis. How does a cell ensure that daughter cells receive a full set of chromosomes?

1. DNA replication origins
2. centromere = primary constriction on chromosome required for mitotic segregation
   - centromeric DNA forms centromere
     1. CENP-A (H3 variant) binds CBF3 complex.
     2. CBF3 complex recruits Ndc80 & Dam1, which initially bind MT's side but finish as end attachment on MT.
     3. Form mitotic spindle from these kinetochore proteins (Ndc80 & Dam1).
   - mitotic spindle checkpoint
3. telomeres = protein-DNA complexes at ends of linear chromosomes containing telomeric sequences (G-rich)
   - TTAGGG ds-repeats = (T-loop) bound by TRF1 & TRF2 (Telomere Repeat Factor); other proteins recruited
   - ss-DNA = D-loop; 3' overhang bound by Pot1 (Protection Of Telomere)
   - T-loop + D-loop = structure that protects telomere

The mitotic spindle checkpoint prevents a cell from leaving metaphase & starting anaphase before 2 conditions are met for the sister chromosomes: (1) under tension & (2) attached to opposite spindle poles. Attachment of sister chromosomes at opposite spindle poles is key to ensuring that daughter cells receive a full set of chromosomes.

2. Describe how DNA is packed in the nucleus (starting from DNA double helix).

short region of DNA double helix

↓

nucleosomes ("beads-on-a-string") form of chromatin

↓

30-nm chromatin fiber of packed nucleosomes

↓

section of chromosome in extended form

↓

section of chromosome in condensed form

↓

only makes up a small section of entire mitotic chromosome

3. What are exons and introns? Does one gene always encode one protein? If not, explain the reason.

Exons

- sequences in protein-coding segment of mRNA that originated from the gene
- still present in mature mRNA
- encode small polypeptides
- CAN code for protein domains
  immunoglobin has several structural domains in exons
  EX: protein domains of antibody = specified in exons

Introns

- sequences present in genes, absent in mature mRNA
- increase length of genes

- this info. is lost during maturation of pre-mRNA (i.e., spliced out of pre-mRNA during maturation)
- frequently contain STOP codons in at least 1 reading frame for protein encoded by gene
- comprise 90% of protein-coding sequences

One gene can encode many proteins due to exon shuffling & alternative splicing.

Exon Shuffling

- having introns b/tw exons increases distance b/tw exons & therefore increases the likelihood of exon recombination
- evolutionary time scale
- occurs at DNA level
- exons from different genes can be combined/joined to form a new gene
- can produce proteins w/ different functions
- EX: LDL receptor (18 exons)
  - 9 = related to EGF pre-cursor
  - 5 = related to C9 complement
  - 3 = unique

Alternative Splicing

- introns have various splicing patterns, allowing 1 pre-mRNA to give rise to multiple proteins
- happens in real time
- 1 pre-mRNA gives rise to multiple proteins
- exons from 1 pre-mRNA are joined in different combos
- cell type specifies combo of exons used
- EX: α-tropomyosin gene → 1 tropomyosin gene gives rise to many different tropomyosin proteins (w/ different functions)

4. Describe the structure of a core promoter.

- shortest sequence of DNA that directs transcription
- modular = has 1 or more of 4 sequence blocks:
  1. initiator (Inr)
     - consensus: $(Py)_2CA(Py)_5$
     - located -3 to +5
     - recognized by TFIID (Transcription Factor II) & other proteins
  2. TATA box
     - consensus: TATAAAA
     - located -25 to -35
     - binding site for TBP (TATA-Binding Protein)
  3. BRE [Transcription Factor IIB (TFIIB)-Binding Element]
     - recognized by TFIIB
  4. DPE (Downstream Promoter Element)

5. How is RNA transcription initiated?

1. TFIIF has an ATP-dependent helicase (in RAP70 subunit) that unwinds duplex DNA to form an open complex.
2. TFIIF RAP30 subunit tightly binds RNA pol. II (this is important for recruitment of RNA pol. II to pre-initiation complex).
3. TFIID makes contacts with core promoter.
4. TFIIA binds TFIID-promoter complex, which increases TBP's affinity for TATA box.
5. TFIID-promoter complex binds TAF (TBP-Associated Factor).

6. TFIIB is a *bridging protein* that brings RNA pol. II to promoter. TFIIB binds RNA pol. II & TFIID-promoter complex. TFIIB helps orient RNA pol. II to select start site.
7. TFIIE binds RNA pol. II & recruits TFIIH to open complex.
8. TFIIH has a Cdk7 kinase subunit that phosphorylates CTD (C-Terminus Domain) of RNA pol. II on *serines*; this marks that RNA pol. II has cleared promoter.

6. How does chromatin rearrangement affect gene expression?

Chromatin rearrangement can change the order of introns & exons in the gene & thus alter which proteins are eventually synthesized; the order of the introns & exons determines protein function.
Alternative splicing & exon shuffling? Or something else?