GENE STRUCTURE AND ACTION

Kenneth Alonso, MD, FACP

Fertilization

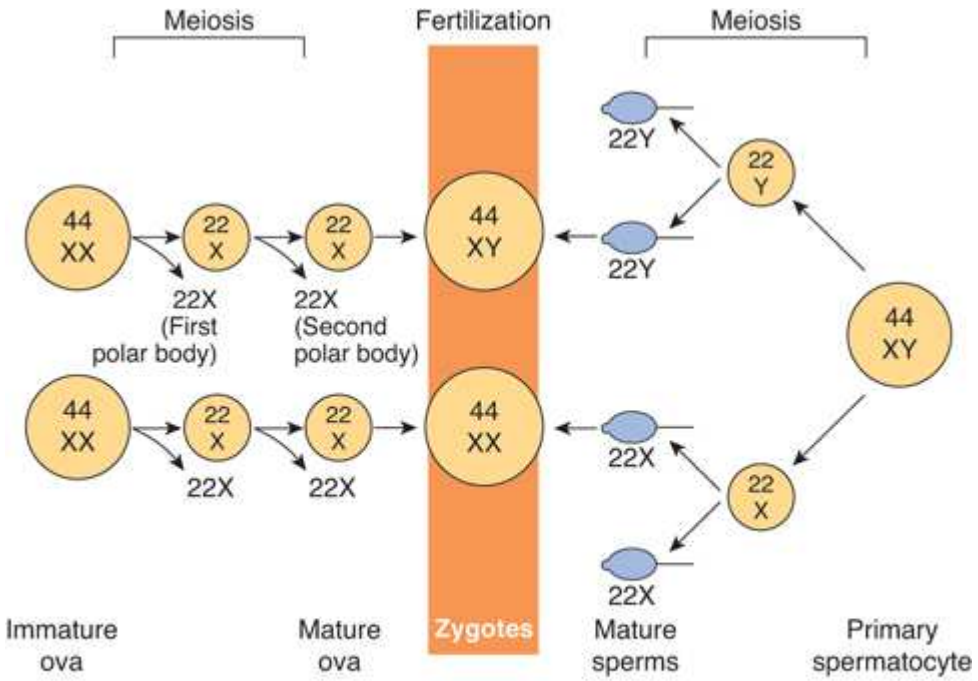
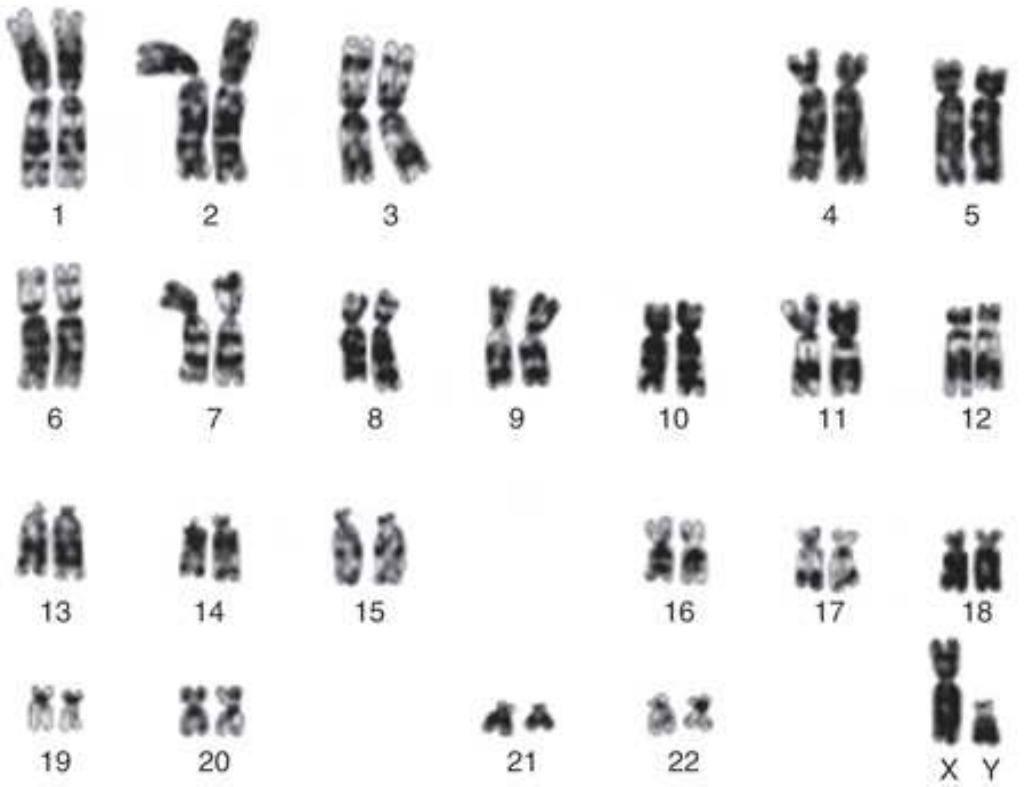


Fig. 25-2 Accessed 07/01/20102000.)

Human male karyotype



Giemsa banded human chromosomes arranged by size and banding

(Reproduced with permission, from Lingappa VJ, Farey K: *Physiological Medicine.* McGraw-Hill, 2000.) Fig. 25-2
Accessed 07/01/2010

Chromosome morphology

- Named for relative length of arms (p, short arm; q, long arm).
- Defined by position of centromere joining sister chromatids of metaphase chromosomes.
- Some short arms are only stalk and satellite.

Pedigree nomenclature

- 46, XY Normal male
- 47,XX,+21/46 XX Female mosiac for trisomy 21
- 46, XY del 4 (p14) Male with distal deletion of the short arm of chromosome 4 band designated 14
- 46, XX, dup (5p) Female with duplication of the short arm of chromsome 5
- 46, XX, t(11:22)(q23:q22) Female with a balanced reciprocal translocation between the long arms of chromosomes 11 and 22
- 46, XY, inv 3 (p21;q13) Male with a pericentric inversion on chromosome 3

Chromosome banding

- Techniques to stain chromosomes differentially by composition after cells cultured and stimulated by mitogen to divide.
- G-banding uses Giemsa and trypsin.
- R-banding is reverse of G-band pattern.
- C-banding stains centromeres.
- Q-banding uses quinacrine fluorescence and stains as do G-bands.
- High resolution banding permits identifications of more bands as it is performed at an earlier state of condensation of chromosome material.

FISH

- Fluorescence in situ hybridization (FISH)
- Hybridize DNA probe labeled with dye. Fluorescent spot corresponds to probe location. Can locate specific genes.
- Can be applied to interphase nuclei (no need to stimulate cell to divide).
- Shows changes in number of chromosomes

Other banding methods

- Spectral karyotyping
- Use probes specific to each chromosome. Each has different fluorescent label. All chromosomes labeled different colors. Easy to see rearrangements
- Competitive genomic hybridization
- Make probes from total DNA of subject and control with different labels. Hybridize both to normal chromosomes. Excess of label shows excess or deficiency of DNA from that sample as compared to control.
- Allele specific serotyping
- Probe hybridizes to DNA of interest.

Nucleic acids

- The genetic code uses 4 letter nuclide alphabet: the purines adenine and guanine; the pyrimidines cytosine and thymidine.
- Glycine, aspartate, and glutamine are amino acids necessary for purine synthesis.

Purine synthesis

- 10 steps
- Begins with ribose-5-phosphate (from the HMP pathway). It reacts with ATP (and pyridoxine) to form pyridoxal ribose-5-phosphate pyrophosphate (PRPP). An amine group is added, releasing the pyrophosphate.
- These two are the rate-controlling reactions for the pathway.
- Glutamine pyridoxal pyrophosphate amidotransferase is the rate limiting step.
- The final product is Inosine monophosphate, which can be converted to AMP and GMP.

Pyrimidine synthesis

- 6 steps
- Begins with the production of carbamoyl phosphate from glutamine, ATP, and bicarbonate. Aspartate is added and the ring is closed, producing orotic acid.
- PRPP is added and the compound decarboxylated, producing uracil monophosphate (UMP) which can be converted to cytidine triphosphate (CTP).
- UMP must be converted to dUMP and a carbon atom transferred (tetrahydrofolate reductase critical) to end with deoxy thymidine monophosphate (dTNP).

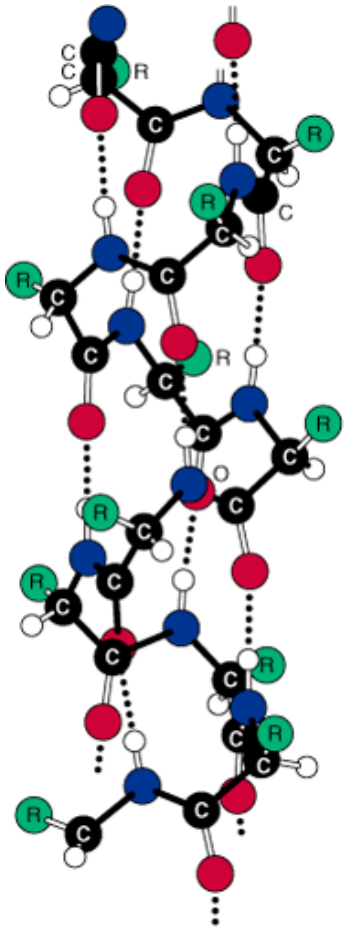
Pyrimidine synthesis

- Aspartate transcarbamylase is the rate limiting step.
- Aspartate alone is necessary for pyrimidine synthesis.
- Uracil arises from the deamination of cytosine.

Nucleic acid structure

- Nucleotides (nucleic acid plus ribose plus phosphate) are linked by 3’-5’phosphodiester bond.
- Deoxyribonucleotides are produced by reduction of ribonucleotides (lose Oxygen at the 2 position). ATP stimulates the reaction. NADH is the final reducing agent.
- Double-stranded DNA forms a helical structure.
- Bases are stacked; ring structures make them essentially flat.
- Offset nature of backbone creates major and minor grooves. (Bases more exposed in major groove.)

α-helix



Hydrogen bonds (dotted lines) formed between H and O atoms stabilize a polypeptide in an -helical conformation. The side chains (R) are on the outside of the helix. The van der Waals radii of the atoms are larger than shown here; hence, there is almost no free space inside the helix.

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Fig 5-4 Accessed 08/01/2010

Nucleic acid structure

- DNA is largely in B form (two antiparallel strands in a right handed helix with the bases on the inside and the phosphodiester backbone on the outside).
- Proteins can interact directly with the bases in the DNA major groove.
- 10 bases per helical turn.
- Guanidine-cytosine base pairs contain 3 Hydrogen bonds; adenine-thymine have 2 Hydrogen bonds, less tightly bound.
- Melting temperature (denaturation) linearly related to guanidine and cytosine content.

Nucleic acid structure

- Metabolically inactive DNA in Z form
- Left handed helix with bases on inside
- A zig-zag structure
- 12 bases per helical turn
- Repeating guanidine-cytosine nucleotides in which the 5-position of cytosine is methylated.
- RNA does not form an extended helix as does DNA.
- Stem and loop forms (A forms).
- Mitochondrial DNA is circular.

Genome

- The organization of genomes is characterized by a high degree or order and non-randomness.
- There is physical segregation of transcriptionally active euchromatin from repressed heterochromatin into distinct regions in the cell nucleus
- Chromatin domains and genes are distributed to preferred locations within the nuclear space
- Many proteins are non-randomly distributed in the nucleus, and are found in sub-nuclear bodies such as the nucleolus, the Cajal body, and splicing factor speckles

Genome

- The two alleles of a gene often differ in their three-dimensional 3D position and their functional status in the same nucleus
- Many chromatin-chromatin interactions only occur with low frequency in individual cells in a population
- The shape and number of nuclear bodies fluctuates considerably among individual cells

Chromosome structure

- The chromatin fiber is made up of units of 146 base pairs (bp) of DNA wrapped around nucleosomes that consist of octamers of core histone proteins
- Histone 1 ties nucleosome beads together in a string.
- The nucleosome core consists of histones H2A, H2B, H3, and H4.
- The chromatin fiber self-interacts to form loops
- At the smallest scale, loops mediate the interaction of regulatory elements, often enhancers with gene promoters, over distances of 10 to several hundred kb

Chromosome structure

- Multiple enhancers may loop to form super enhancer clusters thought to integrate signaling events or to act redundantly on target genes
- Larger loops (up to Mbs in length) contribute to the 3D compaction of the genome
- Used to regulate gene clusters in a precise temporal and spatial fashion via the sequential association of upstream elements with individual target genes
- Aggregates of phase-separated transcription factors bring genome regions into spatial proximity without the need for direct chromatin-chromatin interaction

Chromosome structure

- Chromatin domains that preferentially interact with each other rather than with their surrounding sequencesare referred to as topologically associating domains (TADs)
- They are typically several hundred kb in size and form by a loop-extrusion mechanism
- The cohesin complex drives the formation of chromatin loops via its ATP dependent molecular motor activity
- The extruded loop then folds onto itself to form a domain whose boundaries are defined by the architectural chromatin protein CTCF

Chromosome structure

- Architectural boundaries restrict interactions of regulatory elements
- Euchromatin and heterochromatin elements segregated
- Throughout interphase, the DNA that makes up a single chromosome occupies a relatively compact, spatially restricted territory of the nucleus rather than being dispersed through the nuclear space
- The chromosome has a high surface-to-volume ratio because its interior is permeated by a complex network of anastomosed channels that facilitate the access of regulatory factors to chromatin.

Chromosome structure

- The chromatin fibers of neighboring chromosomes often intermingle, but do not entangle, at their periphery, thus creating a continuum of chromatin throughout the nuclear space
- Inactive genes often locate near the nuclear edge and associate with peripheral heterochromatin, whereas active genes are frequently located in the nuclear interior
- The chromatin fiber is a self-avoiding polymer that can undergo local diffusive motion and is constrained by its polymer structure
- TADs are self-emergent properties of the polymer
- Promoted by cohesion and CTCF

Genome dynamics

- A transcription factor typically spends >95% of its time in a diffusive state or is engaged in non-specific interactions, with dwell-times on the order of several 100 ms
- Only a small fraction of a given transcription factor at any time is bound at specific sites
- The short dwell time of transcription factors ensures that functional complexes on chromatin are not locked into a permanent state
- Despite the transient nature of the individual binding events, the resulting chromatin states are stable due to the continuous replacement of dissociating proteins.

Genome dynamics

- Functional plasticity
- Phase separation is a driver of chromosome organization
- The demixing of distinct protein populations when present above a saturation threshold due to their propensity to preferentially undergo homotypic rather than heterotypic interactions with other biomolecules, leading to the formation of segregated, dynamic phases
- Phase separation mediates the biogenesis of membrane-less nuclear and cytoplasmic compartments

Genome dynamics

- The nuclear envelope acts as a major constraint in the 3D organization of genomes because specific genome regions are tethered to the periphery
- Laminin A prominent role
- Nuclear bodies also act as constraints
- Most genes do not continuously engage the transcription machinery
- Oscillate between dynamic on and off states and undergo ‘‘transcriptional bursting’’
- Stochastic

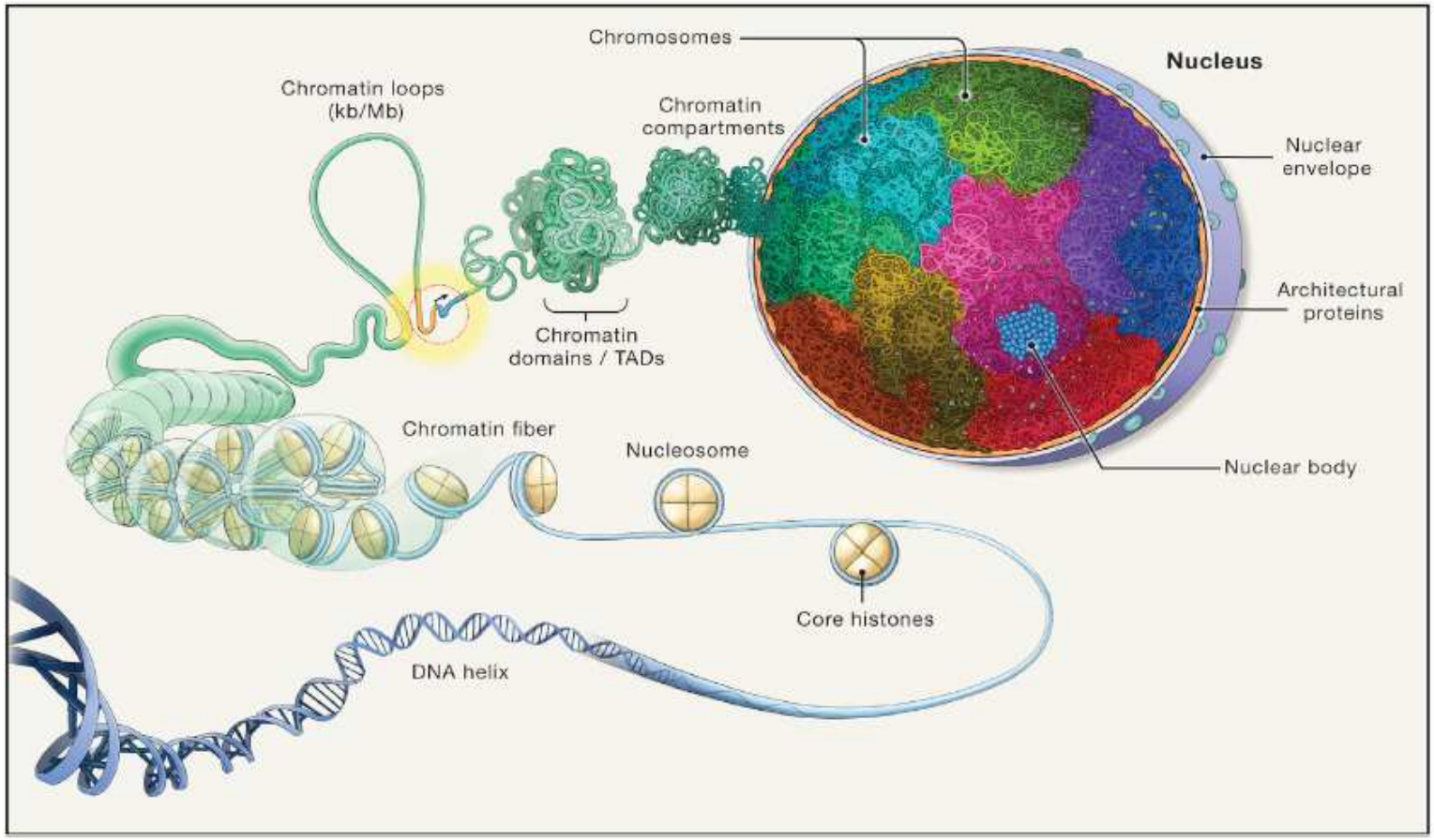
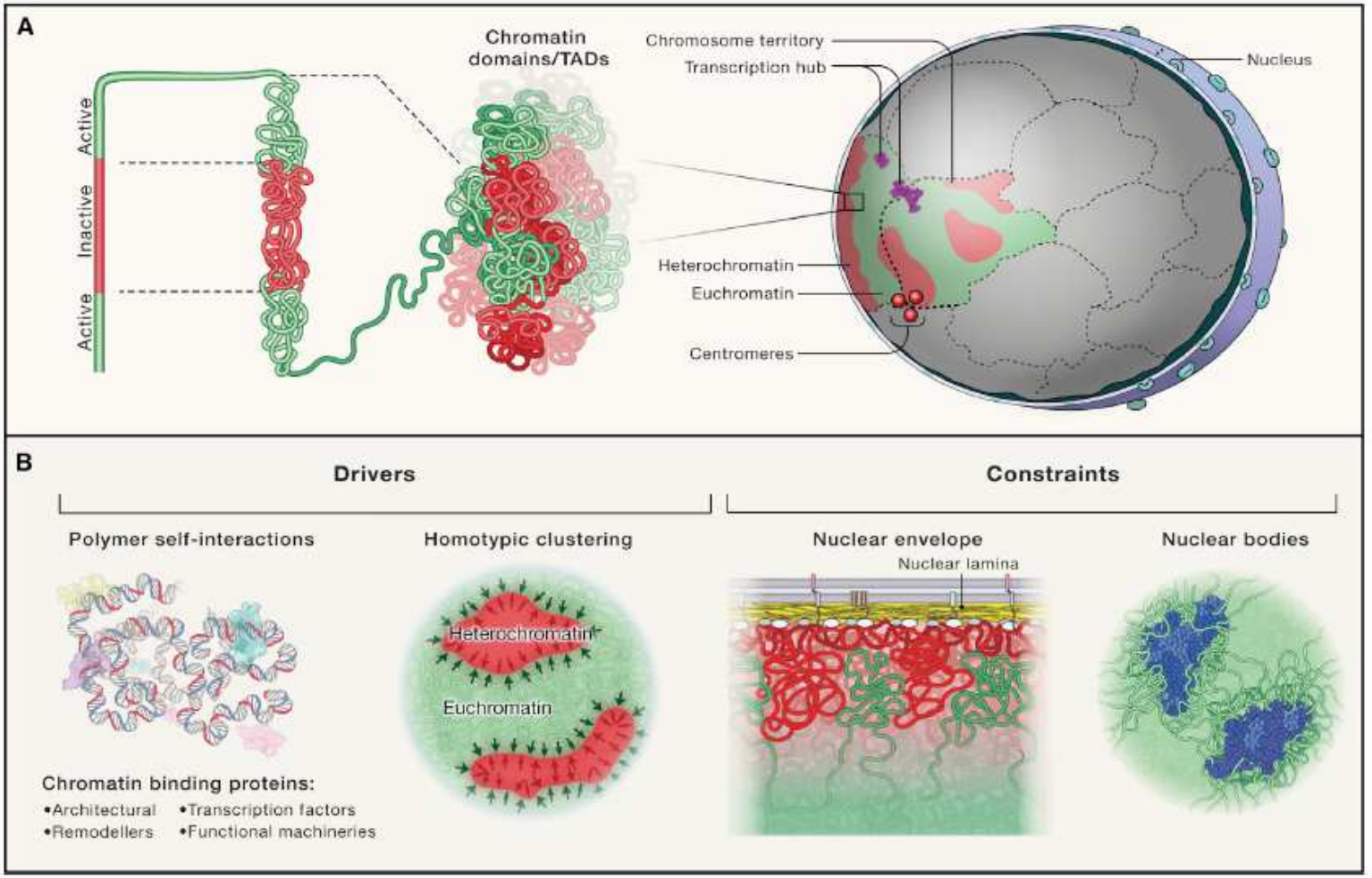


Figure 1. The Organization of the Eukaryotic Genome

Genomes are organized at multiple levels. DNA is wrapped around the nucleosome, which is made up of an octamer of core-histones, forming the chromatin fiber which folds into loops, often bringing upstream gene regulatory elements (yellow), such as enhancers, into proximity to promoters of genes (gold/blue) to control their transcription (black arrow). The fiber then folds into chromatin domains, referred to as TADs, which associate with each other to create chromatin compartments. The DNA of each chromosome occupies a distinct volume, or chromosome territory (multiple colors), within the cell nucleus, generating non-random patterns of chromosome and genes. In the DNA-free space, the nucleus also contains RNA and proteinaceous protein aggregates which form nuclear bodies (blue).

Misteli, The Self-Organizing Genome: Principles of Genome Architecture and Function, Cell (2020), https:// doi.org/10.1016/j.cell.2020.09.014



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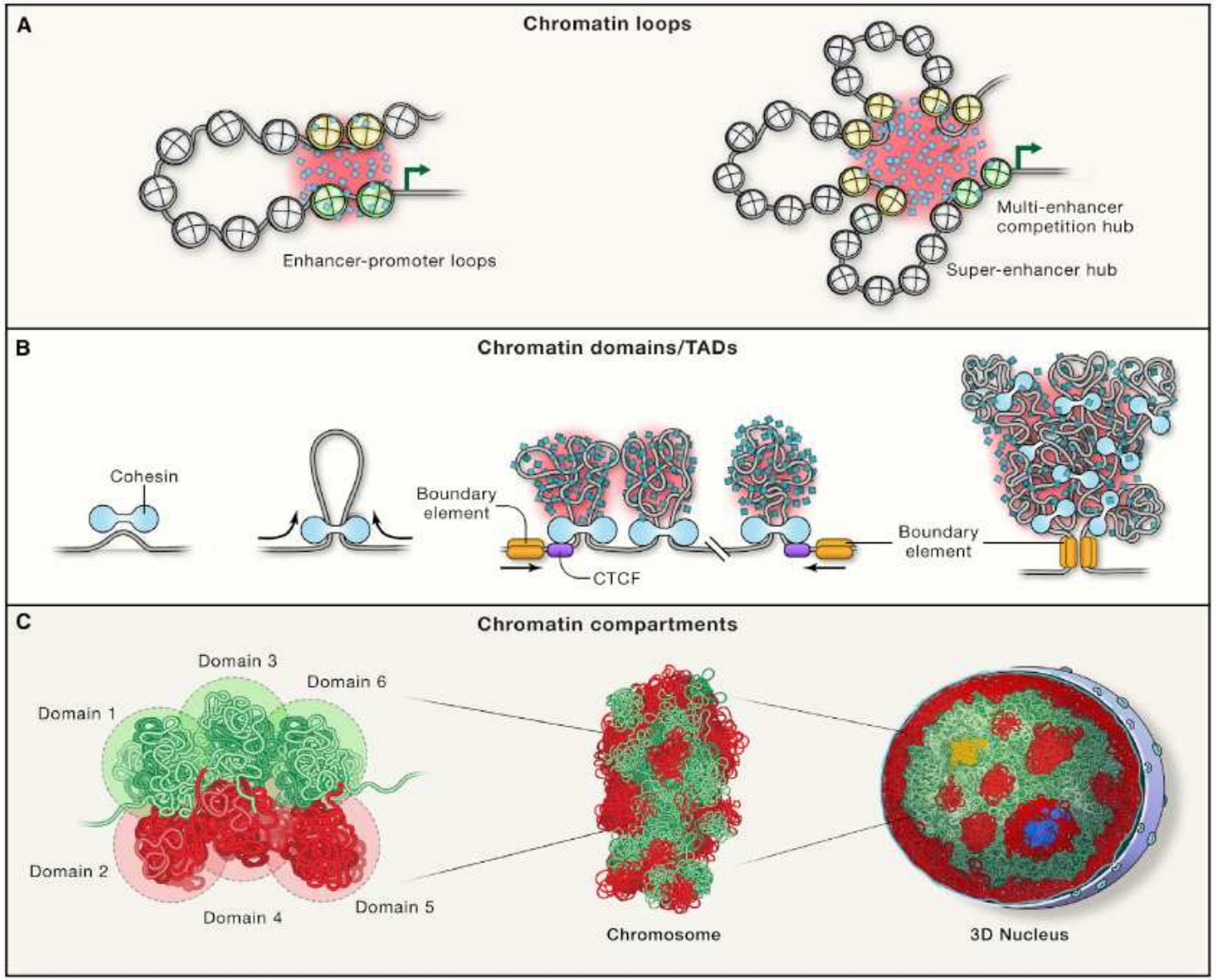
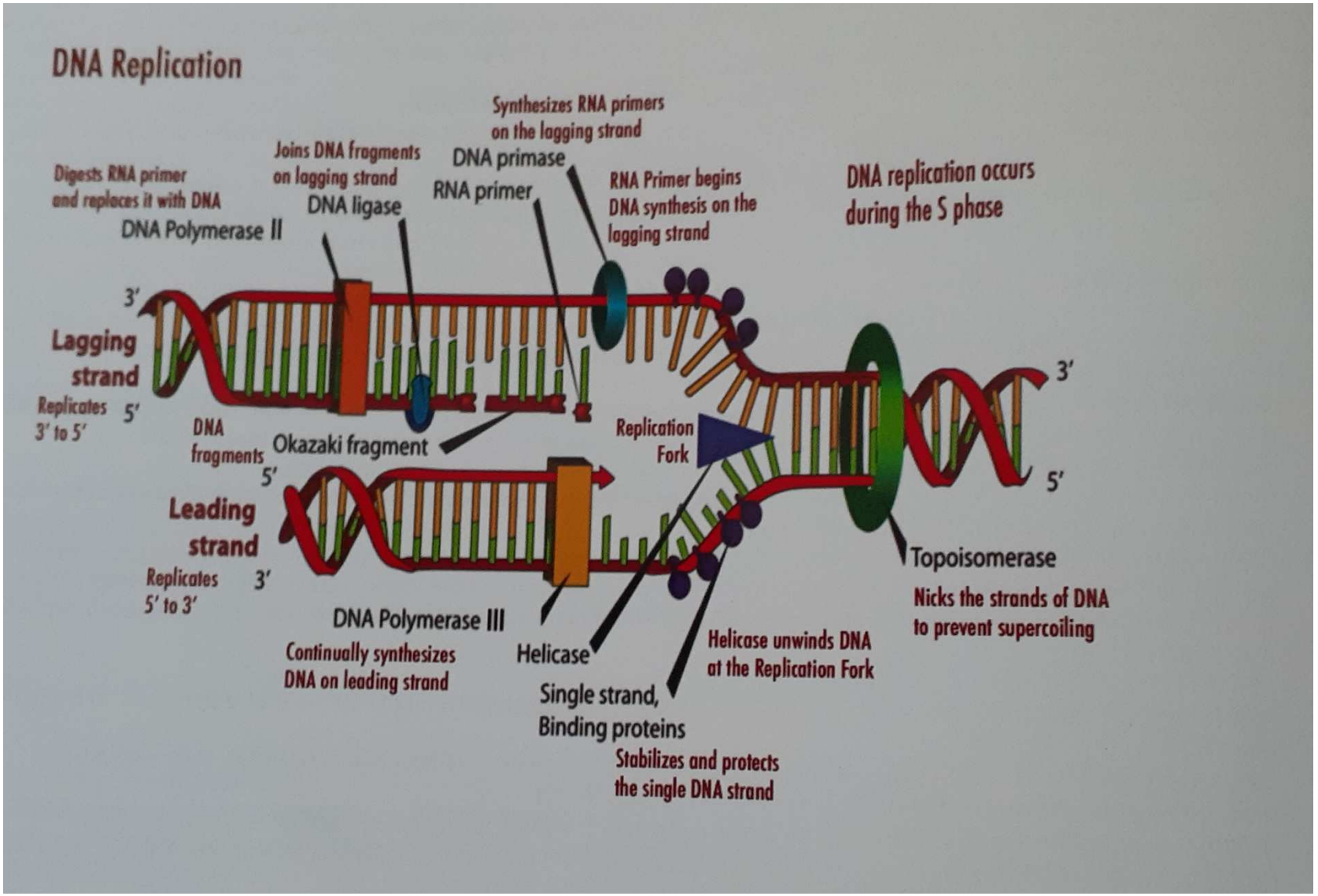


Misteli, The Self-Organizing Genome: Principles of Genome Architecture and Function, Cell (2020), https:// doi.org/10.1016/j.cell.2020.09.014

Genome dynamics

- The dynamic and stochastic nature of the genome, combined with the presence of genetic feedback loops and a strong attractor in the form of cell-type-defining gene expression programs, confers to genomes all major hallmarks of self-organization.

- X chromosome inactivation involves rna coil around chromosome (XIST) with associated proteins to silence chromosome
- Often become target of immune system (autoimmune disease)


DNA Replication
Synthesizes RNA primers on the lagging strand
Joins DNA fragments on lagging strand
DNA primase
Digests RNA primer and replaces it with DNA
DNA ligase
RNA primer
RNA Primer begins DNA synthesis on the lagging strand
DNA replication occurs during the S phase
DNA Polymerase II
3′
Lagging strand
Replicates 3′ to 5′
5′
DNA fragments
Okazaki fragment
Replication Fork
3′
5′
5′
Leading strand
Replicates 5′ to 3′
3′
Topoisomerase
Nicks the strands of DNA to prevent supercoiling
DNA Polymerase III
Continually synthesizes DNA on leading strand
Helicase
Helicase unwinds DNA at the Replication Fork
Single strand, Binding proteins
Stabilizes and protects the single DNA strand

DNA replication

- Replication begins at consensus sequence of adenine-thymidine rich base pairs.
- Semi-conservative.
- Single origin of replication
- Continuous bidirectional DNA synthesis on leading strand
- Discontinuous on lagging strand.
- Replication fork is where leading and lagging strands are synthesized.

DNA replication

- Helicase unwinds the DNA template at the replication fork by breaking Hydrogen bonds.
- Single stranded binding protein prevents reannealing of strands.
- Topoisomerase creates a nick in the helix to relieve tension of supercoils.

Role of topoisomerase

- Double stranded molecule must separate
- Each strand is template for new complement
- Antiparallel nature of strands means new strands are produced in slightly different ways
- Mitochondrial DNA is condensed by wrapping the circular DNA around itself, forming a supercoil.
- Superhelical turns, in the opposite direction, are produced so that the number of turns per base pairs remains unchanged.

Role of topoisomerase

- Toposiomerase I (swivelase)
- Uses no ATP
- Cuts one strand and allows to rejoin ends.
- Operates on positive supercoil.
- Positive supercoiling occurs during strand separation during DNA replication.

Role of topoisomerase

- Toposiomerase II (gyrase)
- Uses ATP
- Produce and remove supercoils (can cut both strands).
- Negative supercoil as wound or unwound
- Energy for unwinding comes in part from reducing strain of negative supercoil.

Transcription to Translation

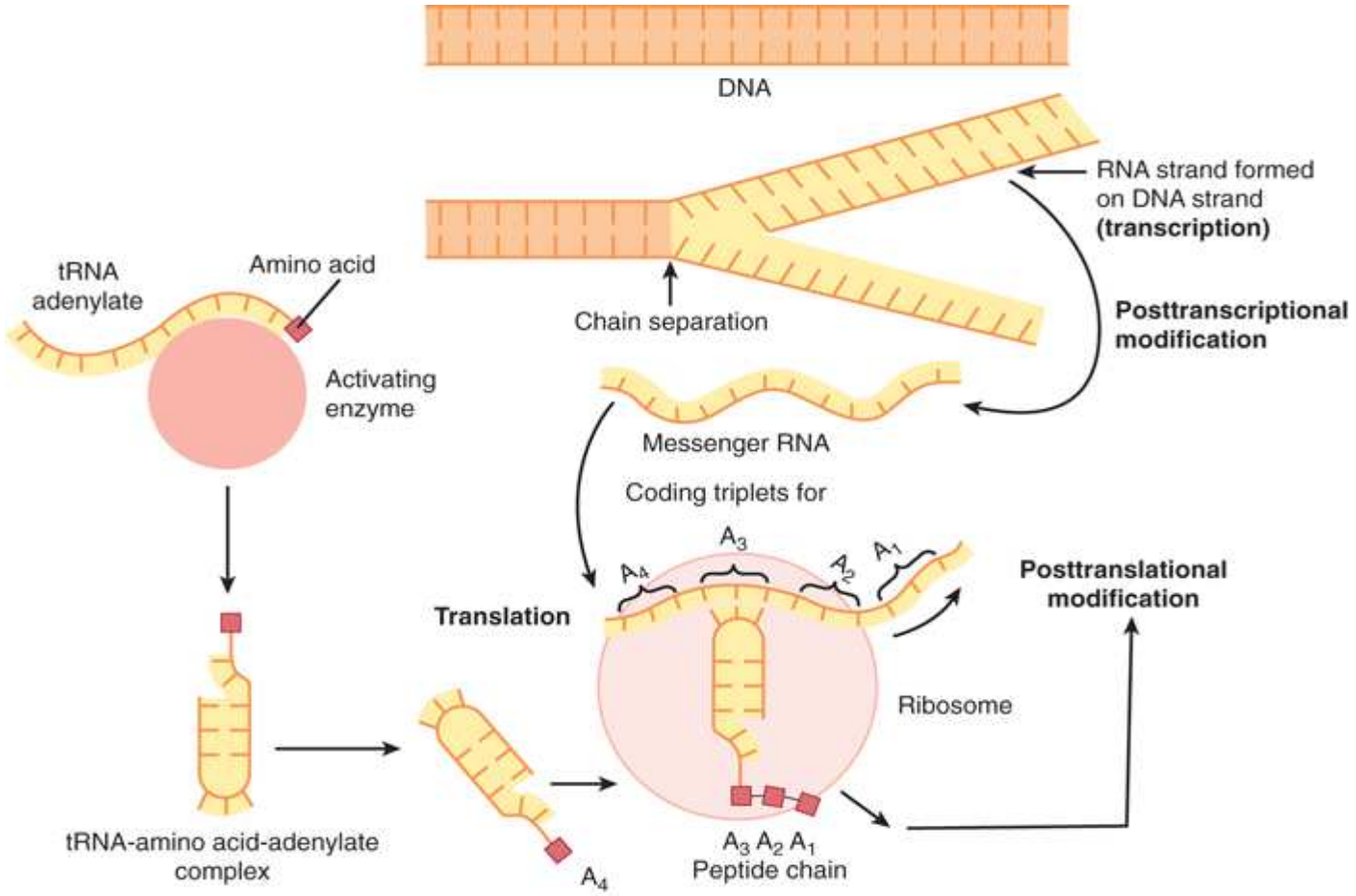


Fig. 1-15 Accessed 07/01/2010

DNA replication

- DNA primase makes an RNA primer on the lagging strand on which DNA polymerase III can initiate replication.
- Strands have a direction set by the backbone.
- Strands are synthesized from a 5’ to 3’ direction.
- 5’-3’ direction of synthesis as the 5’ end bears the triphosphate energy source for the bond.
- Exonuclease activity proofreads in a 3’-5’ direction
- Paired strands run in opposite directions (anti-parallel).
- Necessary for hydrogen bonding to hold strands together.

DNA replication

- One strand properly oriented for continuous synthesis (leading).
- Replication fork opens up ahead of DNA polymerase δ.
- Elongates leading strand at the 3’ end polymerase in proper direction.

DNA replication

- The lagging strand is synthesized in opposite direction, away from fork.
- Replication fork uncovers DNA behind DNA polymerase Iα
- Has to start many times.
- Stops elongation of the lagging chain when it reaches the primer of the preceding fragment.
- Degrades RNA primer
- Lagging strand replicated in short segments, Okazaki fragments, each beginning with a 5’ RNA primer.

DNA replication

- DNA polymerase Iβ excises RNA primer from the lagging strand with 5’-3’exonuclease.
- DNA polymerase Iε is also a repair enzyme.
- DNA ligase seals the DNA of the lagging strand.
- DNA from previous Okazaki fragment serves as primer to fill gaps left by removal of RNA primer (circular chromosome).
- DNA polymerase Iγ replicates mitochondrial DNA.

DNA replicaiton

- With nuclear DNA, telomerase enzyme adds a six base sequence, AGGGTT, to the 3’ end of the DNA template by reverse transcription.
- RNA primer is again formed and a daughter strand synthesized.
- DNA ligase attaches the newly synthesized DNA to the daughter strand.
- Primer and added DNA are cleaved.
- Thus, the chromosome does not increase in size with each replication.

DNA damage

- Heat damages purines preferentially.
- Adenine is also deaminated to hypoxanthine and cytosine to uracil with heat exposure.
- Adenine phosphate endonuclease removes the base.
- UV light dimerizes pyrimidine residues which are adjacent in the same strand of DNA (usually thymidine).
- Benz(a)pyrene from cigarette smoke oxidized in body affects guanine.

DNA repair

- DNA is repaired by excising the damaged base on the 5’ side of the damage, leaving a 3’ hydroxyl group to act as primer for re-synthesis.
- Alterations of a single strand of DNA are the most common aberration.
- Generally endogenous.

DNA repair mechanisms

- DNA single strand breaks are repaired by using the intact complementary strand as a template by:
- BER (base excision repair)
- NER (nuclear excision repair)
- MMR (mismatch repair).

DNA repair mechanisms

- BER is the principal repair mechanism.
- PARP1 binds the broken single strand.
- PARP1 catalyzes the formation of large branched chains of poly-ADP-ribose from its NAD^+ substrate.
- This results in the poly-ADP ribosylation of PARP itself, XRCC1, and β-polymerase.
- This enables the single stranded break to be repaired.

BER

- Removes a single damaged base
- DNA glycolase removes hypoxanthine and uracil from DNA as single base in the G1 phase.

NER

- Replaces a group of damaged base pairs
- An excision endonuclease recognizes thymidine dimers in the G1 phase.
- This enzyme is deficient in Xeroderma pigmentosum.
- No repair upon exposure to UVB light
- O-methyl guanosine demethylase removes guanosine methylated at the sixth Oxygen position as a single base in the G1 phase
- If unrepaired it will pair with thymidine during replication.

NER

- Cockayne's syndrome
- Autosomal recessive
- ERCC6 gene at 10q11.23; ERCC8 gene at 5q12.1
- NER
- Also unable to repair UV damage.
- Photosensitive
- In contrast to xeroderma pigmentosum, no melanoma develops.

NER

- Trichothiodystrophy
- Heterogeneous group
- Autosomal recessive
- ERCC2 gene at 19q13.32; ERCC3 at 2q14.3; GTFHID gene at 6q25.3 are part of general IH transcription complex
- NER defect.
- Sulfur rich brittle hair and nails
- Associated with photosensitivity.
- MPLKIP gene at 7p14.1 not associated with photosensitivity

Other DNA repair

- Fanconi's anemia
- Autosomal recessive disorder
- Bone marrow failure.
- Eleven genes are involved in the pathway.
- Mutation in any one of them renders the cell susceptible to damage by reactive oxygen species as well as affect DNA repair mechanisms.
- Ashkenazi and Afrikaner populations have significantly higher disease incidence.
- FANC gene at 16q24.3

Helicase abnormality

- Replication fork
- Bloom syndrome
- Short stature
- Photosensitive skin rash
- Pigment changes in sun protected areas
- Male infertility
- Increased cancer risk
- Autosomal recessive.
- BLM gene
- RecQ helicase defect at 15q26.1

Helicase abnormality

- Werner's syndrome
- Short stature
- Adult progeria onset in twenties
- Early death from atherosclerosis or cancer
- Autosomal recessive
- WRN gene at 8p12
- Helicase, exonuclease, and telomerase involved in DNA repair.

DNA repair mechanisms

- Double strand breaks can be induced during repair of interstrand cross-links and during replication of single strand breaks at replication forks.
- HR (homologous repair) is the principal repair mechanism and is usually error-free.
- BRCA1 and BRCA2 are essential to homologous repair
- Absence leads to use of error-prone repair mechanisms.

DNA repair mechanisms

- PARP deficiency leads to an increase in persistent single strand breaks which then collapse the replication fork and are repaired by HR (error-free).
- If BRCA1 is also deficient, homologus repair cannot proceed
- Breaks accumulate and the cell dies.
- NHEJ (non-homologus end joining) repair of double strand DNA breaks is more prone to error and may lead to changes in the DNA sequence at the break site.

DNA repair mechanisms

- Post-replication repair involves crossover between the unfinished daughter strand and the parental strand of DNA that has the same sequence and orientation.
- Mismatch repair occurs at the G2 phase
- Which is also the time for final proofreading
- Recognized by lack of methylation
- Hereditary non-polyposis colorectal cancer (Lynch syndrome) as example

DNA repair mechanisms

- Only bacteria contain 3 polymerases.
- Viruses with circular genomes replicate in a fashion similar to bacteria; those with linear genomes replicate one strand at a time.
- DNA Polymerase I excises RNA primer
- DNA Polymerase III elongates leading and lagging strands

RNA synthesis

- Pre-mRNA (rRNA) is generated as two exons are spliced together.
- RNA polymerase binds to DNA promoter site with other transcription factors.
- RNA polymerase:
- I (rRNA) found in nucleolus
- II (mRNA) found in nucleus
- III (tRNA) found in ribosome

RNA synthesis

- The binding site is upstream (5') to the transcription start site for rRNA and mRNA genes
- The binding site is downstream (3') of the start site for tRNA genes.
- RNA is generated

RNA synthesis

- Following transcription, mRNA is transported out of the nucleus.
- 5’end is capped by 7-methylguanosine.
- 3’end is polyadenylated.
- Messenger RNA (mRNA) transfers function coded in DNA.
- Uracil is substituted for Thymidine.
- Actinomycin binds DNA and prevents RNA polymerase from moving along the template.

RNA synthesis

- The codon consists of a 3 letter nuclide alphabet.
- AUG is the usual start codon
- UGA, UAA, UAG are the usual stop codons.
- Methionine and tryptophan alone among the amino acids are designated by a single codon.
- TATA (Hogness box) at position 25
- CAAT at position 75 are promoters.

RNA synthesis

- The DNA template is read in the 3’-5’direction.
- Ribonucleotides added in 5’-3’direction.
- Synthesis continues until a stop signal is reached.
- RNA strand is displaced from 5’end before synthesis completed.
- Transcription factors have four structural motifs
- Bind directly to the DNA in the major groove of the helix by virtue of their positive charge or basic region near the N-terminal end

RNA synthesis

- TATA binding protein and other transcription factors are necessary for initiation of synthesis.
- Coactivators are promoter-specific.
- Those transcription factors that bind hormones or second messengers bind to a response element on the DNA.
- These are upstream from the transcription site and are cis-elements.
- If binding increases transcription rate, enhances
- If binding decreases transcription rate, represses

RNA synthesis

- Cis-element is a DNA sequence that can potentially bind to a protein factor.
- Trans-factor is a protein that can potentially bind to a DNA sequence
- Enhancer element: Region of DNA that binds to transcriptional activator and Increases the transcription rate.
- Silencer element: Region of DNA that binds to repressor and lowers the transcription rate.
- Repressor: Protein that binds to silencer element and lowers the transcription rate.

Types of DNA binding domains

- All have α-helix that binds to major groove.
- Stabilized by other interactions.
- Named for specific structural features.
- Zinc finger: bind Zinc to cysteine or histidine residues to stabilize α-helix
- Facilitates DNA binding.
- Helix-turn-helix and Helix-loop-helix facilitate dimerization and binding of the protein to DNA.

Types of DNA binding domains

- Leucine zipper opens leucine side chains.
- With a leucine zipper, every seventh amino acid is leucine
- In the protein α-helix, every other protein is leucine.
- Form dimer by C-terminal binding to another leucine zipper.
- N-terminal interacts with lysine and arginine
- Regulatory proteins c-fos, c-jun, myc

Secondary structure of RNA

- Single stranded does not mean simple linear form.
- Complementary bases can pair.
- Complex 3-dimensional structures are formed
- Especially true of rRNA.
- Allows parts to be removed.

Splicing of mRNA

- Introns must be removed before mRNA is mature.
- Bases at each end of intron are signals.
- Series of snRNPs perform splicing.
- Forms a lariat structure and rejoins exons.
- Splicing can generate multiple mRNAs.
- May be tissue specific.
- Can significantly change function of product.
- Blocked by polyadenylation upstream.
- Splicing mutations in SLE (anti-snRNP or anti-Smith)
- Splicine mutations in β-thalassemia

Operons

- Operons are polycistronic genes and related control sequences.
- One promoter regulates production of one RNA.
- Single RNA codes for several proteins in a pathway.
- Repressors are proteins produced by regulatory genes.
- Normally bind to Operator region of specific promoters.
- Prevent mRNA transcription and protein production.

Inducers and repressors

- Inducers are small molecules that bind to repressors.
- Repressor becomes inactive, leaves promoter.
- Transcription and translation of genes can proceed.
- Certain repressors are inactive on their own.
- Require another molecule to bind before they bind to promoters and prevent transcription.
- Those molecules are co-repressors.

micro-RNA

- miRNA inhibits gene expression
- 5% of genome encodes miRNA
- Primary transcripts processed within the nucleus
- Exported with a carrier protein to cytoplasm where it is enzymatically “diced” into 20-30 nucleotide lengths
- miRNA unwinds double helix
- Single strands incorporate into a multiprotein RNA-induced silencing complex (RISC)
- Base pairing between miRNA and mRNA directs the RISC to either cleave the mRNA or repress its translation, silencing gene expression.

Regulatory cascade

- One transcription factor can regulate many genes.
- May include another transcription factor.
- That factor can then regulate other sets of genes.
- Promoters may have several transcription factor binding sites.
- Different sites may direct activity in different circumstances.

Co-activation versus co-repression in nuclear receptors

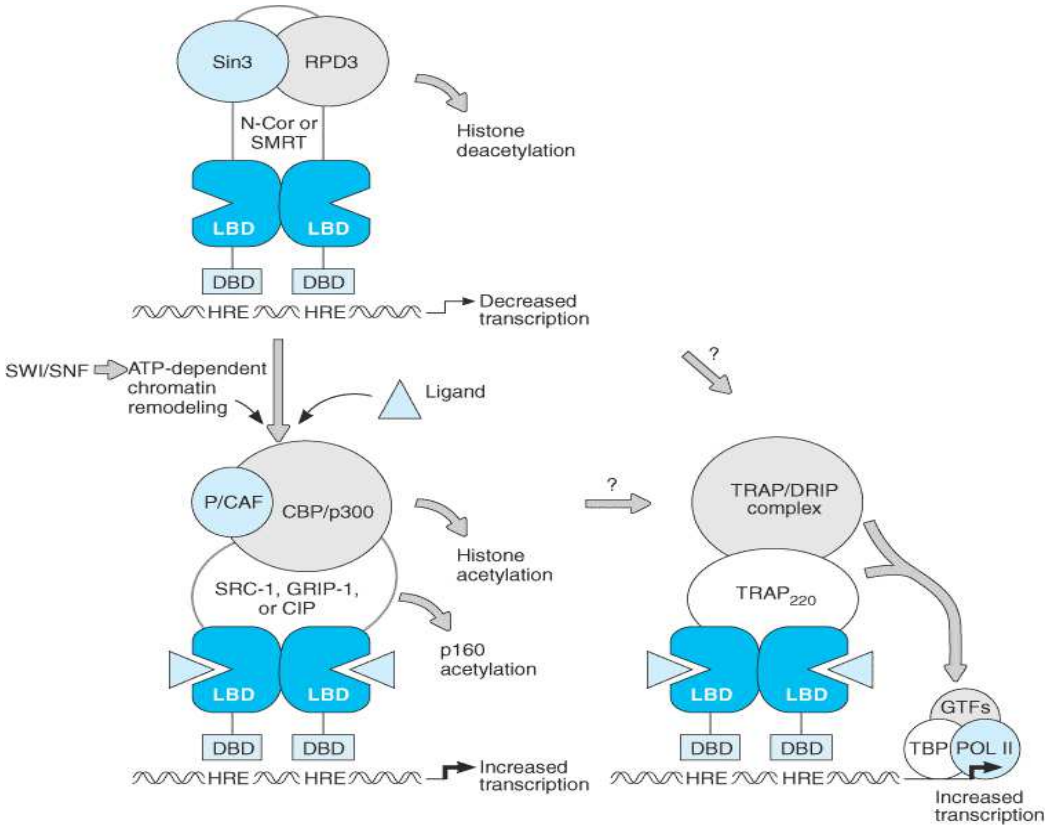


Fig. 2-15 Accessed 07/01/2010

Examples of control

- Catabolites may repress.
- If transcription is not initiated, gene is not expressed.
- For example, in the presence of glucose, the lac operon is repressed
- β-galactosidase is not produced
- Lactose is not metabolized to glucose.

Examples of control

- For example, globin protein made only in presence of heme.
- Heme inactivates kinase that inactivates elongation factor-2.
- Globin mRNA transcription can proceed.

Examples of control

- Ferritin mRNA has iron receptor-E.
- IRE-binding protein binds in the absence of Fe^{2+}
- With more Fe^{2+}, translation occurs.
- Ferritin binds excess iron.
- With low iron, bound IRE-binding protein protects 3' end.
- As more protein made, more iron can enter cell.
- With much iron, IRE-binding protein does not bind
- mRNA degraded faster.

Examples of control

- Thyroid hormone receptor in dimer with RXR on DNA binds corepressor with histone deacetylase.
- Thyroxine changes conformation of the complex.
- Co-activator with histone acetylase can bind to the complex.

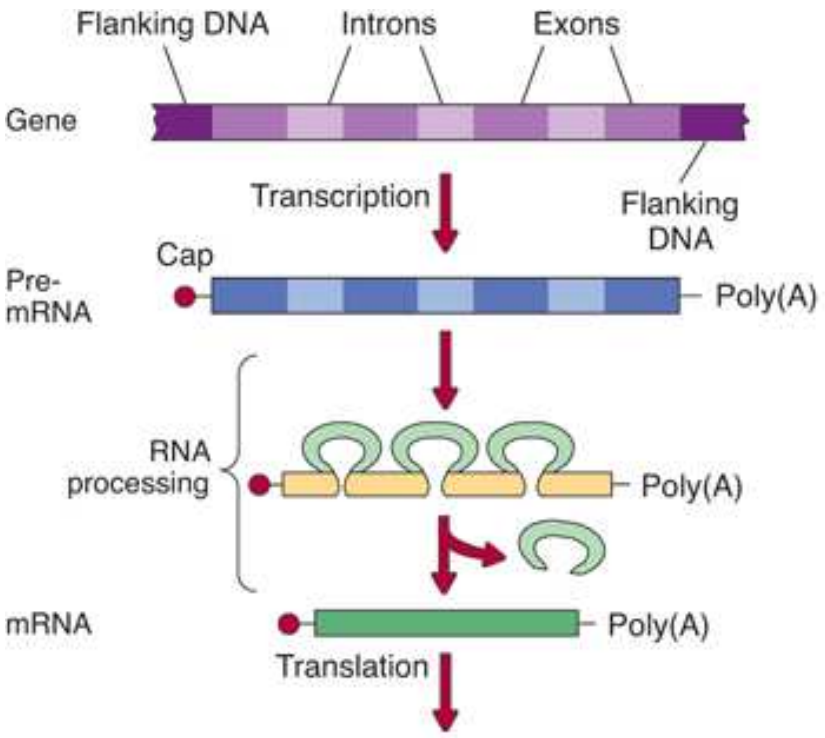
RNA synthesis

- One tRNA for each of the naturally occurring amino acids.
- Transfer RNA (tRNA) “translates” the code.
- Translation is directional and co-linear with the mRNA.
- All tRNA’s have CCA at 3’end where the amino acid binds.
- Folded into cloverleaf structure of 4 stems and 3 loops to maximize hydrogen bonding.

RNA synthesis

- Second loop contains three nucleotide anticodon sequence that interacts with three nucleotide codon sequence in mRNA.
- In its active form, tRNA is L-shaped.

mRNA Transcription



(Modified from Baxter JD: Principles of endocrinology. In: *Cecil Textbook of Medicine,* 16th ed. Wyngaarden JB, Smith LH Jr (editors). Saunders, 1982.) Fig. 1-14 Accessed 07/01/2010

The region that produces introns and exons is flanked by noncoding regions. The 5'-flanking region contains stretches of DNA that interact with proteins to facilitate or inhibit transcription. The 3'-flanking region contains the poly(A) addition site. Transcribed; capped at the AUG end and polyadenylated at the oppositie end (hRNA); spliced .

Transcription to Translation

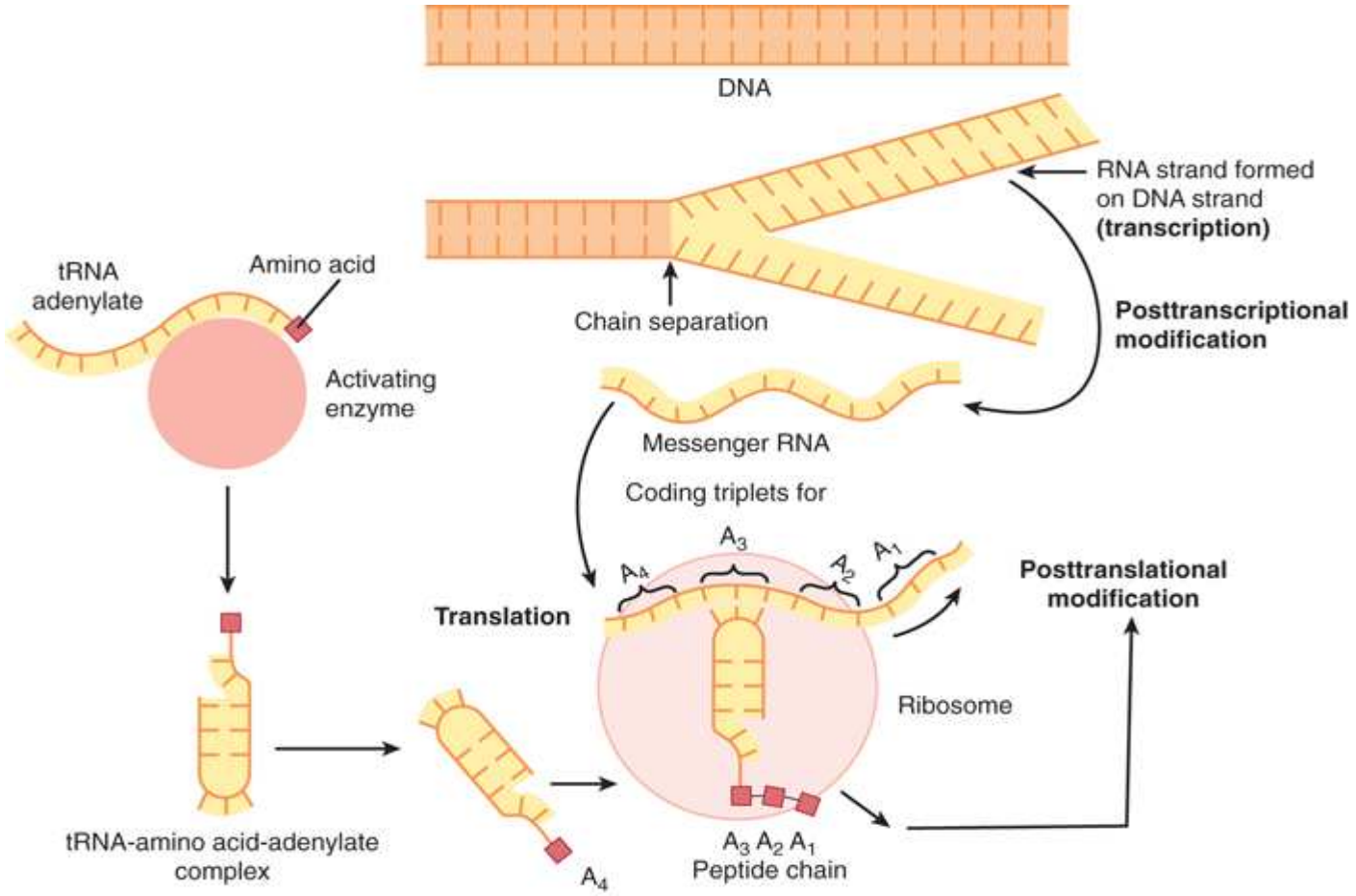
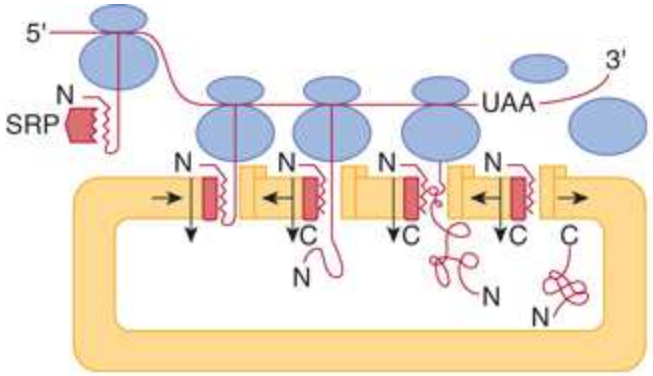


Fig. 1-15 Accessed 07/01/2010

Translation into the Endoplasmic Reticulum



The ribosomes synthesizing a protein move along the mRNA from the 5' to the 3' end. When the signal peptide of a protein destined for secretion, the cell membrane, or lysosomes emerges from the large unit of the ribosome, it binds to a signal recognition particle (SRP), and this arrests further translation until it binds to the translocon on the endoplasmic reticulum.

N, amino end of protein; C, carboxyl end of protein.

(Reproduced, with permission, from Perara E, Lingappa VR: Transport of proteins into and across the endoplasmic reticulum membrane. In: *Protein Transfer and Organelle Biogenesis.* Das RC, Robbins PW (editors). Academic Press, 1988.) Fig. 1-18 Accessed 07/01/2010

Ribosome assembly

- GTP initiates protein synthesis
- Ribosomes are built in the nucleolus.
- RNA precursor cleaved in nucleolus; 5S is from a separate gene.
- Proteins added to RNAs.
- Each subunit exported separately to cytoplasm.

Inhibitors of nucleic acid synthesis

- Drugs that bind to DNA:
- Actinomycin D sterically hinders replication and transcription.
- Agents that bind to RNA polymerase:
- α-amanitin is poison derived from the mushroom, Amanita phalloides.
- It selectively inhibits RNA polymerase II.

Repetitive DNA sequences

- Much of the genome is repetitive DNA.
- Satellite DNA
- 10% of genome
- Few locations, many tandem repeats.
- Three classifications:
- The α-satellite DNA, 171 base pair repeat
- Millions of copies in tandem
- Near centromeres.

Repetitive DNA sequences

- The mini-satellites, 14 to 500 base pair repeat units.
- Few thousand base pairs total,
- The microsatellites, 1 to 13 base pair repeat units
- Total size is a few hundred base pairs.

Repetitive DNA sequences

- Dispersed Repetitive
- 45% of genome
- LINEs
- Up to 7000 base pairs long
- SINEs
- 90-500 base pairs long
- Alu repeats are 300 base pair SINEs with Alu I as part of sequence
- 10% of genome.

Base-pair substitution mutations

- Change in one base pair.
- Silent
- Third position, no change in amino acid.
- Missense
- Changes codon from one amino acid to another.
- Becker muscular dystrophy
- Proximal muscle weakness of legs and pelvis
- Dilated cardiomyopathy
- DMD gene at Xp21.2-p21.1 (dystrophin)
- Live into the 40’s
- Nonsense
- Becomes Stop codon, truncates protein.

mRNA editing

- mRNA sequence can be altered after transcription.
- Classic example, ApoB100 gene at 2p24.1.
- Cytidine deaminated to Uracil
- Produce STOP codon
- Shorter protein product produced.

Insertion and deletion

- Bases added to or removed from sequence.
- Large ones can disrupt more than one gene.
- Small ones can change sequence.
- Frame-shift is special case of small insertion or deletion where the number is not a multiple of 3.
- Changes reading frame of gene.
- ATG usually initiator
- Completely different amino acid sequence follows
- Premature truncation likely.

Frame shift mutation

- Duchenne muscular distrophy
- Proximal muscle weakness of legs and pelvis
- Pseudohypertrophy of calf and deltoid muscles
- Muscle replaced by fat and fibrous tissue
- Dilated cardiomyopathy
- DMD gene at Xp21.2-p21.1 (dystrophin)
- Onset by age 2
- Generally do not survive the twenties

Mutations in non-coding DNA

- Mutations can occur in any DNA sequence.
- May effect mRNA expression rather than protein sequence.
- Promoters,enhancers, and silencers.
- May effect mRNA processing and protein sequence.
- Splice site mutations (GT..AG) rule:
- Donor or acceptor can mutate.
- Cryptic sites can become functional.
- Exon skipping can occur.

Other mutation types

- Duplications
- Large regions or whole genes are duplicated.
- Genes may function and affect gene dosage.
- Transposons
- Mobile genetic elements.
- Can insert in or near gene and alter expression.

Other mutation types

- Expanded repeats
- Trinucleotides (run of one amino acid).
- As number of repeats increases, may disrupt function.
- Germline expansion in females.
- Increased severity and earlier onset in later generations.

Fragile X syndrome

- Phenotype similar across ethnic groups.
- Characterized by long face, prominent jaw, large ears, large testes.
- X-linked dominant
- Male inherits from mother
- Female inherits from either parent.
- Associated with premature ovarian failure
- Most common inherited form of mental retardation
- Downs more common, but not inherited

Fragile X syndrome

- Fragile X as end of long arm breaks in low folic acid environment.
- FMR1 gene at Xq27.3 has CGG repeat in 5' U tandem repeat.
- Methylates gene, blocking transcription.
- Offspring of transmitting males have same number of repeats as father.
- Repeat expands only in female meiosis.

Huntington's disease

- Presents in 30's
- Juvenile form more aggressive
- Chorea
- Depression
- Dementia
- Seizures in 50% (juvenile form)
- Autosomal dominant
- CAG repeats at 4p16.3 (HTT gene)
- Hypermethylation of histone gene
- Atrophy of caudate nucleus
- Neuronal death via NMBA-R binding and glutamate toxicity

Freidrich's ataxia

- Presents between 5-15 years of age
- 25% have late onset disease (ages 26-39) or very late onset disease (age >40)
- Ataxia
- Spasticity and loss of strength in limbs
- In a wheelchair after 10 years
- Hypertrophic cardiomyopathy
- Autosomal recessive
- GAA repeats at 9q21.11 (fraxin gene)

Myotonic dystrophy

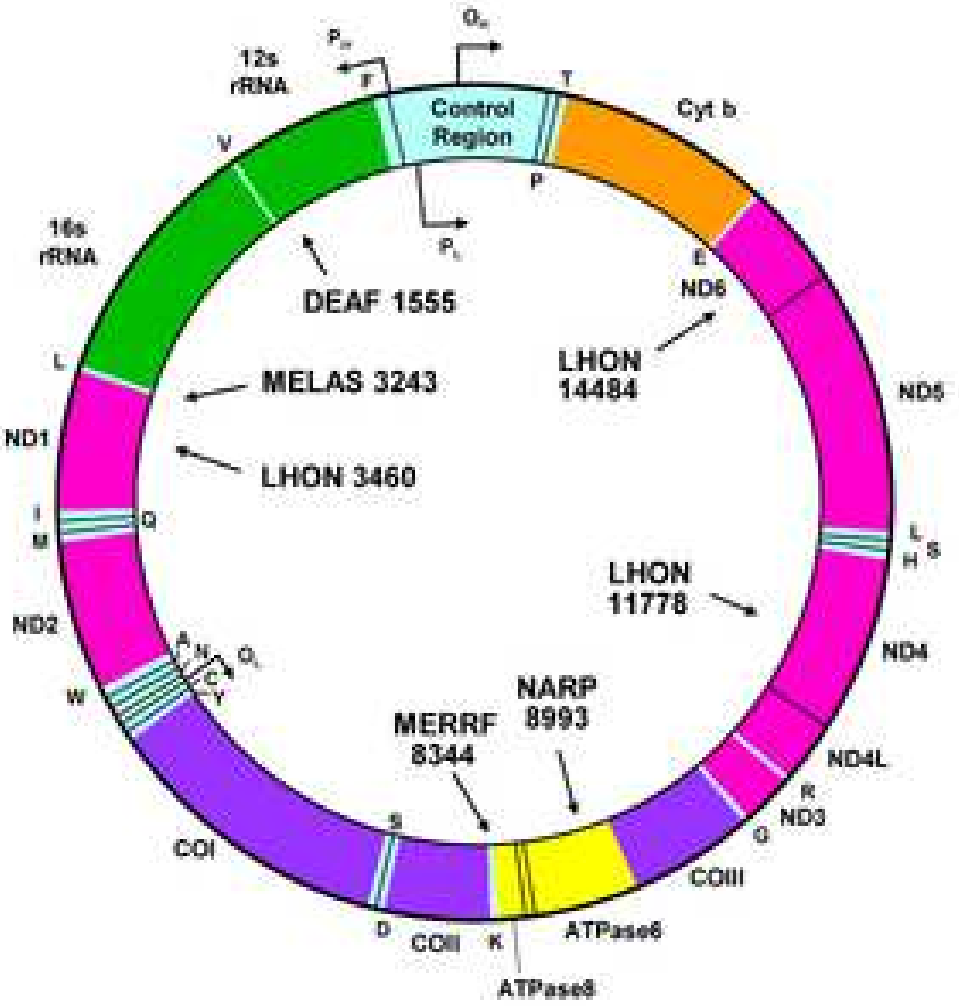
- Presents between ages 20-30
- Myotonia (prolonged contraction after muscle use)
- Cataracts
- Arrhythmias
- Autosomal dominant
- Type I involves muscles of lower legs, hands, neck and face
- CTG repeats at 9q13.32 (DMPK gene)
- Variant presents at birth
- Hypotonia
- Clubfoot

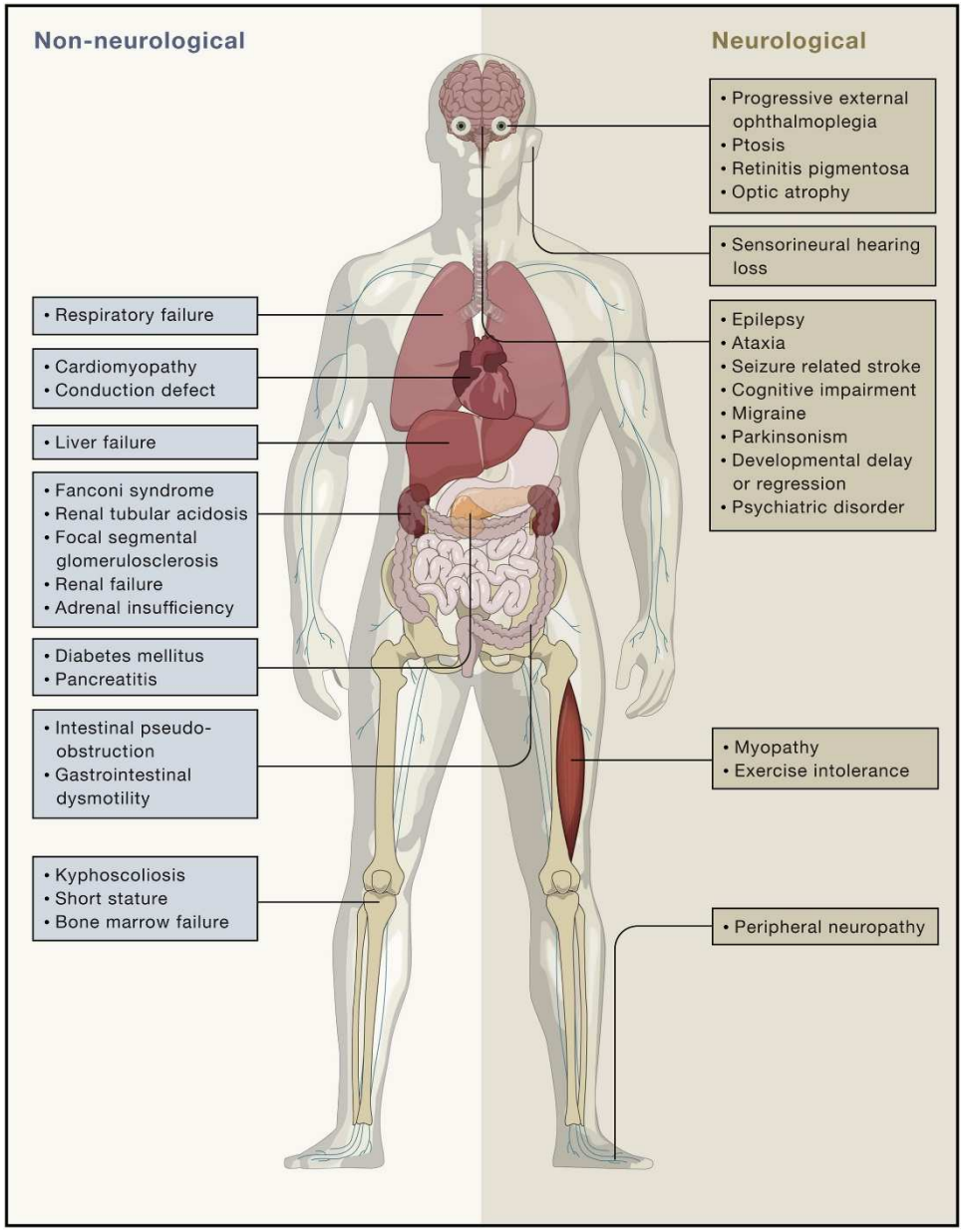
Myotonic dystrophy

- Type 2 involves muscles of the neck, shoulders, elbows, and hips
- CCTG repeats at 3q21.3 (CNBP gene)
- Zinc finger nucleic acid binding protein

Mitochondrial inheritance

- Circular chromosome.
- Inheritance is strictly maternal.
- 37 genes
- 13 control oxidative phosphorylation
- 24 produce rRNAs and tRNAs.
- Mutation rate is high as there is a lack of repair systems and there are many free radicals generated from oxidative metabolism.





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Accessed 03/20/2020

Mitochondrial inheritance

- Single base changes seen in:
- Myoclonic encephalopathy with ragged red fibers (MERRF)
- Presents in childhood
- Myoclonus
- Myopathy
- Red ragged muscle fibers noted on biopsy
- Epilepsy
- Spasticity, ataxia, and dementia develop
- X-linked
- MT-TK gene (tRNA) BP8295-8364

Mitochondrial inheritance

- Mitochondrial encephalomyopathy mitochondrial depletion syndrome
- Rarely survive childhood
- Hypotonia
- Failure to thrive
- Lactic acidosis
- May have cardiac and hepatic abnormalities, white cell depression
- FBXL4 gene at 6p16.1-2

Mitochondrial inheritance

- Mitochondrial myopathy, episodic, with optic atrophy and reversible leukoencephalopathy (MEOAL)
- Autosomal recessive
- Childhood onset
- Rrecurrent episodes of proximal weakness and myalgia
- Precipitated by exercise, infections or low temperature.
- Additional features are optic atrophy, axonal polyneuropathy, and reversible or partially reversible leukoencephalopathy.
- FDX2 gene at 19p13.2

Mitochondrial inheritance

- Leber's hereditary optic neuropathy
- Presents in teens and twenties
- Acute onset of vision loss
- Degeneration of retinal ganglion cells
- May later develop arrhythmias, movement disorder
- MTND genes (NADH dehydrogenase)
- BP 3307-4602 on mitochondrial chromosome
- 50% men and 85% women asymptomatic

Mitochondrial inheritance

- Duplications and deletions noted in:
- Kearns-Sayre disease
- Presents before age 20
- Progressive external ophthalmoplegia
- Ptosis
- Ataxia and arrhythmias develop
- May have pigmented retina
- Ragged red muscle fibers on biopsy
- Large mitochondrial (1,000-10,000) genome deletion

Linkage

- Linkage depends on chromosomal location.
- Chromosome re-organize at meiosis.
- Crossing over may separate linked alleles.
- Distance between genes is important to linkage.
- Multiple crossovers can occur between two chromatids in one meiosis.
- Genes closer in space recombine less.

Linkage disequilbrium

- Mutated and specific linked alleles inherited together.
- Much more likely person with mutant gene carries a specific allele at linked marker.
- Linkage analysis locates which alleles of linked genes are on same DNA strand as a mutated gene to regions of chromosomes.
- Can follow inheritance of linked marker rather than mutation
- Provide information about genome organization.

Linkage disequilbrium

- Over time, recombination will separate mutation from marker, reach equilbrium as recombination separates alleles.
- Can give rough estimate of mutation's "age".

Genetic association

- Genetic association applies when linkage is not known or may not be a factor.
- Some specific allele significantly increases risk of a person being affected.
- May be due to effect of allele rather than linkage disequilibrium.
- Hemchromatosis and HLA-3
- Ankylosing spondylitis and HLA-B27

X-linked dominant disorders

- Males inherit from mother; females, from either parent.
- Hypophosphatemic rickets.
- PHEX gene at Xp22.11
- Kidneys can't reabsorb phosphate.
- Abnormal ossification, bones bend and distort.
- Osteomalacia in adults
- Incontinentia pigmenti.
- Abnormal skin pigmentation and teeth
- Neurological and ocular abnormalities
- Males lost in utero.
- IKBKG gene at Xp28 (NF-kB)

X-linked dominant disorders

- Rett syndrome
- Autism, ataxia, mental retardation.
- Some males survive to term.

X-linked recessive disorder

- Normal vision is trichromatic.
- Opsin proteins absorb colors, responsible for color vision.
- Red and green opsins adjacent at Xq28.
- Share 98% sequence identity.
- Usually one red followed by one or more green.
- Protanomaly (red weakness) and Deuteronomaly (green weakness)
- Protanopia (no red or L cones)
- Deuteronopia (no green or M cones)
- 8% men, 0.5% women

Color vision deficiency

- Autosomal dominant
- Tritanomaly (blue weakness)
- Tritanopia (no blue or S cones)
- Monochromacy (only blue or S cones)
- 7q32.1 (OPN1SW)
- Autosomal recessive
- Achromatopsia (no color perception)
- 1p13.3 (transducin)
- 10q23 and 12p12.3 (phosphodiesterase)
- 2q11.2 and 8q21.3 (cyclic nucleotide gated channel)

Non-disjunction

- Maternal in 90 to 95% of cases.
- About 75% from Meiosis I, rest from Meiosis II.
- Mosaicism seen in some individuals.
- Can improve prognosis.
- Mosaicism can be tissue specific.
- Germline mosaicism can be cause of recurrence in a family.
- Makes calculation of risk of future abnormal pregnancy difficult.

Trisomy 21

- Most common somatic chromosome abnormality.
- Down's Syndrome.
- Variable appearance
- Low set ears, epicanthal folds, palmar simian crease.
- Brushfield spots (white) on iris
- Mental retardation
- Diminshed levels of AFP, Estriol, PAPP-A;
- Elevated β-HCG and inhibin A in maternal blood.
- Nuchal lucency on ultrasound.

Trisomy 21

- Increased risk of:
- Duodenal atresia or obstruction
- Myelogenous leukemia
- Septum primum heart defects
- ASD common
- Life expectancy:
- 80% to age 10
- 50% to age 50
- Dementia develops at age 35.

Trisomy 21

- 95% meiotic (maternal Meiosis I) non-disjunction of homologous chromosomes in stages 1 and 2 of anaphase
- 4% Robertsonian translocations t(13p;21p)
- 1% mosaicism (mitotic nondisjunction).
- Risk of nondisjunction increases with maternal age >35 years old.

Trisomy 21

- Down's patients seldom reproduce.
- Males generally sterile.
- Females can reproduce
- 50% of ova would have 2 copies of 21
- However, about 75% of trisomy conceptuses fail.
- Risk of subsequent pregnancy in affected couples is less than 50%.

Trisomy 18

- Second most common somatic chromosome abnormality.
- Edwards syndrome
- There is a prenatal growth deficit.
- Micrognathia
- Overlapping fingers with clenched fist
- VSD
- Short sternum
- Small mouth
- Short big toes, rocker bottom feet.

Trisomy 18

- Mental retardation
- Spina bifida.
- Ventricular septal defect.
- Omphalocele
- Diaphragmatic hernia.
- Diminished levels of AFP, Estriol, PAPP-A, β-HCG;
- Normal levels of Inhibin A in maternal blood

Trisomy 13

- Patau syndrome
- Cleft lip and palate
- Postaxial polydactyly
- Microcephaly
- Micro-ophthalmia
- Rocker bottom feet
- Diminished levels of β-HCG, PAPP-A in maternal blood
- Increased nuchal lucency on ultrasound

Trisomy 13

- Malformations of central nervous system, heart and kidneys are frequent.
- Only 5% survive to one year.
- About 80% full trisomy
- Some trisomy of long arm due to translocation.

Sex chromosome aneuploidies

- X-inactivation limits affect on phenotype.
- Monosomy X fetus non viable
- Trisomy X
- Some sterility, menstrual irregularity.
- 90% maternal meiotic nondisjunction.
- 47,XYY
- Taller than average
- First described in prison population (high incidence).
- In that population there is described a small reduction in verbal IQ and presence of behavioral disorders.

Turner's syndrome

- Short stature
- Neck web
- May have cystic hygroma
- Dactylitis
- Coarctation of the aorta
- Horseshoe kidney
- Ovarian dysgenesis usual (streak ovaries)
- Amenorrhea
- But may carry fetus if in vivo fertilization
- Some mosaics do have ovaries

Turner's syndrome

- Mosaics:
- 45,X/46,XX, 45,X/46,XY, 45.X/47,XXX, or 45,X/46,X,i(X)(q10)
- Also found are 46,X,r(X) or 46,X,delXq or delXp
- Non-disjunction in paternal Meiosis I
- No Barr body
- Abnormalities in proportion to percentage of 45,X cells

Klinefelter syndrome

- Tall, disproportionately long arms and legs.
- Small testes
- Gynecomastia
- Possibly subtle mental defect
- 47,XXY
- Meiotic disjunction in Meiosis I
- If paternal, is error in chromosome segregation in anaphase in primary spermatocyte stage of spermatogenesis
- Deficiencies increase with extra X.

Klinefelter syndrome

- Histology of testis:
- Dysgenesis of semininferous tubules (no sperm)
- Leydig cell hyperplasia (low testosterone)
- Sertoli cells few
- No germ cells
- Mosaicism increases chance of fertility.

True hermaphrodites

- Have both ovarian and testicular tissue
- 46XX/46XY or 46XX/47XXY
- External genitalia reflect testosterone exposure in weeks 8-16 of gestation
- Pseudohermaphrodites have appropriate ovarian or testicular tissue
- External genitalia reflect excessive androgen exposure (female is virilized)
- OR lack of androgen receptor (male feminized))

Translocations

- Exchange of material between non-homologous chromosomes.
- Are reciprocal or Robertsonian.
- In a reciprocal translocation, part of one chromosome transferred to another.
- It produces derivative chromosomes.
- Meiotic segregation can lead to unbalanced rearrangement (partial trisomy or monosomy).
- Transfer can be unidirectional or bidirectional.
- In a Robertsonian translocation, short arms are lost, and long arms join at centromeres.
- Acrocentric.

Translocations

- Viable translocations involve chromosomes 13, 14, 15, 21, 22.
- Most commonly involved are 14 and 21.
- May have one Robertsonian translocation and one normal chromosome.
- Meiotic segregation can unbalance gamete.
- Alternate segregation
- Either translocation or two normal chromosomes to gametes.
- Adjacent segregation
- All abnormal
- Some cause trisomy, some monosomy.

Inversions

- Chromosome rearrangement in which a segment of a chromosome is reversed end to end.
- Pericentric inversions involve centromere
- Proceed through meiosis.
- Paracentric inversions do not involve centromere
- Does not proceed through meiosis.
- May result in diminished fertility.

Deletion syndromes

- Visible loss of chromosomal material.
- A terminal loss is a loss from end of chromosome
- An interstitial loss is from within chromosome.
- Cri-du-chat syndrome.
- Loss of distal 5p.
- (CTTND2 is a δ-catenin, located in dendrites)
- Small head
- Characteristic cat cry.
- VSD
- Survival impaired.

Deletion syndromes

- Wolf-Hirschhorn syndrome.
- Loss of distal 4p. (NSD, LETMI, NSXI)
- Can also be microdeletion.
- Wide spaced eyes
- “Greek warrior helmet” appearance
- Cleft lip.

Deletion syndromes

- Williams
- Loss of 28 genes of 7q (ELN, the elastin gene included).
- Cheerful, friendly, well developed verbal skills
- Congenital heart disease.
- 22q11 deletion.
- Aberrant development of 3rd and 4th pharyngeal pouches
- Parathyroid and thymus malformation
- Congenital heart disease in DiGeorge's syndrome
- If facial cleft, is velocardial facial syndrome.

Micro-deletion syndromes

- Imprinting causes different expression of maternal and paternal genes in the affected region.
- Micro-deletion leaves only one copy.
- Deletion too small to see in metaphase spread.
- Microdeletion on15q24
- Prader-Willi (maternal)
- Angelman (paternal)

Micro-deletion syndromes

- Prader-Willi
- Mental retardation
- Hyerphagia
- Hypogonadism
- Hypotonia
- Maternal gene silenced by methylation (imprinted)
- Paternal gene deleted
- OCA gene at 15q12-13.1
- Maintains acidity of melanosome

Micro-deletion syndromes

- Angelman
- Mental retardation
- Seizures
- Ataxia
- Inappropriate affect
- Paternal gene silenced by methylation (imprinted)
- Maternal gene deleted on chromosome 15q11.2
- Ubiquitin ligase UBE3A gene

Micro-deletion syndromes

- WAGR
- Microdeletion on11p13
- Affects contiguous genes.
- Wilm's tumor
- Aniridia
- Genitourinary abnormalities
- Retardation (mental)

Microdeletion syndromes

- Neurofibromatosis 1 (von Recklinghausen)
- Autosomal dominant
- 17q11 microdeletion (neurofibromin)
- Café-au-lait spots
- Lisch nodules in iris
- Freckling of axillae (and inguinal regions)
- Neurofibromas
- Associated with pilocystic astrocytomas

Microdeletion syndromes

- Neurofibromatosis 2 (von Recklinghausen)
- 22q12.2 microdeletion (merlin)
- Bilateral acoustic neuromas (Schwannomas)

Uniparental disomy

- One pair of chromosomes derived from one parent, not both.
- May manifest an autosomal recessive condition.
- Abnormal gametes from both, 2 + 0.
- One abnormal, 2 + 1, loss of trisomy in embryo.
- About 30% of Prader-Willi is due to maternal disomy of chromosome15.

Other chromosome abnormalities

- Ring chromosomes. Ends lost, breaks join.
- Inversions. 2 internal breaks that rejoin out of order:
- Pericentric inversion occurs with joining of one break on each side of centromere.
- Paracentric inversion occurs if both breaks are in one arm.
- Cross-overs can lead to duplication-deletion combinations in gametes.
- Isochromosomes are two copies of one arm.

Inheritance paradox

- Mutations that appear to be dominant inheritance pattern in pedigrees
- But are recessive at cellular level
- Require a second copy to be mutated
- Are a paradox.

Drug effects

- Alkylating agents cross-link guanine nucleotides in DNA.
- Cyclophosphamide acts in this fashion.
- Base analogs are similar to one of the four nucleotide bases and can be incorporated incorrectly into DNA during replication and produce mismatch during base pairing.
- Bromodeoxyuridine is an example.
- Antimetabolites share similarity with the nucelotides, but incorporation into DNA inhibits further replication.
- 6 mercaptopurine or 5 flurouracil as examples

Drug effects

- Methylating agents such as ethyl methanesulfonate or thymidine DNA glycosylysase transfer methyl groups to nucleotide bases.
- This may repress or uncover downstream gene action.
- Intercalating agents insert themselves between two nucleotide base pairs.
- This physically interferes with DNA transcription and replication.
- Anthracyclines, thalidomide, aflatoxin, and ethidium bromide are examples.

Drug effects

- Cross-linking agents such as P^{6-} form covalent bonds between nucleotide bases (guanine), blocking transcription and replication.
- Peroxides acquire electrons from DNA.