Genetics

Facts & Stats

- Training adaptations appear to be under predominantly translational and post-translational control (transporting proteins to their positions).
- Steroids and thyroxine pass through the membrane and enter the nucleus and bind to receptors. This complex of hormone-and-protein has the configuration that will allow binding to an acceptor protein (transcription factor) that recognizes the enhancer/promoter region of a gene.
- Peptide hormones and most hormones derived from amino acids are unable to pass through the plasma membrane. They bind to the membrane receptors and cause a second messenger to go to the nucleus. Binding to the membrane receptor causes adenylate cyclase to convert ATP to cAMP which functions as the second messenger. When the hormonal signal ends outside the plasma membrane, Phosphodiesterase converts cAMP to inactive AMP.
- humans have ~ 100,000 genes
- peptides are short chains of 10 fewer amino acids
- polypeptides are 10 - 100 or more amino acids
- proteins are chains of 100 or more amino acids

under very high temperature and very low salt conditions, no hydrogen bonding occurs....thus complementary strands of DNA cannot bond together, they remain single stranded = denatured

- Amino Acyl Transferase activates t-RNA's
- RNA polymerase makes mRNA
- Reverse Transcriptase……converts RNA to DNA
- 1/3 of human genome codes for brain proteins
- nucleotides are held together by phosphate groups
- amino acid = nitrogen-carbon-carbon with an amino group and an acid group
- peptide bond binds an amino acid to another amino acid

General Terms

A gene = codes for all amino acids in a polypeptide chain

A gene is a 3 nucleotide codon, that is a segment on the DNA strand. Codons are the codes for specific amino acids. 3 nucleotides comprise a codon. There are 4 deoxynucleotides from which to choose 3 in making a codon --- adensonsine (A), cytidine (C), guanosine (G), and thymidine (T). Thus a gene, located on DNA, is comprised of information (codon) that is specific to amino acids which used to make the protein that the gene has the blue prints for 4 deoxynucleotides --- adensonsine (A), cytidine (C), guanosine (G), and thymidine (T).

The gene is copied into a messenger that delivers the information of the codon to the protein asemblers in the cell, called ribosomes.

- The start/front-end of the gene is called the 5' end, and the finish/back-end of the gene is called the 3' end. - - In between the start and finish ends are areas that get copied/coded, and areas that do not. The areas that do are called exons, the areas that do not are called introns. Introns are removed from the mRNA prior to translation.
- Enhancer regions are located at the start end. Proteins interact with the DNA here to increase transcription rate. Enhancer regions are called cis regulatory regions. The proteins that interact there are called trans (transcription) factors. Transcription factors bind to regulatory elements in DNA (promoters/enhancers). Transcription factors function in regulatory networks in which several factors interact to regulate gene transcription, rather than functioning in isolation. Phosphorylation triggered by cell surface receptors is a common means of altering the function of transcription factors. External stimuli that affect transcription are neuropeptides, cytokines, and growth factors. The DNA binding areas ussually contain zinc and leucine (an essential amino acid).
- RNA polymerase II is the enzyme that moves along the DNA from the start end to the finish end and produces the mRNA. A series of nucleotides at the start site signal it to start and a series at the end signal it to stop and dissociate from the DNA strand.
- RNA polymerase II binds to the DNA in a part of the start site called the promoter region. Contained in the promoter region is a TATA box, a short sement of thymines and adenines. The polymerase cannot recognize the TATA box, so a transcription factor that recognizes the TATA box binds to the DNA in that area so the polymerase can do so. Once the polymerase binds, it associates with other transcription factors before beginning RNA synthesis.
- A single gene can be transcribed simultaneously by several polymerase molecules following each other like trucks in a convoy. Transcription progresses at a rate of ~60 nucleotides per second. A congregation of many polymerase molecules simultaneously transcribing a single gene allows a cell to produce a particular protein in large amounts.
- The function of tRNA is to transfer amino acids from the cytoplasm's amino acid pool to a ribosome. A cell keeps it's cytoplasm stocked with all 20 amino acids either by synthesizing them from other compounds or by taking them up form surrounding solution. The ribosome adds each amino acid brougth to it by tRNA to the growing end of the polypeptide chain. Each type of tRNA molecule associates a particular mRNA codon with a particular amino acid. As a tRNA molecule arrives at a ribosome, it bears a specific amino acid at one of its ends. At the other end is an anticodon which binds to its complementary codon on the mRNA. Codon by codon the mRNA genetic information is translated as tRNA's deposit amino acids in the order prescribed and ribosome enzymes join the amino acids into a chain. Each tRNA molecule can be used repeatedly, picking up its designated amino acid in the cytoplasm, depositing it at the ribosome, and leaving the ribosome to pick up another one. Some tRNA's can recognize 2 or more different codons.
- Pretranslational control means that mRNA quantity is rate limiting and that some factor such as gene transcription alters mRNA concentration.
- Translational control means that mRNA quantity is in excess and that some factor alters the efficiency of the usage of the mRNA on the ribosome. Thus protein synthesis rate is limited byy the ability of the ribosome and the translational cofactors to utilize the overabundance of mRNA.
- Posttranslational control means that many events happen to a protein after it is synthesised on the ribosome, such as transport or assembly in it's functional site.
- Sites of possible mess-ups
    - the transciption information must be correct
    - the tRNA must get the correct amino acid
    - the codon-anticodon bonding must be correct between tRNA and mRNA so that the amino acids are placed in the correct order
- Environmental agents such as UV light and X-rays, or free radicals can induce a wide range of lesions in DNA strands. Persisting lesions can interfere with essential processes like transcription or replication, which might cause cell death or lead to mutations (cancer). One

method of DNA repair called Nucleotide Excision Repair (NER) which involves 5 steps--- recognition of the DNA injury, dual incision on the damaged strand, removal of the damage-containing patch, gap refilling by DNA synthesis, strand ligation.
- APE (apurinic/apyrimidinic endonuclease) also called Ref-1, is an enzyme that repairs oxidative damage to DNA. It also regulates the redox state of DNA binding proteins which influences the ability of DNA binding proteins to bind to AP-1 complexes (influences binding protein actions).
- Hetero = different heterozygote zygote = cell formed by the fusion of an egg and a sperm
- Homo = identical homozygote
- Pedigree =
- Phenotype = trait that is expressed/observed, a character [the gene for eye color is a phenotype]. The gene for a specific eye color is called an allele.
- Allele = type of gene (ie. of the phenotypes for color = there is a black allele, yellow allele, white allele,etc)
- genotype = gene comes in pairs = 2 dominant, or 1 dominant--1recessive, or 2 recessive dominant = the allele that is expressed to yield a protein. It is dominant over the other allele. recessive = the allele that is not expressed, no protein made.
- Mitosis = cell division (ie. to produce a human from one cell)
    - Diploid = a cell that has two chromosome sets
    Phases of cell division =
        prophase = chromosomes split and shrink
        metaphase = chromosomes move to center of cell
        anaphase = chromosomes split and move to polar ends of the cell
        telophase = nucleus separate into 2, then form new nuclear membrane around each
- Meiosis = cell division that produce sperm or eggs
    - Haploid = a cell having one set of chromosomes gamates = sperm, eggs gonads = testes, ovaries.
Phases of cell division =
    meiosis 1 =
    meiosis 2 =

Translation

Big Picture.....Translation Initiation Factors role in translation (protein synthesis) is to help attach the copy of the gene [mRNA] to the ribosome [builder of the protein].
eIF's attach mRNA to the ribosome, so that translation of mRNA code into protein can take place.
If the mRNA does not attach to the ribosome, protein synthesis cannot take place.
There are several eukaryotic Translation Intitiation Factors

- eIF4A
- eIF4E
- eIF4F
- eIF4G
- eIF2
- eIF2B

The 4 steps of Translation.....
1) 80s Ribosome becomes 40s & 60s subunits
2) mthionyl t-RNA binds to 40s....to make 43s pre-initiation complex
3) mRNA can now bind to this 43s Ribosome
4) this 43s now associates with the 60s to reform the 80s Ribosome

eIF2.....causes step 2 to occur
eIF2 is regulated by eIF2B
eIF4F.....causes step 3 to occur
eIF4F is a complex, comprised by several subunits.
eIF4F has in its complex, eIF4E
Energy production (ATP) processes cause eIF4E to be phophorylated (a phosphate is produced and placed on it)
eIF4E phosphorylation causes it to bind to mRNA [the first thing that must happen in step 3]
A translation repressor.....a "binding protein" called [4E-binding protein-1, (4E-BP1)], can attach to the spot on the eIF's to prevent mRNA attachement to the ribosome, thus preventing translation, thus "repressing" protein synthesis.

When production of phosphates is low, 4E-BP1 is un-phosphorylated, and can thus bind to eIF4E to prevent it from binding to mRNA

eIF4E bound to mRNA can now attach to the combination of eIF4G & eIF4A
This forms an active complex, which is called eIF4F [this completes step 3]
eIF4F active complex can now bind to the Ribosome to initiate Translation.

When glucose supply is low, production of phosphates will be low, thus phosphorylation of 4E-BP1 will be low, thus translation will be repressed. Thus one must supply glucose following workouts. When amino acid supply is low, especially essential amino acids [the branched chain amino acids...leucine, isoleucine, and valine] translation is low as well.

Protein
protein digestion
1. pepsin in stomach, cleaves bonds at tyrosine/phenylalanine, breaks proteins into polypeptides and free amino acids. Inactivated by pH in duodenum
2. trypsin, chymotrypsin, secreted by pancreas into small intestine. Breaks poly's into smaller poly's.
3. carboxypeptidase, secreted by pancreas, brush border enzyme, breaks off one amino acid at a time from poly's. Breaks at carboxl group (starts at end). aminopeptidase (starts at amine end) dipeptidase
Essential Amino Acids -- isoleucine, histidine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine.

Nucleic acids
DNA,RNA
Nucleic acid digestion
1. broken by pancreatic nucleases into nucleotides
2. brush border enzymes (nucleosides, phosphatases) break into pentose sugars and phosphate ions.

RNA Polymerase

-- for humans Transcription is performed by RNA polymerases
-- there are 3…….RNA polymerases
-- RNA polymerase I
-- RNA polymerase II
-- RNA polymerase III
-- they synthesize…….
--ribosomal RNA,.
-- messenger RNA
-- transfer RNA
-- Eukaryotic RNA polymerases are large macromolecular complexes composed of multiple subunits.
-- Among these subunits, five are shared by all RNA polymerases and are essential for cell growth and viability.
-- RNA polymerases (RNAPs) are composed of two large subunits and a number of small polypeptides,

-- RNA pol I is responsible for rRNA synthesis….located in nucleolus
-- RNA pol II synthesizes the mRNAs and some of the small nuclear RNAs (snRNAs) involved in RNA splicing. [splicing is a process to remove introns]…located in nucleo-plasm
-- RNA pol III synthesizes the tRNAs, the 5S rRNA and some snRNAs…..located in nucleo-plasm
-- RNA polymerase proceeds at a rate much slower than DNA polymerase (approximately 50-100 bases/sec for RNA versus near 1000 bases/sec for DNA)

Promoters & Enhancers
Signals are present within the DNA template that act in cis to stimulate the initiation of transcription. These sequence elements are termed promoters. Promoter sequences promote the ability of RNA polymerases to recognize the nucleotide at which initiation begins. Additional sequence elements are present within genes that act in cis to enhance polymerase activity even further. These sequence elements are termed enhancers. Transcriptional promoter and enhancer elements are important sequences used in the control of gene expression.
-- The promoter region contains important sequences that are required for RNA polymerase to bind

---

Nucleotides
--- proteins = bunch of polypeptides
--- polypeptides = bunch of peptides
--- peptides = bunch of amino acids
--- amino acids = bunch of nucleotides
--- nucleic acids = DNA, RNA = bunch of nucleotides
--- nucleotides = nucleosides with phosphate attached
adenosine-5 monophosphate [AMP]
guanosine-5 monophosphate [GMP]
cytidine-5 monophosphate [CMP]
uridine-5 monophosphate [UMP]
--- nucleosides = nucleobases + something
adenosine, guanosine, cytidine, uridine, thymidine
--- nucleobases = adenine, guanine, cytosine, uracil, thymine

--- some of ingested RNA and dietary nucleotides reach the blood stream and are transported to tissues of the body.
--- some of dietary RNA and dietary nucleotides are incorporatted into nuclei acids.
Especially small intestine, liver, muscle
Amount increases during times of stress, trauma, rapid growth, low food supply
--- RNA & DNA are digested in the small intestine, to nucleotides
via action of the pancreatic enzyme ribonuclease, deoxy-ribonuclease producing;
adenosine-5 monophosphate [AMP]
guanosine-5 monophosphate [GMP]

cytidine-5 monophosphate [CMP]
uridine-5 monophosphate [UMP]
thymidine-5 monophosphate [TMP]

--- These nucleotides are then hydrolyzed to the nucleosides
adenosine
guanosine
cytidine
uridine
thymidine

--- These nucleosides may be further hydrolyzed to;
purine bases = adenine, guanine.....then to uric acid
pyrimidine bases = cytosine, uracil, thymine.....then to beta alanine
--- the nucleosides are transported in the enterocytes by both facilited diffusion and sodium-dependent carrier mediated process
--- nucleosides and bases that are not catabolized in the enterocytes are transported via the portal circulation to the liver, where they are also catabolized

Micro-RNA [miRNAs]
--- micro-RNA = small RNA's 22 nucleotides long [21- to 23] non-coding RNA
--- they target mRNA
--- they down-regulate mRNA, thus regulating gene expression
- act as regulators of gene expression along a cellular process known as RNA silencing
- recognition and translational control of specific messenger RNA
- miRNAs are incorporated into miRNA-containing ribonucleoprotein effector complexes to regulate mRNA translation through the recognition of specific binding sites located mainly in the 3′ untranslated region
- regulate up to 90% of genes in human

Important nuclear transcription factors…..
--- Early Growth Response Gene-1 [Egr-1]
--- Specificity Protein-1 [Sp-1]
--- Serum Response Factor [SRF]
--- Mitochondrial Transcription Factor-A [Tfam]

IGF Binding Proteins [IGFBP's]
- IGF's bound to an IGFBP cannot bind to receptors
- there are 6 binding proteins
- greater than 90% of IGF-1 is bound to a IGF binding protein
- IGF-1 is mainly bound to IGFBP-3

Neuronal Transcription Process

- activation stimulus = electro-physiologic……&….second messenger
- the genes that are activated
    - -- neurotransmitter receptors
    - -- neurotransmitter
    - -- ion channels
    - -- cytoskeletal structures
- Transcription factor proteins contain a leucine zipper which…….
    - -- determines their affinity for DNA binding sites
    - -- modulates phosphoralation
    - -- interferes with it's DNA binding by squelching
- 2 categories of transcription factors
    - -- general transcription factors
        - …..bind [w/RNA polymerase II] to the DNA binding site where transcription begins [TATA box] and bridge to the promoter/enhancer transcription factors.
    - -- promoter/enhancer transcription factors

…..are bound to specific sites to the DNA either close or distant to the TATA box.
--- the promoter region is indispensible for transcription initiation
--- enhancers modify promoter activity
- H-reflex = excitability of spinal motornuerons [and presynaptic inhibition]
- V-wave = magnitude of efferent output from alpha motorneuron in descending pathways

---

Translation Processes

Translation initiation factors…….attach the copy of the gene to the ribosome
- eIF's attach mRNA to the ribosome, so that translation of mRNA code into protein, can take place
- 4 steps of translation
Step 1) 80s ribosome becomes 40s & 60s subunits
Step 2) methionyl tRNA binds to 40s…..to make 43s pre-intitiation complex
Step 3) mRNA now binds to this 43s ribosome
- ATP is used to phosphorylate eIF4E, which causes it to bind to mRNA
- eIF4E bound to mRNA can now attach to the combination of eIF4G & eIF4A….this forms the active complex called eIF4F
- eIF4F active comlex now binds to the ribosome
Step 4) this 43s ribosome now associates with the 60s ribosome to form the 80s ribosome

Translation Initation Factors

Eukaryotic Intiation Factors
- eIF4A
- eIF4E
- eIF4F
- eIF4G
- eIF2
- eIF2B

Translation Repressors

- binding protein called 4E binding protein (4E-BP1)
- can attach to the spot on eIF's to prevent mRNA attachment to the ribosome, thus preventing translation
- when production of ATP is low, 4E-BP1 will be unphosphorylated, and can thus bind to eIF4E to prevent its mRNA binding

Translation

- food deprivation is associated with inhibition of mRNA translation
- when glucose supply or essential amino acid supply is low, production of ATP will be low, 4E-BP1 will be unphosphorylated
- one must supply glucose post workout
- one must supply essential amino acids (especially branched chain amino acids….isoleucine, leucine, valine) post workout
- food deprivation is associated with inhibition of mRNA translation
- alterations in translation initiation are associated with changes in the phosphorylation state of eIF4E
- combination carbo & protein meal, but not carbo only meal, increases protein synthesis
- post exercise meal composistion influences recovery via modulation of translation initiation
- zero protein meals fail to stimulate protein synthesis
- leucine stimulates phosphorylation of 4E-BP1
- glucocorticoids cause de-phosphorylation of 4E-BP1
- translation may involve activation of initiation factor eIF2B, which is required for all initiation events

Elongation Factors

- eEF1A

Effects of Insulin on Translation

- insulin induces phosphorylation of the 4E-BP's

Effects of IGF-1 on Translation

- IGF-1 increases amount of eIF4E bound to eIF4G, forming the active complex eIF4F which binds mRNA to the ribosome

- leucine may sensitize the signaling pathway for IGF-1 induced phosphorylation of 4E-BP1

Effects of Alcohol on Translation

- chronic alcohol reduces translational efficiency
  ---- alcohol decreased eIF2B activity
  ---- increases binding of 4E-BP1
  ---- decreases phosphorylated 4E-BP1

Effects of Glucocorticoids (cortisol,etc) on Translation

- causes dephosphorylation of 4E-BP1
- inadequate protein intake causes cortisol to downregulate transcription/translation

---

Protein Synthesis

Time Courses
- Muscle protein synthesis can occur within 12 - 17 hours post exercise.
- New proteins can be made at rate of 1 million per minute.
- Muscle protein synthesis following strength training;
  - 4 hours = 50% elevation
  - 24 hours = 109% elevation
  - 36 hours = back to normal.
- Growth hormone systhesis of IGF-1 = 3 - 9 hours
- Blood pH recovery = 30 minutes (6min bouts at 85-95% Vo2max)
- glut 4 (50% decrease) in 3 - 4 days detraining

Growth factors that initiate satellite cell proliferation are;
- fibroblast growth factor
- macrophage derived growth factor
- platlet derived growth factor
- IGF-1.

Things that inhibit growth are;
TNFB
interferon.
- Prostaglandins E2 and E2a are related to regulation of protein synthesis.
- Resistance training increases basal testosterone levels, with no effect on acute levels.
- Resistance training no effect on basal growth hormone levels, does have acute increases.
- Insulin affects ribosome tRNA, mRNA, and protein synthesis enzyme availability.
- Chronic exercise increases (insulin binding) in muscle. Insulin sensitivity of muscle decreases w/detraining.
- Muscle contraction can be a stimulus for increased amino acid transport into muscle, thus increased translation.
- may be unnecessary to include non-essential amino acids in a protein supplement
- providing essential amino acids after exercise decreases net protein degredation and increases net protein synthesis
  - 40 grams of non-essential mixed with essential amino acids has a large effect
  - 40 grams of essential amino acids has a larger effect than 40 grams of mixed
    - can use 40 grams across 3 hours……13grams per hour is sufficient
  - intracellular amino acid levels increase with amino acid ingestion
    - increased availability of amino acids is the primary mechanism of increase protein synthesis
    - mixed and essential only are both **far** better than non-essential amino acid supplements
- supply of carbohydrates post-exercise does not affect mRNA production, but does affect the level of protein synthesis

---

Tissue Producing Cells

Fibroblast Cells
Produce NGF, and BNDF
- collagen synthesis rate ~ .07% per hour.....is similar to muscle

Osteoblasts
- take up vitamin C in large amounts to use as ground substance to lay down collagen matrix for new bone formation
- unloading causes and increase in glucocorticoid receptor density in osteoblasts and decreases IGF binding proteins in osteoblasts
estrogen increases production of the cytokine TGF-beta by osteoblasts
collagen + calcium = bone
corticosteroids cause avascular necrosis of bone along joint surfaces
Phosonex, a calcium derivative, reverses peridontal disease

Osteoclasts
estrogen promotes apoptosis of osteoclasts (mature and precursors).....limits osteoclast life span.
Dissolves old/weak bone

Chondrocytes
- consists of 90=95% chondrocytes embedded in a matrix of collagen
- chondrocytes do not migrate well into injured areas, due to being embedded in the collagen matrix
-- produce collagen, proteoglycans, noncollagenous proteins

Collagenases
- are matrix metalloproteinases (MMP'S)
- 3 collagenases.....collagenase 1, 2, and 3
- expressed in chondrocytes and osteoblasts
- collagenase is activated by other proteases

Gelatinase
- a matrix metalloproteinase (MMP'S)
- gelatinase A = MMP-2
- gelatinase B = MMP-9
- complements collagenase activity

Tissue Inhibitors of metalloproteinases (TIMP's)
- expressed by osteoblasts
- TIMP-1, 2, and 3

Electro-magnetic fields & healing

increases mesenchymal-cell proliferation and synthetic function
stimulates healing of bone fractures

Ultrasound

- helps healing of fractures (an average of 96 days as compared to 154 days treated by conventional methods)

Mitochondrial Transcription Factors
--- Nuclear Respiratory Factor 1
--- Nuclear Respiratory Factor 2
    -- binding sites on promoter regions of several mitochondrial genes in the nucleaus
    -- regulates Transcription Factor A [for replicaton and transcription of mitochondrial DNA]
--- Silent Information Regulator T1 [SIRT1]
    -- increases mitochondrial biogenesis
    -- activates PGC-1alpha, the master regulator

Mitochondria biogenesis
- Intracellular metabolic signals provoked by contractile activity are the most important in causing mitochondrial biogenesis
- Synthesis of mitochondrial proteins from genes located inside the mitochondria is mainly dependent on DNA replication not transcription, while synthesis of mitochondrial proteins from genes in the muscle fiber nucleus is dependent on mRNA number. In other words, making

more protein is dependent on making more genes in the one case, while making more copies of the gene is the focus in the other.
- termination of mitochondrial gene transcription can be hormonally regulated
- biogenesis begins with an increase in the quantity of phospholipids compising the membrane....expanding the membrane surface area
- this is followed by an increase in the quantity of mitochondrial proteins embedded in the inner and outer membranes as well as the matrix
- Thyroxine may have normal physiological function as an extracellular enhancer of biogenesis.
- thyroxine has been shown to increase transcription of cytochrome c.
- steroid and thyroid hormones act on nuclear gene transcription by activating protein receptors, which then bind to hormone response elements (HRE's).
- mitochondrial genes located in the nucleus of the muscle fiber have been shown to be responsive to thyroid hormone

- mitochondria DNA transcription factors
    --- mitochondrial transcription factor H
    --- this may be the only one
- Nuclear DNA mitochondrial trascription factors
    --- nuclear respiratory factors [NRF-1, NRF-2]
    --- c-jun
    --- c-fos
    --- sp1
    --- mitochondrial transcription factor A [Tfam]
- mitochondrial transcription factor A [Tfam] is imported into mitochondria and controls the expression of mitochondrial DNA
- p43 binds thyroid hormone and DNA sequences
- AMP kinase acts as a energy gauge….when ATP is low, AMPK increases
- the higher the exercise intensity, the higher the AMPK activation
- two forms of AMPK = alpha-1, alpha-2
    --- build-up of AMP
    --- activates AMA Activated Protein Kinase [AMPK]
    --- AMPK causes phosphorylation of transcription factors, which causes Glut4 gene transcription
- AMPK is activated by phosphorylation …..phosphorylized by AMPKK…which is also activated by AMP
- AMPK activates transcription of some mitochondrial genes as well
- AMP kinase actitivity is exercise intensity dependent
- AMPK alpha-1 & 2 are activated by sprint exercise
- AMPK alpha-1……not activated by lower intensity exercise [50-90% Vo2max]
- AMPK alpha-2……is activated exercise [75-90% Vo2max]
- AMPK alpha-2……not activated exercise [50% Vo2max]
-- PPARgamma……peroxisome proliferator-activated receptor gamma……..PGC-1alpha…… peroxisome proliferator-activated receptor gamma coactivator-1 alpha……..mediator of mitochondrial biogenesis
-- production may be stimulated by calcium release during muscle contraction
-- PGC-1alpha activates PPARgamma
-- it activates NRF-1
-- it increased mtDNA copy number
-- its activated by T3 [thyroid hormone]
- genes for mitochondria structural proteins and enzymes are located in the nucleus of the muscle fiber (nuclear DNA)and in the mitochondria themselves (mitochondrial DNA).
- mitochondrial DNA only encodes 13 of the ~100 proteins required to assemble a mitochondria…..mtDNA = 16,659 nucleotides
- All mitochondria posses a circular double stranded DNA molecule inside them that codes for 13 mitochondrial respiratory complexes (enzymes, etc.) found in the inner membrane.
- mitochondrial DNA codes for a RNA and 22 transfer RNA's. This protein synthesis machinery is used to translate the mRNA's transcribed within the mitochondria.
- nuclear DNA includes the codes for all enzymes of the krebs cycle, enzymes for the Beta oxidation of fat, most of the respiratory complexes, and ATP/ADP translocators.
- Glucocorticoid receptors rapidly translocate from the cytplasm into the mitochondria
- mitochondrial DNA is inherited from the maternal oocyte
- mitochondrial DNA codes for;
    - seven subunits of NADH dehydrogenase.....these transfer electrons from NADH to ubiquinone
        MTND1, MTND2, MTND3, MTND4, MTND4L, MTND5, MTND6
    - subunits 6 and 8 of ATPase synthase.....synthesizes ATP from ADP and P in the mitochondrial matrix
    - subunit of cytochrome bc complex.....transfers electrons from ubiquinone to cytochrome c
    - subunits 1,2, and 3 of cytochrome c oxidase
    - 2 ribosome RNA's
    - 22 transfer RNA's

release of calcium via contractile activity…….
--- cytoplasmic calcium regulates mRNA level of nuclear mitochondria genes
--- cytoplasmic calcium regulates mRNA level of genes in mitochondria
--- AMP-activated protein kinase [AMPK] also increases mitochondrial biogenesis
--- PGC-1alpha.....peroxisome proliferator-activated gamma receptor coactivator
    - transcriptional coactivator.....master regulator of mitochondrial biogenesis
    - p53 is a modulator [potential regulator] of mitochondrial biogenesis
    - activated by protein kinase 4
    - activated by calcineurin A
    - high fatty acid content in muscle cause PGC-1alpha down-regulation of mitochondrial biogenesis via methylation of the PGC-1alpha promoter
--- quercetin....a flavonoid, increases SIRT1 and PGC-1alpha, mitochondrial DNA

Angiogenesis…Blood Flow & Angiogenesis Inhibitors,
- Angiogenesis is the culmunation of…
    - dissolution of the extracellular matrix underlying the endothelium of the blood vessel
    - cell migration
    - endothelial cell proliferation
- Growth factors and cytokines can induce and/or promote formation of new vessels by stimulating endothelial cell growth and migration.
- Growth factors include
    - vascular endothelial cell growth factor (VEGF)
    - basic fibroblast growth factor (bFGF)
    - transforming growth factor- beta-1 (TGF-B1)
    - several cytokines
- bFGF is a potent angiogenesis stimulator.....exercise + exogenous bFGF has additive effects
- immobilization prevents exercise induced production of bFGF
- exercise induces and increase in expression of genes encoding angiogenic growth factors (ie. VEGF)
    - the response is quantitatively related to **exercise intensity** and augmented by hypoxia
- Blood flow to active muscle during exercise increases by 30 times. Extraction of oxygen increases by 3 times.
- capillary basement membranes get thicker with sedentary behavior.....exercise training decreases thickness...even in older people
- Cox-2 catalyses formation of angiogenic prostaglandins which can stimulate angiogenesis
- Cox-2 inhibitors can inhibit tumor initiation, promotion, and progression

skin of grapes contains reseratrol....works as a Cox-2 inhibitor

Retenoids are Cox-2 inhibitors (cis-13-retenoic acid, retinol, isotretinoin
- angiostatin is an inhibitor of angiogenesis...and thus is an inhibitor of tumor growth
- in tumors, as you block angiogenesis, you get a 3 - 5 fold increase in apoptosis
- altered arterial oxygen pressure regulates blood flow to active muscles due in part to the release of endothelium-derived dilating and constricting factors
- VEGF mRNA production is activated by nitric oxide
- NSAID's inhibit angiogenesis by inhibition of COX-2 in endothelial cells

Endothelin -1 (ET-1)
-- 21-amino-acid peptide….long-lasting vasoconstrictor
-- can generate a sustained reduction in blood flow
-- can promote generation of superoxide radicals
-- evidence that links ET-1 to oxidative stress is via its ability to reduce blood flow
-- endothelial receptor antagonist, effectively blocks the generation of superoxide anions
-- ET-1generates oxidative stress in the brain through a reduction in blood flow.

Muscle
- increasing muscle temp may increase muscle isoform of IGF-1
- muscle IGF-1 stimulates proliferation and differentiation of satellite cells

embryonic development.....
- uterus temp may affect embryonic development
- myogenesis affected by embryo temp
- increased embryo temp increases myogenesis
- increases mygenic progeny and IGF-1

Myogenesis.....
- controlled by MyoD family of transcription factors
    -- MyoD
    -- mygenic factor-5
    -- myogenin
    -- myogenic regulatory factor-4
- they work with myocyte enhancer factor-2 proteins

perilipin proteins
-- muscle perilipin proteins proteins.....hydrolysis of triglycerides stored in lipid droplets and the passage of fatty acids to the mitochondria
-- in adipocytes, perilipin protein-1 regulates lipolysis by interacting with comparative gene identification-58, an activator of adipose

triglyceride lipase.
-- upon lipolytic stimulation, perilipin protein-1 is phosphorylated, releasing omparative gene identification-58 to activate adipose triglyceride lipase and initiate triglyceride breakdown.
-- perilipin family proteins expressed in skeletal muscle
-- PLIN2, PLIN3, and PLIN5

Muscle atrophy
-- mRNA from muscle protein degradation genes is reduced
-- protein degradation regulated by.....
- ubiquitin ligase enzymes
- F-box [MAFbx]
- muscle specific RING finger-1 [MuRF-1]

Myostatin....inhibitor of muscle mass

alpha-actins
--actin binding proteins
- alpha-actin-2
- alpha-actin-3…at sarcomere Z-line in fast twitch muscles
- absent in 20% of Europeans

Myogenic Regulatory Factors
-- DNA binding proteins that regulate transcription of muscle specific proteins
-- transcription factors
- MyoD
- MRF-4
- myogenin
- Myf-5

- myoD and myogenin are muscle-type specific [myoD specific to fast twitch, myogenin specific to slow]

Myostatin [Growth Differentiating Factor-8….GDF-8]
- member of Transforming Growth Factor beta [TGF-beta] family
- negatively regulates muscle growth
- inhibits proliferation of satellite cells and myoblasts
- glucocorticoids increase GDF-8 production
- inactivity increases GDF-8 production
- strength training increases its production but that is compensated for by other substances

---

Transforming growth factor beta TGF-beta
- during exercise, type 1 increases in cerebral spinal fluid, influences peripheral fat oxidation

11β-hydroxysteroid dehydrogenase (11β-HSD) type 1
-- generates biologically active cortisol from inactive cortisone
-- expressed also in muscle
-- intense physical exercise increases systemic 11β-HSD type 1 activity

Relaxin
-- hormone structurally related to insulin and insulin-like growth factor
-- regulatory effect on the musculoskeletal and other systems through binding to its receptor in various tissues
-- alters properties of cartilage and tendon by activating collagenase
-- involved in bone remodeling and healing of injured ligaments and skeletal muscle

Kartogenin
- stimulated endogenous progenitor cells [mesenchymal stem cells] to become chondrocytes
- no need to inject stem cells into the joint
- increases inhibitors of matrix metalloproteinase
- reduced levels of pro-inflammatory substances

dystrophin
-- expressed in neurons within brain regions including the hippocampus
-- subset of boys exhibit cognitive dysfunction [deficits in memory]

Gene Therapy

- “gene gun” injection of plasmid DNA may be adequate. Using electro-static force or gas pressure, the gun blasts minute metal particles coated with DNA into tissues. Some of the DNA is trapped and expressed in a few cells.
- the efficiencies of non-viral delivery systems are now adequate for the treatment of many diseases.
- safety....use of non-viral vectors.....because there is still some concern that viral vectors may recombine with endogenous viruses or that germ-line cells may be inadvertently modified.

Training

Calcium may be the intracellular signal for gene transcription…….magnitude of calcium increase, frequency, duration, cellular location….all may affect transcription. Different sets of genes can be transcriptionally regulated through distinct patterns of intracellular calcium dynamics

---

DNA Repair

- approx. 30 different gene products (from DNA repair genes) involved in nucleotide excision repair. Impairment of one of these could result in an impairment of the DNA repair process.
- reduced repair capacity is the mechanism of problems.
- DNA repair enzyme polyadenosine diphosphate ribose (riboflavin) polymerase
- DNA repair enzymes
  - ------ Ku70
  - ------ Ku80

Telomeres, Telomerase

- regular DNA polymerases cannot fully replicate the extreme termini (telemeres) of linear chromosomes.
- telomeres are at the ends of chromosomes and comprised of tandemly repeated G-rich sequences.
- the G-rich strand protrudes as a 3’ single strand extension.
- Telomerase is a DNA polymerase that elongates the G-rich strand of of the telomere
- Telomerase synthesizes telomeres using an RNA subunit template that dictates the sequence added onto the chromosome terminus.
- telomerase activity is detected in 85-90% of primary tumors
- telomerase negative tumors have long telomeres
- Some haemopoietic cells are telomerase competent.

Telomerase competent cells can regulate the level of telomerase activity. The presence of telomerase does not necessarily imply stable and unchanging telomere lengths. Functionally active telomerase will slow, but not prevent, telomere shortening.
The most distal telomeric nucleotides cannot be replicated. The most distal telomeric DNA shortens with each cell cycle. The specialised nature of telomeric DNA prevents chromosome ends from activating the cell cycle arrest.
Telomerase is a ribonucleoprotein that stabilizes telomeres by adding TTAGGG repeats to the ends of chromosomes. Telomerase enzyme enables the cell to maintain the integrity of its chromosomes each time it divides. Without it, the ends of the chromosomes, called telomeres, are whittled away until too much is lost for the cell to maintain normal function. At that point, it ceases to replicate. The gene that expresses the enzyme appear to be turned off sometime before or shortly after birth. In most cancer cells the gene appears to have been switched back on. The mechanism of DNA replication in human chromosomes is different for each of the two strands, called the leading and lagging strands. On the lagging strand, a gap occurs at the very end that cannot be filled in by enzymes that replicate the remainder of the DNA. Therefore, the telomeres become shorter after each cell division. The presence of telomerase in cancer cells prevents telomere shortening and allows the cells to divide indefinitely. Telomerase contains an RNA that it uses as a template to synthesize TTAGGG repeats directly onto telemere ends. This extension of the 3"-end in turn, permits additional replication of the 5"-end of the lagging strand, thus compensating for the telomere shortening that would occur in its absense.

- TA-65....compound extracted from the Astragalus plant
- TA-65 can turn on telomere gene and cause telomerase to be produced
- telemere length controlled by telemerase, htert, and shelterin
-- telomerase preferentially elongates short telomeres
-- Tel1p.....yeast ATM-like checkpoint kinase.....was highly enriched at short telomeres
-- Tel1p targets telomerase to the DNA ends most in need of extension
-- Tel1p binds preferentially to short telomeres and modifies one or more telomeric substrates to facilitate access of telomerase to DNA ends
-- Cdc13p is phosphorylated by Tel1p, and this phosphorylation is critical for its ability to promote telomerase-mediated telomere lengthening
-- telomeres are replicated by a specialized reverse transcriptase called telomerase
-- telomerase preferentially lengthens short telomeres
-- elimination of any telomerase subunit results in the progressive loss of telomeric DNA and eventual death of most cells
-- telomere binding proteins Rif1p and Rif2p.....associate with telomeres throughout the cell cycle via interaction with the duplex telomeric DNA binding protein Rap1p
-- Lack of either protein causes telomere lengthening, whereas deletion of both results in synergistic lengthening and this lengthening is telomerase dependent

-- the Rif proteins are negative regulators of telomerase
-- Rif2p, but not Rif1p also inhibits telomere addition
-- telomerase lengthens only a subset of telomeres with preferential elongation of --- less than 10% of long telomeres are lengthened
--- 50% of short telomeres are elongated
-- In the absence of the negative regulators Rif1p or Rif2p, the frequency of elongation for all telomeres increases about 2-fold
-- Two telomerase subunits, Est2p and Est1p, bind preferentially to the short telomere
-- When Tel1p was lost from short telomeres, preferential binding of Est1p and Est2p to short telomeres was lost

Shelterin

- protein that regulates telemere length
- shelterin complex = 6 proteins....
    - telemere related factors-1 [TRF1]
    - TRF2
    - TRF1- interacting protein 2 [TIN2]
    - Protection of Telemeres-1 [Pot--1]
    - Pot-1 and Tin2-organizing protein repressor/activator protein 1 [RAP1]
    - Tripeptidyl peptidase 1 [TPP1]

- increased production is induced by physiological stress [exercise]
