NUTRITIONAL GENOMICS (Nutrigenomics) "NTGE 2422"

Level II Human nutrition

Mutayomba Sylvestre

SUBJECT UNIT

1. Genetics variability
2. Nutrition and gene

GROUP ASSIGNMENTS

1. Preventive medicine and personalized diet
2. Metabolic syndrome
3. Genetics influence on cancer prevention.
4. Environmental influence on cancer prevention.

INTRODUCTION

- The **link between food and health** is a long and a well documented one.
- With over 24,000 people worldwide dying from hunger each day and **obesity** reaching epidemic proportions in developed counties, **the consequences of too little or too much food are easily seen**.
- People no longer view food as merely **a source of calories** but rather as a complex mixture of **dietary chemicals, some of which are capable of preventing, mitigating, or treating disease**.
- With the sequencing of the human genome, **a new genetic dimension h**as been added to the equation **linking the foods we eat to the good health we all hope to enjoy**.
- We bring **two things to the dinner table**: our appetite and our genotype.

- Genetic diversity makes **each of us uniquely different**, we are also beginning to understand **why** we respond to our nutritional environment differently and how **these differences can, over time, lead to health or disease**.
- Genomic analysis reveals that humans are **99.9% identical at the DNA level**.
- This implies that the remaining **0.1% of the human genome** (or about three million single nucleotide polymorphisms (SNPs)) **is responsible for** all the morphological, physiological, biochemical and molecular **differences between any two individuals.**
- Certain genotypes are more severely **affected by specific types of dietary factors** than other genotypes (although **no genotype is completely immune to the deleterious effects of poor diet**).
- Diet–gene interactions are strongly influenced by **epigenetic, environmental**, **socio-economic**, and **lifestyle** filters that modify or potentiate genetic effects.

- Nutrigenomics adhere to the following precepts:

(1) **Poor nutrition** can be a risk factor for diseases;

(2) Common dietary chemicals can act on the human genome, either **directly** or **indirectly**, to **alter gene expression** and/or **gene structure**;

(3) **The degree** to which diet influences the balance between health and disease **depends on an individual's genetic makeup**;

(4) Some diet-regulated genes (and their common variants) play a **role in the onset, incidence, progression, and/or severity of chronic diseases**, and

(5) Dietary intervention based on knowledge of **nutritional requirement**, **nutritional status**, and **genotype** can be used to prevent, mitigate, or cure chronic disease.

- Good nutrition has been, and will continue to be, the cornerstone of good health and disease **prevention—but good nutrition comes at a price**.

GENETIC VARIABILITY: INTRODUCTION TO GENETICS

THE GENETIC MATERIAL

Discoveries: The Chemical Nature of DNA

- 1869—Fredrich Miescher named the chemical nuclei contained **nuclein**. Other chemists discovered it was acidic and named it ***nucleic acid*.**

- It was soon realized that there were **two types of nucleic acids**: DNA and RNA.

- Early in the 20th century, **4 types of nucleotides** were discovered.

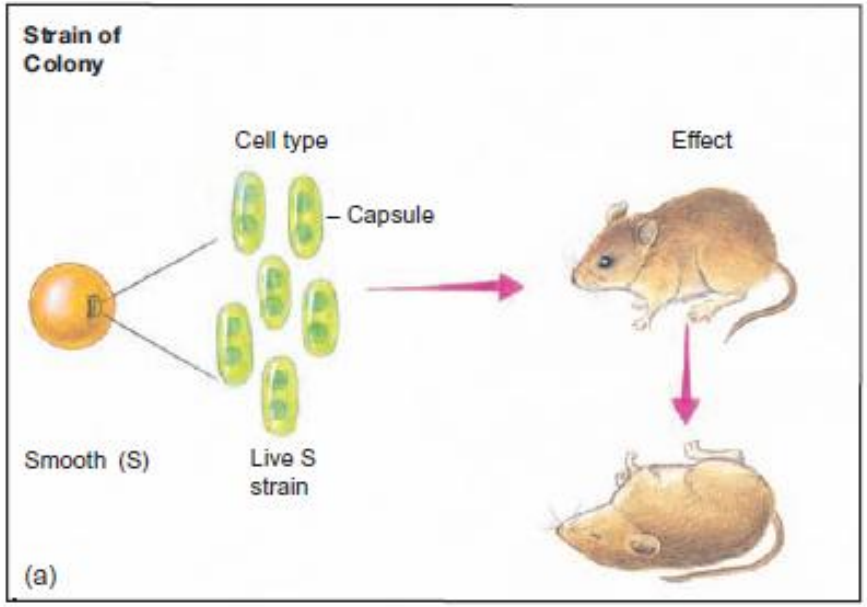
DNA proven as Genetic material

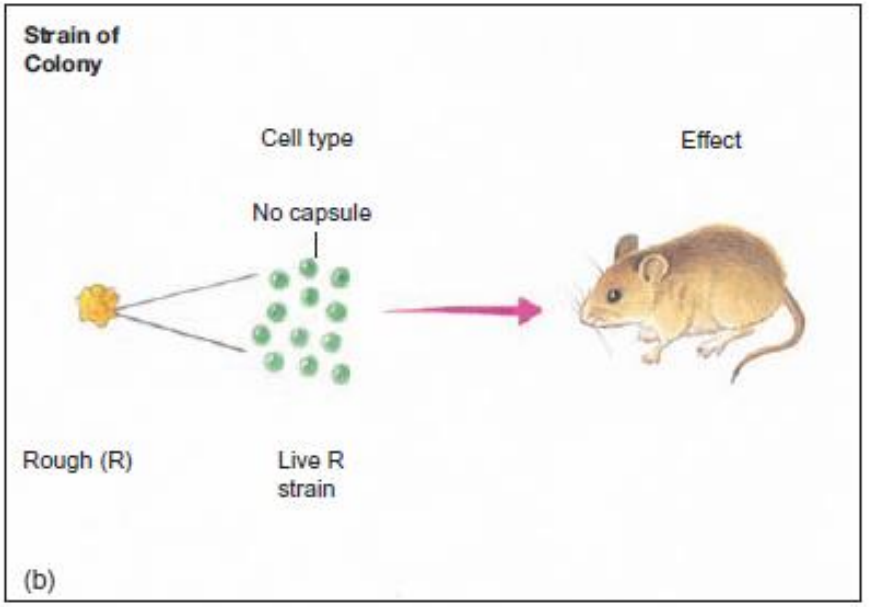
- Once Morgan hat genes are showed located on chromosomes, proteins and DNA were the candidates for the genetic material.

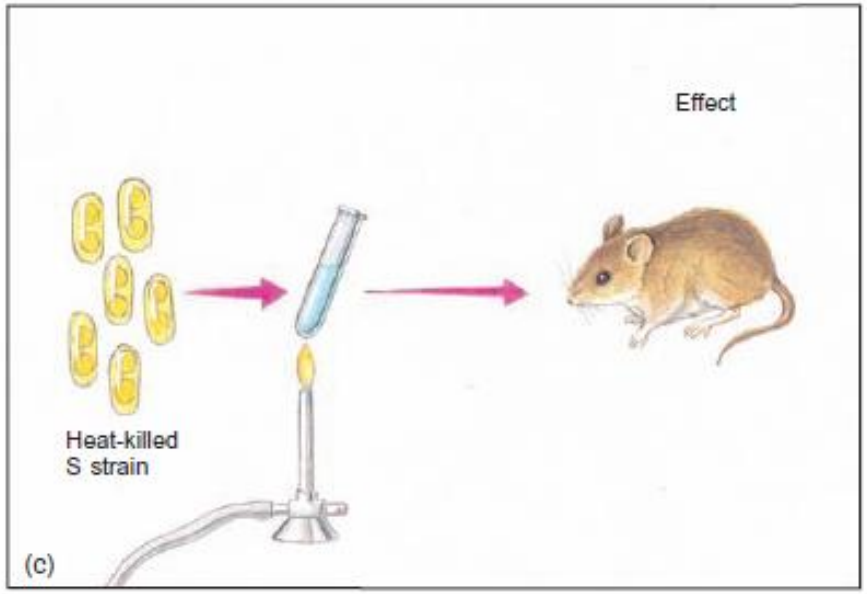
- Until the 1940s, the specificity of function of proteins seemed to indicate that they were the genetic material.

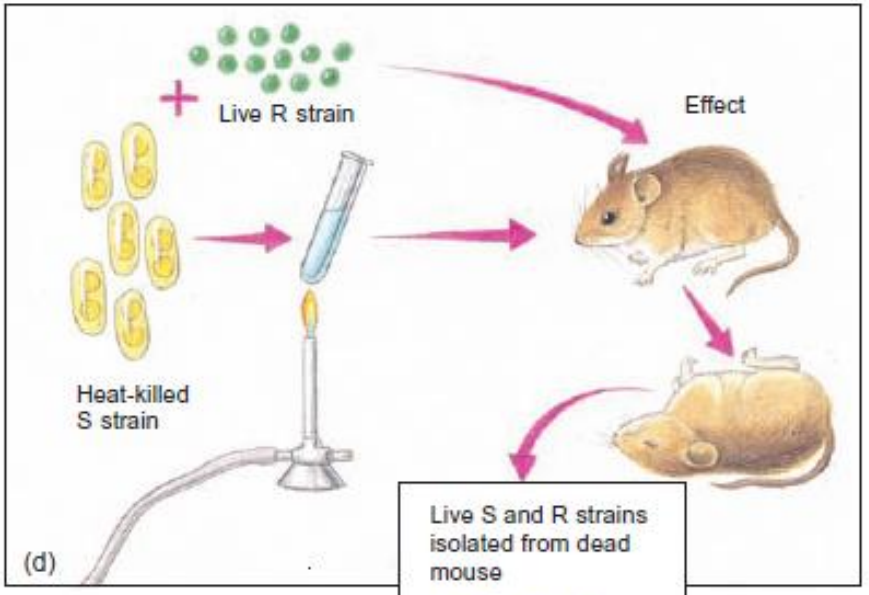
- However, this was **not consistent with** experiments with microorganisms, like **bacteria and viruses**.
- The Griffith Experiment, 1928
- Oswald Avery, Maclyn McCarty and Colin MacLeod (1944) announced that they found that only DNA transformed the cells.
- Additional evidence supporting Avery's conclusion was provided in 1952 by Alfred Hershey and Martha Chase.

The Griffith Experiment









Hereditary Information Can Pass between Organisms.

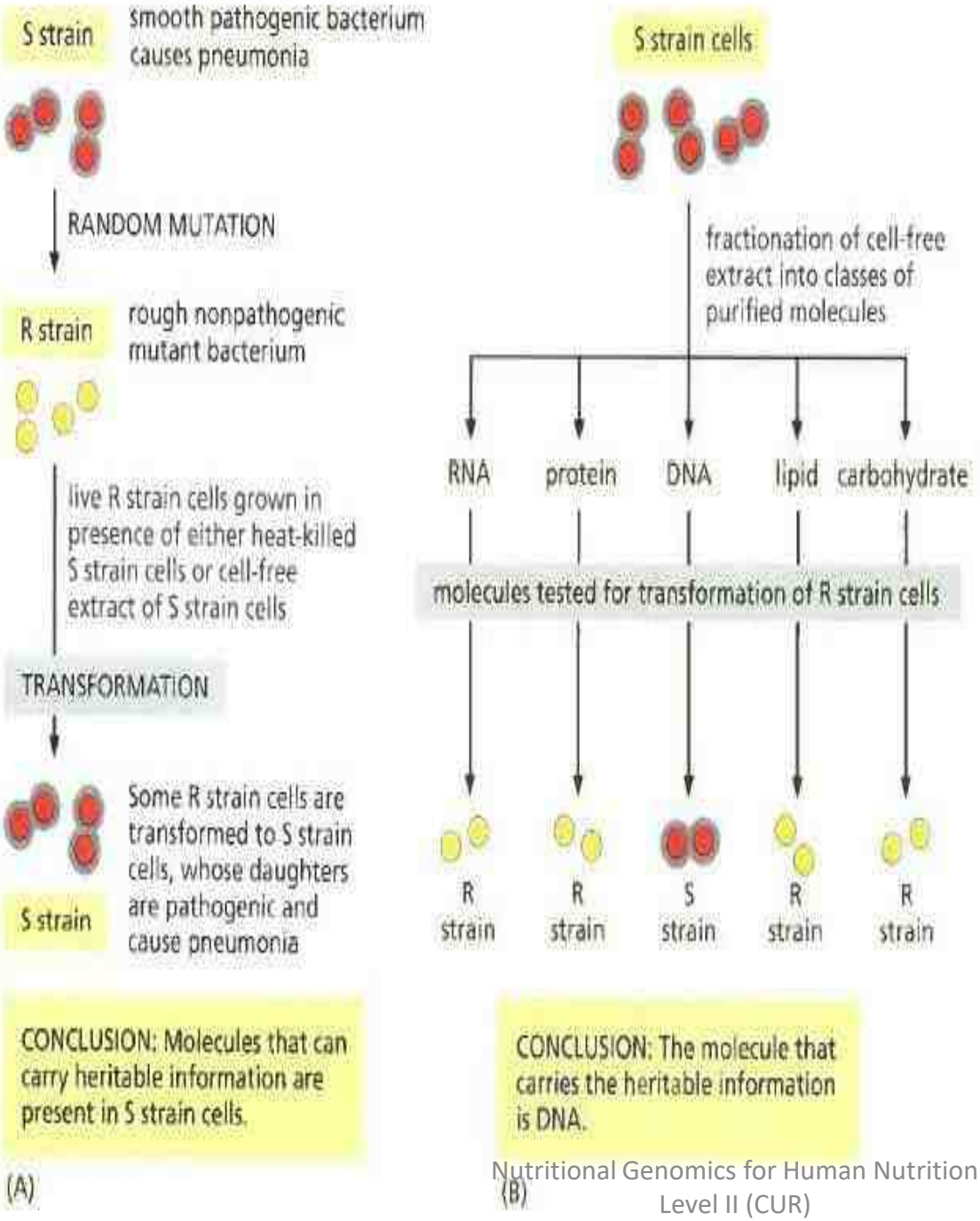
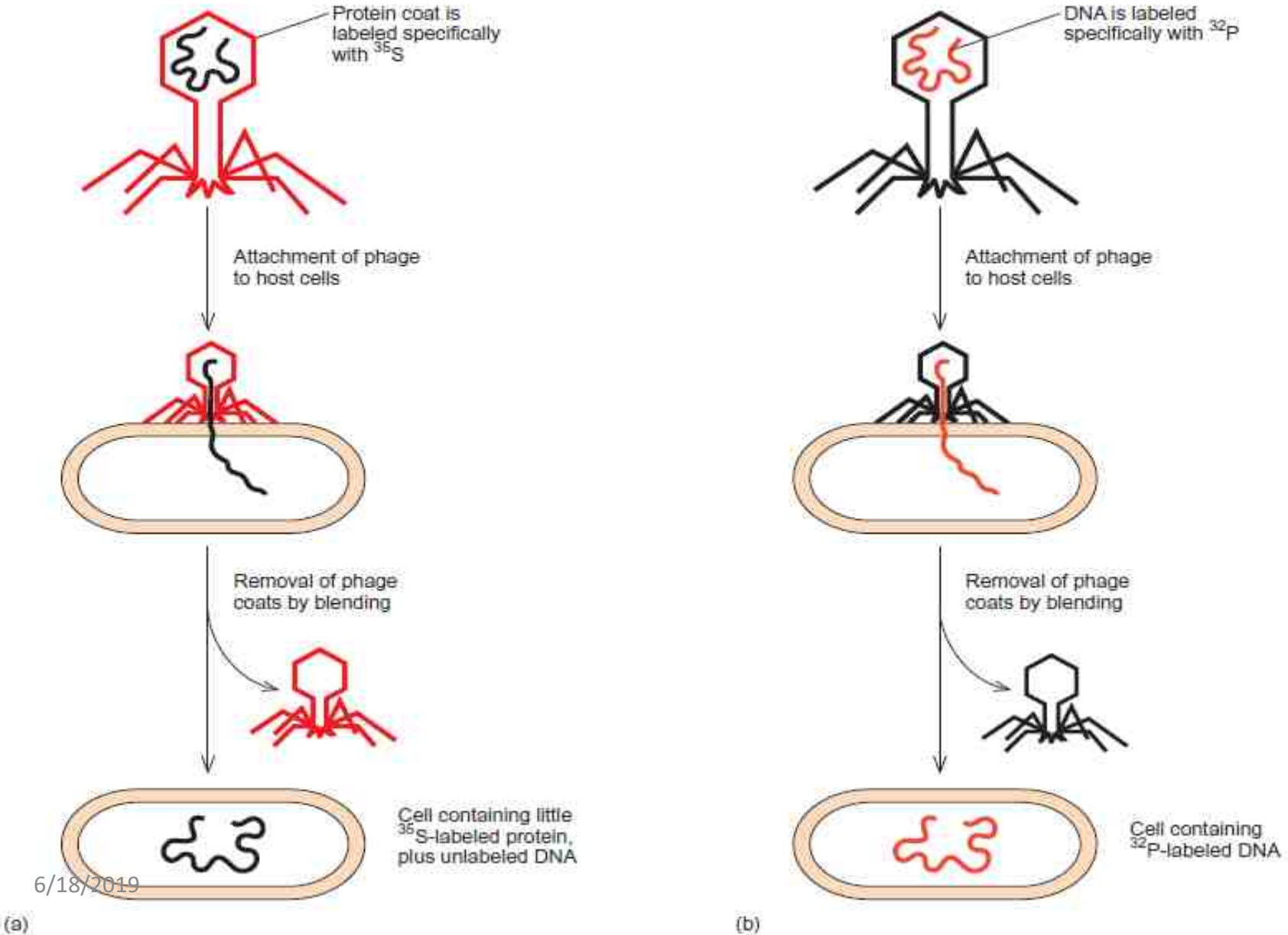


Figure 4-2 The first experimental demonstration that DNA is the genetic material. These experiments, carried out in the 1940s, showed that adding purified DNA to a bacterium changed its properties and that this change was faithfully passed on to subsequent generations. Two closely related strains of the bacterium *Streptococcus pneumoniae* differ from each other in both their appearance under the microscope and their pathogenicity. One strain appears smooth (S) and causes death when injected into mice, and the other appears rough (R) and is nonlethal. (A) An initial experiment shows that a substance present in the S strain can change (or transform) the R strain into the S strain and that this change is inherited by subsequent generations of bacteria. (B) This experiment, in which the R strain has been incubated with various classes of biological molecules purified from the S strain, identifies the substance as DNA.

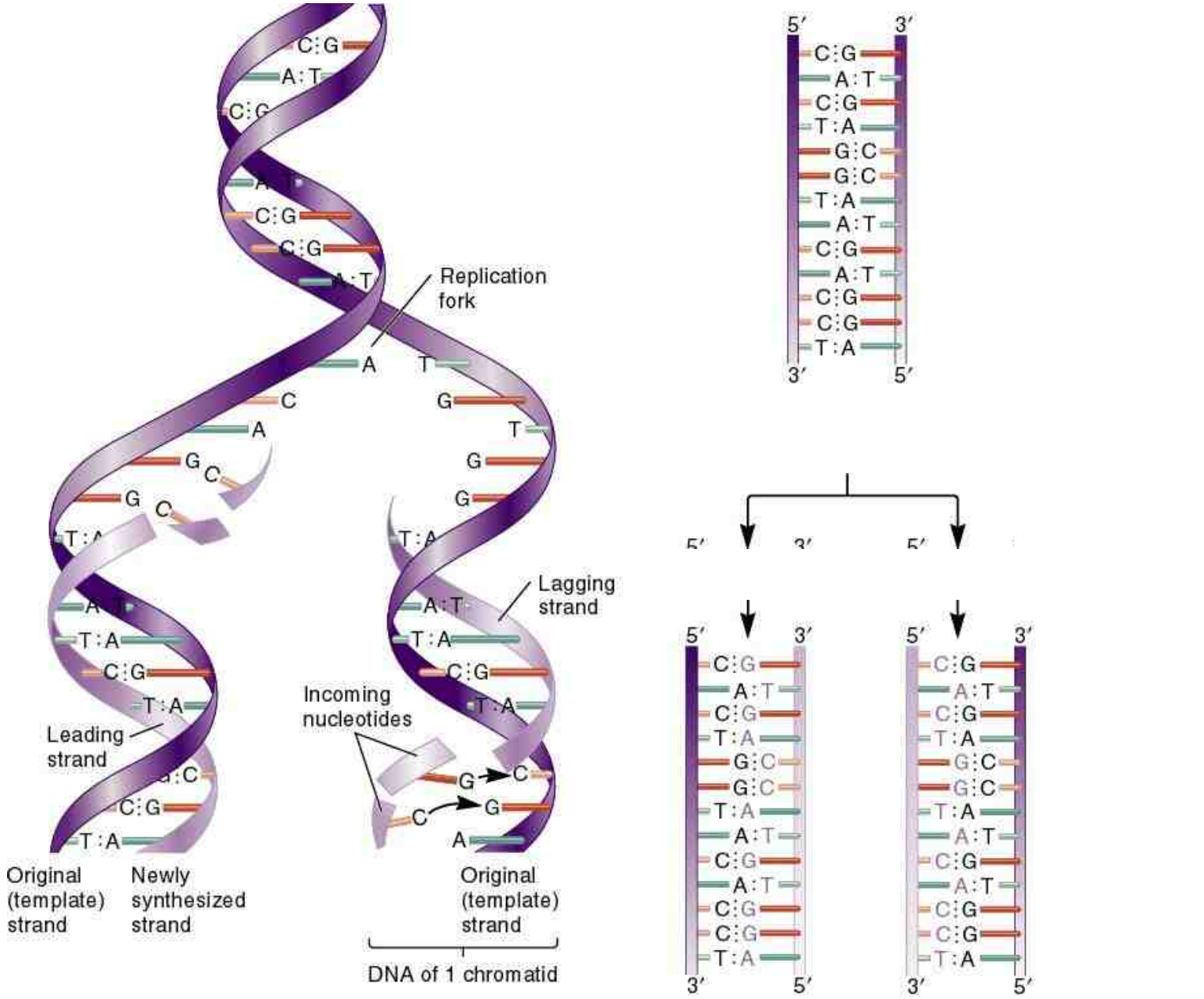
The Hershey —Chase experiment

The phage T2 is composed of DNA and protein only



DNA REPLICATION IN EUKARYOTES

- **Timings are highly regulated.**
- **Occurs during the S phase of the cell cycle, preceding mitosis or meiosis**
- **Begins at multiple sites along the DNA helix.**
- **Having multiple origins of replication provides a mechanism for rapidly replicating the great length of the eukaryotic DNA molecules.**

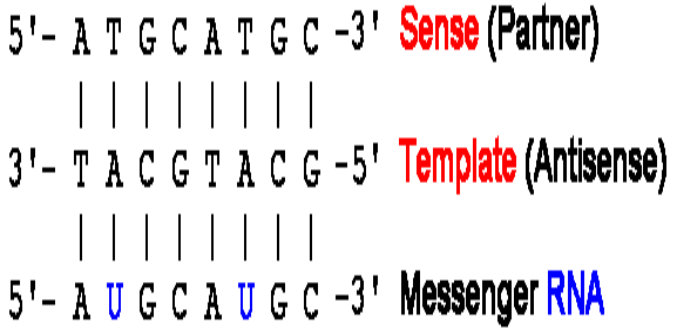


Identical base sequences

Sense and Antisense

- **A DNA sequence is called ‘sense’ if its sequence is the same as that of a mRNA copy that is translated into protein.**
- **The sequence on the opposite strand is complementary to the sense sequence and is called antisense sequence.**

– A **mRNA transcript** is base-complementary to the **template strand** of **DNA** & thus **co-linear** with the **sense strand** of **DNA.**



– **Colinear**: **mRNA** and **DNA** sense strand have “same” sequence. (Except substitute **U** for **T**)

– **Both sense and antisense sequences can exist on different parts of the same strand of DNA.**

INTRODUCTION TO GENETICS

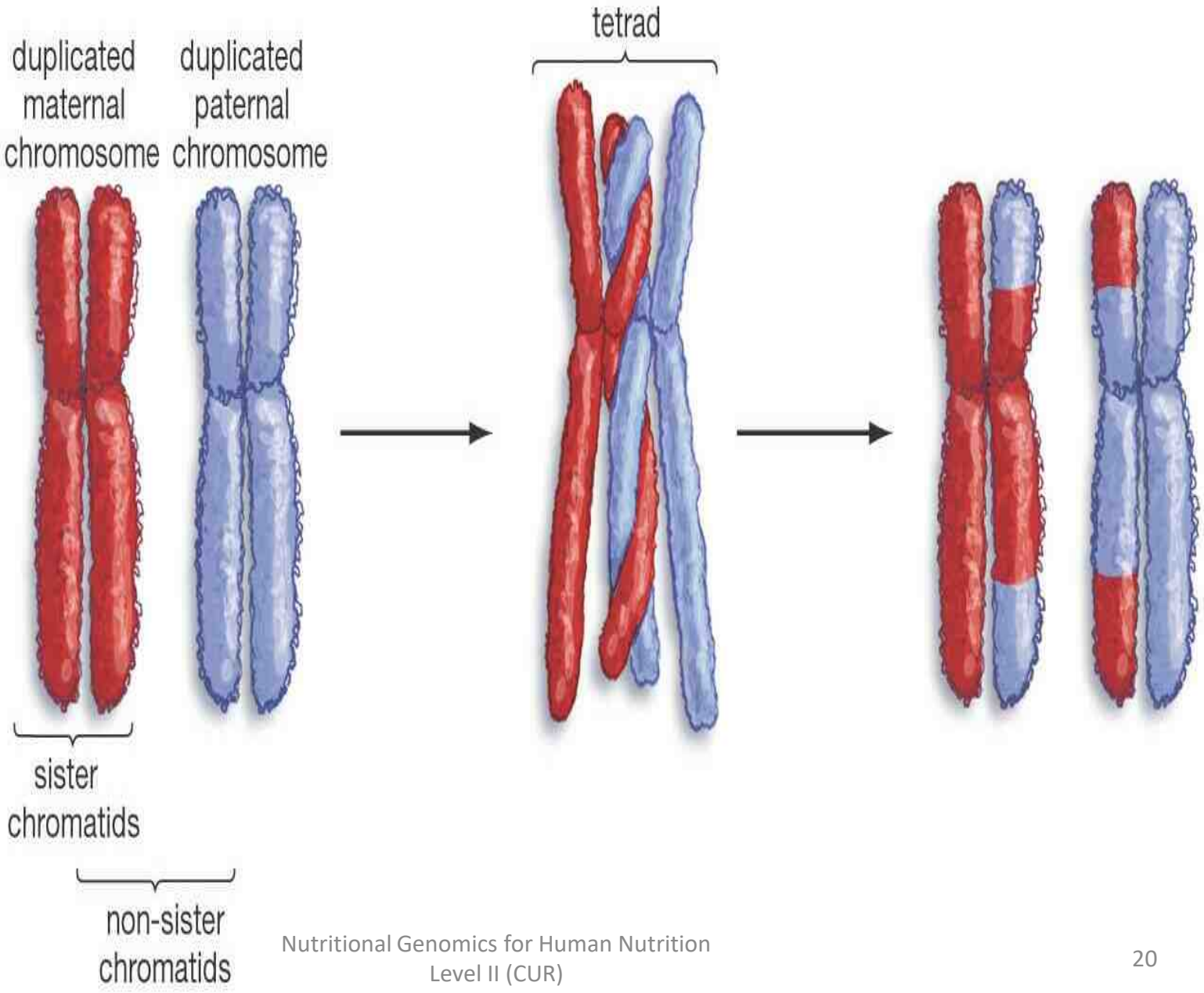
- The science of genetics is the study of **two contradictory aspects of nature**: Heredity and variation.
- The process of transmission of characters from one generation to next is called **inheritance or heredity**.
- Heredity is **the cause of similarities** between individuals.
- This is the reason that brothers and sisters with the same parents **resemble each other and with their parents**.

- **Variation** is the cause of differences between individuals.
- This is the reason that **brothers and sisters who do resemble each other are still unique individuals**.
- The science of genetics attempts to explain **the mechanisms and the basis for both similarities and differences between related individuals.**

Chromosomes

- The **genetic information** lies within the cell nucleus of each living cell in the body.
- The genes lie **within the chromosomes**.
- Humans have 23 pairs of these small thread-like structures in the nucleus of their cells.
- 23 or half of the total 46 comes **from the mother** while the other 23 comes **from the father**

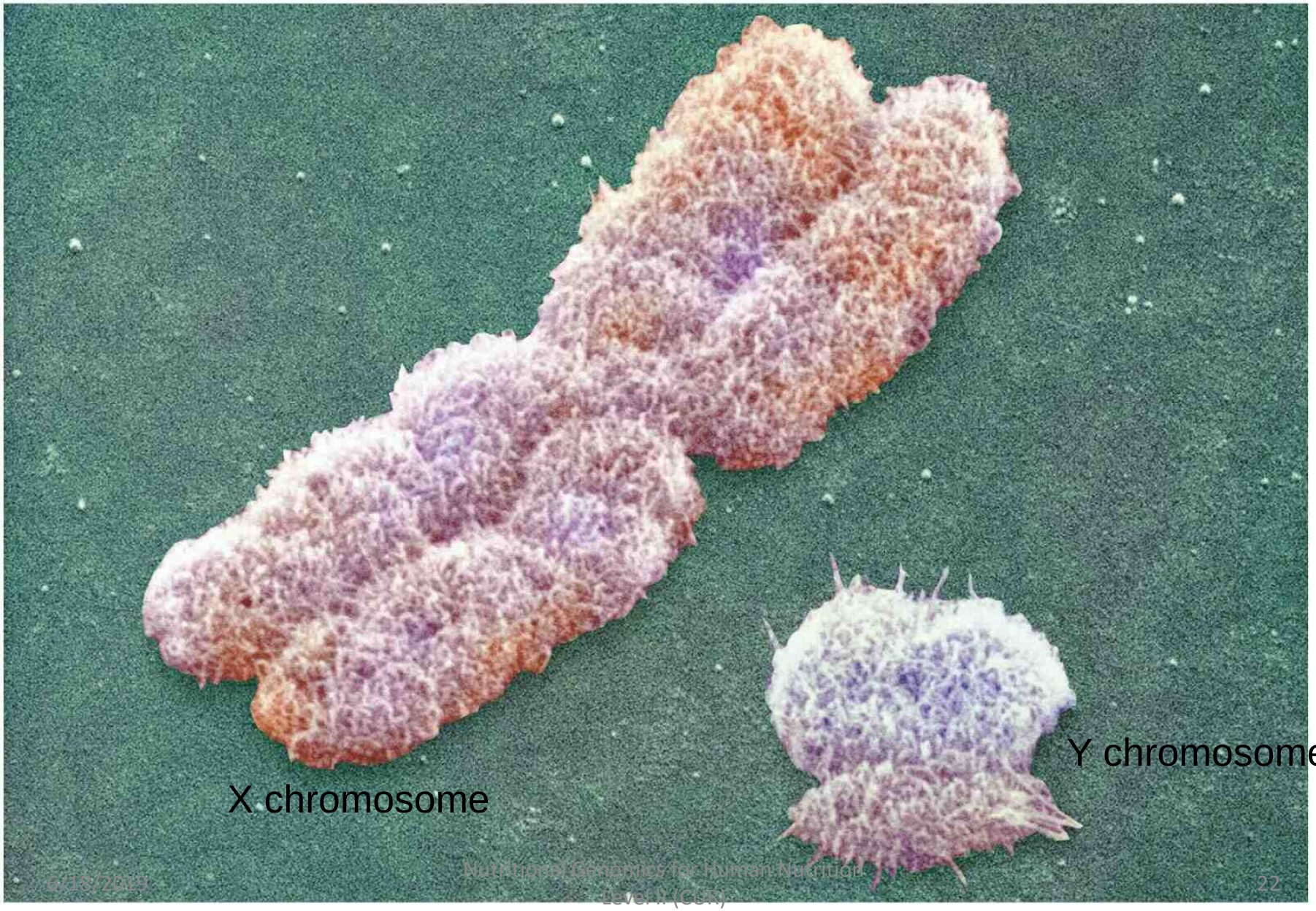
Exchange of parts of non-sister chromatids.



Males and females

- Women have 46 chromosomes (44 autosomes **plus two copies of the X** chromosome) in their body cells. They have half of this or 22 autosomes plus **an X chromosome in their egg cells**.
- Men have 46 chromosomes (44 autosomes **plus an X and a Y** chromosome) in their body cells and have half of these 22 autosomes **plus an X or Y** chromosome in their sperm cells.
- When the egg joins with the sperm, the resultant baby has 46 chromosomes (with either an XX in a female baby or XY in a male baby).

Boy or Girl? The Y Chromosome "Decides"



Reproduction

- Reproduction is **the most important function** of living organisms from bacteria to mammals, **providing the conservation of species in a number of generations**.
- Forms of reproduction of organisms are very diverse and complex.
- In nature, **there are two basic methods of reproduction**: **asexual** and **sexual** reproductions.

Sexual reproduction

- Sexual reproduction is reproduction **involving sexes**. During sexual reproduction, the sex cells of parent organisms unite with one another and form **a fertilized egg cell**.
- In this situation, each **sex cell** is a **gamete**.
- The gametes of human cells are haploid, from the Greek **haplos, meaning "single**."
- The term implies that each gamete contains **a single set of chromosomes**—23 chromosomes in humans.
- When the human gametes unite with one another, **the original diploid** condition of 46 chromosomes is reestablished

Sexual vs asexual reproduction

http://highered.mheducation.com/sites/0072495855/student_view0/chapter28/animation__random_orientation_of_chromosomes_during_meiosis.html

Type	Advantages	Disadvantages
Asexual reproduction	No mate needed. Many offspring produced quickly	No variation in the offspring.
Sexual reproduction	Genetic variation in the offspring.	Requires both sexes to participate.

Meiosis – A Source of Distinction

This makes for a lot of genetic diversity. This trick is accomplished through independent assortment and crossing-over.

Meiosis vs Mitosis

	Meiosis	Mitosis
Definition:	A type of cellular reproduction in which the number of chromosomes are reduced by half through the separation of chromosomes, producing four haploid cells.	A process of asexual reproduction in which the cell divides in two producing a replica, with an equal number of chromosomes in each resulting diploid cell.
Function:	sexual reproduction	Cellular Reproduction & general growth and repair of the body
Type of Reproduction:	Sexual	Asexual
Occurs in:	Humans, animals, plants, fungi	all organisms
Genetically:	Different	identical

Mendelian Genetics

- Humans have understood genetics **on some level** for thousands of years.
- They have been **improving crops and animals** through breeding for quite some time, selecting desirable traits and attempting to propagate them.
- Mendel began by doing **controlled experiments** **with peas** and is often regarded as **the father of modern genetics**,
- He did demonstrate that a combination of dominant and recessive traits determine the physical appearance of an organism.
- The importance of Mendel's work **did not gain wide understanding until the 1890s**

Laws of inheritance

- Mendel stated that **each individual has two factors for each trait**, one from each parent.
- The two factors **may or may not contain the same information**.
- If the two factors are identical, the individual is called **homozygous** for the trait.
- If the two factors have **different** information, the individual is called **heterozygous**.
- The alternative forms of a factor (gene) are called **alleles**.
- The **genotype** of an individual is made up of the many alleles it possesses.
- An individual's physical appearance, or **phenotype**, is determined **by its alleles as well as by its environment**.

Mendel's monohybrid cross

- The simplest experiments Mendel performed involved only **one pair of contrasting traits**.
- He mated individuals from two parent strains, each of which **exhibits one of the two contrasting forms of the character**.
- The cross between true breeding peas with **tall** stems and **dwarf** stems is representative of Mendel's monohybrids crosses. *Tall* and *dwarf* represent contrasting forms or traits of the character of **stem height**.

Mendel's dihybrid cross

- A natural extension of performing monohybrid crosses was for Mendel to design experiments where two **characters were examined simultaneously**.
- For example, if pea plants having **yellow** seeds that are also **round** were bred with those having **green** seeds that are also **wrinkled**.

Law of Dominance

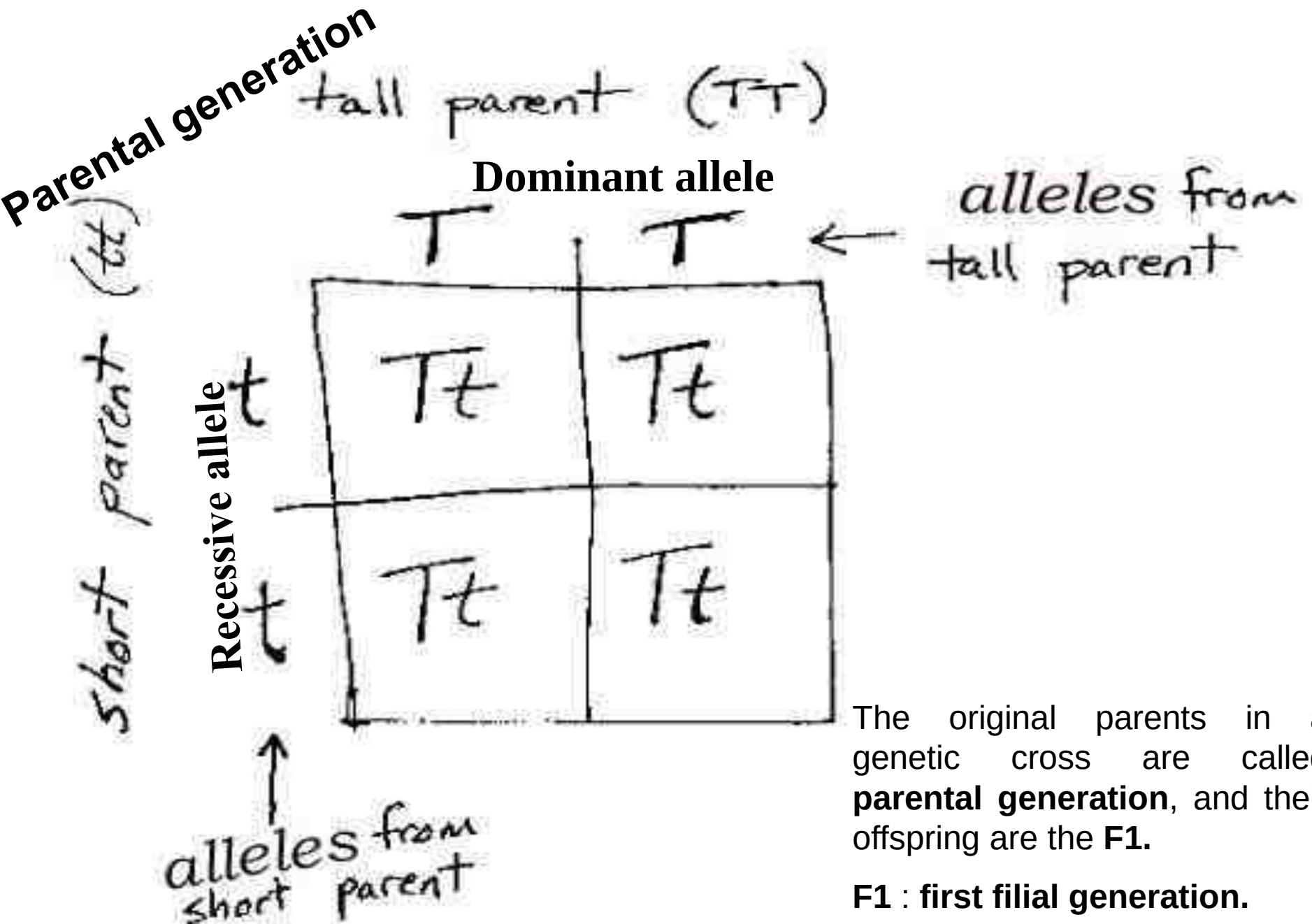
- In a cross of **parents that are pure** for contrasting traits, only one form of the trait will appear in the next generation. Offspring that are **hybrid** for a trait will have **only the dominant trait in the phenotype**. http://www.dnaftb.org/4/animation.html (8/11/2015)
- While Mendel was crossing (reproducing) his pea plants (over & over & over again), he noticed something interesting.
- When he crossed **pure tall** plants with **pure short** plants, all the new pea plants (referred to as the F1 generation) **were tall**.
- Similarly, crossing **pure yellow** seeded pea plants and **pure green** seeded pea plants produced an F1 generation of all yellow seeded pea plants. The same was true for other pea traits.

- What he noticed was that when the parent plants had contrasting forms of a trait (tall vs short, green vs yellow, etc.) the phenotypes of the offspring resembled only one of the parent plants with respect to that trait.
- "Law of dominance" states that **one of the factors for a pair of inherited traits will be dominant and the other recessive, unless both factors are recessive**.

The PUNNETT SQUARE (P-Square for short)

- We will start by using a P-Square to illustrate **Mendels Law of Dominance**

F1 individual (pure tall plants x pure short plants)

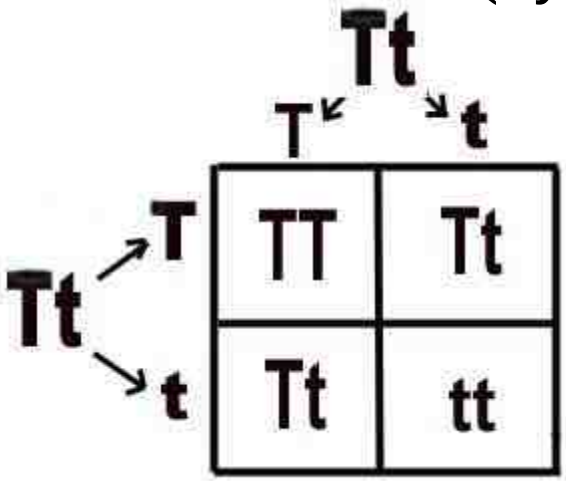


The original parents in a genetic cross are called **parental generation**, and their offspring are the **F1.**

F1 : **first filial generation.**

Law of Segregation

F2 individual (hybrid tall plants of f1x hybrid tall plants of f1)



Parent Pea Plants (Two Members of F1 Generation)

Offspring (F2 Generation)

Genotypes: Tt x Tt

Phenotypes: tall x tall

Genotypes: 25%TT 50%Tt 25% tt

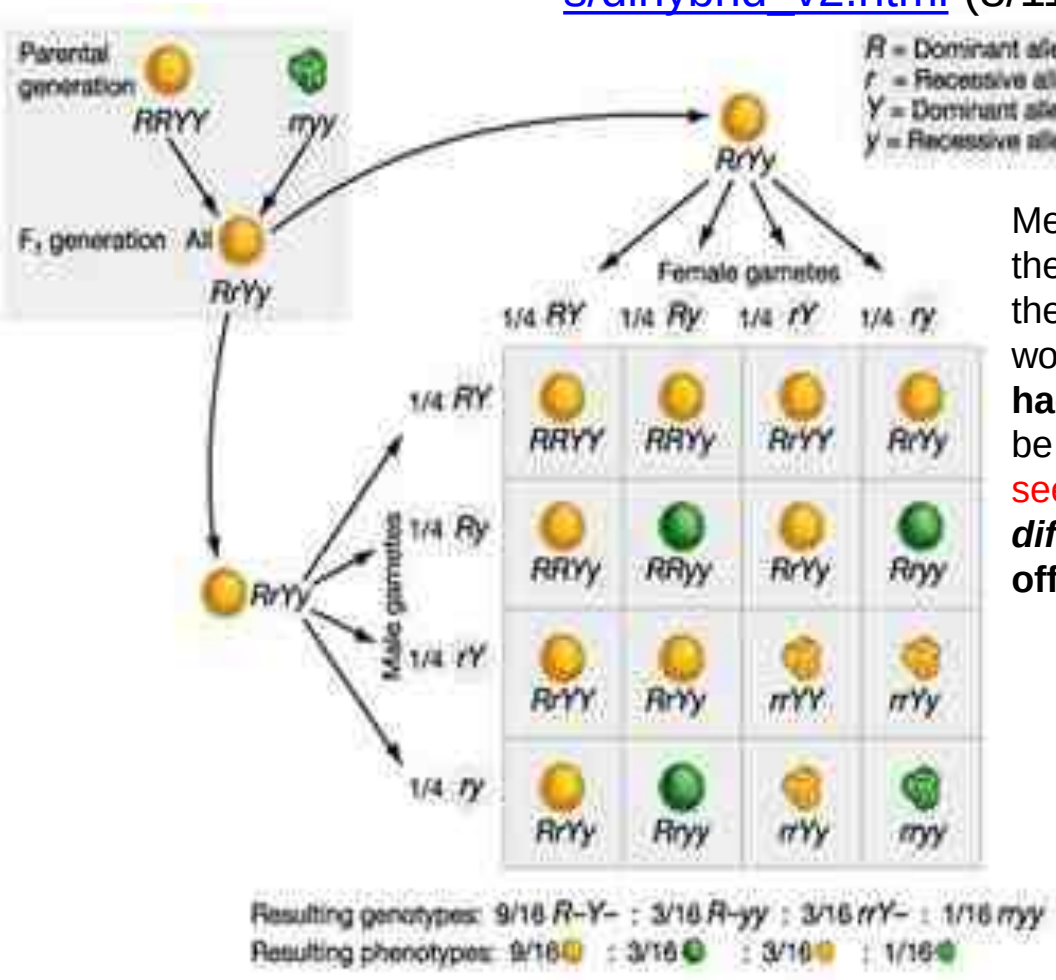
Phenotypes: 75% tall 25% short

According to the p-square, any time you cross two hybrids, 3 of the 4 boxes will produce an organism with the dominant trait ("TT", "Tt", & "Tt"), and 1 of the 4 boxes ends up homozygous recessive, producing an organism with the recessive phenotype ("tt“)

- The law “**2 alleles for each gene separate during gamete formation, and are distributed to different gametes so that every gamete receives only one member of the pair**”.

The law of independent assortment

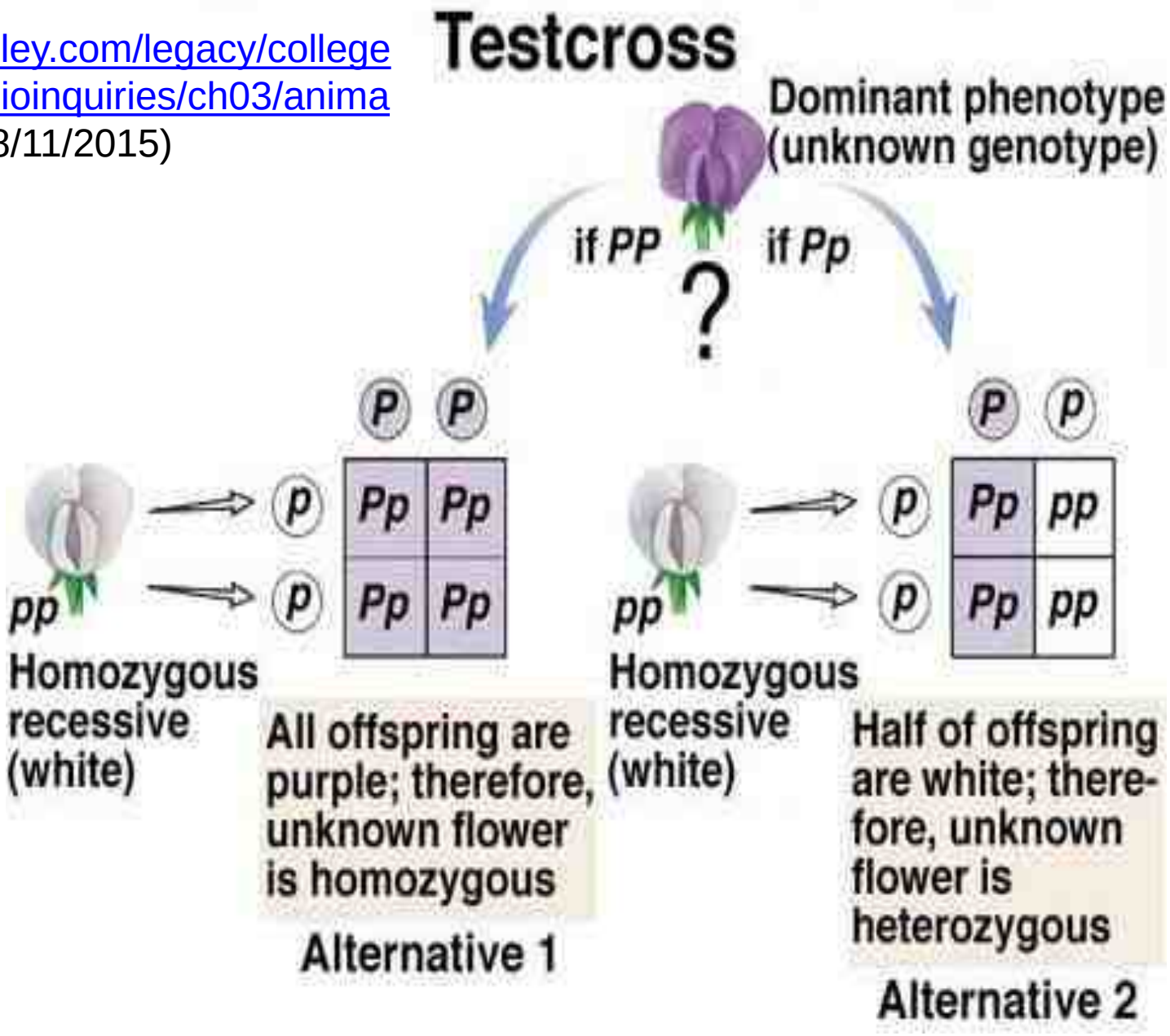
- Mendel performed **dihybrid crosses** (a breeding experiment between P generation (parental generation) organisms that *differ in two traits*).
- In this cross, the traits for Round seed shape (RR) and yellow seed color (YY) are dominant. Wrinkle seed shape (rr) and green seed color (yy) are recessive. http://www.siskiyous.edu/class/bio1/genetics/dihybrid_v2.html (8/11/2015)



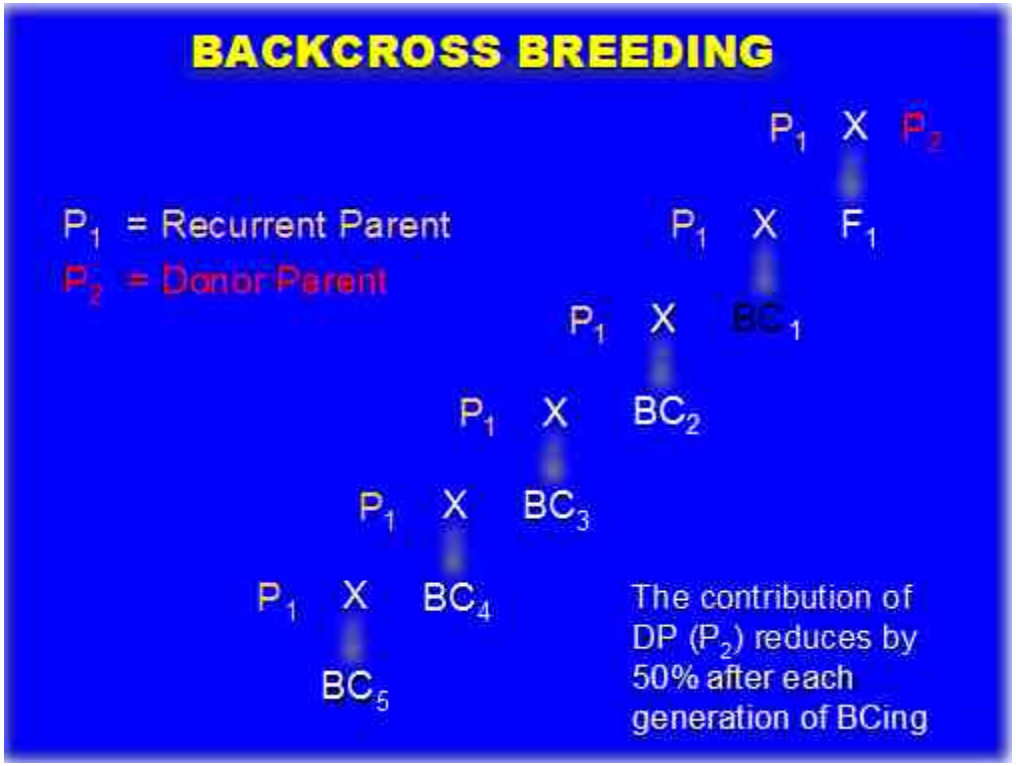
Mendel noticed during all his work that the **height** of the plant and the **shape** of the seeds and the **color** of the pods had no impact on one another. In other words, **being tall** didn't automatically mean the plants **had to have green pods**, nor did green pods have to be filled only with wrinkled seeds, the different traits seem to be inherited independently. **Alleles for *different* traits are distributed to sex cells (& offspring) independently of one another.**

- The law "allele pairs separate independently during the formation of gametes. Therefore, traits are transmitted to offspring independently of one another"

http://higheredbcs.wiley.com/legacy/college/pruitt/0471473219/bioinquiries/ch03/animations/p0310_b.htm (8/11/2015)



- **Backcrossing** is a crossing of a **hybrid with one of its parents** or an individual genetically similar to its parent, in order to achieve offspring with a **genetic identity which is closer to that of the parent**.
- Backcrossed hybrids are sometimes described with acronym "BC", for example, an F1 hybrid crossed with one of its parents (or a genetically similar individual) can be termed a BC1 hybrid, and a further cross of the BC1 hybrid to the same parent (or a genetically similar individual) produces a BC2 hybrid.



EPISTASIS

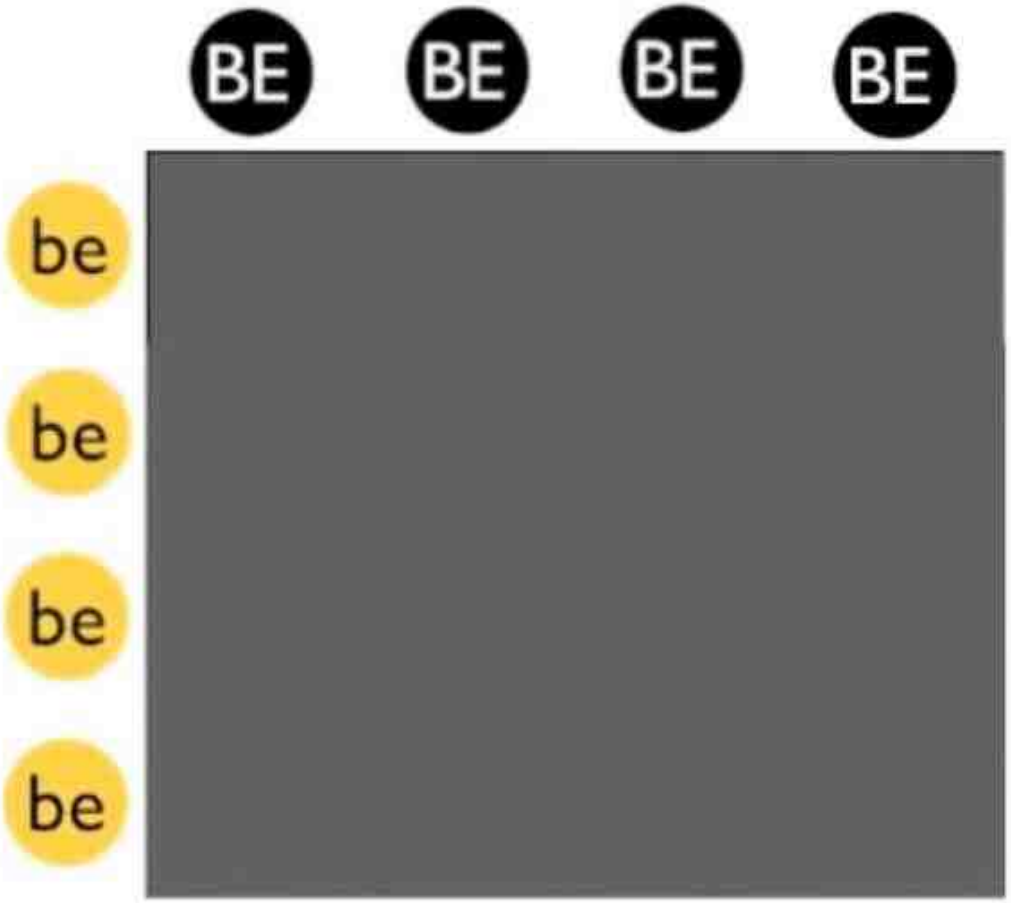
- In its **strictest classical genetic definition**, is **the interaction of genes where one gene** (or locus) **masks the effect of another**.
- A gene is defined as **a stretch of continuous DNA that occupies a specific location on a chromosome and that codes for a protein or functional RNA product**.
- This stretch of DNA **can have minor changes in sequence and still lead to a product**, though this product may be slightly, or significantly, different as a consequence of the altered sequence.
- **These alternate sequences** of DNA at a specific locus (region on a chromosome) are called **alleles**.
- Each gene can have **many alleles throughout a population**, but each individual can **only have two alleles of a gene**.

- Some alleles are deleterious and **cause disease**, others are **harmless**, and some may be **beneficial**.
- If a person has a harmless allele and a disease allele, **what determines the outcome of whether this person will have a disease is whether the disease allele is dominant or recessive.**
- If two copies of the deleterious allele are necessary for the disease to manifest, the disease allele is said to be recessive, or masked by having one copy of a normal allele.
- What is important to remember about dominance and recessiveness is that these terms describe the effect that a *single* gene has on the measurable trait, **not the effect of two different genes at different chromosomal loci**.
- In contrast to dominance and recessiveness, the classical definition of epistasis refers to at least ***two*** **genes that are not alleles of each other, interacting to have a measurable effect on a trait**.

- An example in the animal world is that of coat color of Labrador retrievers, which can be **yellow**, **black**, or **chocolate**.
- Coat color in this example is **not caused by alleles of a single gene**, but rather by the interaction of alleles of two different genes.
- In this respect, **epistasis can be defined in a manner similar to dominant and recessive, except that instead of one allele being dominant to another allele of that same gene, alleles of one gene can be dominant to alleles of a different gene.**

BE	BE	BE	BE
be			
be			
be			
be			


BB EE
BB Ee
Bb EE
Bb Ee
bb EE
bb Ee
bb ee
Bb ee
BB ee


BE
BE
BE
BE
be
be
be
be

Dihybrid Cross

	BE	Be	bE	be
BE	BBEE	BBEe	BbEE	BbEe
Be	BBEe	BBee	BbEe	Bbee
bE	BbEE	BbEe	bbEE	bbEe
be	BbEe	Bbee	bbEe	bbee

BB EE

BB Ee

Bb EE

Bb Ee

BBEE	BBEe	BbEE	BbEe
BBEe	BBee	BbEe	Bbee
BbEE	BbEe	bbEE	bbEe
BbEe	Bbee	bbEe	bbee

9/16 (56%) Black

bb ee

Bb ee

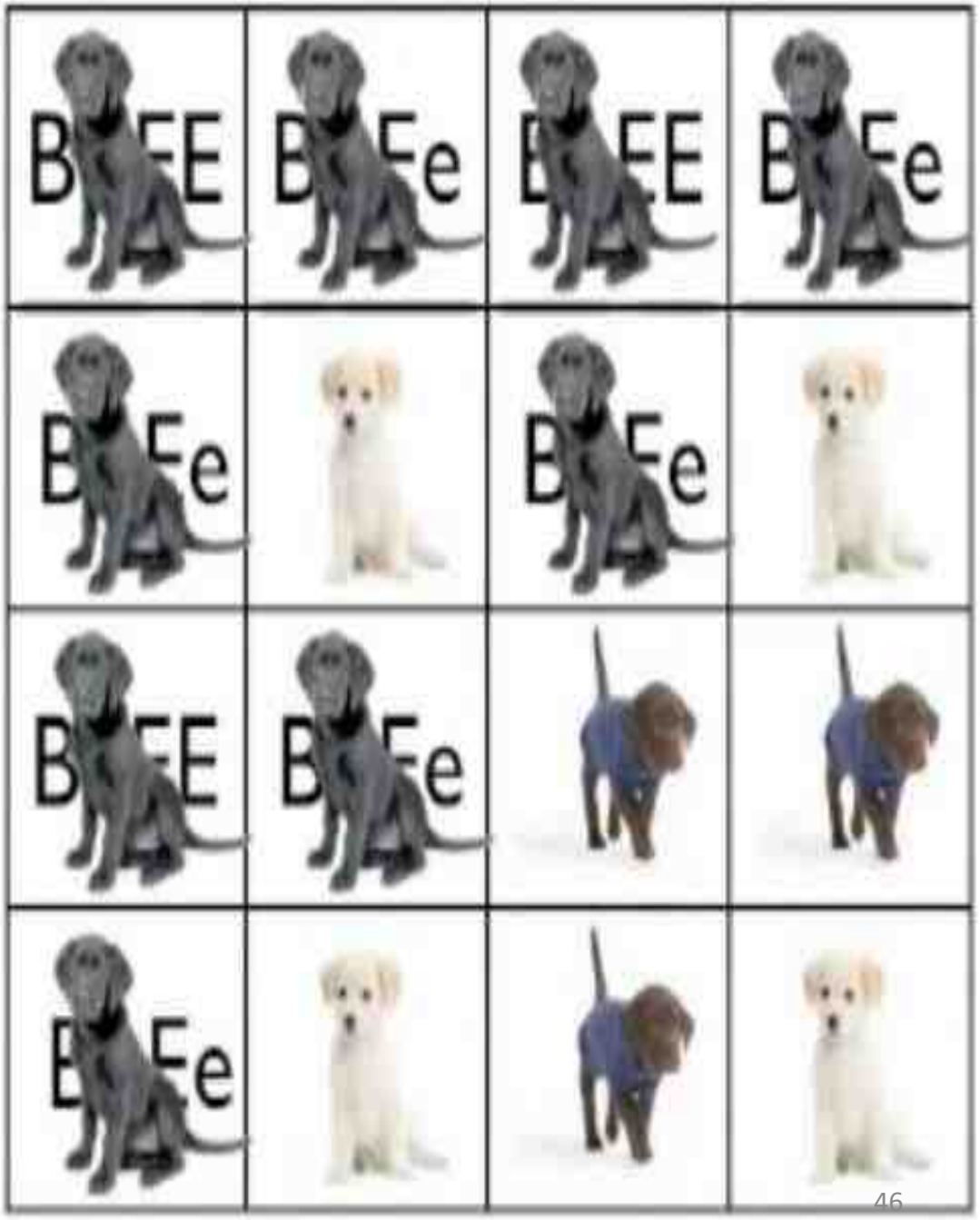
BB ee

B_EE	B_Ee	B_EE	B_Ee
B_Ee		B_Ee	
B_EE	B_Ee	bbEE	bbEe
B_Ee		bbEe	

9/16 (56%) Black

bb EE

bb Ee



SEX-LINKED INHERITANCE

- Most animals and many plants show sexual **dimorphism**; in other words, an individual can be either **male or female**.
- In most of these cases, sex is determined by special **sex chromosomes**.
- In these organisms, there are two categories of chromosomes, **sex chromosomes** and **autosomes** (the chromosomes other than the sex chromosomes).
- The rules of inheritance considered so far, with the use of Mendel's analysis as an example, are **the rules of autosomes**. Most of the chromosomes in a **genome** are autosomes.
- The sex chromosomes are fewer in number, and, generally in **diploid** organisms, there is just **one pair**.
- The expectations of **sex-linked inheritance** in any species depend on how the chromosomes determine sex

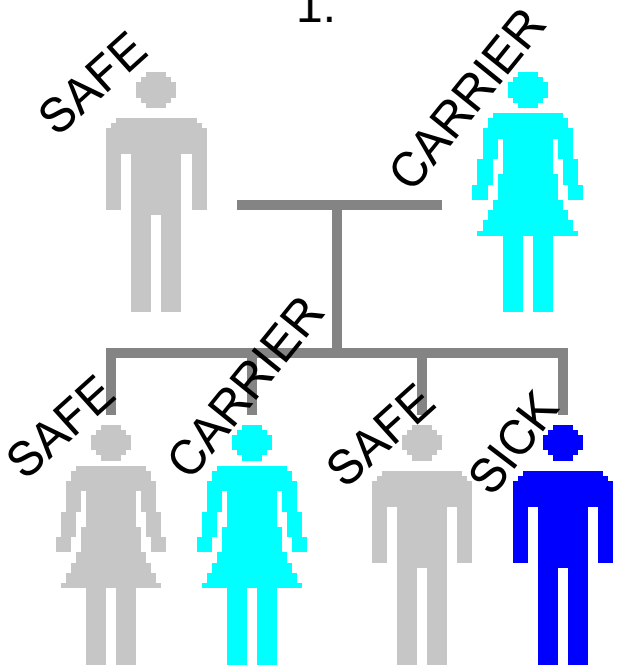
- In general terms, traits determined by genes on sex chromosomes are not different from traits determined by autosomal genes.
- In humans it is preferable to speak in terms of **X-linked** or **Y-linked** **inheritance**.
- Genes linked to the X and Y chromosomes show **distinctive patterns of inheritance**.
- The Y chromosome contains **few identified genes**, while the larger X chromosome contains **several thousand genes**
- Y-linked traits are passed on the Y chromosome, and X-linked traits on the X. **Males are hemizygous for X-linked traits.**

- They have **one copy of X-linked genes and these are expressed in the phenotype**.
- **Females express X-linked traits like those that are autosomally inherited**. Two copies of homozygous recessive alleles are required for expression.
- A sex-influenced allele is **dominant in one sex but recessive in the other**. **Hormonal differences** between males and females typically cause the differential expression.
- Genes that are carried by either sex chromosome are said to be **sex linked**.
- Since only men inherit Y chromosomes, they are the only ones to inherit **Y-linked** traits.
- Men and women can get the **X-linked** ones since both inherit X chromosomes.

X-Linked Recessive Inheritance

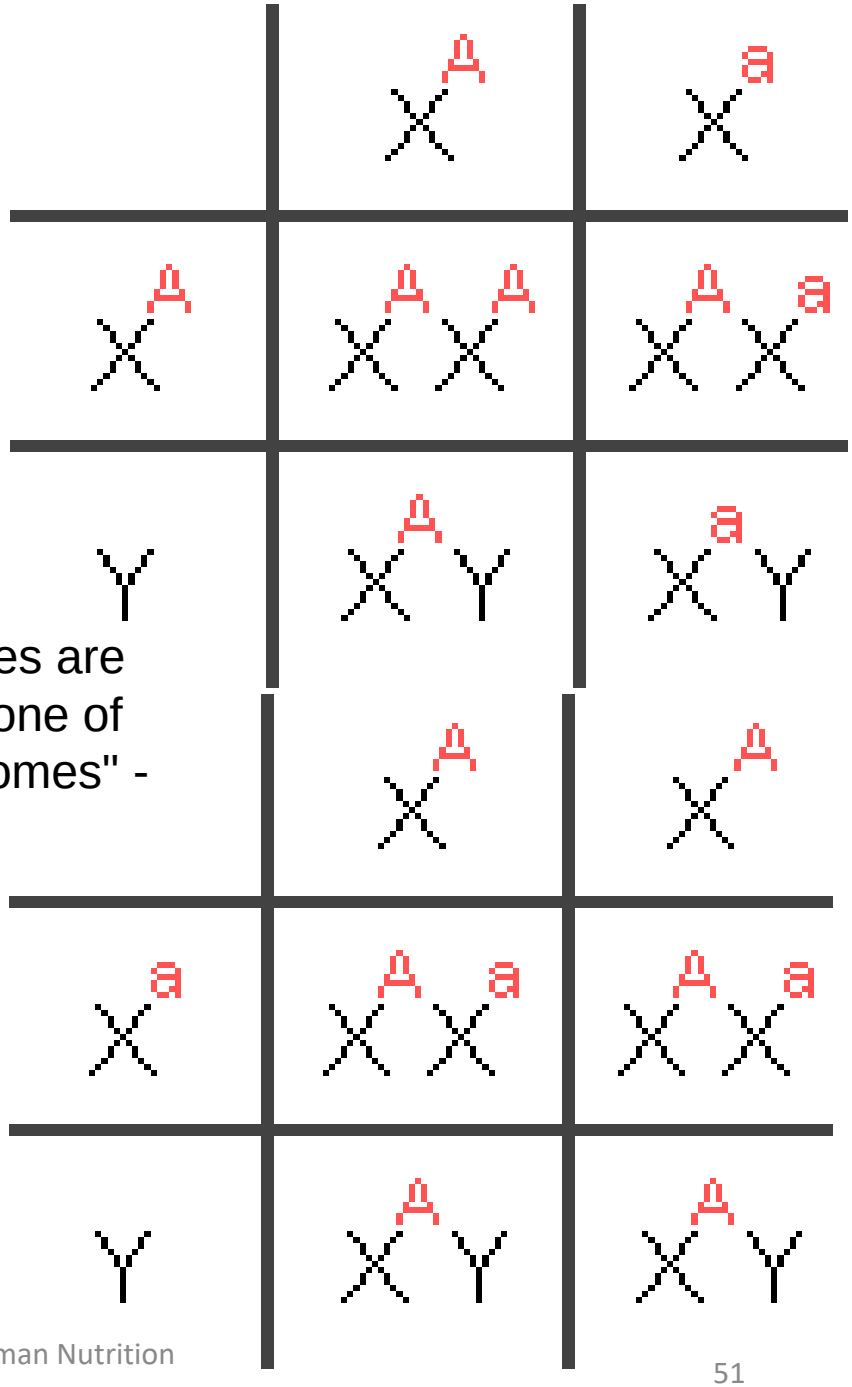
- **X-linked recessive** traits that are **not related to feminine body** characteristics are primarily expressed in the observable characteristics, or phenotype, of men.
- Genes on chromosome X **not coding for gender** are usually expressed in the male phenotype even if they are recessive **since there are no corresponding genes on the Y chromosome** in most cases.
- In women, a recessive allele on one X chromosome is often **masked in their phenotype by a dominant normal allele** on the other.
- This explains **why women are frequently carriers of X-linked traits but more rarely have them expressed** in their own phenotypes.

1.



2.

Sex-linked diseases are inherited through one of the "sex chromosomes" -- the X or Y chromosomes.



http://content.bfwpub.com/webroot_pubcontent/Content/BCS_5/phelanphys2e/Q%20Animations/0701/_START_C.html

WHEN THE ABNORMAL GENE DOMINATES.

- Dominant inheritance occurs when an abnormal gene from one parent is able to cause disease even though the matching gene from the other parent is normal.
- The abnormal gene dominates.
- Recessive inheritance occurs when both matching genes must be abnormal to produce disease.
- If only one gene in the pair is abnormal, the disease does not show up or is mild.

- X-linked recessive diseases are sometimes referred to as "**male only**" diseases. However, this is not technically correct.
- Females can get an X-linked recessive disorder, but this is very rare.
- Female carriers can have **a normal X chromosome that is abnormally inactivated**. This is called "**skewed X-inactivation**." These females may have symptoms similar to those of males.

X-Linked Dominant Inheritance and Y-linked inheritance

- X-linked dominant conditions are expressed in both males and females. These conditions are generally **more severe in males**.
- In mammals, **Y-linkage**, also known as **holandric inheritance,** is the determination of a **phenotypic trait by an allele (or gene) on the Ychromosome**.
- Because the Y-chromosome is **small** and **does not contain many genes**, few traits are Y-linked, and Y-linked diseases are rare.
- Since the only humans who have a Y chromosome are males, Y-linked traits are passed only from father to son.

MULTIPLE GENES AND ALLELES

- In classical Mendelian genetics, each gene has two possible alleles. However, **some genes have more than two alleles.**
- The gene for the blood type protein has three alleles (A, B, and O). One eye color gene in fruit flies has many alleles.
- Human blood types are determined by proteins on the surface of the red blood cells.
- Alleles A and B, for A type and B-type glycoprotiens, are co-dominant; that is, a person who inherits a A allele from one parent and a B allele from the other parent will have type AB blood. The o allele is recessive.

- The O allele produces no glycoproteins. Thus a person with the genotype AO will make some type A glycoproteins, and have type A blood.
- A person with the genotype OO will make neither the A-type nor the B-type glycoproteins, and will have type O blood.
- Most human traits are controlled by several genes. Some, such a skin color, eye color, and hair color, are controlled by multiple copies of the same gene.
- In skin color, for example there are **several pairs of genes that code for the pigment melanin**. The more copies of the dominant allele a person has, the darker their skin.
- Some traits, such as human height, are **controlled by the activities of many different genes**.

QUESTIONS

1. Mr. and Mrs. Smith have a daughter, Samantha. Mr. Jones, their neighbor, is suing for custody of the child, claiming that he had an affair with Mrs. Smith and that Samantha is his daughter. The judge in the case orders blood tests to determine blood types of all the people involved. The results are:

- Mr. Smith: Type AB; Mrs. Smith: Type B; Mr. Jones: Type A; Samantha: Type O.
- Is it possible that Mr. Jones could be Samantha's father?

2. What if Samantha had type AB blood? Who could be her father in that case?

LETHAL GENE

- Cuénot and Baur discovered first recessive lethal genes because they altered Mendelian inheritance ratios.
- **Recessive lethal** do not actually cause death unless an organism carries two copies of the lethal allele.
- Examples of human diseases caused by recessive lethal alleles include cystic fibrosis, sickle-cell anemia, and achondroplasia

CONDITIONAL LETHAL GENES

- Conditional lethal genes are expressed under certain conditions.

- Favism is a sex-linked, when affected individuals **eat fava** beans, they develop hemolytic anemia.
- Affected individuals may also develop anemia when administered **therapeutic doses of antimalarial** medications and other drugs.
- They are resistant to malaria, because it is more difficult for malaria parasites to multiply in cells with deficient amounts of **glucose-6-phosphate dehydrogenase**.

- A mutant protein may be genetically engineered to be fully functional at 30°C and completely inactive at 37°C.
- By developing a conditional lethal version of a dominant lethal gene, **scientists can study and maintain organisms carrying dominant lethal alleles.**

Dominant Lethal Genes

- Dominant lethal genes are **expressed in both homozygotes and heterozygotes**.
- But how can alleles like this be passed from one generation to the next if they cause death.
- One example of a disease caused by a dominant lethal allele is **Huntington's disease**, a neurological disorder in humans, which **reduces life expectancy**.
- Because **the onset of Huntington's disease is slow**, individuals carrying the allele can pass it on to their offspring.
- This allows the allele to be maintained in the population.

SYNTHETIC LETHAL GENES

- Some mutations are **only lethal when paired with a second mutation**.
- These genes are called synthetic lethal genes.
- Synthetic lethality can indicate that:

❖ Two genes **function in parallel pathways** that **share information** with one another.

✓ Each of the **two pathways could compensate for a defect in the other**, but when both pathways have a mutation, the combination results in synthetic lethality.

❖ **Two affected genes have the same role**, and therefore, lethality only results when both copies are nonfunctional and one gene cannot substitute for the other.

❖ **Both genes may function in the same essential pathway**, and **the pathway's function may be diminished** by each mutation.

- When an allele causes lethality, this is evidence that the gene must have a critical function in an organism.
- The discoveries of many lethal alleles have provided information on the functions of genes during development.
- Additionally, scientists can use conditional and synthetic lethal alleles to study the physiological functions and **relationships of genes under specific conditions**.

Transcription

- **The process by which genetic information from DNA is transferred into RNA.**
- **Produces mRNAs that are translated into sequences of amino acids (polypeptide chains or proteins), and**
- **rRNAs,**
- **tRNAs, and**
- **additional small RNA molecules that perform specialized structural, catalytic, and regulatory functions and are not translated.**
- **The final product of gene expression, therefore, can be** RNA or protein**, depending upon the gene.**

General features of RNA synthesis

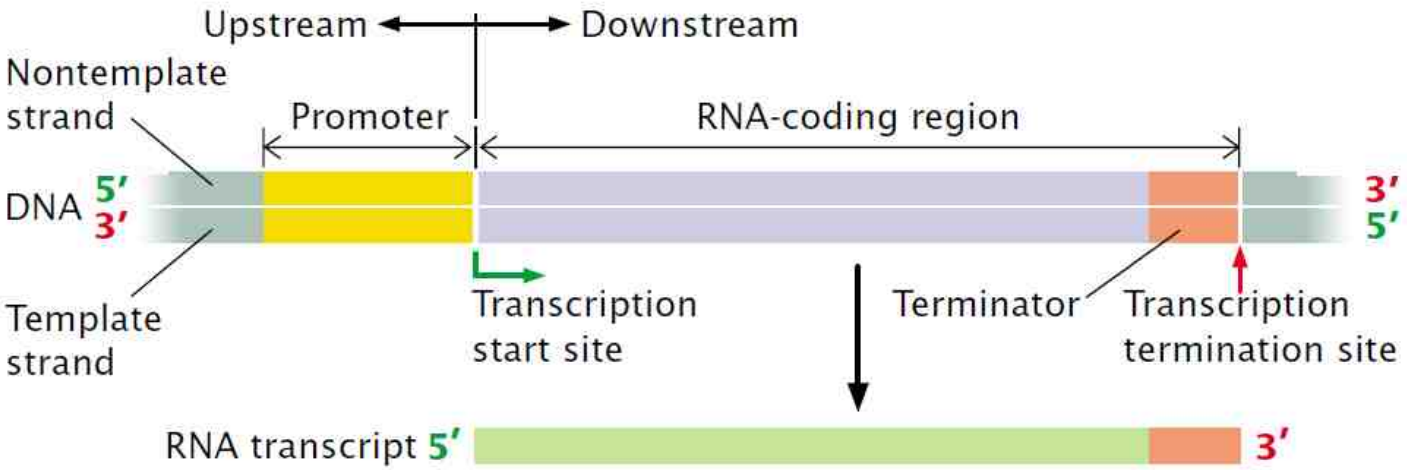
- **Synthesized on a DNA template, catalyzed by DNA dependent RNA polymerase (usually just called RNA polymerase).**
- **ATP, GTP, CTP, and UTP are required, as is Mg2+**
- **no RNA primer is required**
- **ALL transcription creates new RNA in a 5' to 3' direction. The nucleotide at the 5' end of the chain retains its triphosphate (ppp) group**
- **Existing chain must have a 3' OH group.**
- **Newly added rNTP must base-pair with template DNA.**
- **The DNA base sequence contains signals for initiation and termination of RNA synthesis.**
- **The enzyme binds to and moves along the DNA template in the 3' -> 5' direction**
- **The DNA template is unchanged**

The Transcription Unit

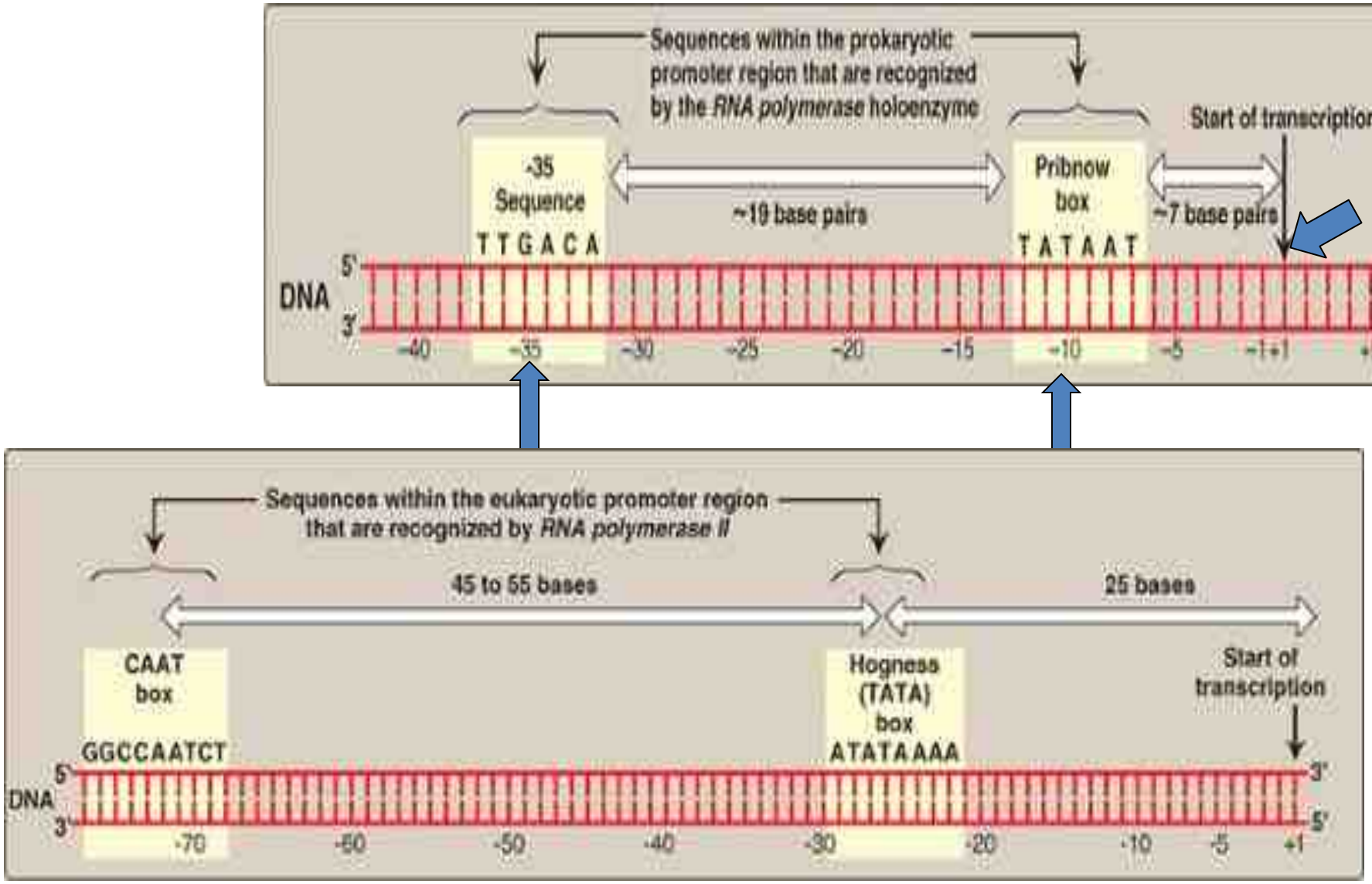
- **Is the stretch of DNA that codes for an RNA molecule and the sequences necessary for transcription.**

Contains 3 critical regions:

1. **PROMOTER**
2. **RNA CODING REGION**
3. **TERMINATOR**



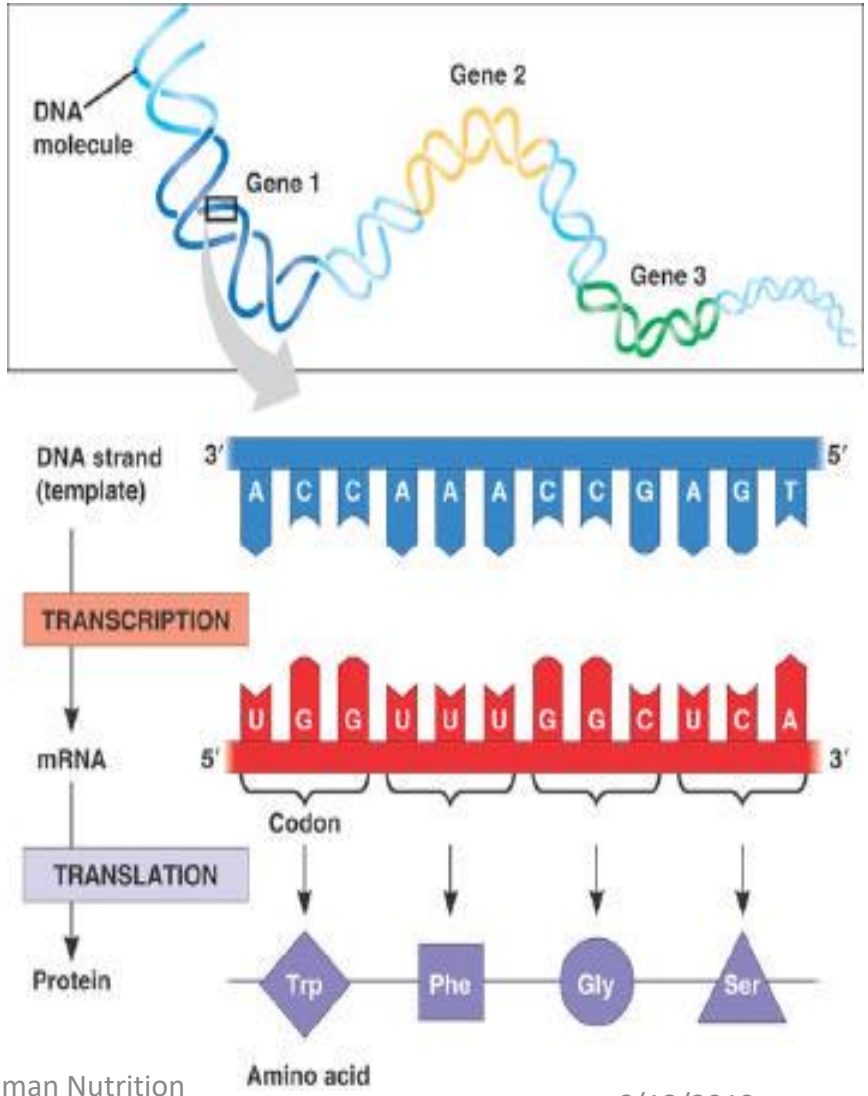
Promoters and Consensus Sequences



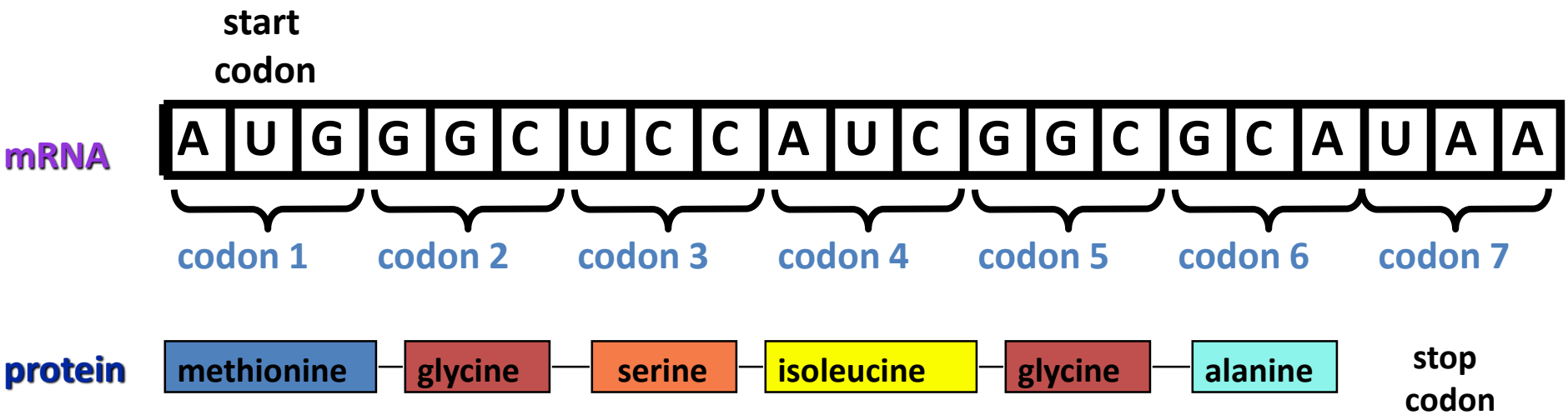
A Consensus Sequence is a short stretch of DNA that is conserved among promoters of different genes.

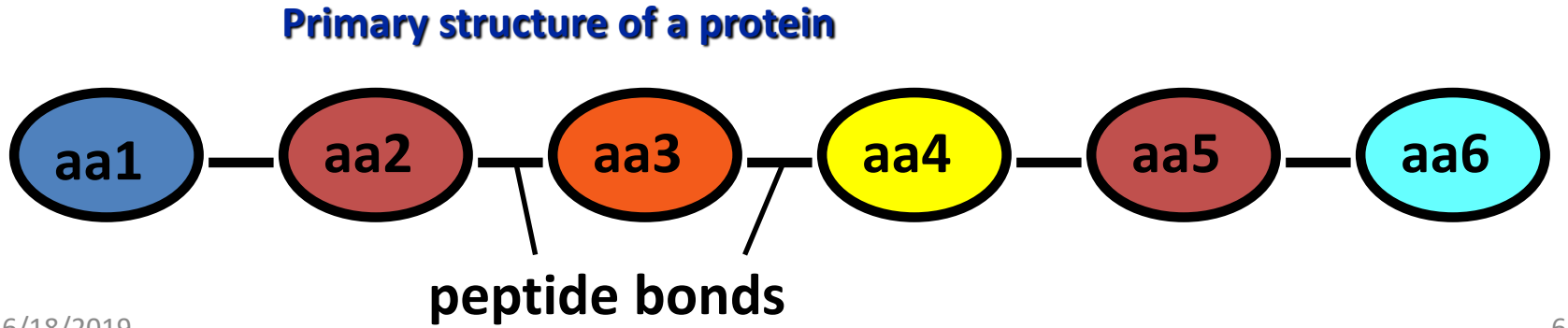
PROTEIN SYNTHESIS: FROM GENE TO PROTEIN

- Genes are stretches of nucleotides **organized in *triplets.***
- Different arrangements or DNA triplets encode for each one of the 20 amino acids that make proteins.
- During transcription, **a DNA triplet will produce an mRNA *codon.***
- During translation, a **codon** will constitute an **amino acid**



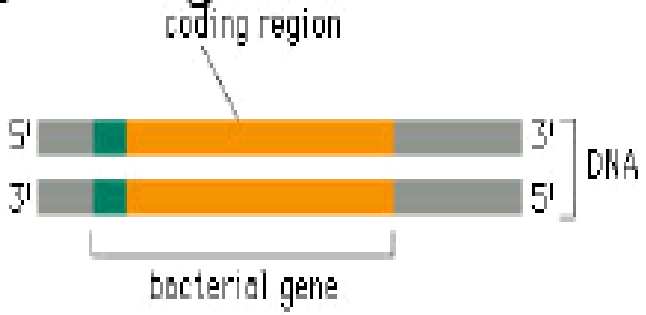
A. Messenger RNA (mRNA)



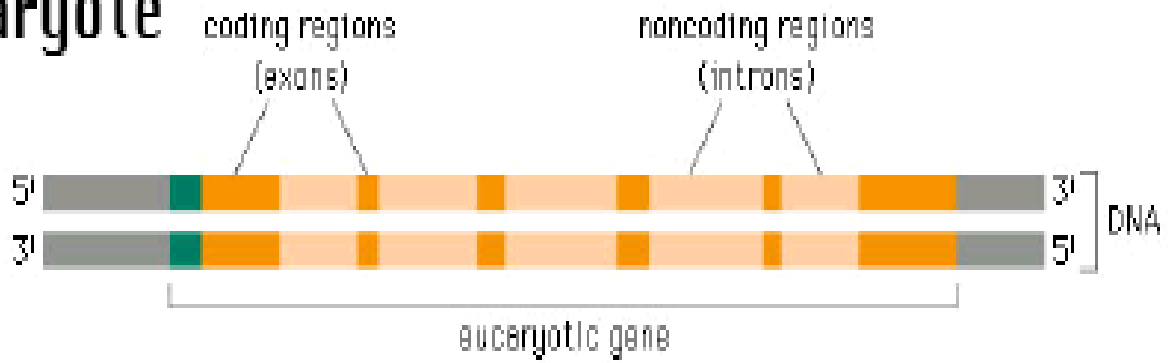


non-coding regions (introns)interspersed in the coding regions (exons) of the gene

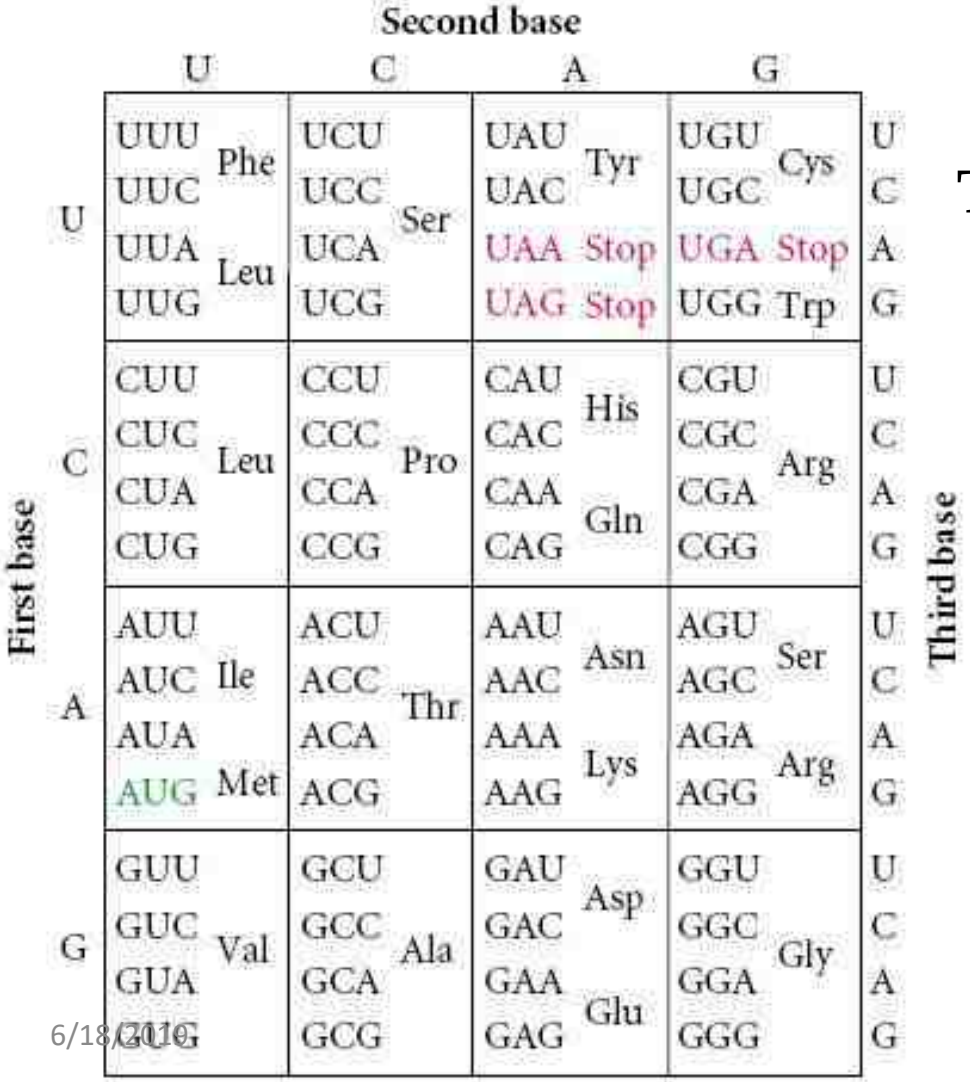
Eubacteria (prokaryote)



Eukaryote



One amino acid is encoded by three consecutive nucleotides in mRNA, and each nucleotide can have one of four possible bases (A, G, C, and U) at each nucleotide position thus permitting **4^3 = 64 possible codons** (see Figure below).

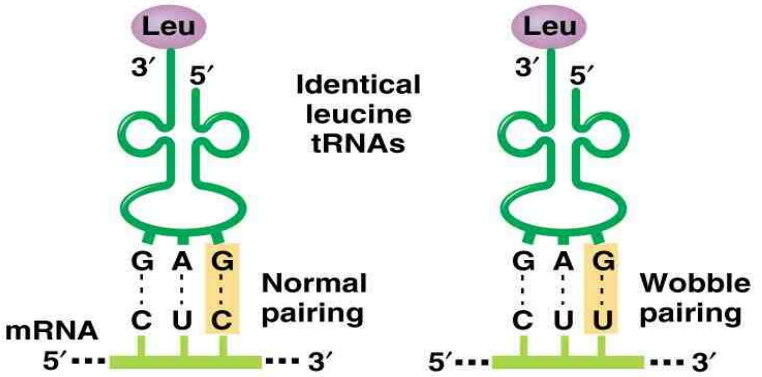


THE GENETIC CODE

The genetic code consists of 64 codons and the amino acids specified by these codons. The codons are written 5′→3′, as they appear in the mRNA. AUG is an initiation codon; UAA, UAG, and UGA are termination codons.

Table The wobble rules, indicating which bases in the third position (3′ end) of the mRNA codon can pair with bases at the first (5′ end) of the anticodon of the tRNA

First Position of Anticodon	Third Position of Codon	Pairing
C	G	Anticodon 3′–X—Y—C–5′ / 5′–Y—X—G–3′ Codon
G	U or C	Anticodon 3′–X—Y—G–5′ / 5′–Y—X—U–3′ C Codon
A	U	Anticodon 3′–X—Y—A–5′ / 5′–Y—X—U–3′ Codon
U	A or G	Anticodon 3′–X—Y—U–5′ / 5′–Y—X—A–3′ G Codon
I (inosine)	A, U, or C	Anticodon 3′–X—Y—I–5′ / 5′–Y—X—A–3′ U C Codon



WOBBLE

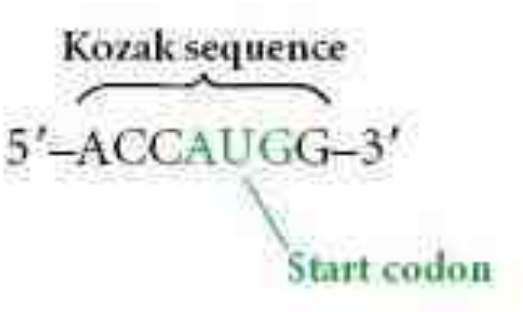
Occurs when the third base (5'end) of the tRNA anticodon has some play or wobble, so that **it can hydrogen bond with more than one kind of a base in the third position** (3' end) of the codon.

Concepts

The genetic code consists of 61 sense codons that specify the 20 common amino acids; the code is degenerate and some amino acids are encoded by more than one codon. Isoaccepting tRNAs are different tRNAs with different anticodons that specify the same amino acid. Wobble exists when more than one codon can pair with the same anticodon.

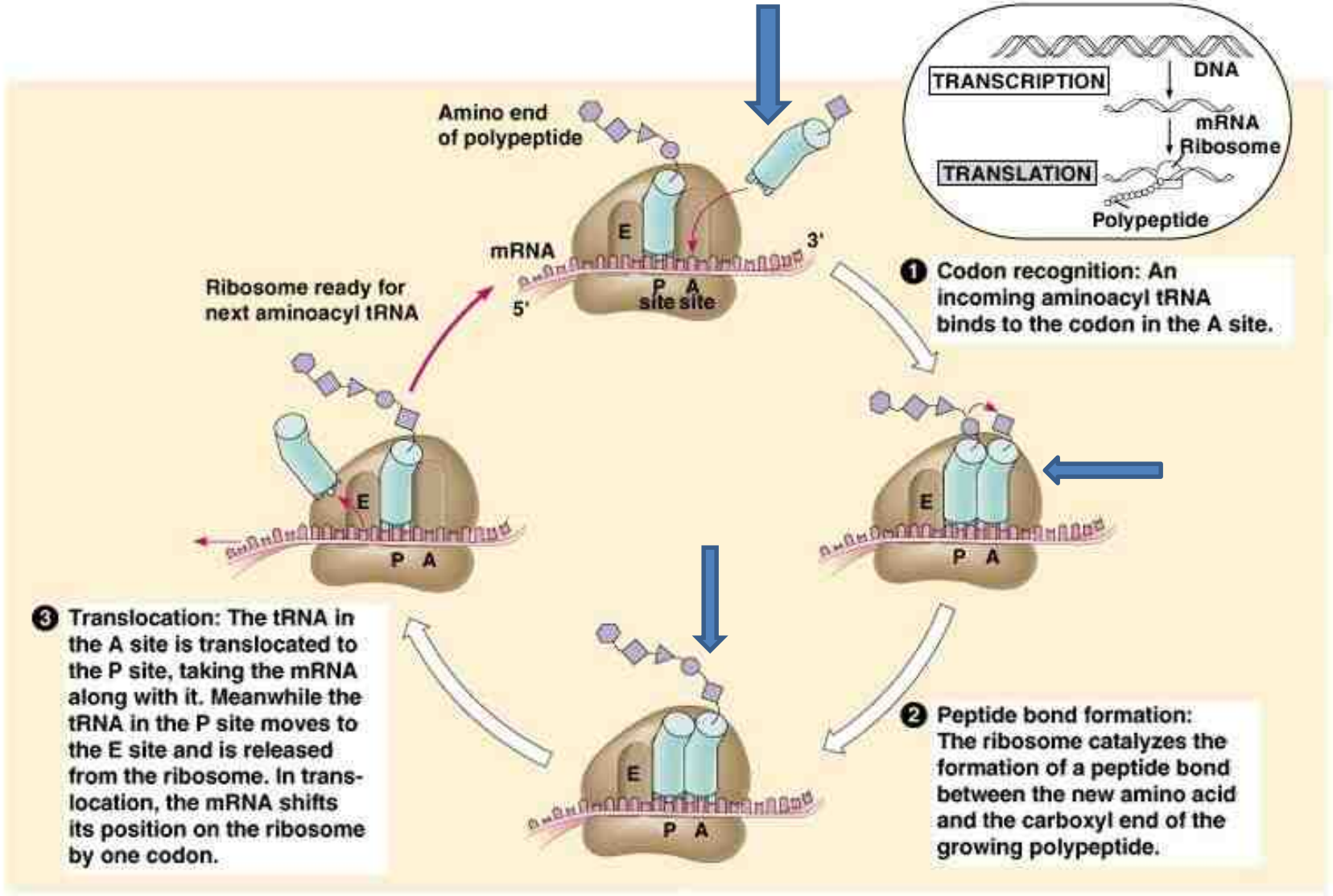
INITIATION

- **In bacteria, the process initiates when 30S ribosome subunit + IFs/GTP bind to AUG start codon and Shine-Dalgarno sequence.**
- **Shine-Dalgarno sequence – a short target site, consensus sequence composed of 8-12 purine-rich nucleotides eg. 5'-AGGAGGU-3' in *E. coli*, located about 3-10 nucleotides upstream the initiation codon, where the translation begins.**
- In eukaryotes, the initiation codon, the 5'-AUG-3' is within the consensus sequence – 5'-ACCAUGG-3', called Kozak sequence.



Elongation in eukaryotic cells takes place in a similar manner.

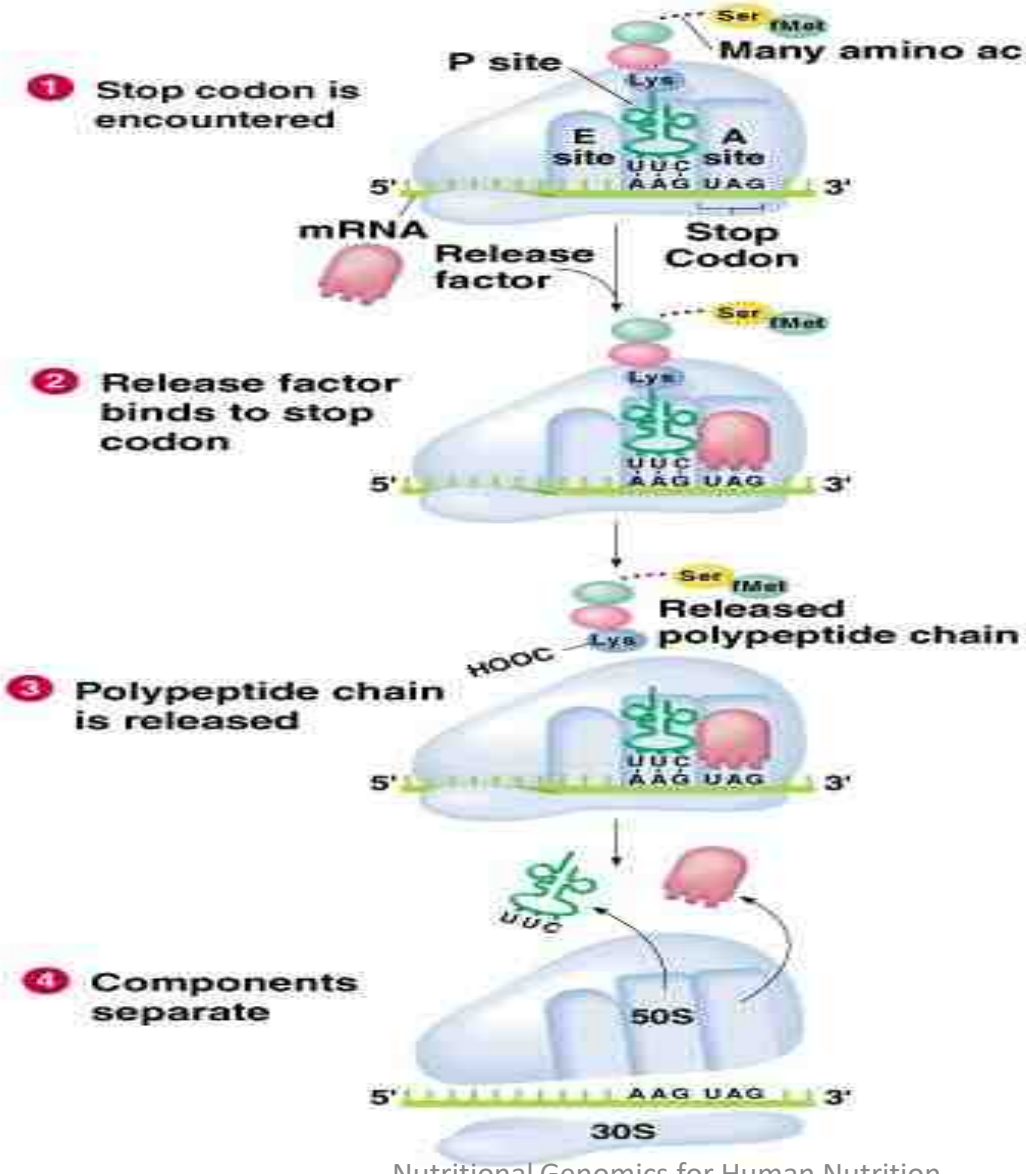
THE ELONGATION CYCLE OF TRANSLATION - OVERVIEW



Concepts

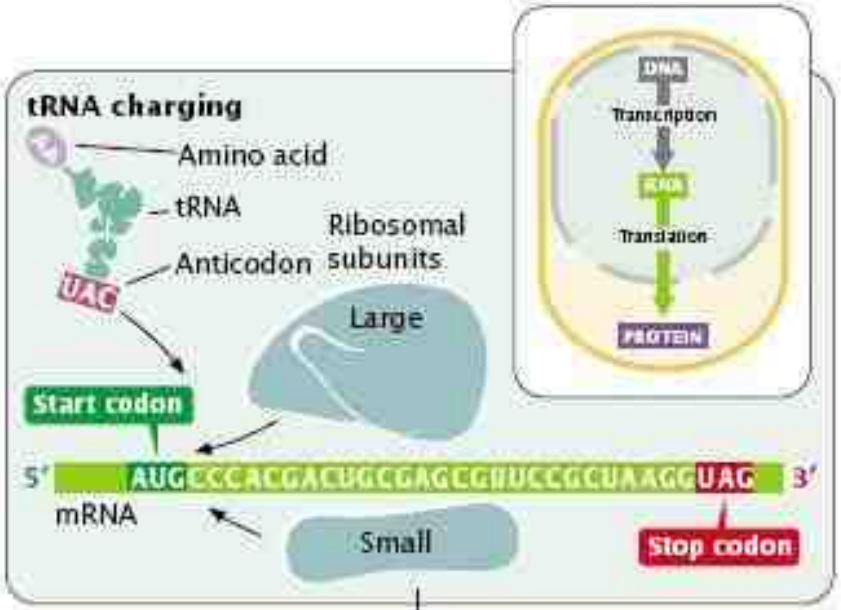
Elongation consists of three steps: (1) a charged tRNA enters the A site, (2) a peptide bond is created between amino acids in the A and P sites, and (3) the ribosome translocates to the next codon. Elongation requires several elongation factors (EF-Tu, EF-Ts, and EF-G) and GTP.

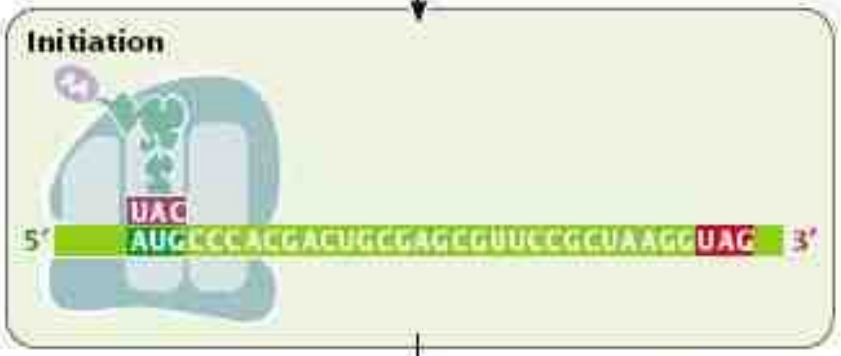
TERMINATION

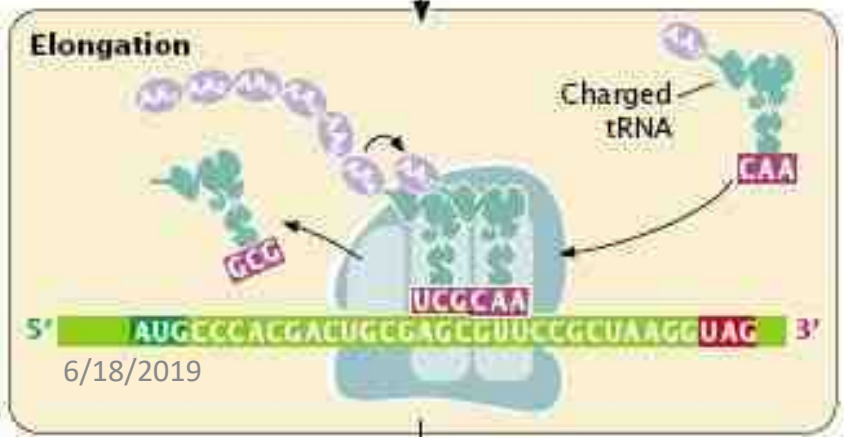


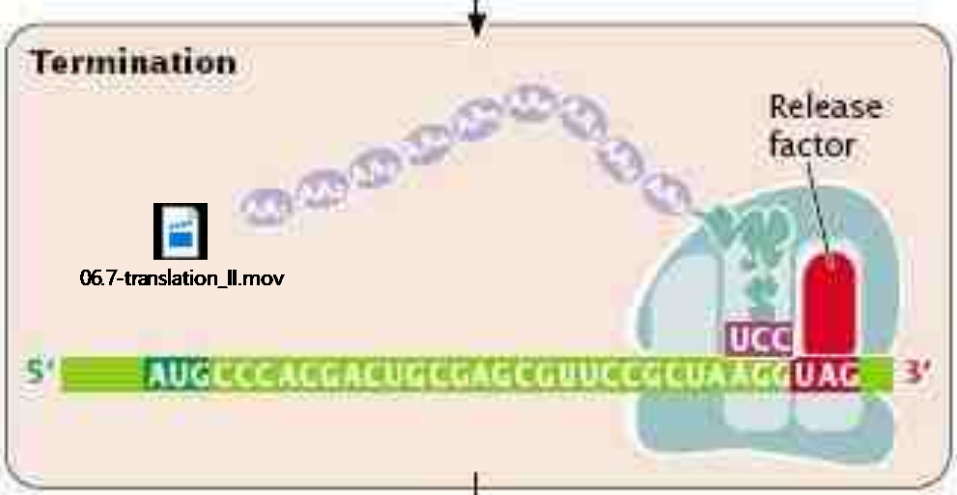
Concepts

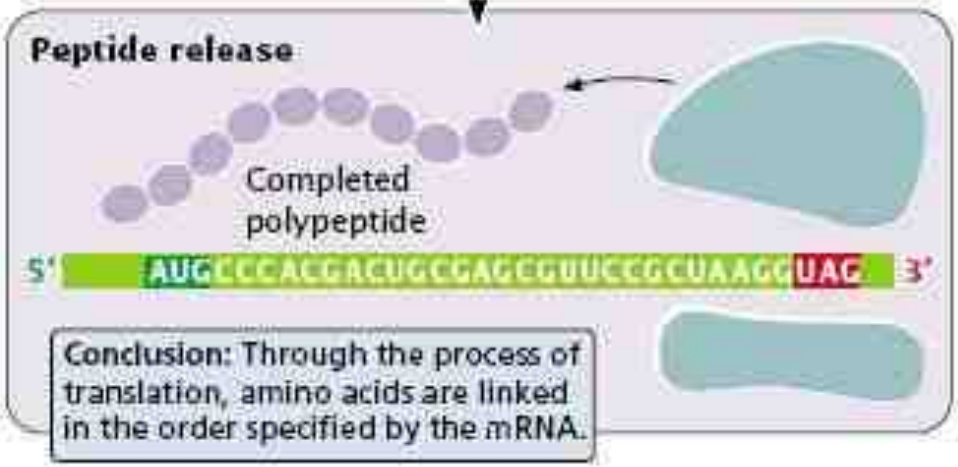
Termination takes place when the ribosome reaches a termination codon. Release factors bind to the termination codon, causing the release of the polypeptide from the last tRNA, the tRNA from the ribosome, and the mRNA from the ribosome.







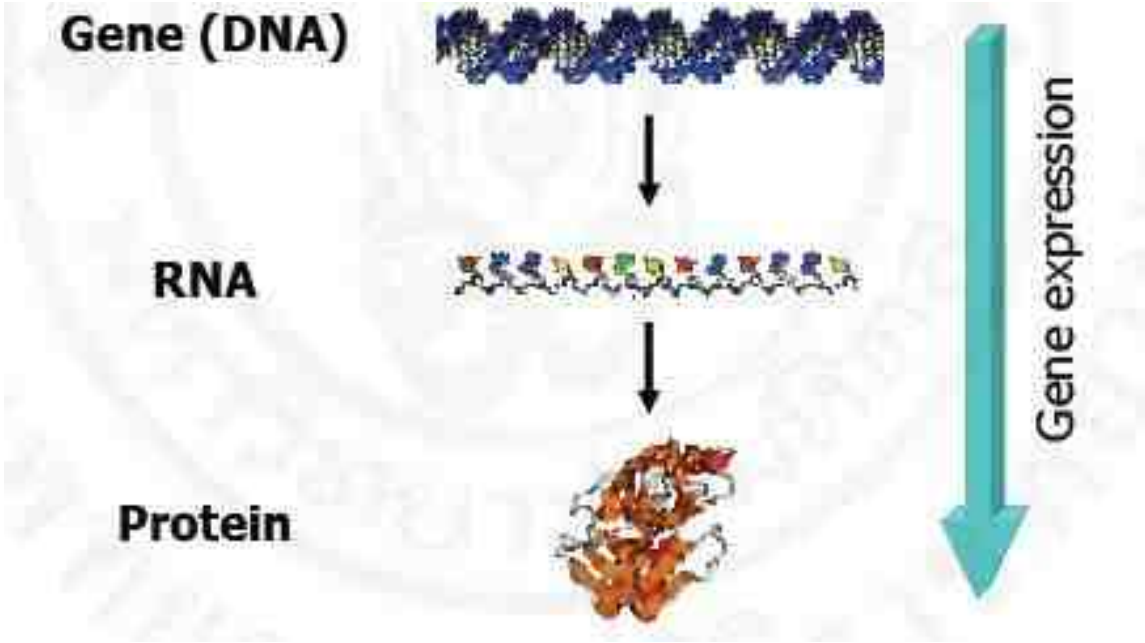




The four steps involved in translation are tRNA charging (the binding of amino acids to tRNAs), initiation, elongation, and termination. In this process, amino acids are linked together in the order specified by the mRNA to create a polypeptide chain. A number of **initiation**, **elongation**, and **release factors** take part in the process, and **energy** is supplied by ATP and GTP.

GENE EXPRESSION

- **Overall process by which the information encoded in a gene is converted into an observable phenotype (most commonly the production of a protein).**



GENE EXPRESSION

Gene expression can be either:

- **Constitutive- Genes that are actively transcribed (and translated) under all experimental conditions, at essentially all developmental stages, or in virtually all cells. Are *expressed at a fixed rate, irrespective to the cell condition*. Their structure is simpler. K/A housekeeping gene. e.g. in citric acid cycle.**

- **Inducible- A gene is expressed at higher level under the influence of some signal. These genes must be rapidly "switch on" or "off" depending on the temporary needs of the organism for their products.**

- **Repressed- Genes whose transcription and translation decreases in response to a repressing signal**

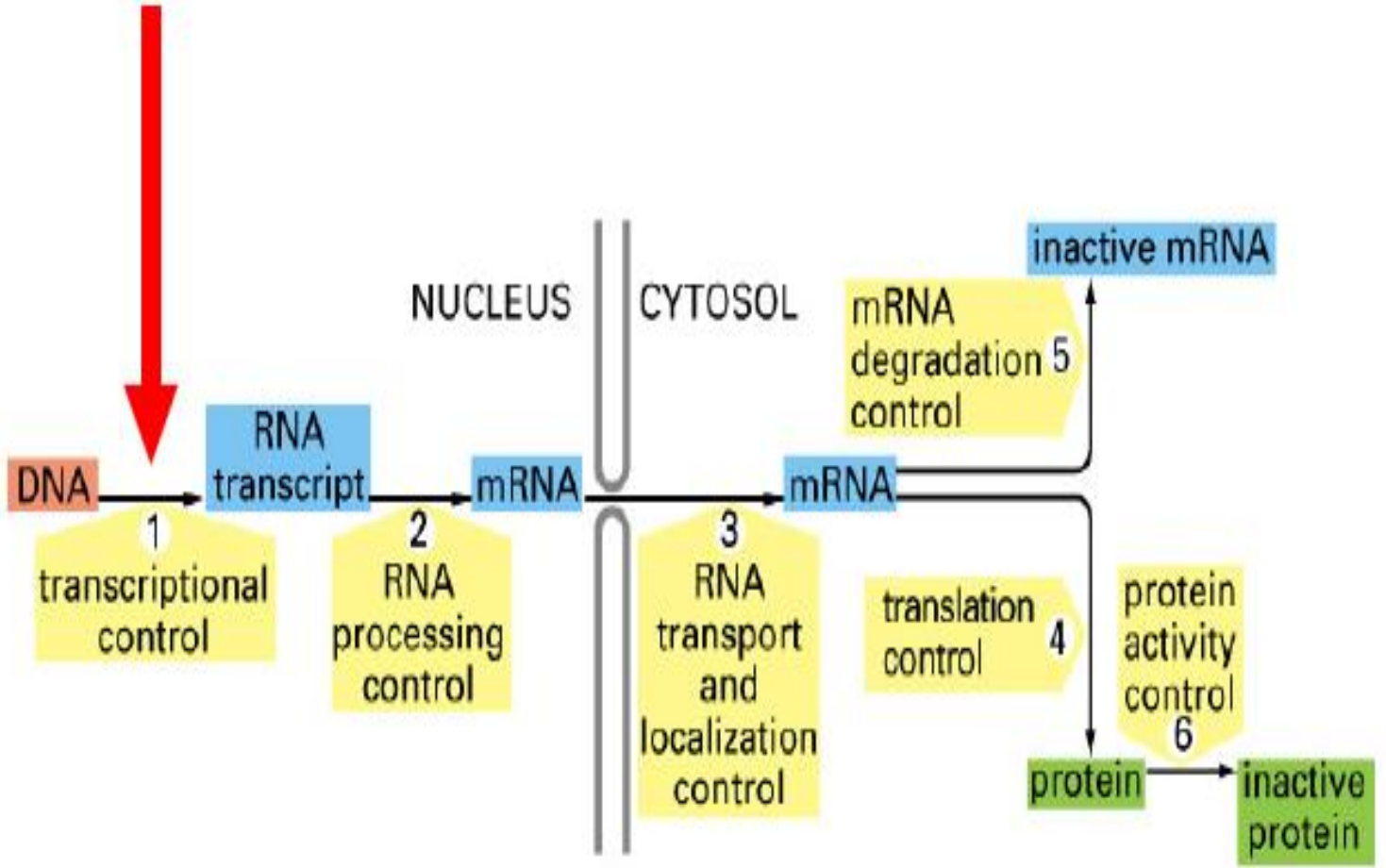
REGULATION OF GENE EXPRESSION

- **Virtually every cell in body contains a complete set of genes.**
- **But they are not all turned on in every tissue**
- **Each cell in body expresses only a small subset of genes at any time.**
- **During development different cells express different sets of genes in a precisely regulated fashion.**
- **Estimated number of human genes ~ 21,000 or more.**
- **At any given time, only a fraction of these genes (about 5,000 in most cells) are estimated to be active at a time.**

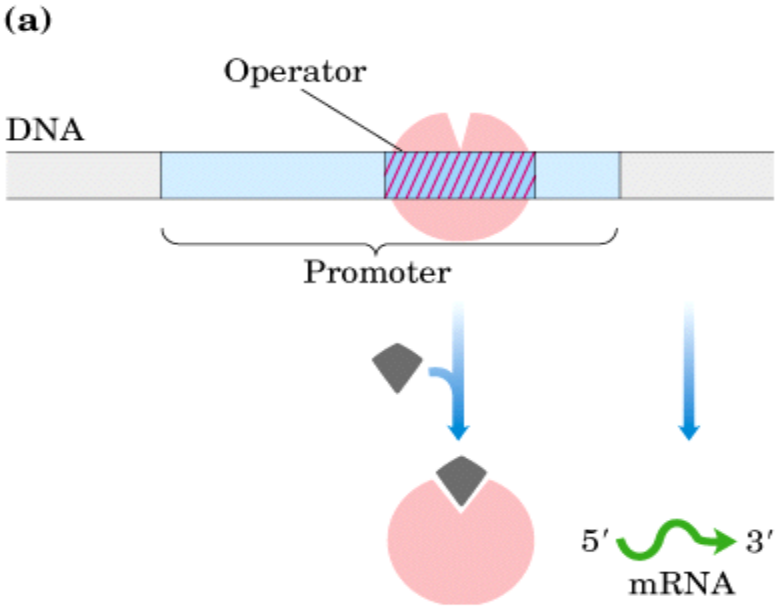
Genes are expressed through transcription and translation, but what decide which gene, when, where and how it is expressed ?

→ The expression of a gene (or a part of the genome) can be regulated in many ways depending on cell organization and needs of the organism.

Gene expression is regulated at many levels

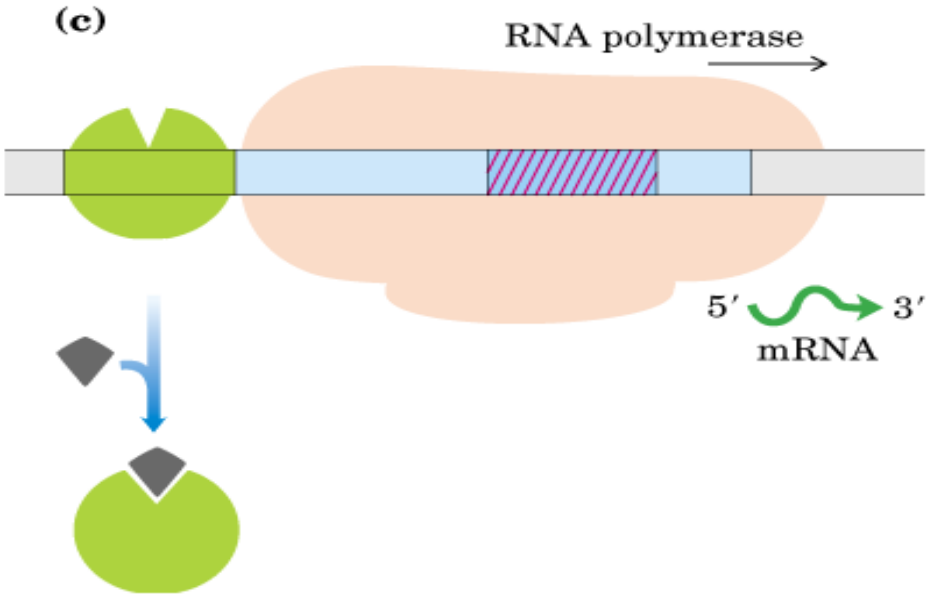


Negative regulation
(bound repressor inhibits transcription)



Molecular signal (◆) causes *dissociation* of regulatory protein from DNA

Positive regulation
(bound activator facilitates transcription)



Molecular signal (◆) causes *dissociation* of regulatory protein from DNA

EFFECT OF NUTRIENTS ON THE GENE EXPRESSION:

- The gene expression in response to changes in the nutritional status is one of the well established events in Prokaryotes.

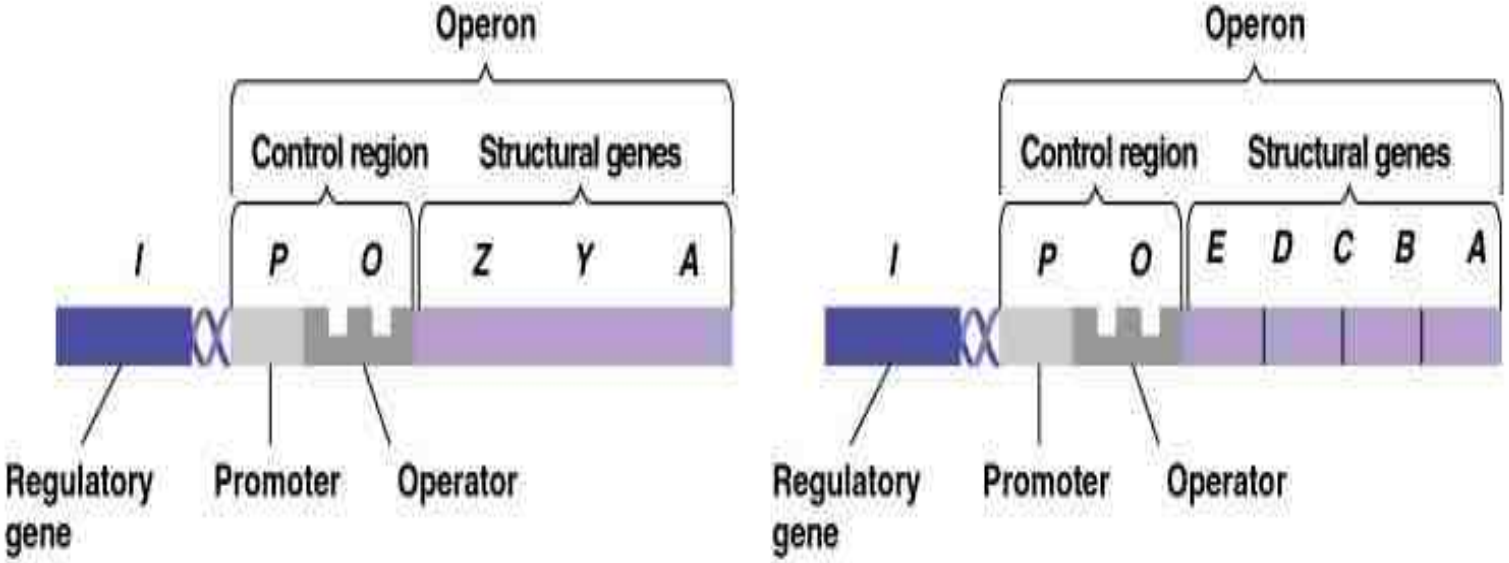
- Single cell organisms are able to adjust their metabolic capacity in response to variation in the nutrient supply in the culture medium e.g. **nutrient dependent regulation of the lactose, histidine and tryptophane operons by their respective substrates has been well characterized in bacteria.**

- In multi-cellular organism, the control of gene expression differs in many aspects from that operating in single cell organism, and involves complex interactions of hormonal, neural and nutritional factors.

TRP AND LAC OPERONS, EXAMPLE OF HOW GENE EXPRESSION RESPOND TO NUTRIENTS

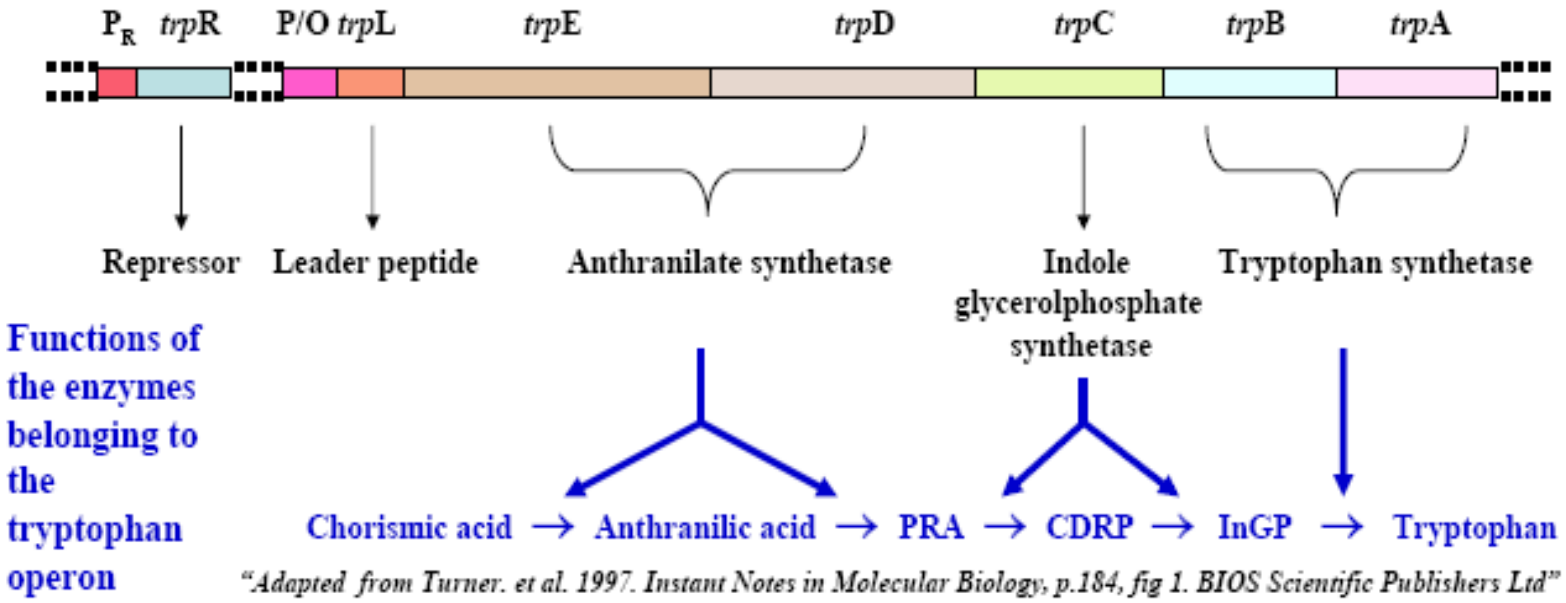
Operons

- **Its structure: Each Operon is consisted of few structural genes (cistrons) and some cis-acting element such as promoter (P) and operator (O).**
- **The control sites, promoter and operator genes are physically adjacent to the structural gene in the DNA.**
- ***the regulatory gene* can be quite far from the operon**



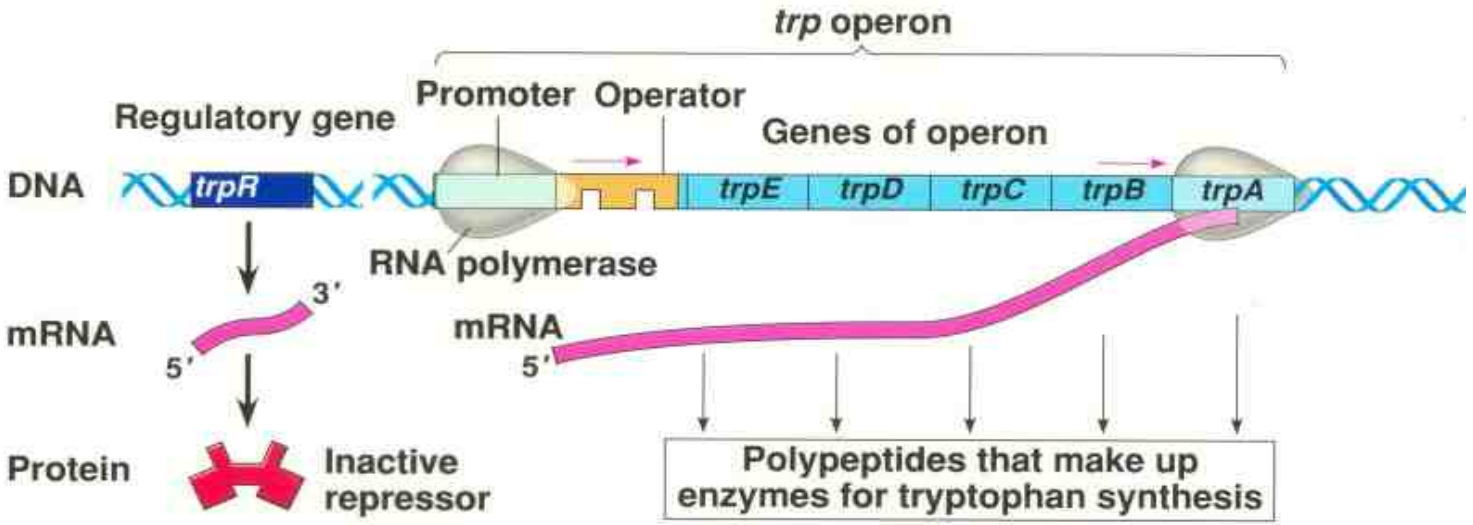
Structure of the operon. The operon consists of the promoter (*P*), and operator (*O*) sites, and structural genes which code for the protein. The operon is regulated by the product of the regulatory gene (*I*).

Tryptophan (*Trp*) Operon

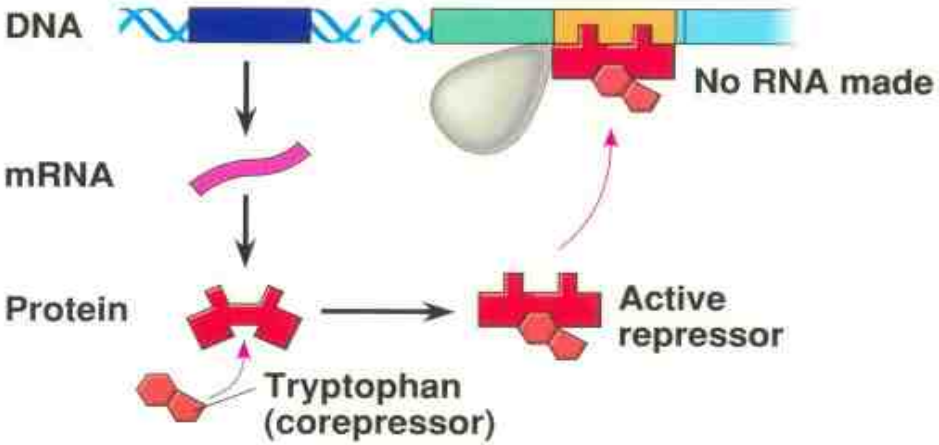


- **The tryptophan (*trp*) operon is a negative repressible operon, composed of :**
- **1. Regulatory sequences : the operator lies inside the promoter region**
- **2. Structural genes include *trp*E, D, C, B, A involved in the synthesis of tryptophan**
- **3. Regulatory gene : the coding sequence (*trp*R) for the repressor and its promoter (PR) is not adjacent to the operon.**
- **A special gene, *trp*L, encodes the Leader peptide which underlies a regulation mechanism called "attenuation"**

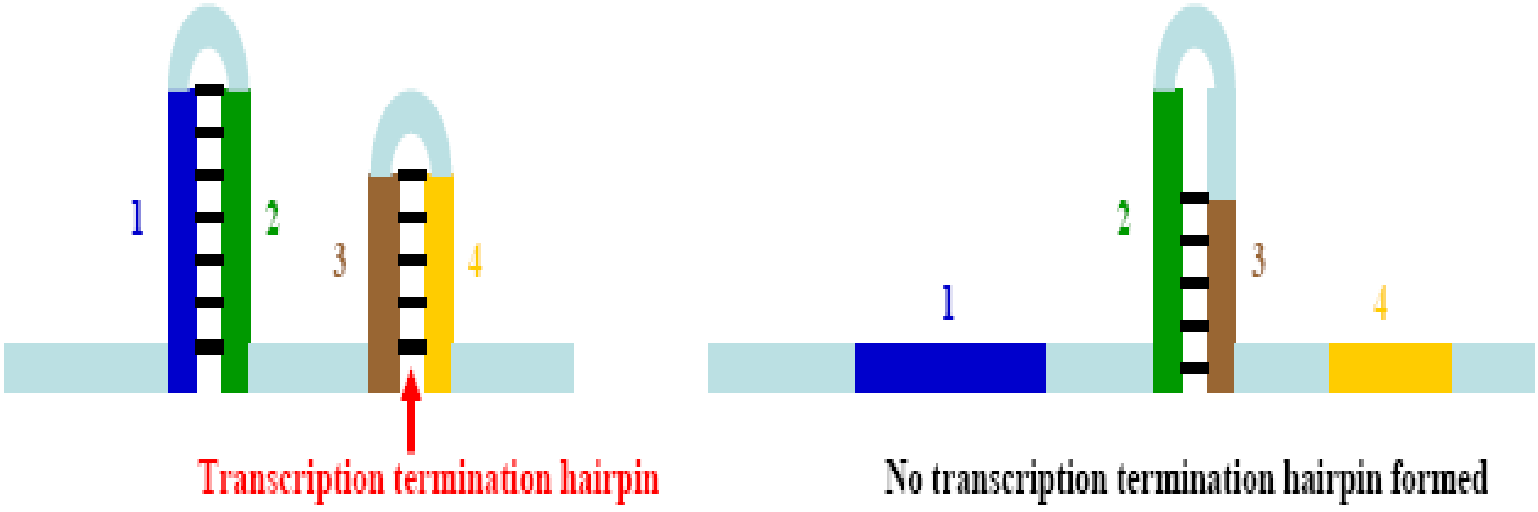
Figure 18.19 The *trp* operon: regulated synthesis of repressible enzymes

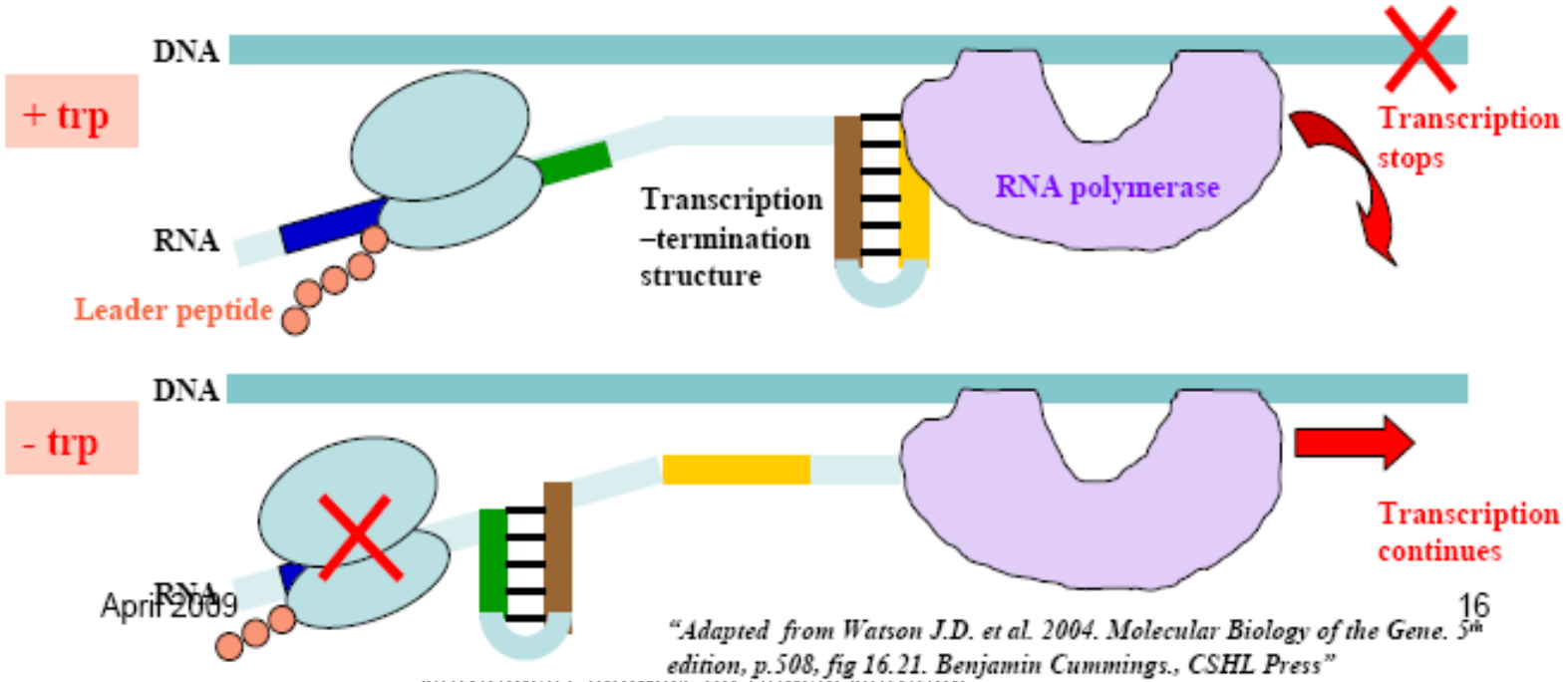


(a) Tryptophan absent, repressor inactive, operon on

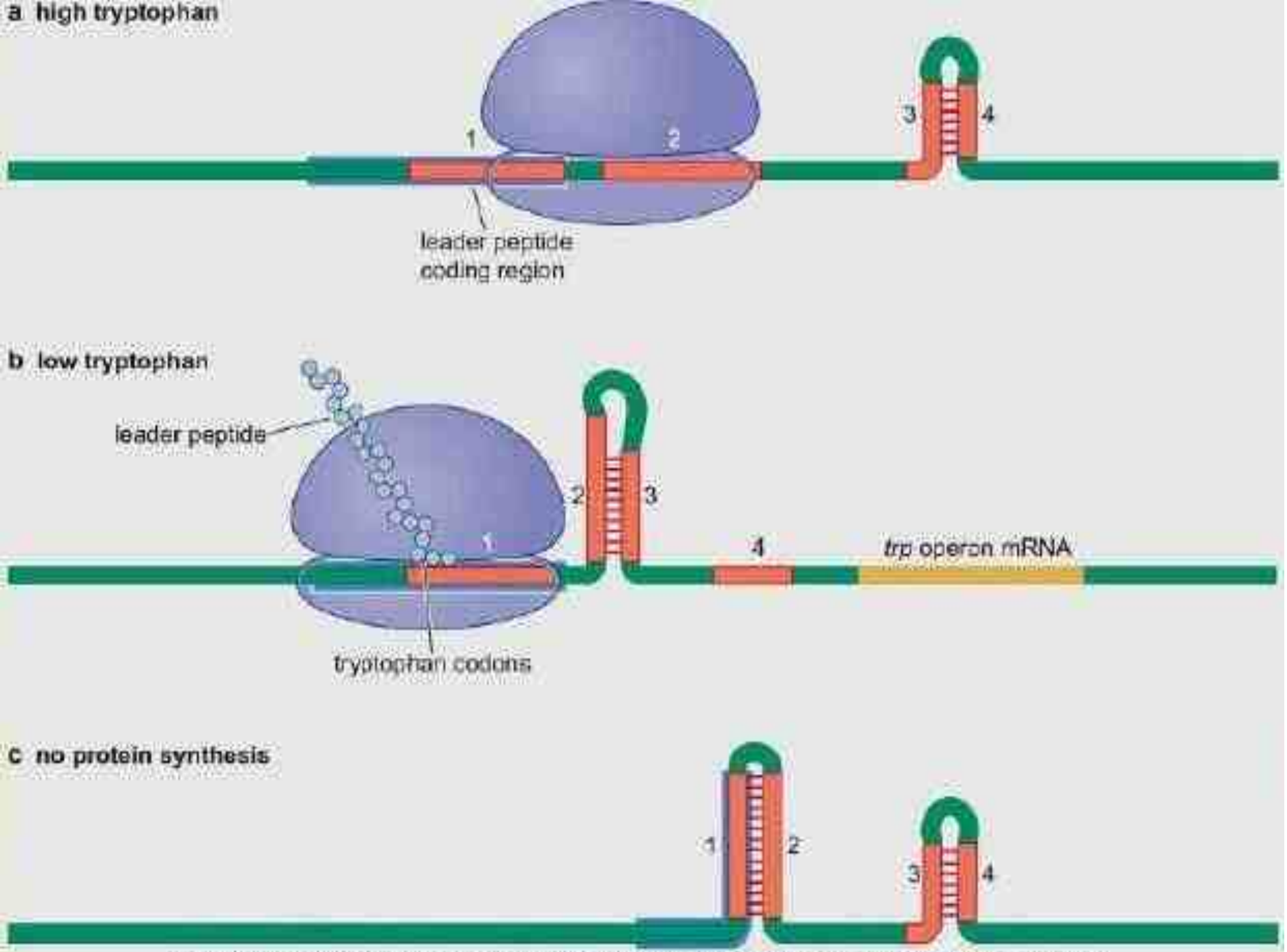


(b) Tryptophan present, repressor active, operon off

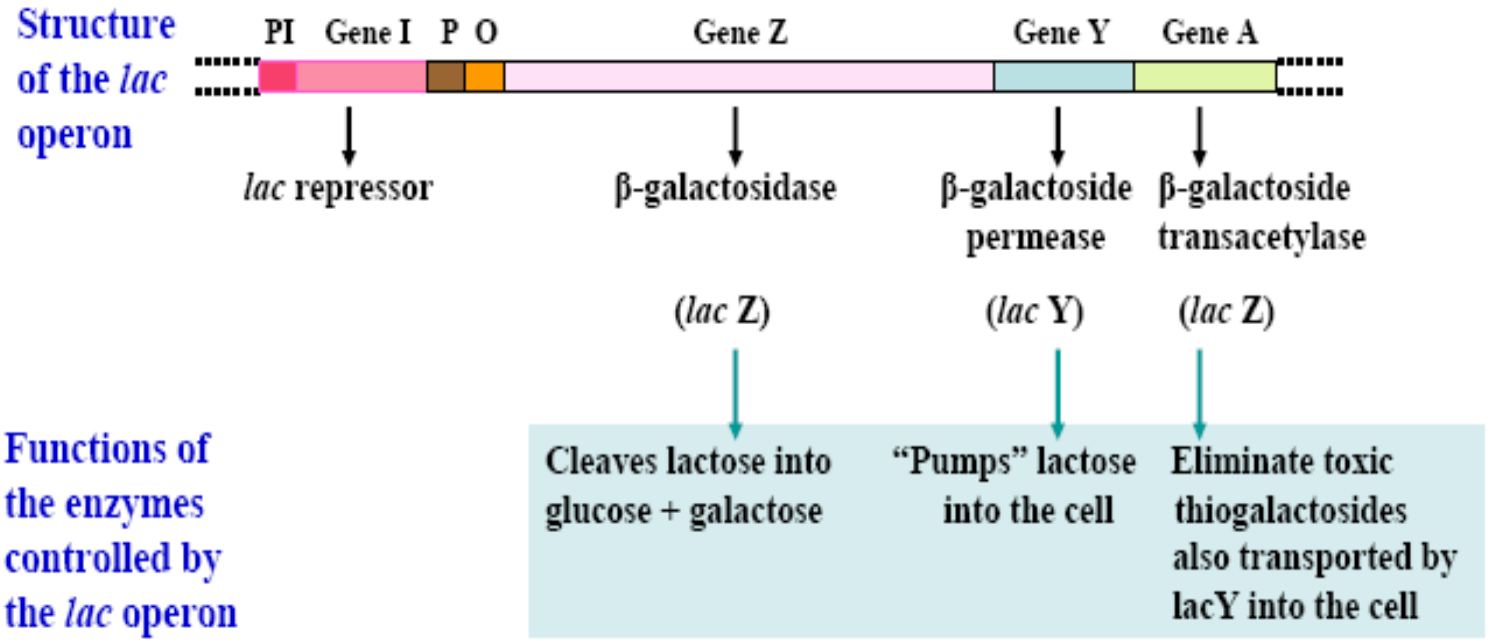




"Adapted from Watson J.D. et al. 2004. Molecular Biology of the Gene. 5th edition, p.508, fig 16.21. Benjamin Cummings., CSHL Press"



THE *LAC* OPERON



"Adapted from Turner. et al. 1997. Instant Notes in Molecular Biology, p.180, fig 1. BIOS Scientific Publishers Ltd"

The *lac* operon is a negative inducible operon, composed of :

1. Regulatory sequences : (1) the operator (O) which binds the repressor protein, (2) the promoter (P) containing two binding sites, one for the RNA polymerase, the other for CAP-cAMP complex

2. Structural genes involved in lactose metabolism : gene Z, Y and A

3. Regulator gene : the promoter (P_I) and the coding sequence (gene I)

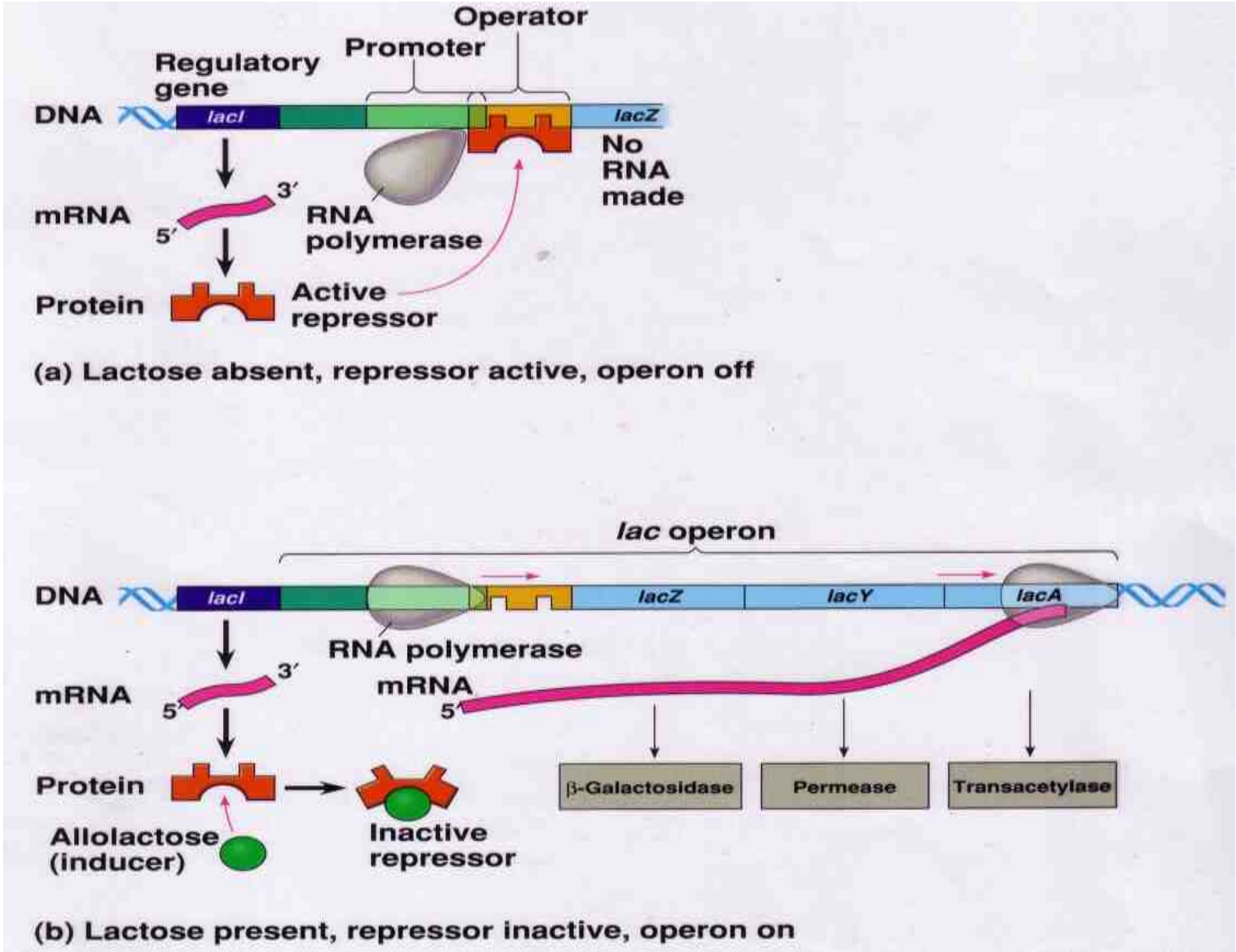
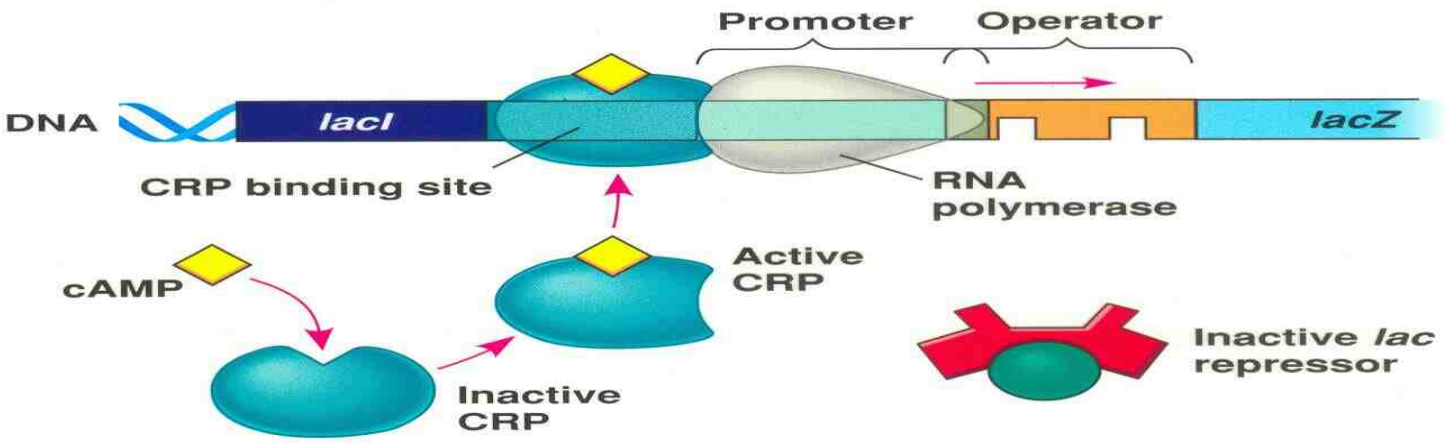
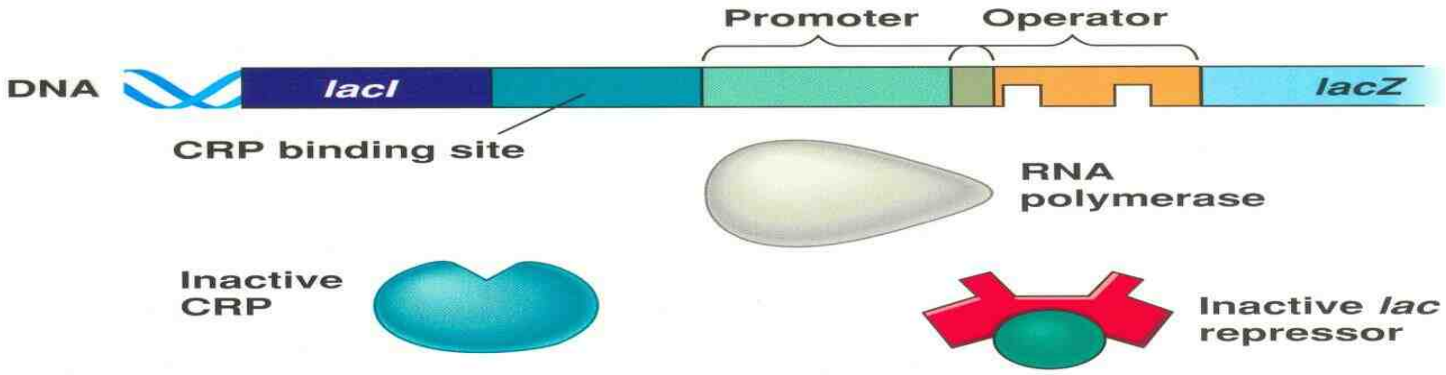

Operator
Promoter
Regulatory gene
DNA
lacI
lacZ
No RNA made
mRNA
3′
5′
RNA polymerase
Protein
Active repressor
(a) Lactose absent, repressor active, operon off
lac operon
DNA
lacI
lacZ
lacY
lacA
RNA polymerase
mRNA
3′
5′
mRNA
5′
Protein
β-Galactosidase
Permease
Transacetylase
Allolactose (inducer)
Inactive repressor
(b) Lactose present, repressor inactive, operon on

Figure 18.21 Positive control: cAMP receptor protein

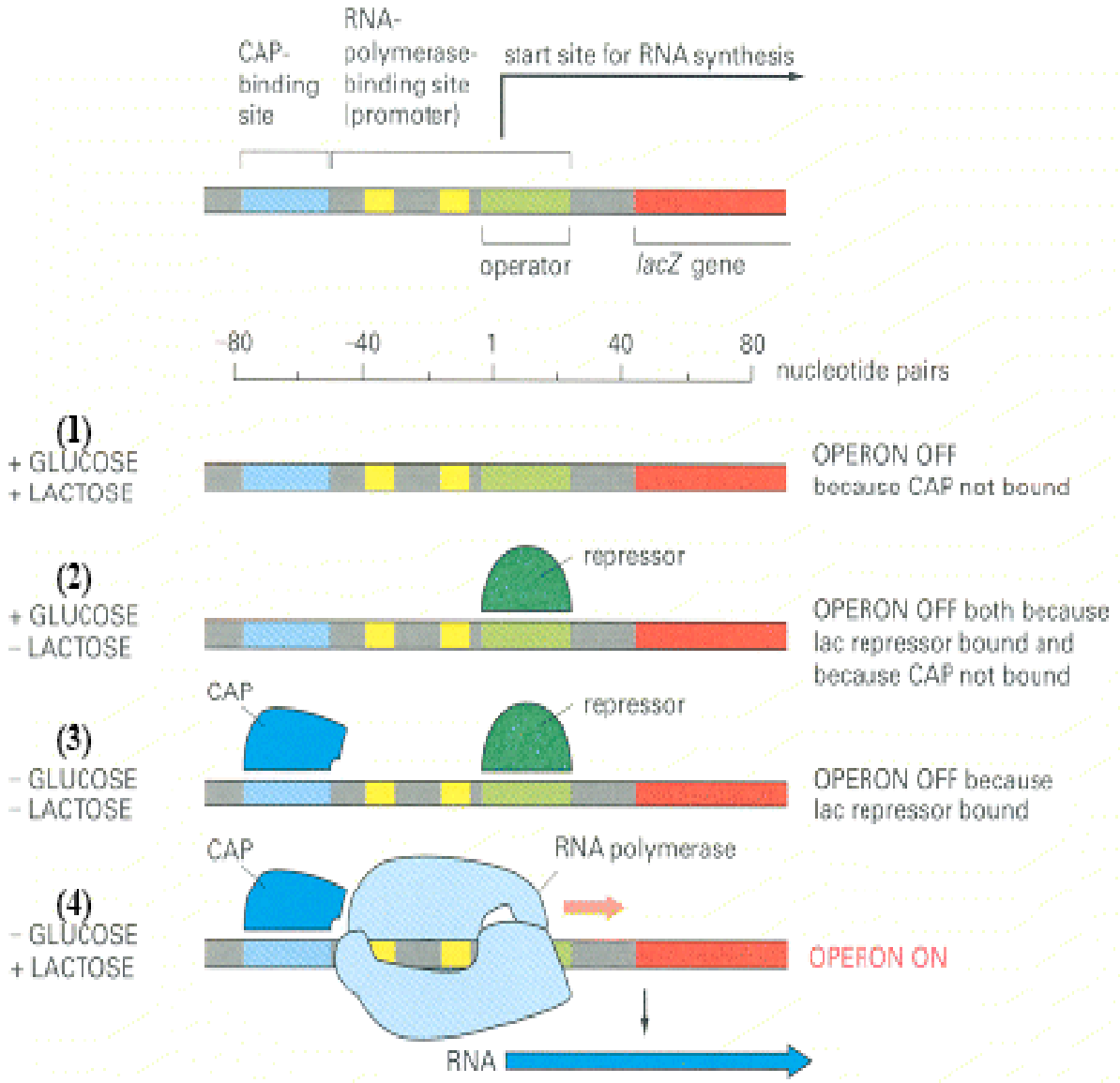


(a) Lactose present, glucose absent (cAMP level high): abundant *lac* mRNA synthesized



(b) Lactose present, glucose present (cAMP level low): little *lac* mRNA synthesized

THE *LAC* OPERON – A SUMMARY



(1) : Actually, a very little amount of *lac* mRNA is produced

(2), (3) : no *lac* mRNA produced

(4) : Abundant production of *lac* mRNA

Gene Regulation in Eukaryotes

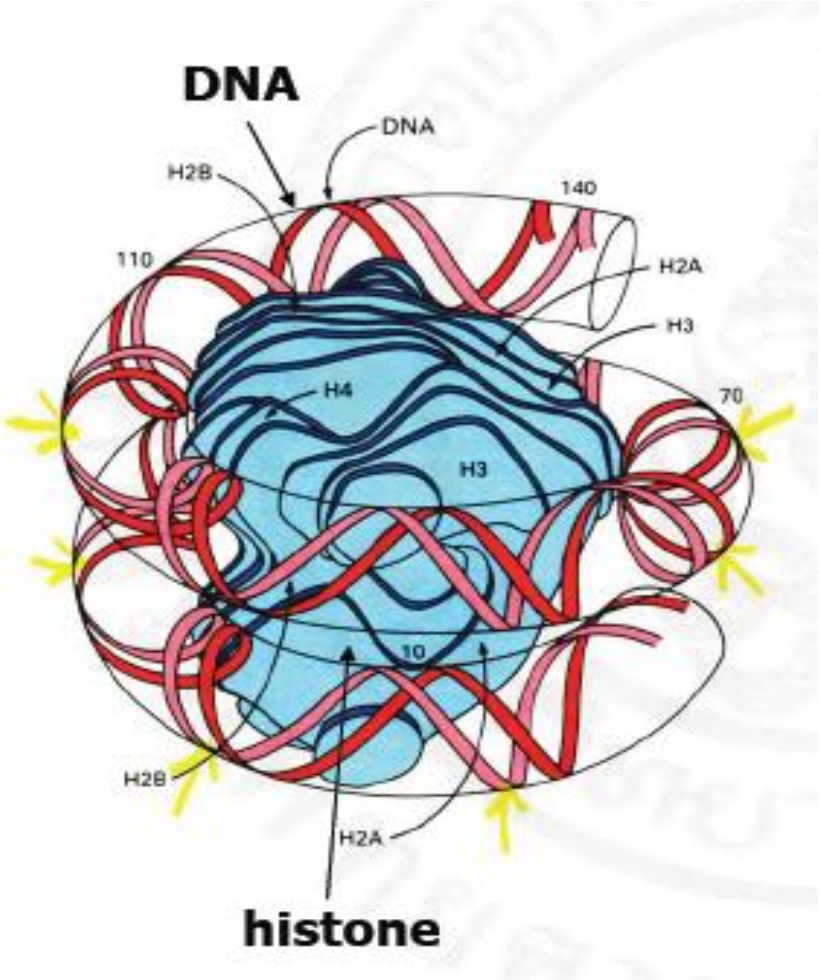
Control at DNA level

By Chromatin remodeling

By DNA methylation

By Gene amplification

REGULATION OF TRANSCRIPTION VIA CHROMATIN REMODELING



- **Eukaryotic Chromosomes: Linear, organized and complex – not just a DNA molecule**
- **Nucleosome consists of a histone core octamer (two subunits of H2A, H2B, H3, and H4) and 146 bp of DNA wrapped 1.75 turns around the core.**
- **Histones are small (102 to 135 amino acids) proteins that contain a very high proportion of positively charged amino acids such as lysine and arginine. Thus, they have high affinity for DNA (negatively charged molecules).**

Levels of Packaging

1 **Nucleosome**

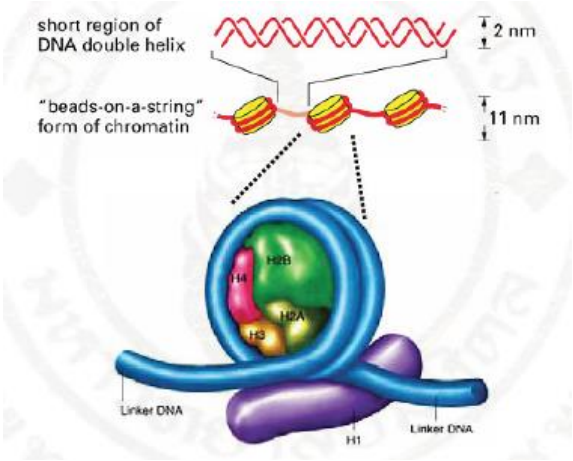
2 **30 nm fiber/Solenoid**

3 **Loops**

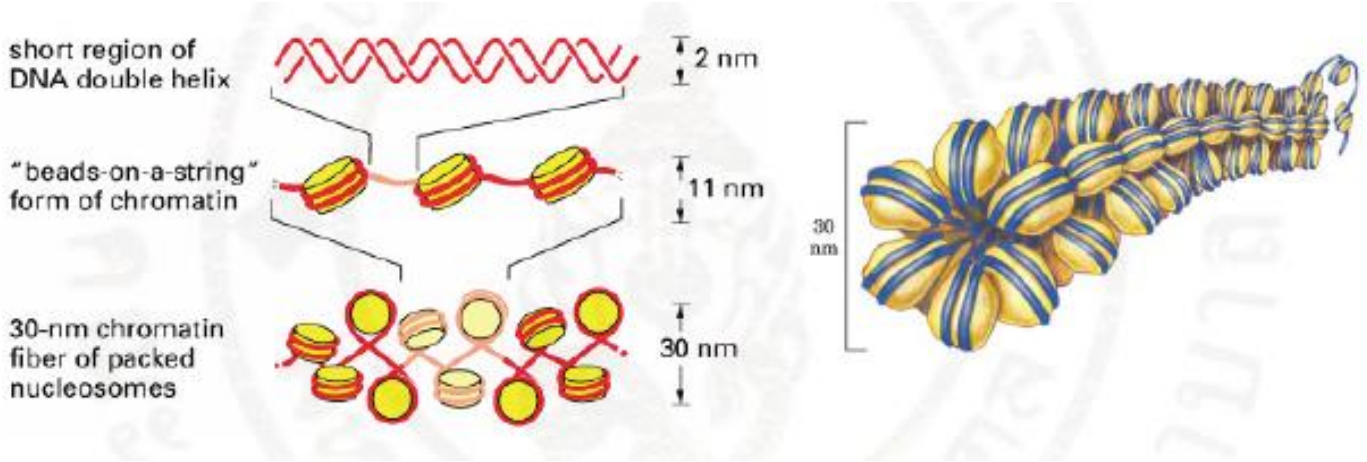
4 **Rosettes**

5 **Chromosome**

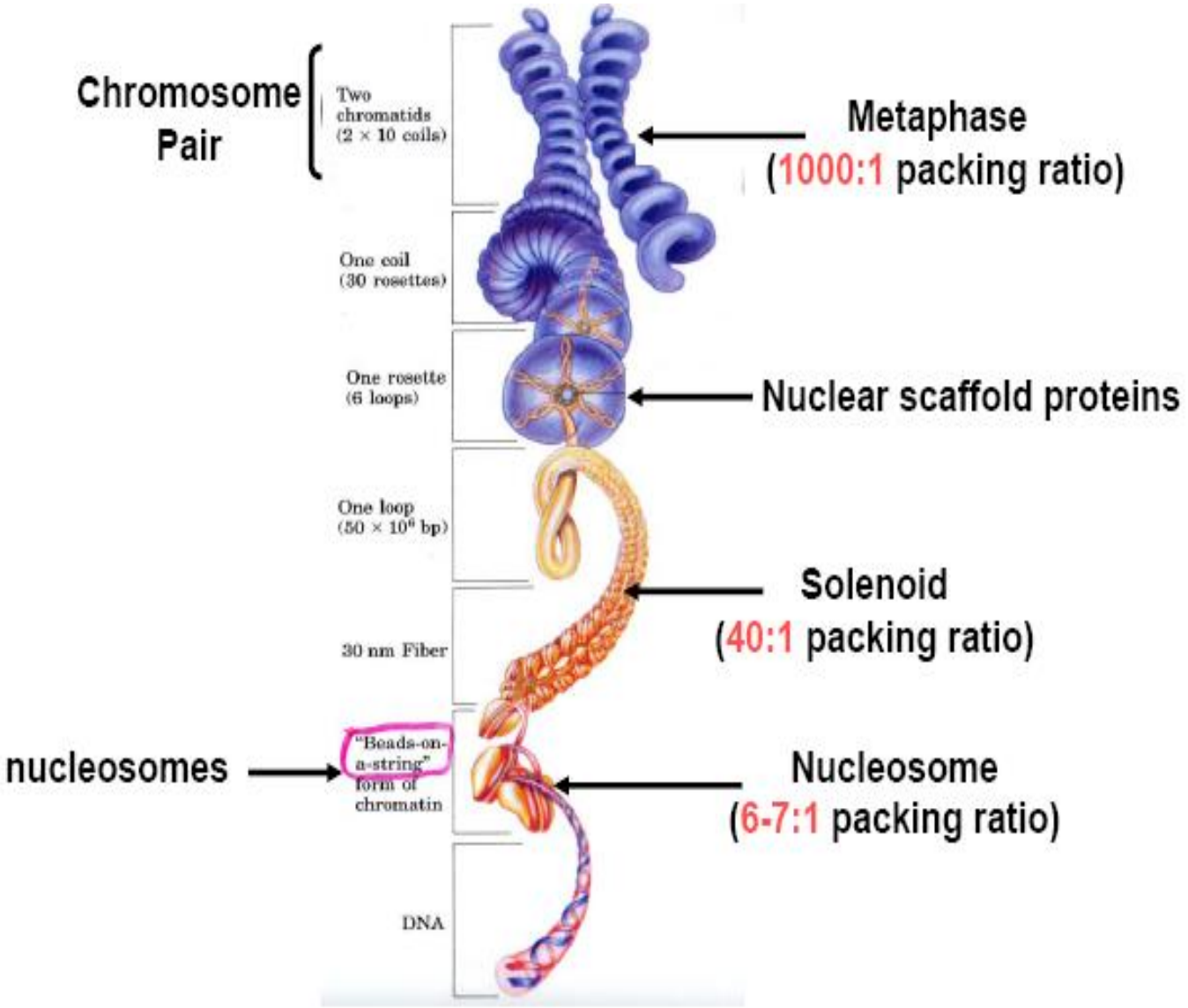
Nucleosome - the most fundamental unit of packaging



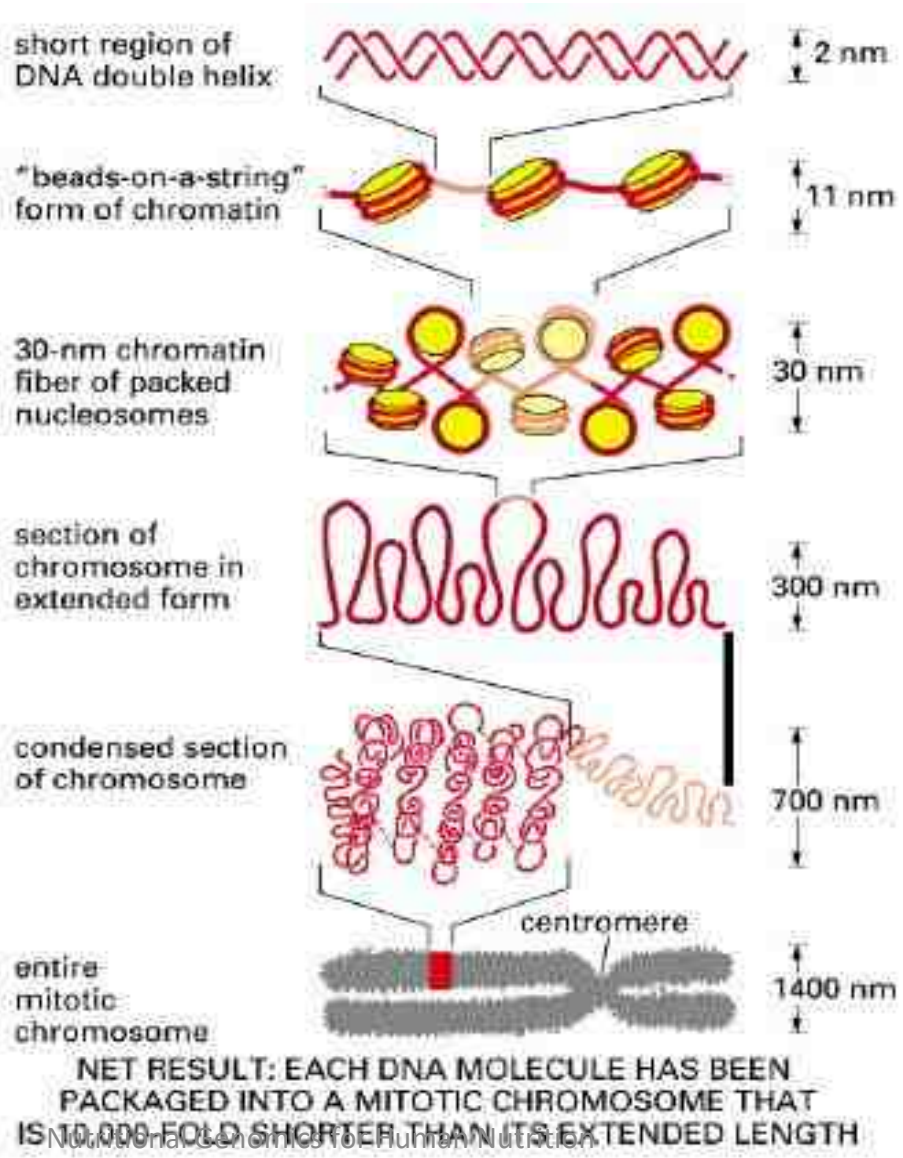
- **Nucleosome is consisted of a histone core octamer (two subunits of H2A, H2B, H3, and H4) and 146 bp of DNA wrapped 1.75 turns around the core.**


short region of
DNA double helix
2 nm
"beads-on-a-string"
form of chromatin
11 nm
30-nm chromatin
fiber of packed
nucleosomes
30 nm
30
nm

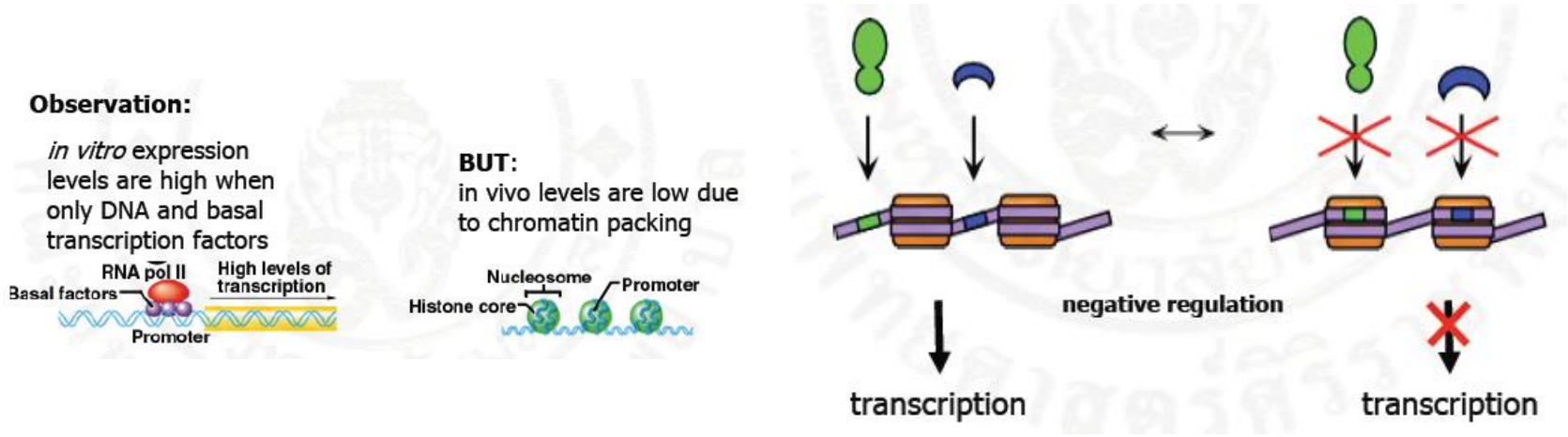
Levels of Complexity of Chromosome Structure



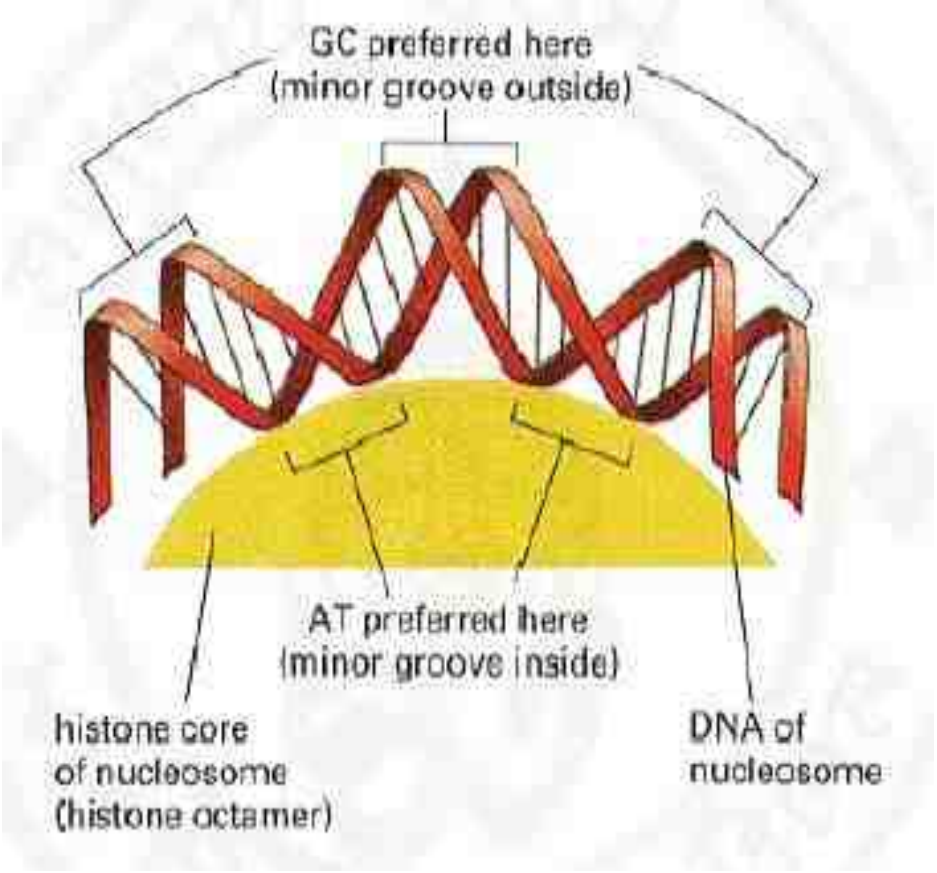
Levels of Complexity of Chromosome Structure



Transcription factor binding to DNA is inhibited within nucleosomes

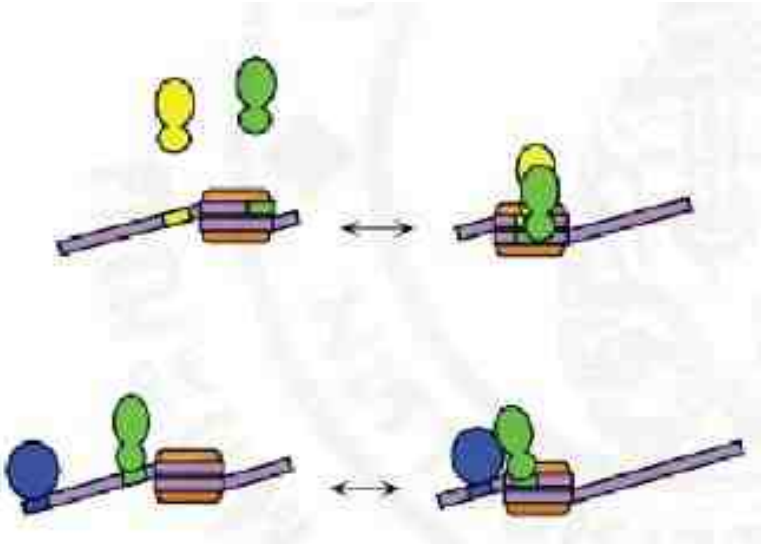


- **Affinity of transcription factor for its binding site on DNA is decreased when the DNA is reconstituted into nucleosomes.**
- **The packaging of DNA into nucleosomes is generally regarded as a block of transcription, presumably because the nucleosome interferes with binding of activators or elements of the transcription machinery.**



- **Minor groove faces protein surface every 10 bp and touches arginine side chains.**
- **Major grooves are accessible to sequence-specific regulatory proteins.**

Positive regulation by nucleosomes

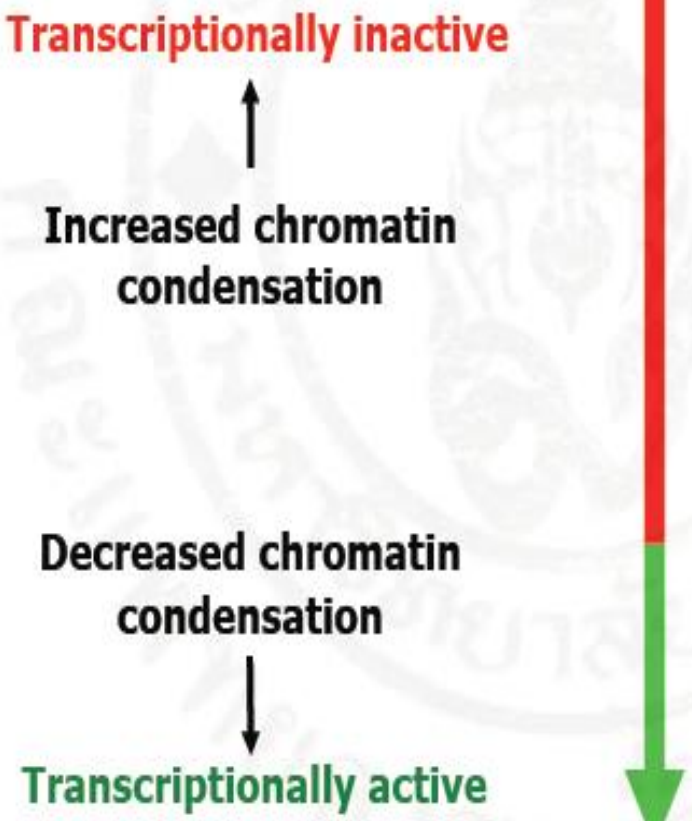


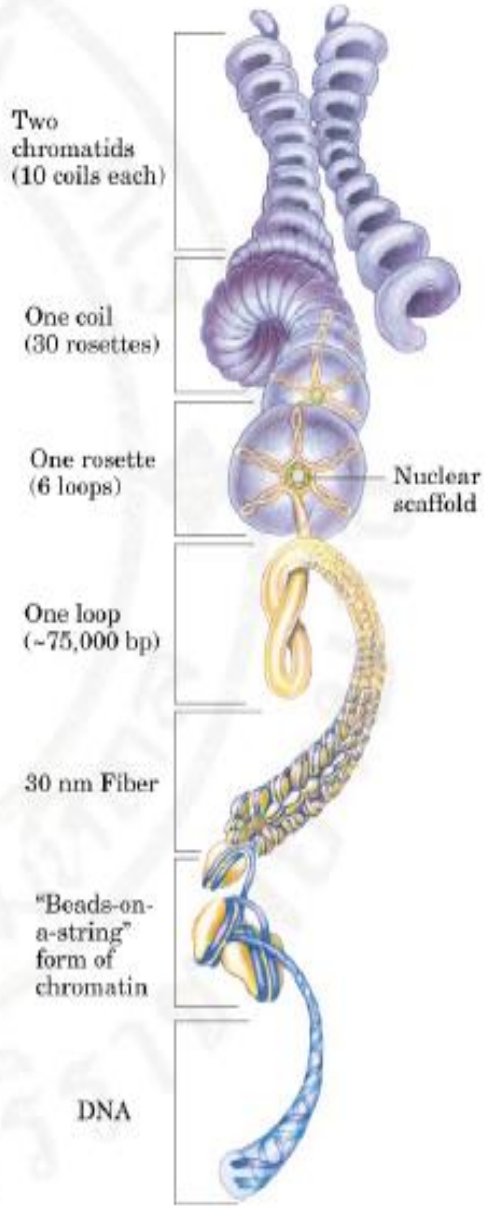
- **Sometimes transcription can be positively regulated by packaging of DNA into nucleosomes.**

(a) Nucleosomes may position independent activator binding sites to permit synergistic binding of activators.

(b) Nucleosomes may alter the orientation or distance between factors, thereby stimulating interactions required for transcription.

- **Heterochromatin - Most condensed form**
  1. **Replicated Late**
  2. **Not Transcribed**
- **Euchromatin - most relaxed form**
  1. **Replicated early**
  2. **Easily Transcribed**





How can chromatin structures be modulated?

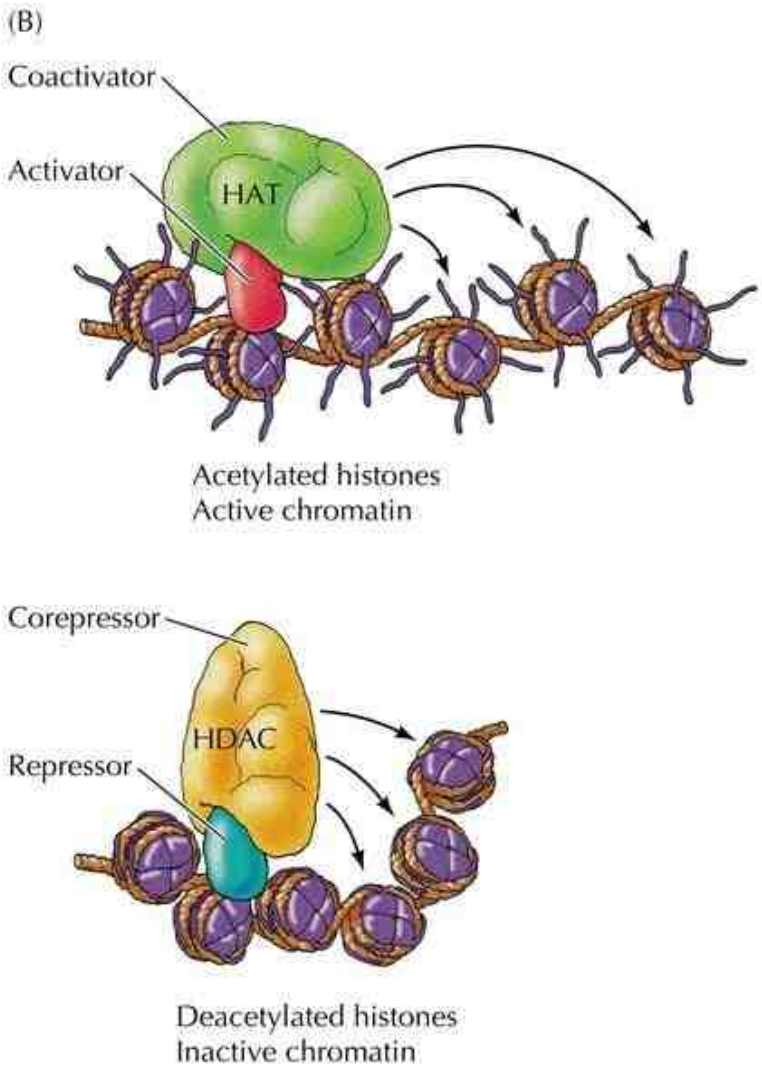
1. Histone modifying enzymes
2. Chromatin remodeling complexes

1. Histone modifying enzymes

- Two key enzymes involving in chromatin structure regulation

a. Histone acetyl transferase (HAT): Acetylation by HATs and coactivators leads to euchromatin formation

b. Histone deacetylase (HDAC) Methylation by HDACs and corepressors leads to heterochromatin formation



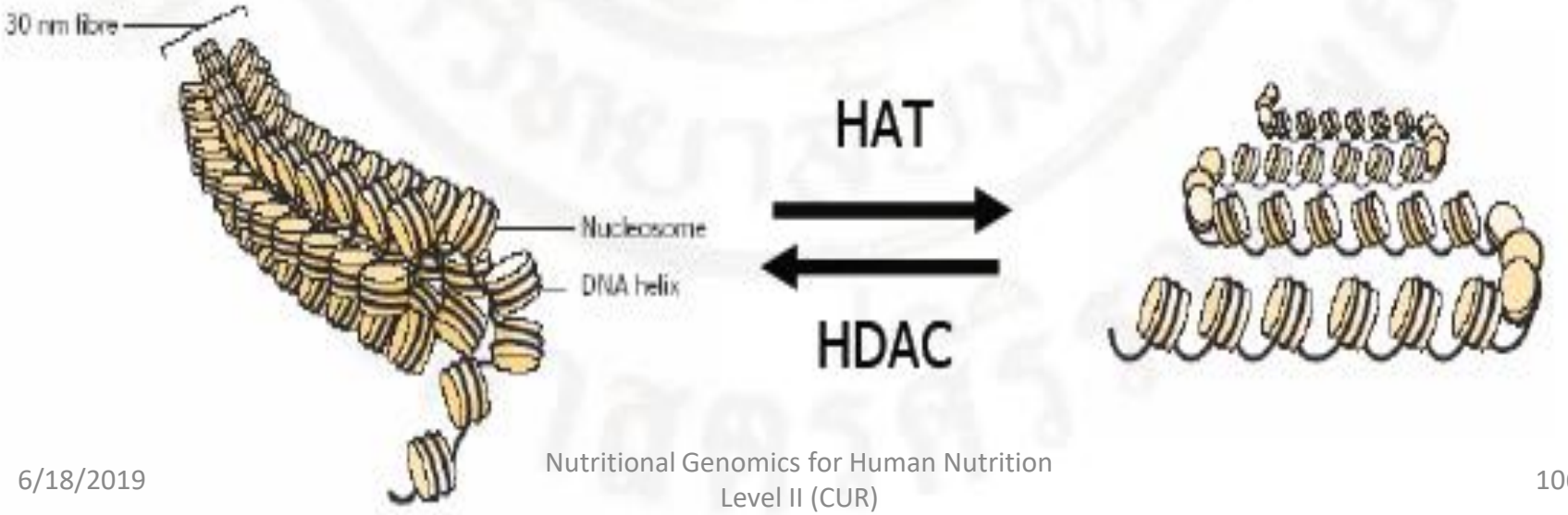
- **Histones have Lys-rich tails whose interactions mediate histone-histone interactions in chromatin**
- **Histone Acetyl transfer complexes acetylate these Lys.**
- **HATs Weaken histone-histone & histone-DNA interactions to expose DNA for transcription**
- **Tails also Methylated & Phosphorylated.**

• Histone modifying enzymes (e.g. histone acetylase/deacetylase) can cause a change in chromatin structures.

Acetylated histone → Decreased chromatin condensation

- decondenses 30nm chromatin fibers
- increases access of transcription factors to DNA in nucleosomes.

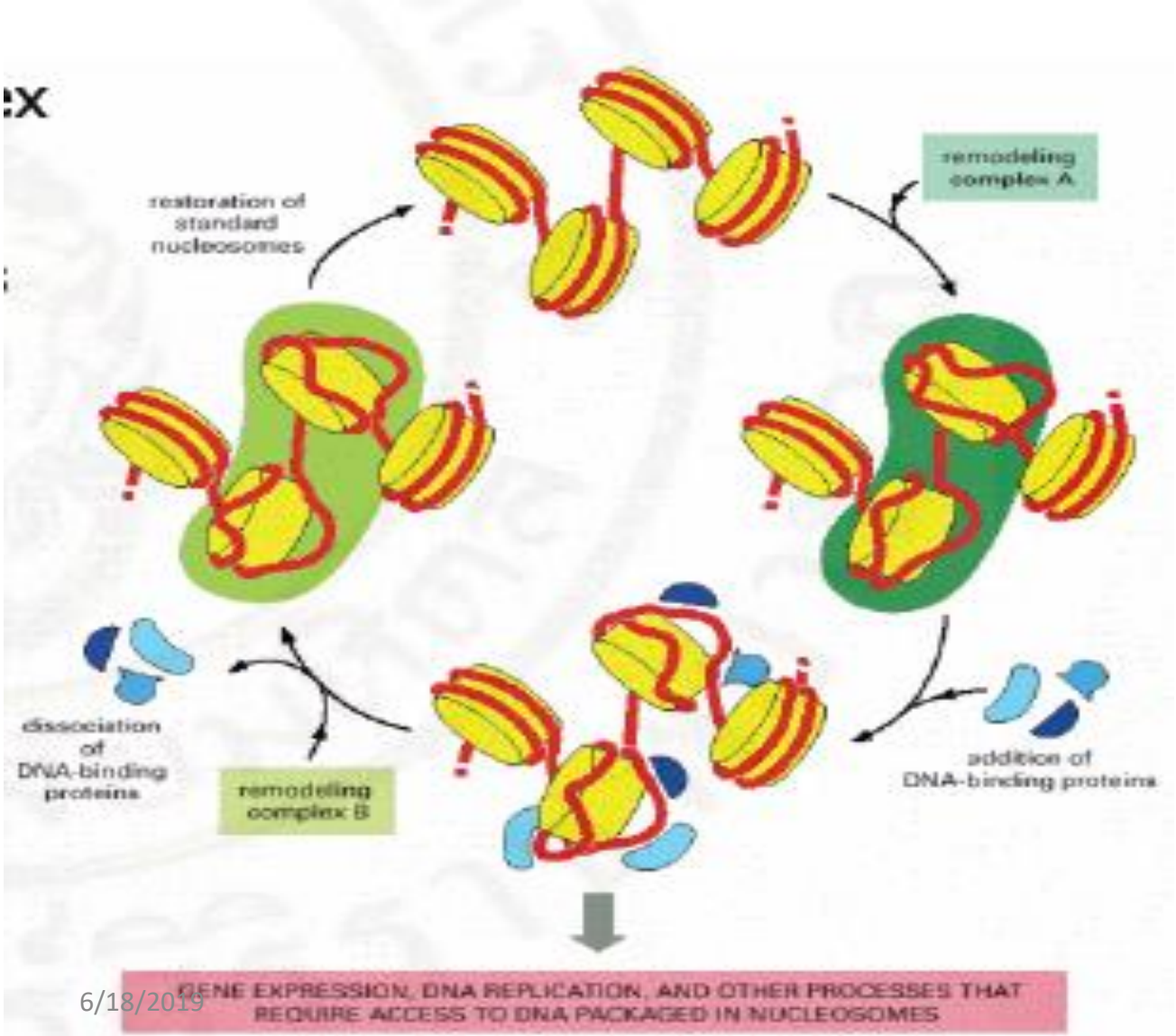
Deacetylated histone → Increased chromatin condensation



2. Chromatin remodeling complexes

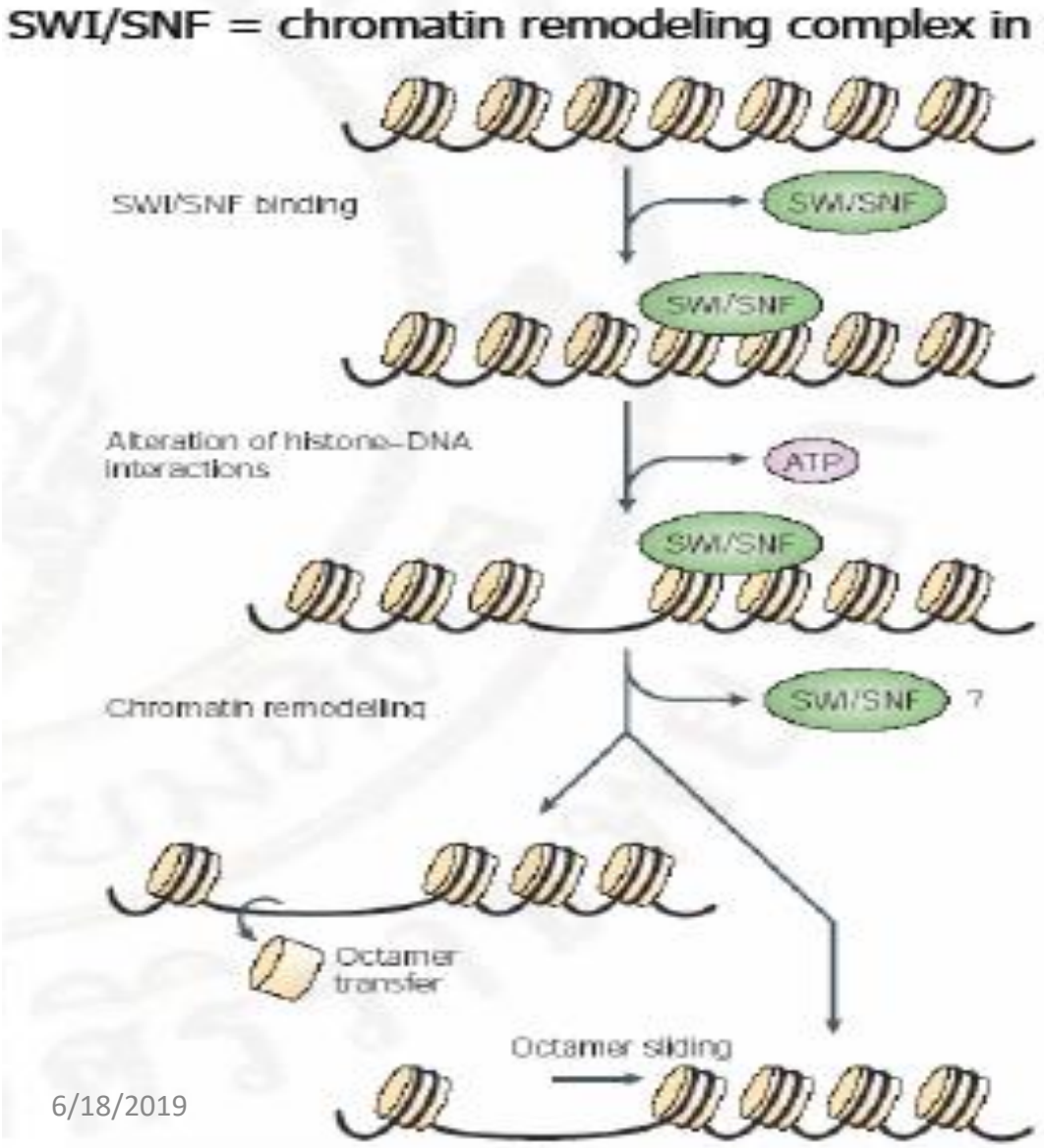
- **Polypeptide complexes that use the energy of ATP hydrolysis to compact or relax the chromatin structure.**
- **There are several different types of chromatin remodeling complexes.**
- **Most of them are large, multi-subunit complexes (>10 subunits).**

Actions of chromatin remodeling complex on chromatin structure

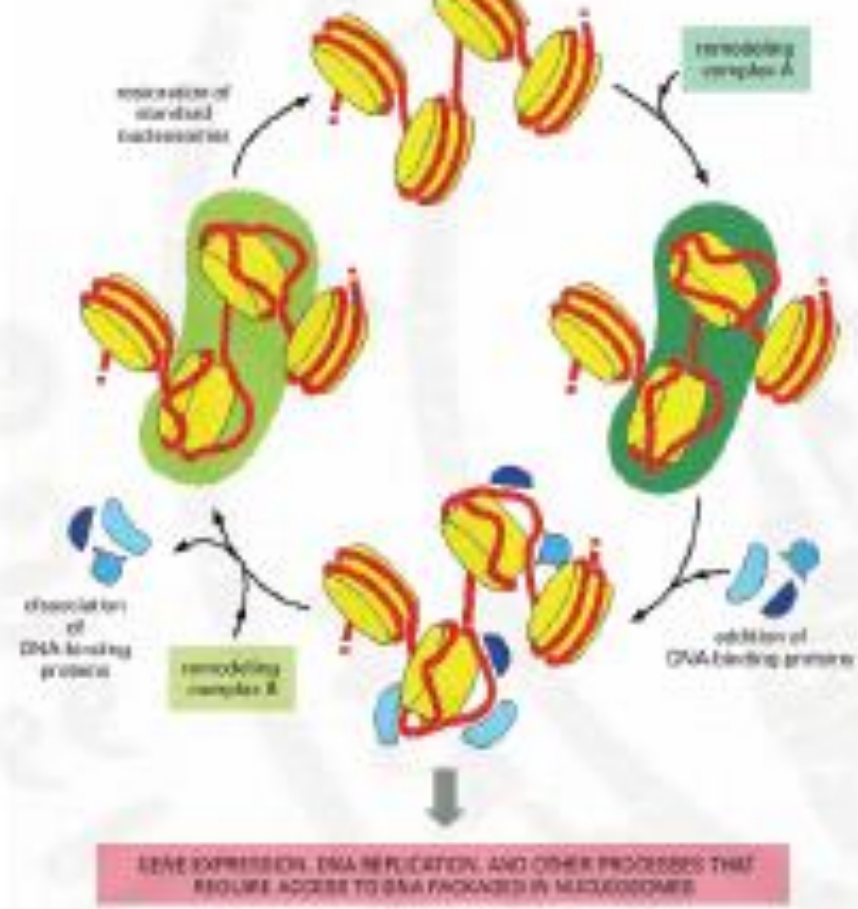


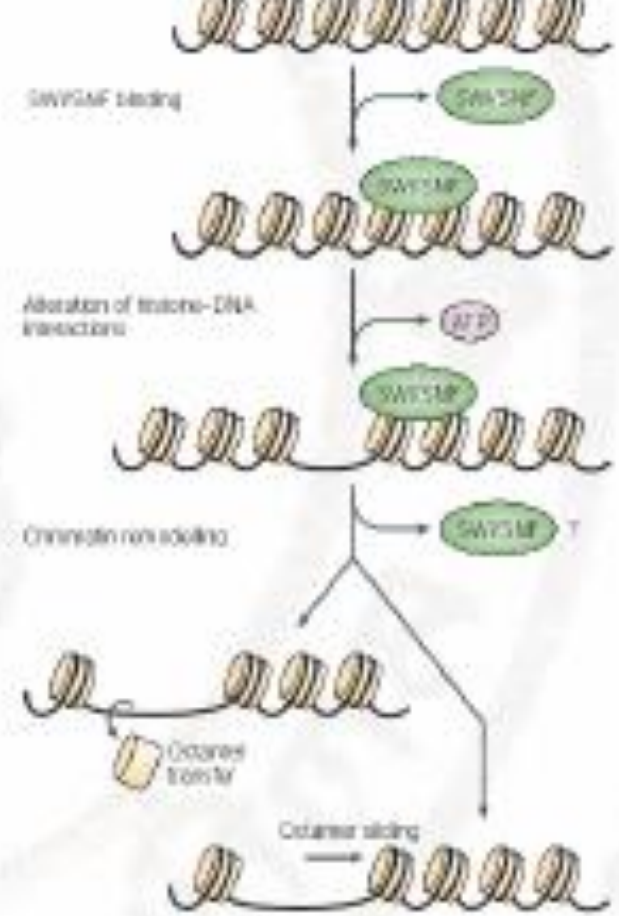
1. **Change the structure of nucleosomes temporarily so that DNA becomes less tightly bound to the histone core.**

Actions of chromatin remodeling complex on chromatin structure



2. Catalyze changes in the positions of nucleosomes along DNA.





- **Remodeling of chromosome permits ready access to nucleosomal DNA by other proteins in the cell, particularly those involved in gene expression, DNA replication, and repair.**

Mutations

- A **stable change** of a gene such that the changed condition is **inherited** by offspring cells.
- The altering of one DNA sequence to another .
- **The rate of naturally** occurring mutations, is quite low and varies widely between individual genes and organisms. Mutational changes are passed from generation to generation as the cells divide. This is known as **traditional mutagenesis**.

❖ Mutations within DNA generally fall into one of two categories.

- **Point mutations**
- **Frame shift mutations**

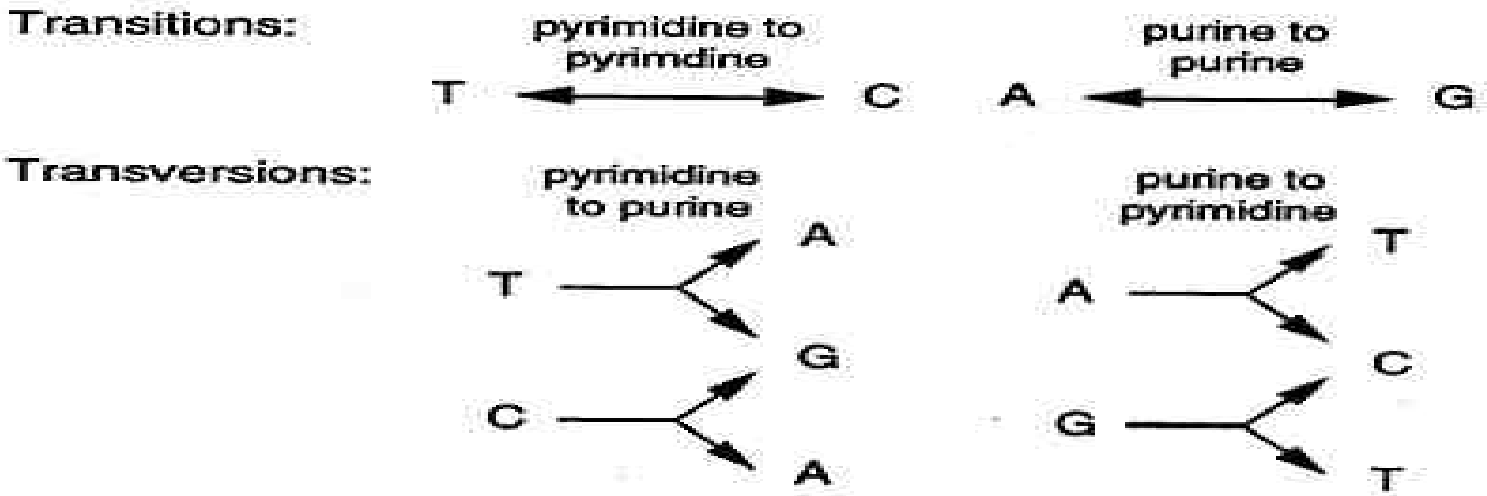
POINT MUTATION

- A point mutation is a type of mutation that causes the **replacement of a single base nucleotide with another nucleotide of the genetic material**. It is of two types:

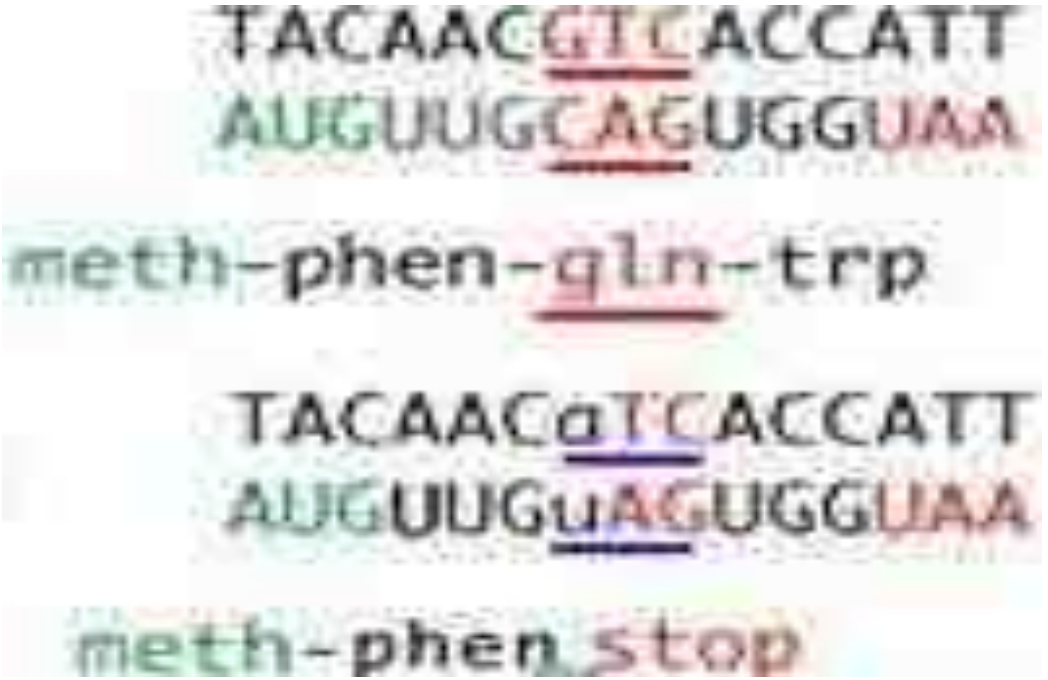
1) Transition mutations

2) Transversions

- **Transition mutations**:-The replacement of a purine base with another purine or replacement of a pyrimidine with another pyrimidine.
- **Transversions**: - replacement of a purine with a pyrimidine or vice versa. Transition mutations are more frequent than transversion mutations.

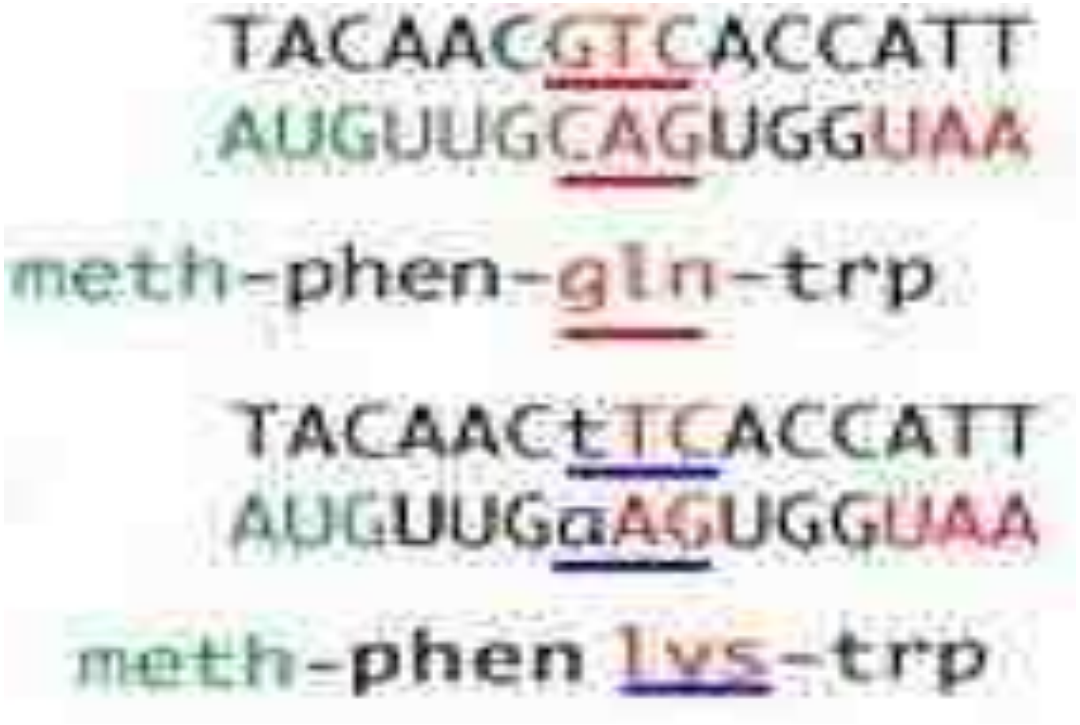


- Point mutations can also be categorized functionally:
  - Nonsense mutations
  - Mis-sense mutations
  - Silent mutations
- A mutation results in a formation of a **new stop codon.** Therefore translation is stopped prematurely and a shortened protein is made.



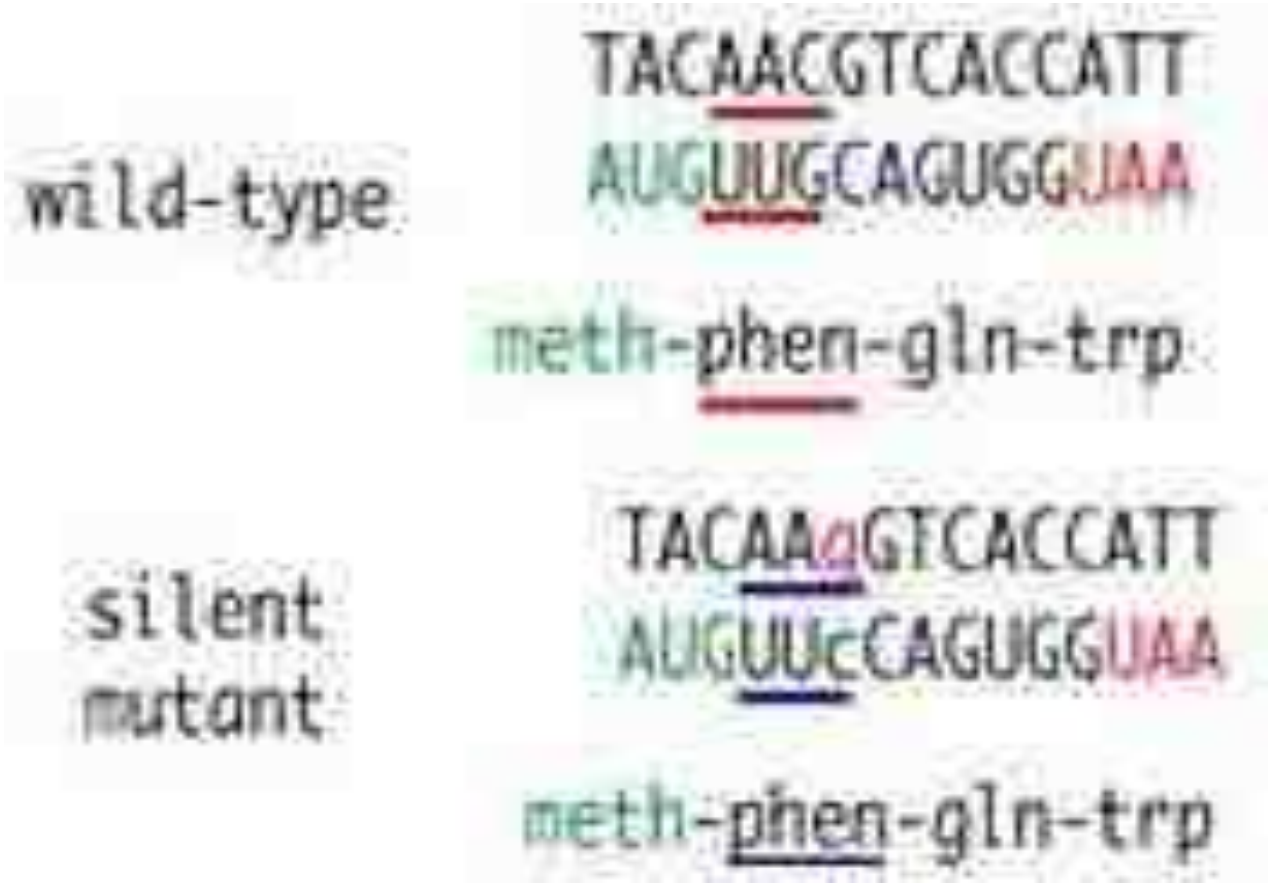
Mis sense mutation

- A mutation results a change in an **amino acid**, where the new amino acids has a different property than the old amino acid.



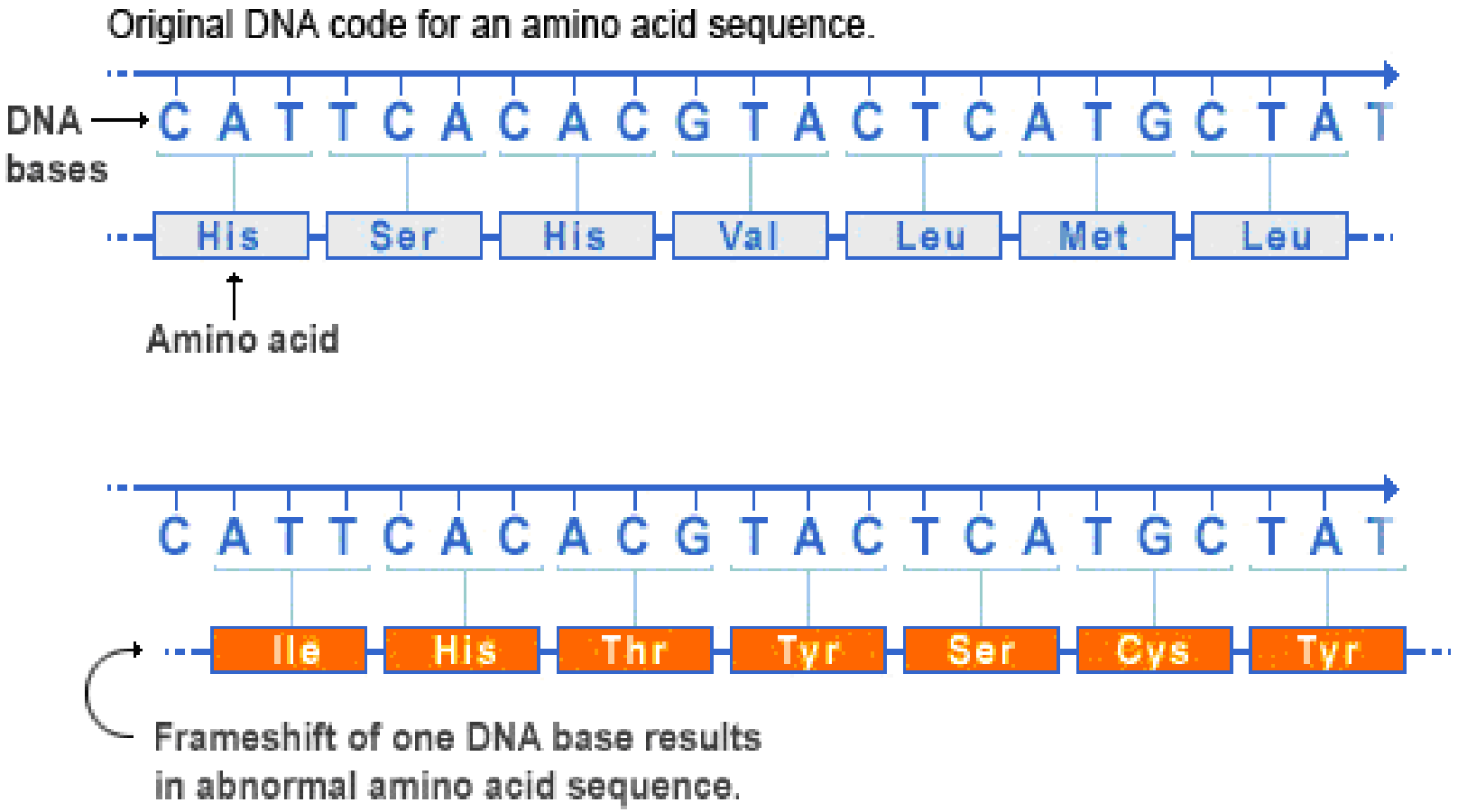
Silent mutation

- A change in a base pair does not result in a change of amino acid.



Frame shift mutation

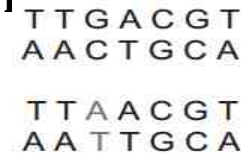
- Results due to **deletion or insertion** of nucleotides in DNA structure.
- During translation, it shifts the reading frame beyond the mutation thus forms a different set of codons.
- As the result of this lot of amino acids in sequence are changed..



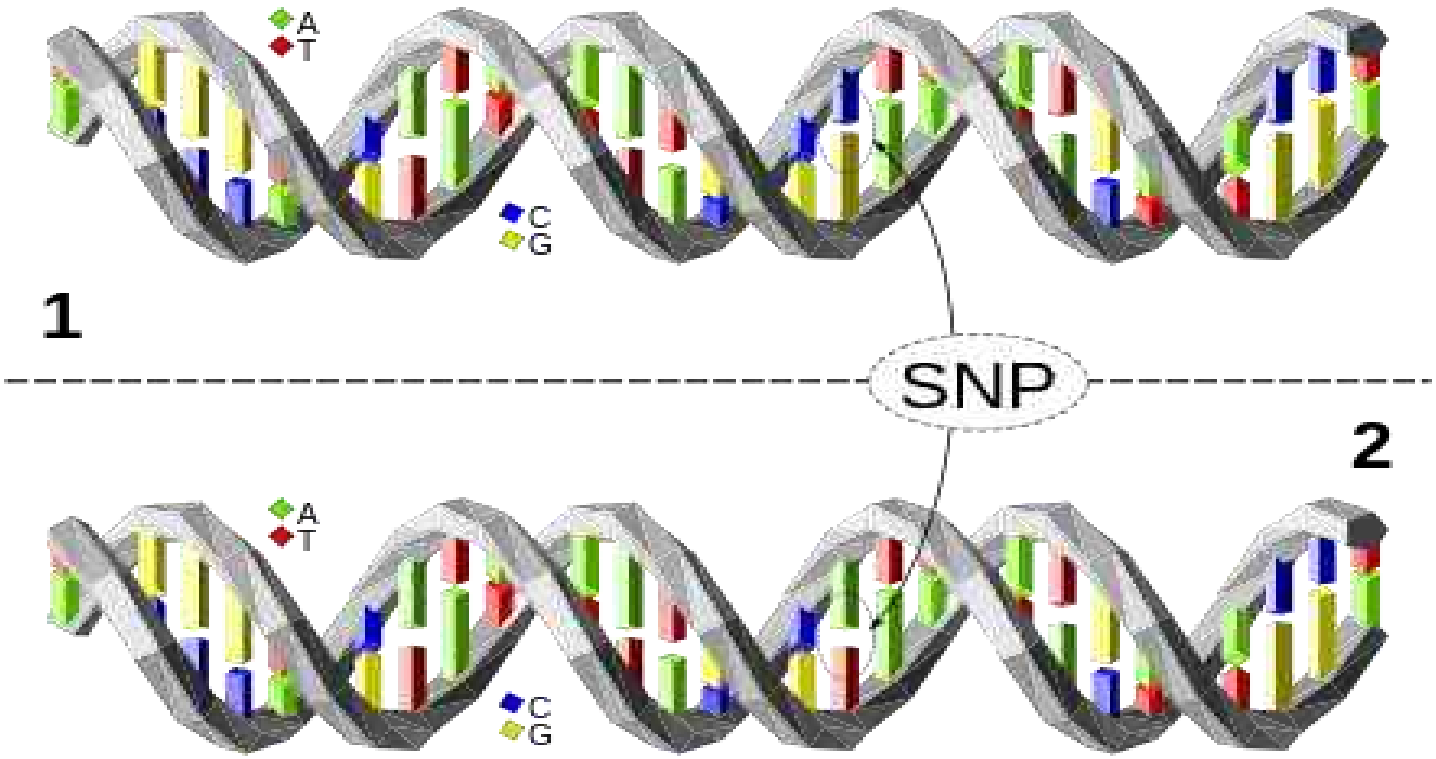
SINGLE NUCLEOTIDE POLYMORPHISM AND ITS IMPACT ON FOLATE AND HOMOCYSTEIN METABOLISM

SINGLE NUCLEOTIDE POLYMORPHISM

- Most of the genes have small sequence differences – polymorphisms – that **vary among individuals**.
- Single nucleotide polymorphisms (SNPs) arise from differences in a single base unit and are **the commonest form of genetic variation**.
- Polymorphic regions of the genome **control individual phenotypic differences among the human population**.
- They are **found throughout the genome**, including the X and Y sex chromosomes.
- Everyone has **his or her own distinctive pattern of SNPs** and this, therefore, provides a **means of identification**.



- Single nucleotide polymorphism (SNP), **results from** a single base mutation replacing one base for another.
- SNPs are estimated to represent about **90% of all human DNA polymorphisms**. SNPs can result from either the **transition or transversion of nucleotide bases**.
- ***Specific genetic polymorphisms*** in human populations change their ***metabolic response to diet*** and influence the risk patterns of disease as **SNPs are similar to variations in a recipe**.



DNA molecule 1 differs from DNA molecule 2 at a single base-pair location (a C/A polymorphism)

- Each gene is ***a recipe for a specific protein or group of proteins*** that either **regulate biological functions** or serve as **structural building blocks** for tissues (e.g., collagen).
- **Some SNPs change the recipe for the gene so that either *a different quantity of the protein* is produced or *the structure of the protein molecule is altered*.**
- These genetic polymorphisms lead to ***alteration of the response to the dietary components*** **by influencing absorption and metabolism**.
- **One of the best-described examples of the effect of SNPs is the *relationship between folate and the gene for MTHFR*– 5,10-methylenetetrahydrofolate reductase**.
- MTHFR has a role in **supplying 5-methyltetrahydrofolate**, which is necessary for the re-methylation of homocysteine to form methionine.
- **Methionine** is essential to many metabolic pathways including **production of *neurotransmitters* and *regulation of gene expression***.

- **Folate is essential** to **the efficient functioning of this MTHFR**.
- There is a common polymorphism in the gene for MTHFR that leads to two forms of protein: the wild type (C), which **functions normally**, and the **thermal-labile** version (T), which has a significantly **reduced activity**.
- **People with two copies of the wild-type gene (CC) or one copy of each (CT) appear to have normal folate metabolism.**
- Those with two copies of the unstable version (TT) and low folate **accumulate homocysteine and have less methionine**, which increases their risk of **vascular disease and premature cognitive decline**.
- Variations in the genomic sequence (polymorphisms) affect an individual's response to diet and susceptibility to disease.
- SNPs are scattered throughout the genome and are **found in both coding and noncoding regions** leading to **functional alterations** of **regulating proteins** or **modifying enzymes** at the **top of biological cascades** or at **rate-limiting steps** in intermediary metabolism.
- Nucleotide substitutions occurring in protein coding regions can also be classified as **synonymous** and **nonsynonymous** according to their effect on the resulting protein.

- A substitution is synonymous if it causes no amino acid change, while a nonsynonymous substitution results in alteration in the encoded amino acid.
- **The latter** type can be further classified into **missense** and nonsense **mutations**.
- A missense mutation results in amino acid changes due to the change of codon used, while a nonsense mutation results in a termination codon.
- SNPs occur with a very high frequency, with estimates ranging from about 1 in 1000 bases to 1 in 100–300 bases.
- Overall, it has been estimated that the human genome contains **about ten million SNPs**
- Once a SNP is discovered, **association studies are conducted to assess statistical associations between the SNP and the specific phenotypes known or suspected to be affected by the gene variant**.

POLYMORPHISMS IN THE APOE GENE

- *APOE* is polymorphic with **three major alleles**: *ApoE2* (cys112, cys158), *ApoE3* (cys112, arg158), and *ApoE4* (arg112, arg158)
- **APOE** (**Apolipoprotein E)** polymorphisms is associated with **plasma LDL-C** concentrations in that in different populations worldwide, **carriers of the E4** allele have **higher LDL-C** concentrations than subjects **homozygotic for the E3** allele, whereas **carriers of the E2** allele have **much lower LDL-C concentrations**.
- Individuals with an **E2/E2 combination** may **clear dietary fat slowly** and be at greater risk for early vascular disease and the genetic disorder type III hyperlipoproteinemia (high chylomicrons and IDL)—**94.4%** of such patients are E2/E2, while **only ~2% of E2/E2** develop the disease, so other environmental and genetic factors are likely to be involved (such as **cholesterol in the diet and age**).

https://en.wikipedia.org/wiki/Hyperlipidemia

- However, there is much to be learned about these APOE isoforms, including the **interaction of other potentially protective genetic polymorphisms**, so caution is advised **before making determinant statements about the influence of APOE polymorphisms**; this is particularly true as it relates to how APOE isoforms influence cognition and the development of Alzheimer's Disease.
- Among the most extensively studied polymorphisms within nuclear receptors are those in the genomic regions of PPARs, VDR (vitamin D receptor), and ERs (Estrogen receptors).
- **Polymorphisms in these genes may be linked with altered risk to develop obesity, osteoporosis, and cancer.**

POLYMORPHISMS IN THE PPARɣ GENE

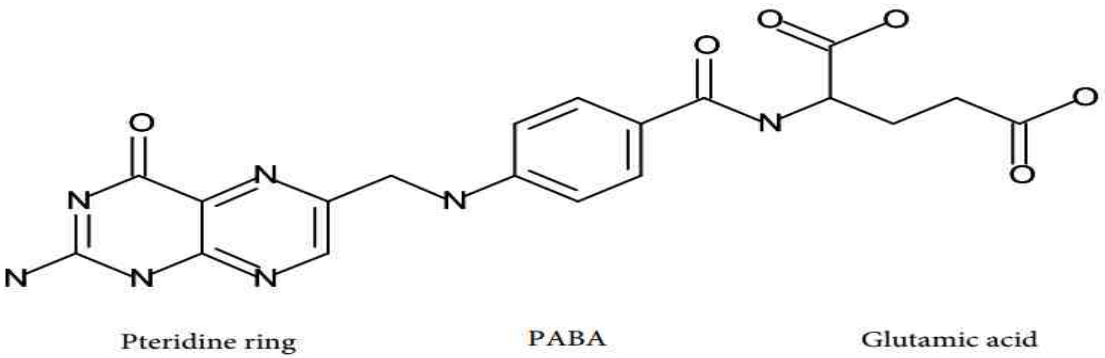
- Several naturally occurring **genetic receptor variants** of PPARγ have been identified and associated with changes in insulin **sensitivity**, insulin **secretion**, or **susceptibility for obesity**, all of which influence the risk for ***non-insulin-dependent diabetes mellitus*** (NIDDM).
- A relatively common polymorphism **within the amino terminus** of the PPARγ2 isoform is an **alanine for proline** change at codon 12 (Pro12Ala), which appears to **reduce the risk of developing NIDDM**.
- This allele, which occurs with a frequency of almost 15% among Caucasians, was originally reported to be **associated with a lower body mass index** (BMI) and insulin sensitivity.
- **A rare gain-of-function missense** mutation at codon 115 (**Pro115Gln**) in PPARγ2 was found to be associated with **severe obesity, but not with insulin resistance**.
- Individuals with **one or two heterozygous loss-of-function** mutations within the LBD of PPARγ (**Val290Met**, **Pro467Leu**), exhibit **marked insulin resistance with early onset of NIDDM**, symptoms associated with the **metabolic syndrome** including dyslipidemia (high triglyceride and low HDL cholesterol) and hypertension.
- At a molecular level, both mutant receptors revealed **impaired transcriptional activity** due to **attenuated ligand binding** and failure to recruit transcriptional coactivators.

FOLATE AND HOMOCYSTEINE METABOLISMS

- Folate metabolism is one of the **basic metabolisms of biochemistry** in which **one-carbon units are transferred to homocysteine**.
- Homocysteine is a molecule that stands **at the junction point of the remethylation cycle and the transsulfuration pathway.**
- Because they provide **detoxifcation of homocysteine**, the remethylation cycle and the transsulfuration pathway **together** can be evaluated as '**homocysteine metabolism**'.

FOLATE AND ITS METABOLISM

- **Folic acid** is a water-soluble vitamin from the B group (**vitamin B9**), which is also referred to as **folate** and **pteroyl monoglutamate** (PteGlu).
- Its chemical structure consists of 3 parts: a **pteridine ring**, **paraaminobenzoic acid**, and **glutamic acid**.



DIETARY SOURCES AND ABSORPTION

- **Humans cannot synthesize folic acid** and thus are **dependent on dietary sources**.
- **Yeast extracts, liver, kidney, leafy green vegetables, and citrus** fruits are **folate-rich foods**.
- **Bread, potatoes, and dairy products** are middle-grade sources, but **because they are consumed in large quantities, they contribute significantly to total folate intake**.
- Folates in foods are **transported via an ion-exchange mechanism** that is carried out against the pH gradient **along the brush membrane of enterocytes**.
- **Folate is anionic at intraluminal pH** and it is **exchanged with a hydroxyl anion**.
- Although absorption is **higher proximally**, it is absorbed all along the jejunum.
- **Polyglutamyl folates** are hydrolyzed **into monoglutamyl folates** by **gamma-glutamyl hydrolase (GGH)** and then all monoglutamyl folates are **converted into 5-methyltetrahydrofolate-monoglutamate (5-MTHFGlu 1)** ***in enterocytes***.
- **5-MTHF-Glu1 is the plasma form** of this vitamin and it is transported to the peripheral tissues, where it is **converted into tetrahydrofolate-monoglutamate (THF-Glu1)** via a reaction catalyzed by **methionine synthase** that uses **vitamin B12 as a cofactor (see figure on slide 16)**.

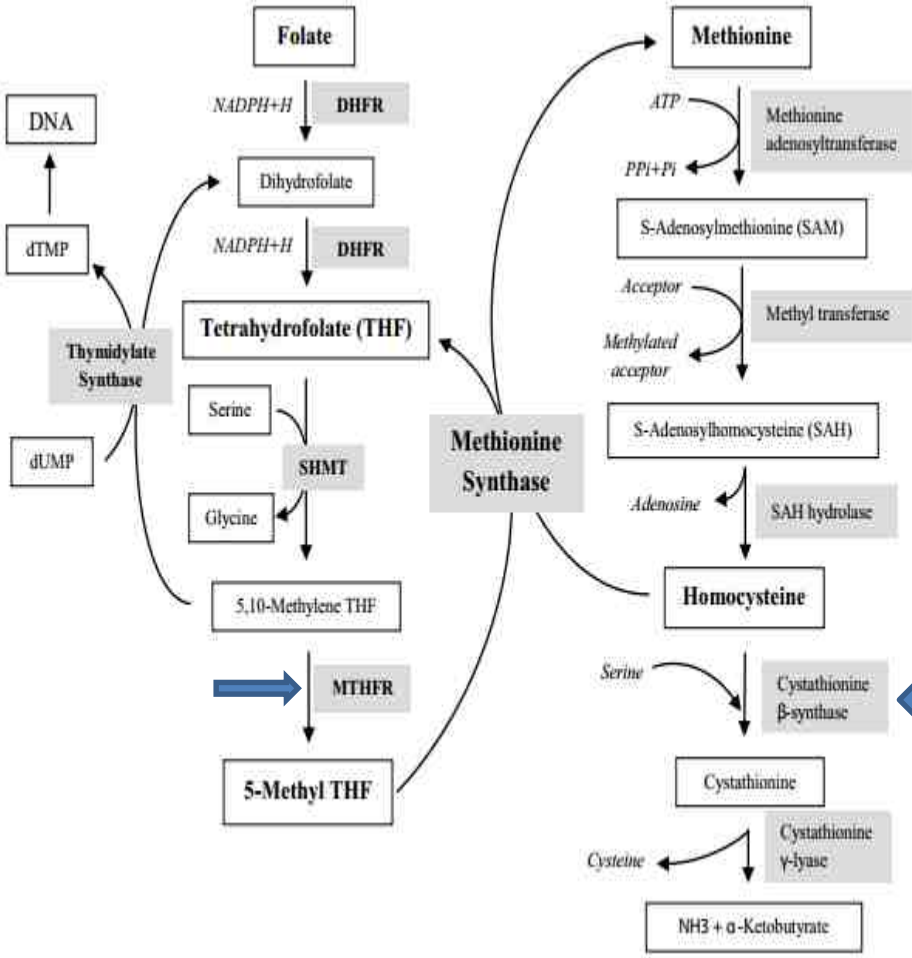
- After absorption, **5-MTHF-Glu1 enters the portal circulation**. The liver takes up **most of this folate** and some of it is released into the enterohepatic cycle by **secretion into the bile**.
- 5-MTHF-Glu1 is **reabsorbed from the renal proximal tubules via receptor-mediated endocytosis** and contributes to plasma 5-MTHF-Glu1 levels.
- Due to the **GGH** enzyme in plasma, **folylpolyglutamate is not found in plasma**.
- During erythropoiesis in the bone marrow, **folate is found in growing erythroblasts**.
- Most of the folate in erythrocytes is in the form of **5-MTHF- Glu_n** and 5-formyltetrahydrofolate **(5-FTHF)- Glu_n**.
- There is **no known role for erythrocyte folate**, but **it is thought that this folate is involved in long-term folate homeostasis and reserves**.
- In contrast to plasma concentrations, it **is not affected by recently eaten foods**.
- Thus, measurement of erythrocyte folate **can provide information about long-term folate status**.
- Folic acid in old erythrocytes is **rescued by the *reticuloendothelial system* and then transported to the liver**

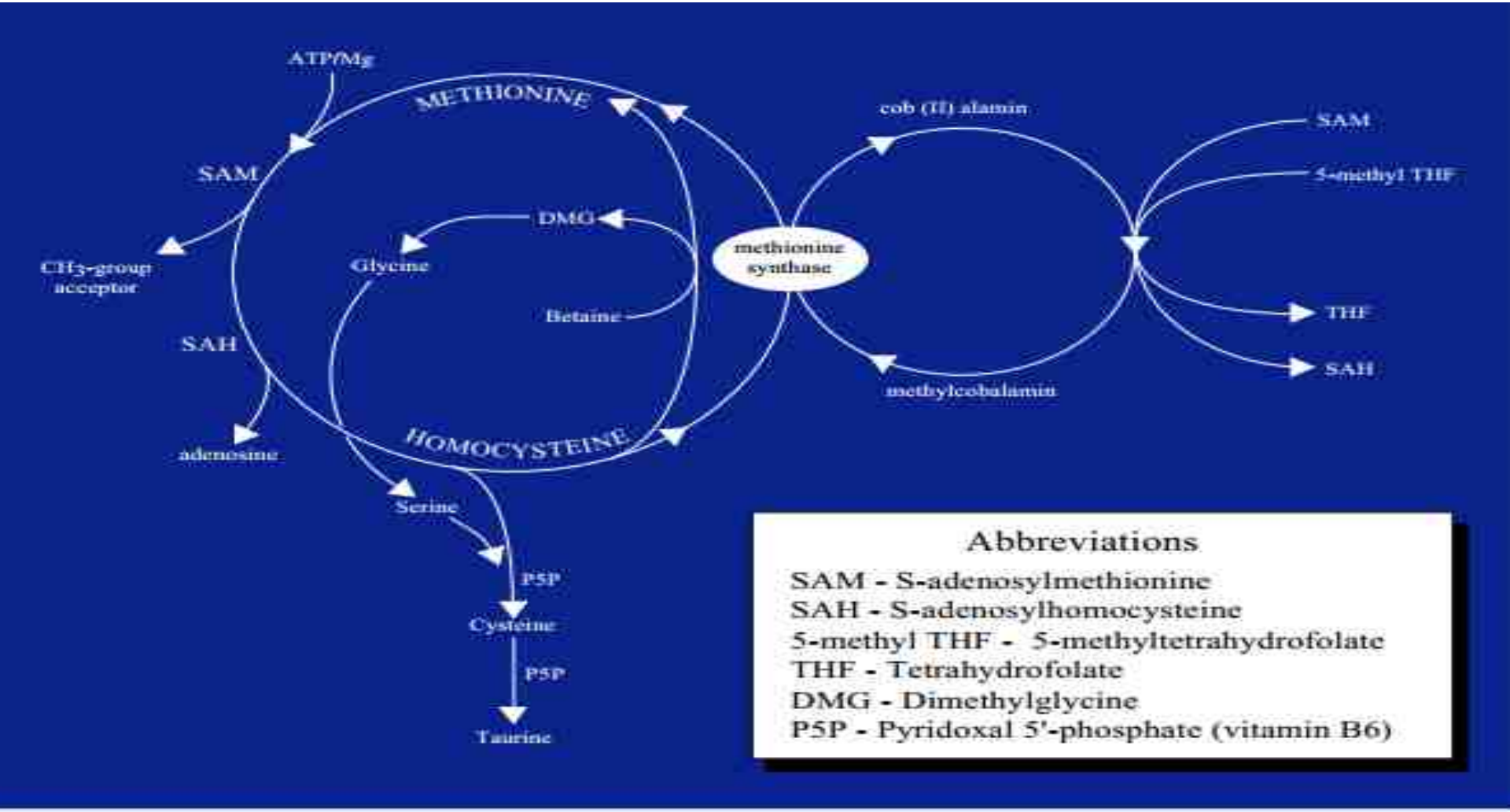
- There are **3 physiological mechanisms for transportation from the cell membrane**.
- The first is transport by the **folate-binding proteins/folate receptors (FRs)** family, which **moves folate into the cell unidirectionally**.
- FRs operate **by receptor-mediated endocytosis**.
- Certain tissues are rich in the components of this transport system, such as the **choroid plexus, vas deferens, renal proximal tubules, erythropoietic cells, ova, and placental trophoblasts**.
- The second mechanism is transport via **reduced folate carrier** (RFC).
- Unlike receptor-mediated transport, **this allows bidirectional transportation**.
- The RFC serves as an **anion exchanger** that **increases intracellular folate concentrations**.
- This is the **major folate uptake pathway in mammalian cells**.
- This transporter-mediated process is **more effective than receptor-mediated transport**.
- As a third mechanism, it has been shown that there can be folate transportation via passive diffusion across cell membranes.

- Folate enters a cell as 5-MTHF-Glu 1 on a large scale.
- At the same time, **transformation of 5-MTHFGlu1 into tetrahydrofolate by vitamin B12-dependent methionine synthase leads to the transformation of dietary-origin 5-MTHF Glu1 into the biologically more useful intracellular vitamin form, which can also be used in nucleotide biosynthesis**.
- In cells, the vitamin **B12-dependent methionine synthase** acts as the rate-limiting step for the intracellular accumulation of folate.
- This reaction is a unique step, **demethylating** 5-MTHF-Glu1 into **THF-Glu1** (see figure on slide 16).

HOMOCYSTEINE AND ITS METABOLISM

- **Homocysteine** (2-amino-4-mercapto butyric acid) is **a sulfur-containing amino acid** from **a nonprotein source**; it is not included in the structure of any protein and is **synthesized from methionine.**
- Most of it is **bound to albumin with a disulfide bond**.
- The rest is in unbound **free disulfde and *sulfhydryl forms***.
- Homocysteine exists **at the junction** point of 2 important pathways (remethylation and transsulfuration) and is **regulated by many different enzymes**.
- In remethylation, homocysteine ***acquires a methyl group from N-5-methyltetrahydrofolate*** or from betaine to form methionine.
- The reaction with N-5-methyltetrahydrofolate occurs **in all tissues** and is vitamin B12 dependent, whereas **the reaction with betaine is confined mainly to the liver and is vitamin B12 independent.**




ATP/Mg
METHIONINE
SAM
CH3-group acceptor
SAH
adenosine
DMG
Glycine
Betaine
HOMOCYSTEINE
Serine
P5P
Cysteine
P5P
Taurine
methionine synthase
cob (II) alamin
methylcobalamin
SAM
5-methyl THF
THF
SAH
Abbreviations
SAM - S-adenosylmethionine
SAH - S-adenosylhomocysteine
5-methyl THF - 5-methyltetrahydrofolate
THF - Tetrahydrofolate
DMG - Dimethylglycine
P5P - Pyridoxal 5'-phosphate (vitamin B6)

- The destiny of homocysteine, between de novo methionine biosynthesis and the transsulfuration pathway, is **determined by S-adenosylmethionine** (SAM serves primarily as a universal methyl donor to a variety of acceptors) allosterically.
- In the homocysteine remethylation cycle, **at the beginning, 5,10-methylene-THF is reduced to 5-MTHF** by **5,10-methylene tetrahydrofolate reductase** (MTHFR).
- Vitamin B12-dependent methionine synthase then converts the homocysteine into methionine; in this process, **5-MTHF is transformed into THF**.
- This final step **requires methionine synthase reductase** (MSR), which **activates the methionine synthase by reducing it**.
- In addition, **homocysteine can be converted into methionine by betaine-homocysteine** methyltransferase (**BHMT**), which is not dependent on vitamin B12.

- Subsequently, **synthesized methionine is transformed into SAM**, which is a general **methyl donor for some important biomolecules**, such as **adrenaline**, **phosphatidylcholine**, and **carnitine**, by **methionine adenosyl transferase and ATP**.
- The cycle is then completed with the transformation of SAM into **S-adenosyl homocysteine** (SAH), and later, back into **homocysteine**.
- The trans sulfuration (The exchange of **sulfur**, or **sulfur-containing moiety**, between two different compounds) of homocysteine involves **uniting with serine to produce cystathionine**.
- This vitamin **B6-dependent reaction** is catalyzed by **cystathionine-β-synthase** (CBS).
- After passing this step, **homocysteine can no longer be a precursor for methionine**.
- Next, cystathionine is **hydrolyzed into cysteine and α-ketobutyrate** by another vitamin B 6-dependent enzyme, **cystathionine-γ-lyase** (CGL).
- In vitamin B12 functional deficiency, **homocysteine** and **methylmalonic acid** (MMA) levels increase in plasma and urine.
- Because **folate is another coenzyme of methionine synthase, MMA measurements are more selective for vitamin B12 status alone**.

METHYLENETETRAHYDROFOLATE REDUCTASE GENE (MTHFR) POLYMORPHISMS

- **Folate** and **methionine** are important nutrients in the "one-carbon" metabolism that is **closely associated with DNA synthesis and DNA methylation**.
- **Genetic variation** in these pathways may **change susceptibility to cancer development.**
- DNA methylation of various **oncogenes or tumor suppressor genes** may **induce selective growth transformation of cells or its inhibition**.
- **Alterations in metabolism** may occur with genetic **variation of any of the enzymes directly involved in maintaining homeostasis**.
- The one-carbon metabolism features **two main branches**: **one** consists of *reactions involving purine and thymidine synthesis*, and the **other** is responsible for ***synthesis of methionine and S-adenosylmethionine*** **for polyamine and protein synthesis and methylation reactions.**

- **MTHFR** catalyzes the reduction of 5,10-methylenetetrahydrofolate (THF) to 5-methyl-THF, the predominant circulatory form of folate and **a carbon donor for the remethylation of homocysteine to methionine**.
- **MS** catalyzes the transfer of methyl bases from 5-methyl-THF to homocysteine, thereby **producing methionine and tetrahydrofolate**.
- **Two polymorphisms** in the gene of MTHFR gene (**MTHFR C677T and MTHFR A1298C**) and **one** in the gene for MS (**MTR A2756G**) are known to have functional importance and are directly linked with levels of homocysteine.
- MTHFR **activity** was reported to be lower in the MTHFR 677T and **1298C alleles**.
- The MTRG allele was correlated with homocysteine levels indicating lower MS enzyme activity.
- Several groups have reported associations between MTHFR polymorphisms and **acute lymphoblastic/myeloblastic leukemia**.
- Two biological mechanisms, **DNA instability and DNA methylation**, have been hypothesized to explain **the association between one-carbon metabolism and carcinogenesis**.
- In terms of DNA instability, reduced MTHFR activity may lead to accumulation of methylene-THF.

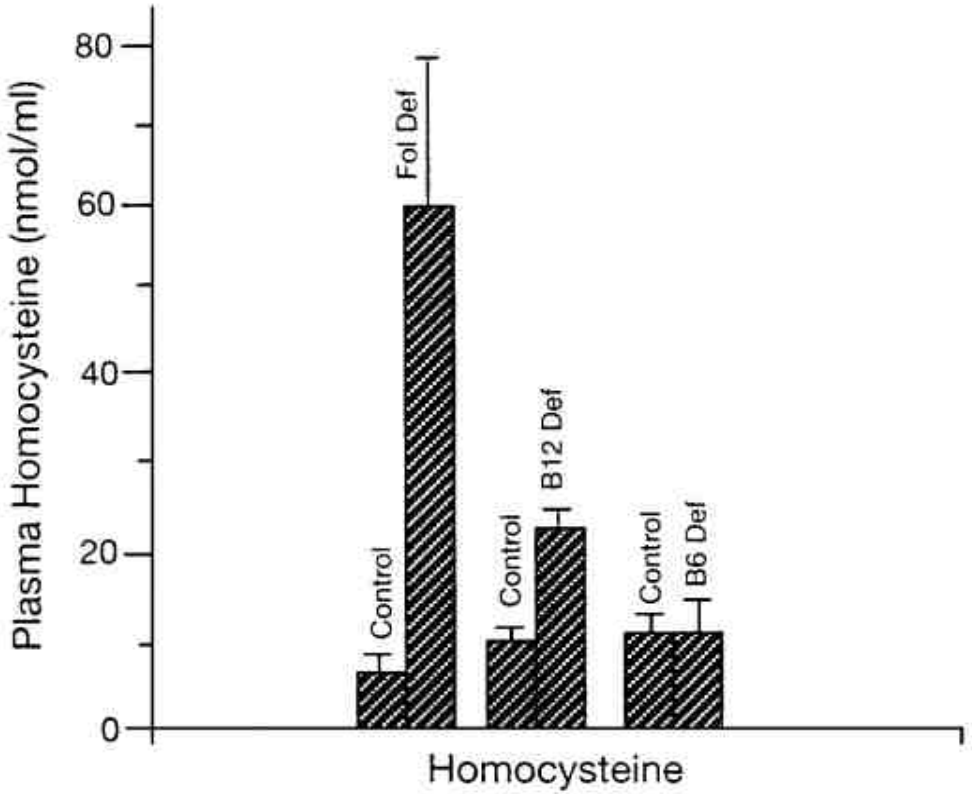
- As to DNA methylation, there have been several reports of a **close association with the one-carbon metabolic pathway**.
- Decreased activity of MTHFR leads to reduction of 5-methyl-THF, a methyl donor (for homocysteine), and thus to **hypomethylation**.
- An association between the **MTHFR C677T polymorphism and genomic DNA hypomethylation was reported**.
- If impacting on a tumor suppressor gene, such as TP15 or TP16, which are often **hypermethylated in malignant lymphomas**; **any tendency for hypomethylation would be expected to be protective**.
- Possible hypomethylation inducible alleles (MTHFR 677T, 1298T) were **protective for malignant lymphoma**.
- The most common polymorphism on the MTHFR gene is C677T.
- In **homozygous individuals**, there is **mild homocysteinemia that may increase with decreased dietary folate intake**.
- Genetic defects in MTHFR lead to **psychomotor growth delay**, serious mental retardation, and other psychiatric problems in infancy.

- One-third of **psychiatric** and **psychogeriatric** patients have **low serum or erythrocyte folate levels**.
- Folate deficiency **affects normal brain development** by different mechanisms.
- **First**, because **folate is an essential precursor for DNA synthesis**, ***deficiency disrupts the division of neural progenitor cells***.
- Related to this, in a study of **pregnant mice**, it was found that **folate deficiency disrupts the replication of progenitor cells in the ventricular germinal zones of the brain**.
- Second, folate deficiency may **affect normal fetal brain development because of elevated homocysteine levels in the mother**.
- During the third trimester of pregnancy, **maternal hyperhomocysteinemia** is associated with a 2-fold **increased risk for schizophrenia in babies**.

SUPPORTING EVIDENCE FOR NUTRITIONAL CAUSES OF HYPERHOMOCYSTEINEMIA

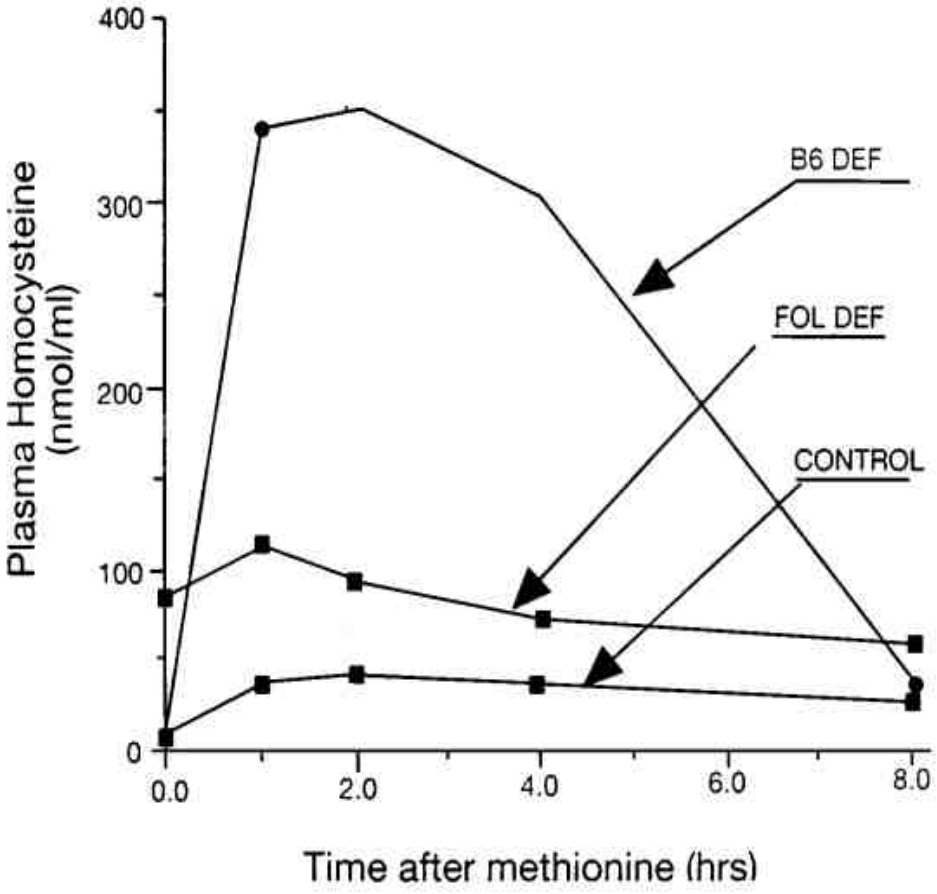
- Using rats as models, it was found that fasting plasma homocysteine concentrations increased **8- to 10-fold** in folate-deficient rats and **2.5-fold** in vitamin **B12- deficient** rats.

Effects of **folate**, **vitamin B12-**, and **vitamin B6-**deficiencies on fasting plasma homocysteine levels.



- When the **folate-deficient rats** were administered intraperitonial (i.p.) injections of ethionine, a **methionine analog**, plasma homocysteine concentration ***decreased*** **almost to its normal level.**
- This decrease is thought to be due to ***S*-adenosylethionine, which, like SAM, is an effective activator of cystathionine *β*-synthase, but unlike SAM is less likely to be deethylated and subsequently metabolized to homocysteine**.
- This activation by *S*-adenosylethionine reintroduced the **coordination between the two pathways that was interrupted in folate deficiency because of diminished SAM synthesis**.
- In **vitamin B6-deficient rats**, fasting plasma homocysteine concentration was not elevated.
- Moreover, an oral gavage of **methionine caused a marked increase in plasma homocysteine concentration in the vitamin B6-deficient rats** that was **accompanied by a significant elevation in hepatic SAM concentration**.
- This is contrasted with **folate-deficient rats** in which **methionine loading caused *no significant change in plasma homocysteine* concentration from preload levels**.

Plasma homocysteine concentrations after **methionine loading** in control, folate deficient, and vitamin B6-deficient rats.



HYPERHOMOCYSTEINEMIA IN HUMANS

- The more severe cases are due to **homozygous defects in genes encoding for enzymes of homocysteine metabolism**.
- In such cases, a defect in an enzyme **involved in either** homocysteine **remethylation** or **transsulfuration leads to large elevations of homocysteine in the blood and urine**.
- The classic form of such a **disorder—congenital homocystinuria**—is caused by homozygous defects in the gene encoding for **cystathionine beta synthase (CBS).**
- In these individuals, fasting plasma homocysteine concentrations can be as **high as 400 *μ*mol/liter**.
- Homozygous defects of other genes that lead to similarly severe elevations in plasma homocysteine include those encoding for **MTHFR** or for any of the enzymes **that participate in the synthesis of methylated vitamin B12**.

Classification of hyperhomocysteinemia[a]

Severe hyperhomocysteinemia High tHcy levels at all times; caused by deficiencies in CBS, MTHFR, or in enzymes of B_{12} metabolism
Mild hyperhomocysteinemia Fasting; moderately high tHcy levels under fasting conditions; reflects impaired homocysteine methylation (folate, B_{12} or moderate enzyme defects, e.g. thermolabile MTHFR)
Post-methionine load Abnormal increase in tHcy after methionine load. Abnormal net increase reflects impaired homocysteine transsulfuration (heterozygous CBS defects, B_6 deficiency)

[a]Cystathionine beta synthase (CBS), methylenetetrahydrofolate reductase (MTHFR), total homocysteine (tHcy).

- An **impairment in the remethylation pathway**, even if it is mild, **will lead to a substantial increase in plasma homocysteine concentrations** under fasting conditions.
- Such an impairment may be due to **inadequate status of either folate or vitamin B12 or to defects in the gene encoding for MTHFR**.
- **MTHFR contains FAD** as a prosthetic group, which **raises** the possibility that vitamin **B12 status** is also a determinant of fasting plasma homocysteine levels.
- **In contrast**, **a mild impairment in the transsulfuration** pathway will lead, at most, to **a very slight increase** in fasting plasma homocysteine levels.
- This mild impairment, which may be due to **heterozygous defects in the CBS gene or inadequate levels of vitamin B6**, is normally **identified by an abnormal increase in plasma homocysteine after a methionine loading test**

or

following a meal.

- **Vitamin B6 was** found to be effective in lowering post–methionine-load plasma homocysteine.

MTHFR THERMOLABILITY

- Mutations that result in severely **reduced MTHFR activity** and hyperhomocysteinemia **are rare**.
- However, two unrelated patients with moderate **hyperhomocysteinemia** and **low folate levels** were reported to have **a variant of MTHFR that was distinguished from the normal enzyme by its lower specific activity** (50%) and its **thermolability**.
- That MTHFR thermolability is an **inherited recessive trait**, which is present in approximately **5% of the general population and 17% of patients with proven coronary artery disease**.
- It has been shown that MTHFR thermolability is caused by a point mutation (677 C to T transition) at a polymorphic site, resulting in a **valine substitution for an alanine in this enzyme**.
- **The frequency of homozygotes for the 677CT mutation** may vary significantly in populations from different geographic areas (from 1.4% to 15%)
- **The occurrence of an interaction between MTHFR thermolability genotype and folate status was demonstrated.**

- When **plasma folate** concentrations were **above the median** (15.4 nmol/liter), **plasma homocysteine** levels were **low** and unrelated to the MTHFR genotype.
- However, when plasma folate concentrations were **below the median**, plasma homocysteine levels were significantly **higher in homozygotes for the 677CT mutation than in those with the normal genotype**.
- **These data imply that the *phenotypic expression of the MTHFR genotypes is dependent on the availability of folate*.**
- **This suggest that homozygotes for the thermolabile genotype might have a higher folate requirement than do individuals with a normal genotype.**

NUTRITIONAL REGULATION OF HOMOCYSTEINE METABOLISM

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- The utilization of homocysteine molecules by the transsulfuration and remethylation pathways is **nutritionally regulated**.
- When the intake of **labile methyl groups** (i.e. methionine and choline) is modified, the **de novo synthesis of methionine methyl groups is affected**.
- When a ***basal*** **methionine-containing diet** was administered, homocysteine moieties were found to cycle through the remethylation pathway approximately 1.5–2.0 times **before being catabolized** through the transsulfuration pathway.
- When **dietary methionine was *halved***, the number of cycles per homocysteine moiety **increased twofold**.
- Conversely, **when *excess* dietary methionine** was administered, homocysteine cycling **fell below basal levels**.
- This **capacity of the body to discriminate** between the remethylation and transsulfuration pathways as ***a way to adapt to varying amounts of methionine*** in the diet strongly implies **the existence of a coordinate regulation between these two pathways**.

- This coordination is **achieved by at least *two mechanisms***.
- ***The first*** mechanism is a **function of SAM's propensity/tendency to act as an allosteric inhibitor of methylenetetrahydrofolate reductase (MTHFR) and as an *activator* of *cystathionine β-synthase*.**
- As such an effector, SAM **suppresses the synthesis of an important substrate (N-5-methyltetrahydrofolate) required for remethylation** and **promotes** the initial reaction of transsulfuration (cystathionine synthesis).
- Thus, ***intracellular SAM concentration*** is an important **determinant of the fate of homocysteine molecules**.

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- The **second** mechanism by which remethylation and transsulfuration are coordinated consists of **the *regulation of intracellular SAM concentration, itself***.
- In the liver, **SAM synthesis is catalyzed by two enzymes** peculiar to this organ that are **immunologically similar but different in other respects**.
- **One** enzyme, a tetramer of high molecular weight, exhibits a **high affinity** for methionine and is thought to **function at normal physiological conditions**.
- The ***second*** enzyme is a dimer of a lower molecular weight, has a ***low affinity*** for methionine, and is thought to **function under conditions of *high methionine*** intake.

- Thus, ***changes in intracellular methionine*****, particularly due to dietary intake, will** ***affect the rate of SAM synthesis based on the activity of the SAM synthetase enzymes***.
- It is also **thought that** the **utilization of SAM is regulated specifically by a reaction in which the methyl group of SAM is transferred to the amino group of glycine, forming sarcosine.**
- This reaction is catalyzed by glycine N-methyltransferase (GNMT), which is abundant in the liver and strongly **inhibited by N-5-methyltetrahydrofolate polyglutamates**.
- Thus, *along with* ***intracellular methionine****,* ***N-5-methyltetrahydrofolate*** ***participates in the regulation of intracellular SAM concentrations***.
- When the two mechanisms of regulation are considered together, the following scenarios can be predicted.

1. When dietary methionine is high, **the low-molecular-weight SAM synthetase will rapidly convert the incoming methionine to SAM**.

- The resulting rise in intracellular SAM concentration will be associated with

(*a*) inhibition of methylenetetrahydrofolate reductase resulting in ***suppressed N-5-methyltetrahydrofolate*** synthesis, thereby allowing the **GNMT enzyme to act near full capacity because of suppressed inhibitor** (N-5-methyltetrahydrofolate) concentration; and

(*b*) activation of the cystathionine *β*-synthase enzyme, thus **increasing the rate of homocysteine catabolism**.

- ✓ In this way, **homocysteine transsulfuration is promoted over remethylation**, consistent with **the reduced need for de novo methionine synthesis due to the high dietary supply of methionine**.

2. Conversely, when the dietary methionine supply is low, **SAM concentration is insufficient for the inhibition of MTHFR**, resulting in an **elevated rate of N-5-methyltetrahydrofolate production**.

- The resulting rise in intracellular N-5-methyltetrahydrofolate concentration will be associated with

(*a*) Inhibition of GNMT and thereby **conservation of SAM**, and

(*b*) An increase in the availability of substrate for homocysteine remethylation.

Thus, remethylation will be favored over transsulfuration because the concentration of SAM is too low to activate the cystathionine *β*-synthase enzyme.

This process is consistent with the **increased need for de novo methionine synthesis attributed to the low dietary input of methionine**.

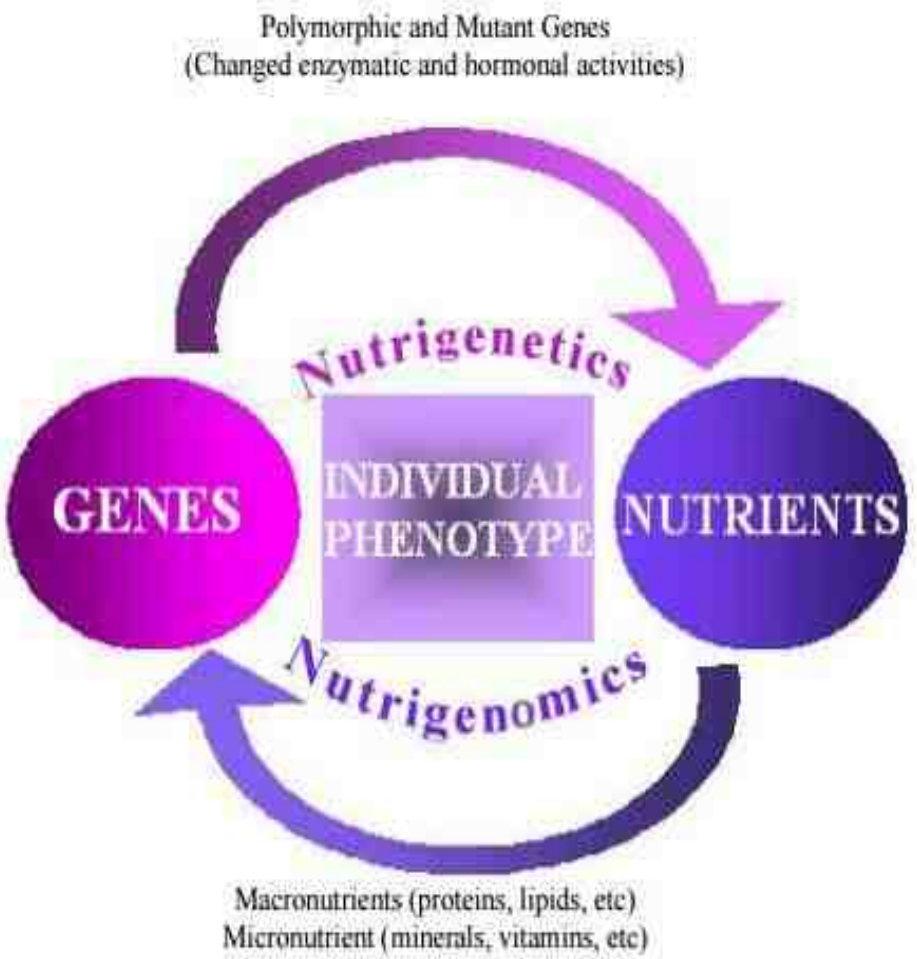
NUTRITIONAL GENOMICS AND PERSONALIZED DIET

- **Nutritional genetics** is not a single field, but is considered as the combination of two: nutrigenomics and nutrigenetics.
- Nutrigenomics is **establishing the effects of ingested nutrients and other food components on gene expression and gene regulation**, i.e., to study **diet-gene** interaction in order to identify the **dietetic components having beneficial or detrimental health effects**.
- It also determine the ***individual nutritional requirements*** **based on the genetic makeup of** the person.
- Nutrigenomics also identify the **genes involved in physiological responses to diet** and the genes **in which small changes, called polymorphisms, may have significant nutritional consequences** and the **influence of environmental factors on gene expression**.
- Eventually nutrigenomics will lead to **evidence-based dietary intervention strategies for restoring health and fitness and for preventing diet-related disease**.

GENE-DIET-DISEASE-INTERACTION

- Modifying the dietary intake can prevent some monogenetic diseases, e.g., **in phenylketonuria (PKU) food containing the amino acid phenylalanine, including high protein food** such as fish, chicken, -eggs, milk, cheese, dried beans, nuts, and tofu must be avoided.
- In case of defective **aldehyde dehydrogenase enzyme**, alcohol must be avoided.
- Patients having **galactosemia** should avoids diets which contain lactose or galactose, including all milk and milk products while in case of **lactose intolerance** (shortage of the enzyme lactase) patients should avoid milk and milk products.
- Diseases and conditions that are **known to have genetic and/or nutritional components** are **candidates for nutrigenomic studies to determine whether dietary intervention can affect the outcome**.
- Differences in genetic makeup or genotype are factors in gastrointestinal cancers, other gastrointestinal conditions or digestive diseases, inflammatory diseases, and osteoporosis.
- Diseases that are **known to involve in the interactions between *multiple genetic and environmental factors*** such as diet **include** many cancers, diabetes, heart disease, obesity and some psychiatric disorders

- Nutrigenetics points to understanding how the **genetic background** of an individual impact to the diet.
- Nutrigenomics aims to identify the **effects of several nutrients**, including macronutrients and micronutrients **on the genome** and explores the interaction between genes and nutrients or food bioactives and their effects on human health.
- Nutrigenomics will **unravel the optimal diet from within a series of nutritional alternatives**, whereas nutrigenetics will yield critically important information that will **assist clinicians in identifying the optimal diet for a given individual**, i.e., personalized nutrition.



NUTRIGENOMICS AND CHRONIC DISEASE

NUTRIGENOMICS AND OBESITY

- **Obesity is the commonest nutrition-related disorder** and is the core element of a group of metabolic abnormalities.
- **Also obesity and associated metabolic anomalies** dramatically increase the risk of developing a variety of chronic diseases including CVD and cancer.
- However, **individual susceptibility to obesity strongly depends on the genetically determined patterns of energy balance regulation**.
- Multiple polymorphic genes encoding central and peripheral determinants of energy intake and expenditure have been revealed.

NUTRIGENOMICS AND CVD

- CVD is the primary diet-related chronic disease of the modern time and the **inflammation** is emerging as underlying many chronic disorders including CVD.
- CVD can be characterized **as a group of multifactorial conditions associated with obesity, atherosclerosis, hypertension, and thrombosis**.

- All of these pathologic entities are known to be **closely related to both genetic factors and environmental influences**.
- **Diet is considered as one of the environmental influences** and **a strong relationship between *diet composition and CVD risk is well established***.
- ***Obesity*** per se is a major cardiovascular risk factor, **thus polymorphic genes involved in energy balance control certainly provide "favorable" or "unfavorable" background for the development of CVD**.
- ***Atherosclerosis*** constitutes the key element in the pathogenesis of CVD and it can be regarded as **a complex combination of lipid transport and metabolism disorder with chronic inflammation**.
- ✓ **Permanently elevated plasma levels** of **total cholesterol**, **LDL cholesterol**, and **triglycerides** predispose to the development of atherosclerotic plagues, whereas increased high density lipoprotein (HDL) cholesterol levels appear to be protective.
- ✓ Genetic **variation in genes encoding for *apolipoproteins*, *some enzymes*** and ***hormones*** can **alter individual sensitivity to develop the cardiovascular diseases.**

- Some of these variants are susceptible for dietary intervention, for example: Individuals with the E4 allele in the apolipoprotein E gene show higher low-density lipoprotein-cholesterol (bad cholesterol) levels with increased dietary fat intake compared with those with the other (E1, E2 and E3) alleles receiving equivalent amounts of dietary fat.
- Also specific polymorphism in genes encoding ***lipid transport proteins***, their *receptors*, and ***lipid-processing enzymes*** **and inflammation related proteins** were shown to be **associated with the characteristic changes in blood lipid concentrations.**
- One **polymorphism** (**-504 CC**) **in the hepatic lipase gene** is **associated with an increase in protective HDL levels** compared with the TT genotype (common in certain ethnic groups such as African–Americans**)** ***in response to high fat diet.***

part 1

HYPERTENSION

- ***Hypertension*** is one of the **components of the obesity associated metabolic syndrome**, and influence of **dietary factors altering energy homeostasis appears to predispose to blood pressure elevation**.
- It is well known that the ***loss of weight*** **in hypertensive obese individuals usually leads to simultaneous blood pressure decrease**.
- ***Sodium chloride*** is the only dietary risk factor well defined to predispose to hypertension.
- However, blood pressure responses to increases and decreases in dietary salt intake may be heterogenous, as **only about 15% have sodium-sensitive hypertension**.
- For the **other 85%, eliminating salt from the diet has no effect on their blood pressure**.

- Polymorphic genes implicated in blood pressure regulation include **renin-angiotensin system genes** including those encoding **angiotensinogen** (AGT), **angiotensin converting enzyme** (ACE), and **aldosterone synthetase** (CYP11B2).
- However, *no evidence of the interactions between polymorphic variants of these genes and* ***dietary factors is available***.
- On the other hand **sodium transport/metabolism-related genes** such as those encoding **epithelial sodium channel** (ENaC) subunits, adducin, and 11B-hydroxysteroid dehydrogenase **are certainly *of interest*, given well-proven association between dietary salt intake and hypertension**.
- There are also **some reports** associating human hypertension with polymorphisms in **some G-proteins** and **adrenergic receptors** but **evidence is not sufficient**.
- **So nutrigenomics is addressing *why some people can control their hypertension with diet, whereas others require drugs***.

ARTERIAL THROMBOSIS

- **Thrombosis** of arteries affected by atherosclerosis constitutes the main mechanism leading to **acute coronary and cerebrovascular syndromes**.
- **Impaired balance of *multiple factors constituting blood coagulation system* can lead to hypercoagulative state increasing thrombosis probability**.
- Both the environmental and genetic factors are involved.
  - Diet, especially **excessive fat** ingestion can trigger postprandial (after meal) hypercoagulative state.
  - **Gene polymorphisms affecting hemostasis** (as genes encoding platelet surface glycoproteins, and coagulation factors) have been implicated.
  - Blood coagulation is counterbalanced by the anticoagulant and fibrinolytic systems that **also include polymorphic factors**.

NUTRIGENOMICS AND CANCER

- Cancer is a process composed of **multiple stages** in which **gene expression**, and **protein** and **metabolite** function begin to **operate aberrantly**.
- **Inherited mutations in genes** can increase one's susceptibility for cancer.
- The risk of developing cancer can be **markedly increased if there is a gene diet interaction**.
- ***The likelihood of identical twins developing the same cancer is less than 10%,* indicating that the environment plays an important role in cancer susceptibility**.
- Evidence of **genome and epigenome *damage biomarkers,* in the absence of overt exposure of *genotoxins***, are themselves sensitive indicators of **deficiency in micronutrients required** as cofactors or as components of ***DNA repair enzymes***, for ***maintenance of methylation of CpG sequences*** and ***prevention of DNA oxidation and/or uracil incorporation into DNA***.
- Diet is considered as **a source of either carcinogens** (intrinsic or cooking-generated) present in certain foods or **constituents acting in a protective manner** (vitamins, antioxidants, detoxifying enzyme-activating substances, etc.)

- It is clear that carcinogen metabolism affecting **polymorphisms** may **modify probability of contact between carcinogens and target cells**, thus acting at the stage of cancer initiation.
- Influences of **polymorphisms of gene encoding factors involved in hormonal regulation are most strongly manifested in hormone dependent tumors such as breast, prostate, ovarian and endometrial cancers**.
- **Polymorphisms in sex hormone receptor genes** comprising those encoding estrogen receptor, progesterone receptor, and androgen receptor **have been shown to be *associated with cancer risk modulation***.
- ✓ **Dietary factors** can certainly interact **with hormonal regulation.**
- ✓ Obesity strongly affects hormonal status.
- ✓ At the same time **some food components**, such as **phytoestrogens are known to be processed by the same metabolic pathways as sex hormones**, thus their cancer-preventive effect can be **modulated by the polymorphisms** mentioned above.

DIET AND INCREASED RISK OF CANCER

- There is an **increase risk of colorectal cancer with high consumption of *red meat***.
- N-Acetyl transferase (NAT) is a phase II metabolism enzyme that exists in two forms: NAT1 and NAT2.
- **Several polymorphisms** exist in NAT1 and NAT2, some of which have been associated NAT capabilities of **slow**, **intermediate**, or **fast acetylations**.
- NAT is involved in **acetylation of the heterocyclic aromatic amines found in *heated products* especially well cooked red meet**.
- During cooking of muscle meat at high temperature **some amino acids may react with creatinine to give heterocyclic aromatic amines** (HAA).
- **HAA can be activated through acetylation to reactive metabolites which bind DNA and cause cancers**.
- Only NAT2 fast acetylators can perform this acetylation.
- ***NAT fast acetylator genotype* had a higher risk of developing colon cancer in people who consumed relatively large quantities of red meat**.

- A combination of ***excess body weight*** **and** ***physical inactivity*** **are estimated to account for** ***one fifth*** **to** ***one third*** **of several of the most common cancers, specifically cancers of the breast** (postmenopausal), colon, endometrium, **kidney** and **esophagus**.
- Specific **dietary irritants**, such as salts and preservatives have been suggested as being **carcinogens for gastric cancer**.
- **C667T polymorphism** in MTHFR gene which reduces enzymatic activity **is inversely associated with occurrence of colorectal cancer**.
- **Low intake of folate, vitamin B12, vitamin B6 or methonine are associated with increased risk for cancer in** ***CC or TT*** **phenotype of MTHFR gene**.

DIET AND CANCER PREVENTION

- Cancer prevention studies have shown that **all of the major signaling pathways deregulated in different types of cancer, are *affected by nutrients***.
- So far, more than **1000 different phytochemicals** have been identified with cancer-preventive activities.
- ***Dietary fibers* have a protective effect against bowel cancer.**
- Long chain polyunsaturated fatty acids (**LC-PUFA**) **beneficially affect physiological processes.**
- **Fish oil**, rich in **omega-3 fatty acids**, **inhibits the growth of colonic tumors in both in vitro and in vivo systems**.
- **Bioactive components present in fruits and vegetables** can prevent carcinogenesis by several mechanisms such as **blocking metabolic activation** through **increasing detoxification**.
- ✓ **Reactive oxygen species (ROS) attack DNA bases**, resulting in **potential mistranscription** of DNA sequence.
- ✓ Such disruptions can interfere with DNA replication and thus **produce mutations in oncogenes and tumor suppressor genes**.
- ✓ ROS can also result in breakage of DNA strand, resulting in mutations or deletions of genetic material

NUTRIENT–GENE INTERACTIONS INVOLVING SOY PEPTIDE AND CHEMOPREVENTIVE GENES IN PROSTATE EPITHELIAL CELLS

- Epidemiological studies repeatedly show **associations between food intake and the incidence and severity of chronic diseases**.
- **The idea**, however, **that foods contain bioactive molecules that can affect physiology and gene expression is a relatively new one**.
- Dietary molecules are now known **to affect gene expression directly or indirectly after modification by primary or secondary metabolism**.
- Some components of foods can act (1) **as ligands for transcription factors**, (2) as **positive or negative activators of signal transduction pathways** or (3) as **ligands** for nuclear receptors.
- Genistein, vitamin A, and hyperforin (a phytochemical produced by some of the members of the plant genus hypercum, notably hypercum perforatum) are just a few **well documented examples of dietary chemicals that can bind directly to nuclear receptors and influence gene expression.**

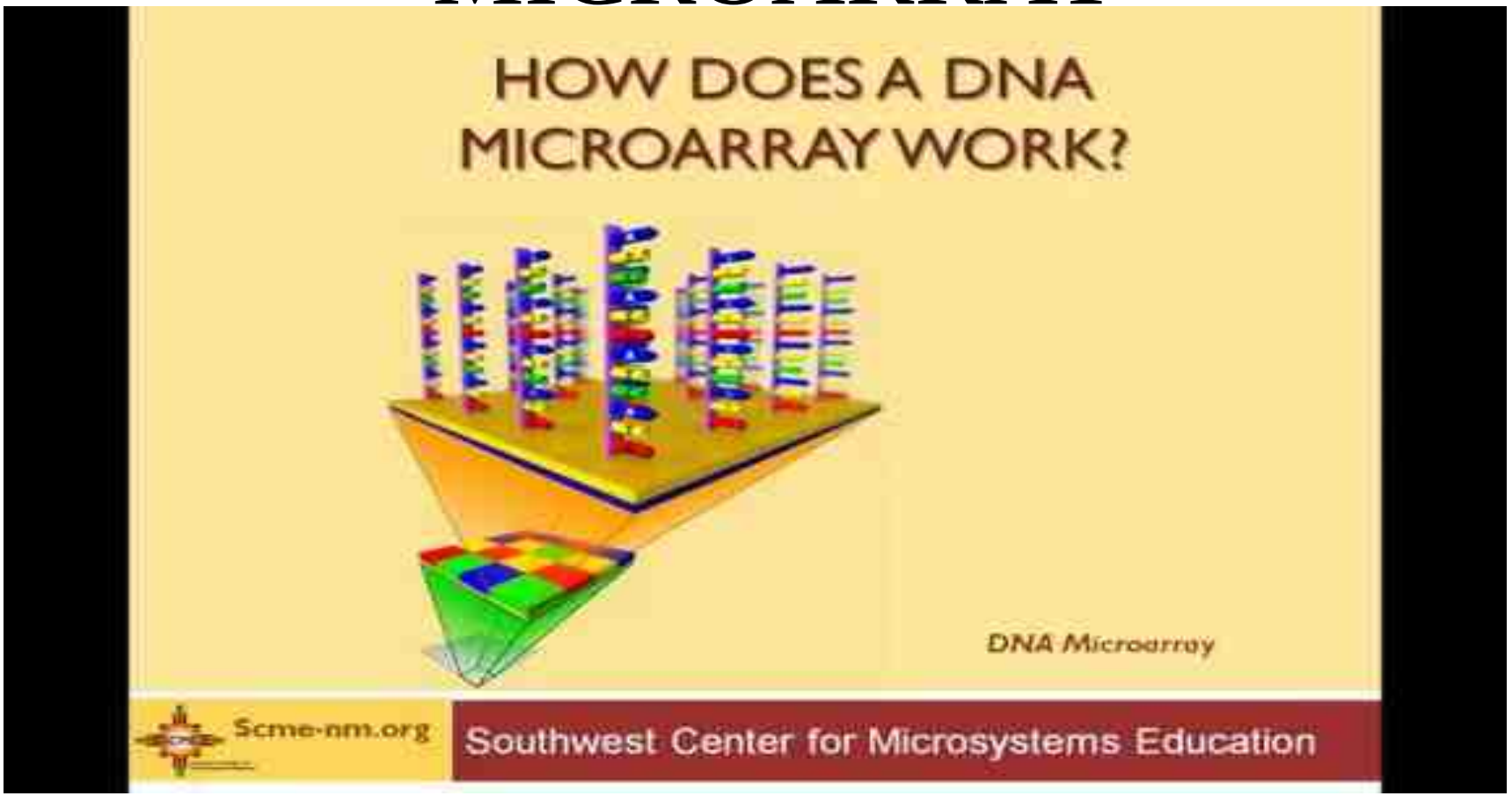
- The **role of dietary factors in the etiology of different types of cancer** is also well documented.
- For example, **catechins** such as epigallocatechin-3-gallate (EGCG) **found in green tea** block signaling pathways to cell proliferation by binding to membrane receptors and **inhibiting tyrosine kinases**
- **Diets high in EGCG are associated with reduced risk of proliferative heart disease and various types of cancers**.
- **Diets rich in soy** are also associated with **lower cancer mortality rates, particularly for cancers of the colon, breast, and prostate**.
- Isoflavones and the Bowman–Birk protease inhibitor (BBI) are some of the **components in soybeans** believed to be responsible for suppressing carcinogenesis.

- In the case of BBI, extensive studies at the University of Pennsylvania have clearly shown that **this 8 kDa inhibitor of chymotrypsin and trypsin prevents *chemical carcinogen and X-ray induced tumor* formation in mammalian cells and in laboratory animals**.
- Purified BBI, or its soy concentrate called **BBIC**, has comparable suppressive effects on the carcinogenic process in a variety of in **vitro and in vivo systems**.
- **Carcinogenesis suppression by BBI or BBIC can be achieved through different routes of administration**, **including the diet**.
- To date, however, BBI's mechanism of action has not been elucidated.
- Recently, **lunasin**, a small peptide also found in soybean seeds, has shown promise as a chemopreventive agent.
- Lunasin is a 43 amino acid **small subunit of a soybean 2S albumin**.
- The carboxyl end of lunasin contains **a chromatin-binding domain**, a cell adhesion motif Arg-Gly Asp (RGD) **followed by eight Asp residues**.

LUNASIN FUNCTION

- Studies in animals and mammalian cells have shown that **lunasin may play a role in *cell cycle control***.
- For example, **transfection of the *lunasin gene into mammalian cells* results in mitotic arrest and subsequent cell death**.
- Lunasin is capable of **preferentially binding to deacetylated histones**.
- When chemically synthesized lunasin was added exogenously to mammalian cells, it is observed to **colocalize with hypoacetylated chromatin and prevent histone H3 and H4 acetylation in vivo in the presence of a histone deacetylase inhibitor**.
- When (C3H/10T1/2 and MCF-7) **human breast cancer cells** were treated with lunasin **in the presence of the histone deacetylase inhibitor**, sodium butyrate, a 10- to 95-fold **reduction in acetylation** of core histones H3 and H4 was observed.
- The genome-wide reduction in core histone acetylation suggests an **epigenetic mechanism of action for lunasin**, which can influence expression of genes required for carcinogenesis.

MICROARRAY


HOW DOES A DNA MICROARRAY WORK?
DNA Microarray
Scme-nm.org
Southwest Center for Microsystems Education

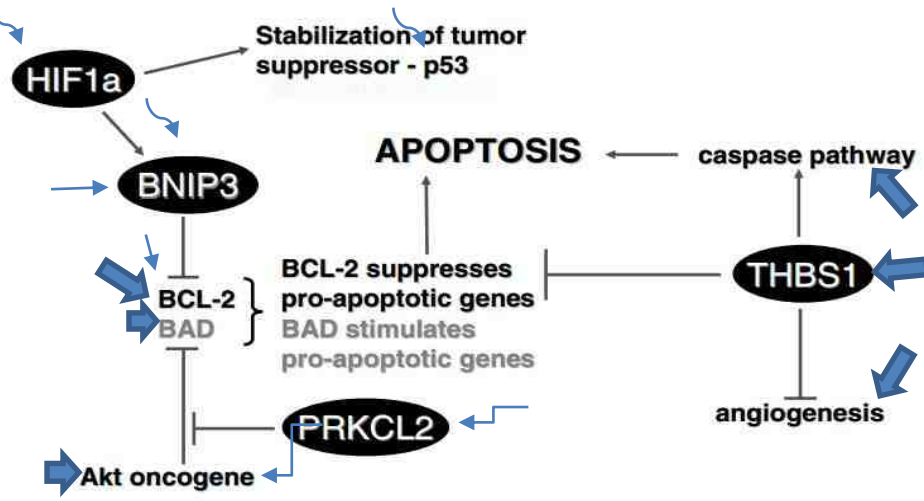
LUNASIN TREATMENT OF PROSTATE CANCER AND GENE EXPRESSION PROFILING

- This type of cancer is **the second leading cause of death among American men**.
- The effects of **anticancer agents on gene expression profiles of prostate** cell lines using **cDNA microarray** analysis have been reported.
- Microarray analysis has been a useful tool for **simultaneously determining changes in gene expression levels of tens of thousands of genes**.
- The gene expression profiles of **non-tumorigenic** (RWPE-1) and **tumorigenic** (RWPE-2) cells treated with **lunasin were assessed using microarray analysis**.
- The results of this analysis indicated that of the 14,500 genes interrogated, **123 genes had a greater than twofold** change in expression in the cells exposed to 2 μM lunasin for 24 hours.
- Of these genes, **121 genes were upregulated in RWPE-1** cells while only **two genes were upregulated in RWPE-2 cells**.
- **No genes were downregulated** in either non tumorigenic or tumorigenic epithelial cells treated with 2 μM lunasin.
- Genes upregulated in RWPE-1 cells include **those involved in tumor suppression, apoptosis, and the control of cell division**.

GENES FOR APOPTOSIS

- Transfection of lunasin into mammalian cells results in **arrest of mitosis leading to apoptosis**.
- The antimitotic effect of lunasin is believed to be due to the binding of its polyaspartyl carboxyl end **to regions of hypoacetylated chromatin**, like that found in **kinetochores in centromeres**.
- Apoptosis is triggered **when the kinetochore complex does not form properly and the *microtubules fail to attach to the centromeres***, leading to mitotic arrest and eventually cell death. Exogenous addition of lunasin **upregulates genes that are known to play a direct or indirect role in the induction of a**

Lunasin upregulation of genes involved in apoptosis: Apoptosis is controlled by **genes that suppress (bcl-2)** or **promote** (bad and the caspases) the apoptotic process.
In addition to promoting apoptosis, lunasin upregulates genes (**black ellipses**) that can also **stabilize tumor suppressors like p53 and suppress genes involved in angiogenesis**.



- **THBS1** induces apoptosis by **activating the caspase cell death pathway**.
- In addition, THBS1 has also been shown to have **a potent antiangiogenic activity** and the induction of apoptosis by THBS1 is associated with **decreased expression of the antiapoptotic gene, bcl-2**.
- Lunasin also upregulates the expression of BNIP3, **a gene that inhibits the antiapoptotic activities of BCL-2**.
- **BNIP3**, formerly known as Nip3, is **a mitochondrial protein that activates apoptosis and overcomes BCL-2 activity**.
- **BNIP3 causes cell death through opening of the mitochondrial permeability transition pores, resulting in mitochondrial dysfunction and plasma membrane damage.**

- Another gene upregulated by lunasin is the **hypoxia-inducible factor-1**, alpha subunit (HIF-1A), a basic helix–loop–helix **transcription factor that regulates the expression of BNIP3.**
- The BNIP3 promoter contains **a functional HIF-1A responsive element** and is potently **activated by both hypoxia and forced expression of HIF-1A**.
- Studies have also shown that HIF-1A **binds and stabilizes p53**, a tumor suppressor.
- On the other hand, PRKCL2, also termed PRK2, **promotes apoptosis by inhibiting the antiapoptotic activities of the oncogene Akt**.
- Akt **exerts its antiapoptotic effects by inactivating BAD, a proapoptotic BCL-2 family protein, by phosphorylation.**
- However, a PRKCL2 **C-terminal fragment** generated during the early stages of apoptosis **binds Akt, resulting in the inhibition of the Akt-mediated phosphorylation of BAD, thereby allowing apoptosis to occur**.

- **Lunasin primes the precancerous cell for apoptosis by upregulating proapoptotic genes like bad and inhibiting the antiapoptotic activities of Akt and some members of the bcl-2 gene family**.
- Lunasin may be indirectly involved in stabilization of tumor suppressor p53 via its interaction with HIF-1A.

GENES INVOLVED IN SUPPRESSION OF CELL PROLIFERATION

- Lunasin's inhibitory effects on carcinogenesis can be explained by the **upregulation of genes that play a role in *tumor suppression* or *anti proliferation***.
- For example, **lunasin upregulates** a tumor suppressor gene **encoding the cyclic AMP dependent protein kinase A type I-**α regulatory subunit, **PRKAR1A**.
- Mutations in the PRKAR1A result in the Carney complex (CNC), **a multiple neoplasia syndrome that is associated with thyroid tumorigenesis**.
- It is proposed that PRKAR1A mutant cells have deregulated control of gene expression, which results in the **activation of cAMP signaling pathways and abnormal growth and proliferation**.
- Another gene upregulated by lunasin, *BTB (POZ) containing domain 1 (ABTB1 or BPOZ)*, is thought to be **one of the mediators of the growth-suppressive signaling pathway of the tumor suppressor PTEN**.
- **Inactivation of the PTEN gene** is extremely common in human cancer, including cancer of the prostate.

- **Overexpression of BPOZ** inhibits cell cycle progression and **suppresses growth of cancer cells while the transfection of BPOZ antisense accelerates cell growth.**
- **Another antiproliferative gene** upregulated by lunasin is Tob (also referred to as Tob1).
- **Tob** is a member of the **antiproliferative** BTG/Tob family and **mice that are Tob deficient are prone to spontaneous formation of tumors**.
- The gene **erbb2** interacting protein (ERBIN), **a novel suppressor of *Ras signaling*, is upregulated by lunasin**.
- ERBIN, a leucine-rich repeat-containing protein, **interacts with Ras and interferes with the interaction between Ras and Raf**, **resulting in the negative regulation of Ras-mediated activation of extracellular signal regulated kinases (Erk).**
- **The Ras oncogene** is one of the most common mutations occurring in about 30% of human cancers.
- Mutations that cause ***constitutive activation*** of Ras result in **a continuous signal that tell the cells to grow regardless of whether or not receptors on the cell surface are activated by growth factors**.

MITOTIC CHECKPOINT GENES

- Other genes upregulated by lunasin include **the mitotic checkpoint genes** like **budding uninhibited benzimidazoles 1 homologue beta** (BUB1B or BubR1), TTK protein kinase (a homologue of yeast MPS1) and mitotic arrest deficient 2-like 1 (MAD2L1).
- Mitotic spindle checkpoint proteins **monitor proper microtubule attachment to chromosomes prior to progression through mitosis**, allowing *correct segregation of chromosomes into progeny cells*.
- TTK is a protein kinase that phosphorylates **MAD1p—a process essential for the activation of the mitotic checkpoint**.
- In yeast, MPS1 is required early in the spindle assembly checkpoint. It is considered to be a limiting step in checkpoint activation, since **it can activate the pathway when overexpressed**.
- Overexpression of MPS1 is able **to delay cell cycle progression** into anaphase in a manner similar to checkpoint activation by spindle damage.

- MPS1 is also **required for the essential process of spindle pole body duplication**.
- **BubR1** is a protein kinase required for checkpoint control.
- Evidence shows that **inactivation of BubR1 by microinjection of specific antibodies abolishes the checkpoint control**.
- Another study revealed **that endogenous BubR1 protein levels are reduced in some breast cancer cell lines**.
- Furthermore, the breast cancer-specific gene 1 (**BCSG1**), coding for an oncogenic protein, directly **interacts with BubR1**, resulting in the degradation of BubR1 through the proteosome machinery.
- It is speculated that **BCSG1-induced reduction of the BubR1** protein allows **breast cancer to progress at least in part *by compromising the mitotic checkpoint control through the inactivation of BubR1***.
- Another checkpoint gene **MAD2L1** was reported to have **reduced expression in a human breast cancer** cell line exhibiting **chromosome instability and aneuploidy**
- **Lunasin** might upregulates these mitotic checkpoint genes **to allow a heightened level of molecular surveillance** to **prevent premature cell division**, **chromosome instability**, and **aneuploidy**.

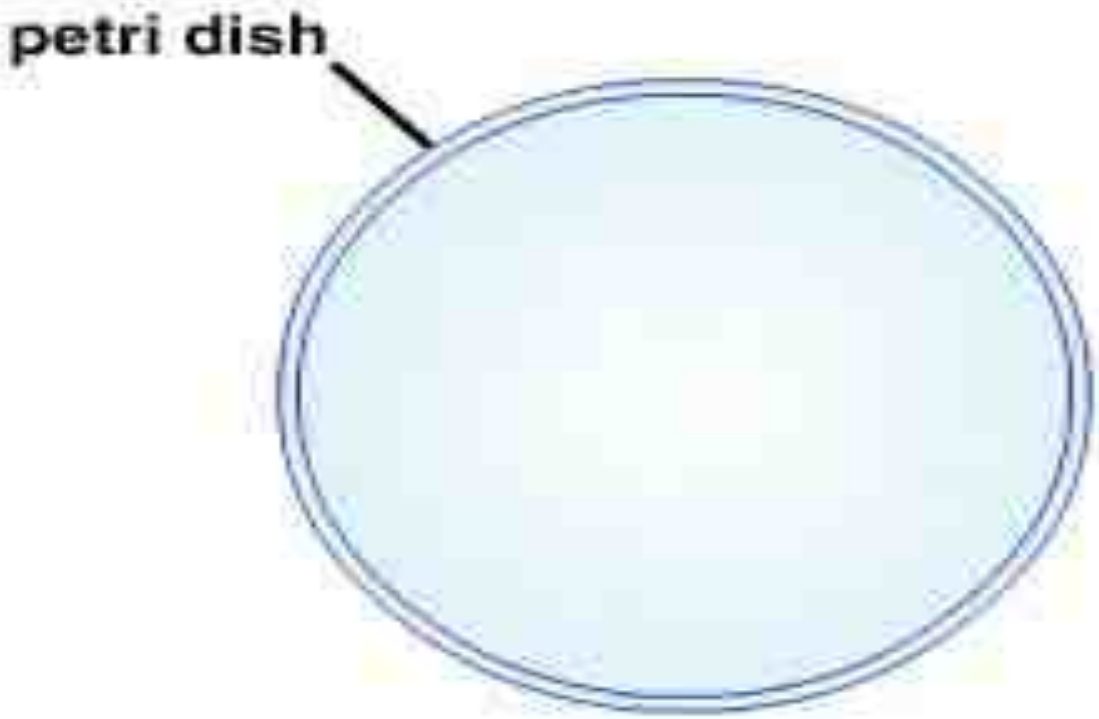
GENES INVOLVED IN PROTEIN DEGRADATION

Experiments show that **lunasin upregulates several genes involved in protein degradation and turnover via the ubiquitin pathway**.

It is possible that **lunasin upregulates these genes to mediate the *degradation of proteins that are required for the onset of cell transformation and foci formation***.

CONNEXIN 43 GENE FOR THE GAP JUNCTION PROTEIN

- Adjacent cells communicate with each other through **gap junctional channels that allow the passage of small molecules**.
- This process is referred to as "***gap junctional intercellular communication***"(GJC) and is **blocked in many cancer cells**, including **malignant human prostate cells**. Gap junctional channels are composed of proteins called connexins.
- **Lunasin** upregulates the expression of a gap junction protein called connexin 43, **which has been shown to have a tumor suppressive role**.
- **Decreased expression and impaired post-translational modification** of connexin 43 were **observed in several prostate tumor cell lines but not in normal cells**, suggesting that **the loss of junctional communication is a critical step in the progression to human prostate cancer**.
- Studies have shown that **the viral oncogene Src** disrupts cell growth regulation by **adding a phosphate group to a tyrosine residue in connexin 43**, thereby blocking gap junction communication.
- **Transfection of a functional connexin 43 gene into tumorigenic mouse cells results in the restoration of GJC, normal growth regulation, and cell-to-cell communication, as well as suppression of tumorigenesis**


petri dish

T2DM: FROM BIRTH ONWARD

- Inheriting **different combinations of genes and specific epigenetic modifications that may occur in utero means that each individual is born with a unique susceptibility to T2DM and other chronic diseases**.
- Once born, **the external environment greatly affects the risk** of developing chronic diseases.
- Genes that cause chronic diseases must be **regulated directly or indirectly by calorie intake and/or by specific chemicals in the diet because *diet alters disease incidence and severity***.
- The **progressive** and sometimes **slow change in phenotype** from health to disease must occur, at least in part, **through *changes in gene expression*.**
- The precise, statistical definition of gene–environment interaction is "**a different effect of *an environmental exposure* on disease risk in persons with different genotypes**" or, alternatively, "**a different effect of *a genotype* on disease risk in persons with different environmental exposures**".
- **In other words, nutrients affect expression of genetic information and genetic makeup affects how nutrients are metabolized.**

- Gene–diet interactions have been found **in experimental animals and in humans**.
- ✓ Krauss and co-workers **relied on phenotype to show genotypic differences**.
- Individuals with **small, dense LDL particles** (phenotype B) have an **increased risk** of coronary artery disease relative to those individuals exhibiting **large**, **less dense LDL particles** (phenotype A).
- The expression of phenotype A depended on diet: 12 out of 38 men who switched **from a 32% fat diet to a diet containing 10% fat developed the phenotype B pattern**.
- At least **three distinct genotypes were present in this group**, one genotype each for the A or B phenotype and **a third genotype that is responsive to low fat/high carbohydrate diets.**
- **This genotype produces the A phenotype when these individuals eat a diet containing 32% fat, but a B phenotype when fed 10% fat—a result that can be explained by genotype–environment interactions.**

- Although much attention in the nutrigenomics community is **focused on *gene regulation by dietary factors*.**
- Dietary chemicals **also alter the *activity of proteins and enzymes directly***.
- Ames and colleagues noted that mutations or polymorphisms in genes **often result in the corresponding enzyme having an increased *K*m (Michaelis constant) for a coenzyme.**
- The *K*m is a measure of affinity of ligand for its protein.
- Increases in *K*m result in **decreased affinity of coenzyme and therefore enzymes with increased *K*m have a decreased activity**.
- **Increasing the concentration of the coenzyme, which may come from diet, can ameliorate the effect of the decreased *K*m**.
- This concept is called the *K*m constant and is **an example of how alterations in diet may influence individuals differently depending on their genetic makeup.**

T2DM: METABOLOMICS

- The concentrations of **some transcriptional ligands and coenzymes** are **controlled by their in vivo metabolism from a dietary chemical precursor**.
- **Steroids**, for example, are produced through a core of ten linked reactions from **cholesterol**.
- The concentration of any given steroid ligand will be greatly **influenced by *specific combinations* of alleles for the enzymatic steps in biosynthetic** branch, and degradative pathways.
- Alleles of these interacting and linked genes **may be different among individuals from the same or different ancestral groups, creating differences in the levels of steroids and therefore expression of genes that these steroids regulate**.
- **Hence, analyzing genotypes and haplotypes within genes of a pathway is unlikely to provide a reliable association with the amount of the end product.**
- **High-throughput analysis of metabolites and the effect of changes in their concentrations is a new field called metabolomics**

THE NEED FOR DEFINED AND CONTROLLED EXPERIMENTAL SYSTEMS.

- Identifying **genes**, **proteins**, or metabolites regulated by diet and **involved in or marked by chronic disease processes in humans is challenging** because of the genetic variation among individuals, their long lifespan, and difficulty controlling and monitoring dietary intakes.
- **Cell cultures**, on the other hand, don't have **livers**, **microflora in the alimentary tract**, or the full metabolic repertoire of their complementary in vivo counterparts.
- **Animal studies are therefore necessary to *verify the results* from human studies and cell culture experiments.**
- A distinct advantage of using animal models İS **the array of genetically defined mouse strains, the result of a 100 year effort to produce and characterize inbred strains for biomedical research.**
- Comparative genomic analyses have demonstrated that **mice and rats share *genes and diseases that are similar in other mammals***.
- **For example, 99% of mouse genes have human homologues and obesity-induced diabetes (T2DM) occurs in mice and dogs.**

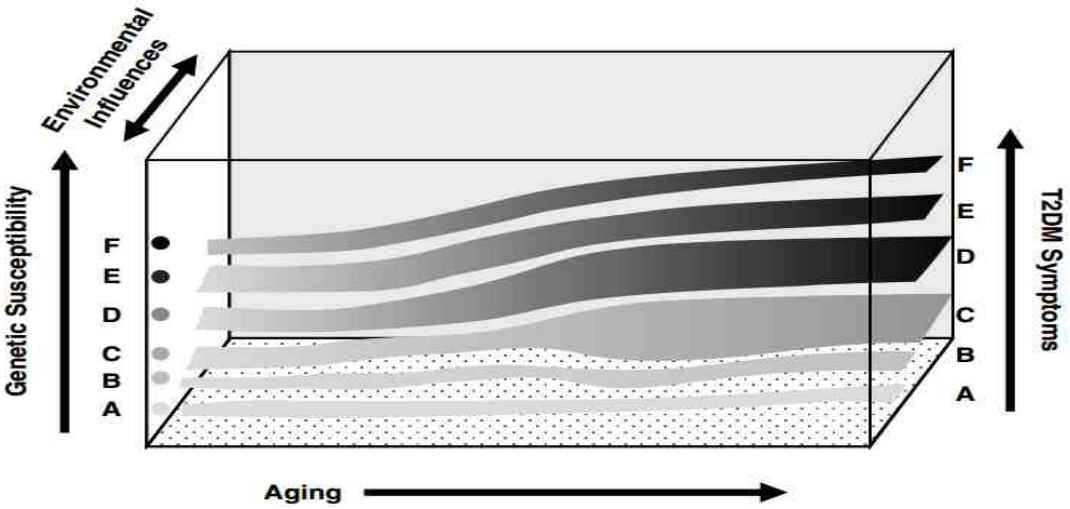
- Experimental laboratory animals are well suited for the study of nutrient–gene interactions because **the genotype and environmental factors can be controlled and systematically altered**.
- The use of **diets with known compositions** is of critical importance in nutritional genomics research.

EPIDEMIOLOGY

- The incidence and severity of chronic diseases **varies among countries**: **Stomach** cancer is higher while **breast** and **prostate** cancer are lower in **Japan** compared with the **United States**.
- These disparities **cannot be explained by either genes or diet alone** because the **average genetic makeup and environment** varies among countries.
- **Disease incidences change when individuals migrate** from one country and begin adopting the host country's **diet and culture**.
- **Second generation descendants** typically have disease incidences similar to the members of the principal culture, **a time too short for Mendelian genetic changes to occur**.
- By identifying food intake and cultural practices among individuals of different genetic ancestries and countries, **epidemiological research aids in the identification of nutritional and environmental contributors to disease and provides essential information for guiding the design of molecular and genetic research**.

- The analysis of nutrient–gene interactions in humans became apparent even before the completion of the human genome project.
- For example, the **G75A** (a G to A change, 75 base pairs upstream of the transcription start site) polymorphism in ***APOA1*** is associated with **increased HDL-cholesterol (HDL-C)** concentrations in the serum in **certain individual who consume higher amounts of polyunsaturated fatty acids** (PUFAs).
- Individuals **homozygous for the more common G SNP** have **lower levels of HDL-C** with increased intake of PUFAs.
- This is a classic example of genotype–diet interactions: **HDL-C levels are dependent on the genotype (G or A at 75) and the intake of a specific nutrient (PUFA).**
- The advent of SNP–nutrient–phenotype analyses has led to the realization of **the need for genomic controls**—that is, **testing whether individuals in a study have similar genetic makeup**.
- Although **the primary focus of nutritional genomics is the understanding of nutrient–gene interactions**, expression of genetic information also is influenced by numerous environmental factors.

- Many genetic and environmental influences **change *during life and aging* with the net result that health and chronic diseases are not discrete, dichotomous states** but are rather processes.
- Figure on the right above schematically shows **the theoretical paths of six individuals** (A through F in Table) **during aging**.



- Individuals are **born with differing susceptibilities to disease** (*y* axis, location of starting ribbon).
- **Life** and **aging** (*x* axis) exposes each individual to environmental influences (*z* axis) that have differing effects (width) depending on genetic makeup.

Hypothetical Genotypes of Six Individuals at Seven Disease Loci[a]

QTL	A	B	C	D	E	F
1	+	+	+	–	–	–
2	+	o	o	–	+	–
3	+	+	–	o	–	–
4	+	+	+	+	o	–
5	+	+	–	–	+	–
6	+	–	+	–	–	–
7	+	+	–	+	–	–

[a] Each individual inherits one of three alleles of each of the seven genes at QTLs 1 through 7. +, –, and o indicate protective allele, allele that contributes to disease, or allele that is neutral, respectively. Genetic susceptibility increases with increasing number of alleles that contribute to disease.

- The **different heights of the initial** condition (left axis) reflect **the differences in genetic susceptibility** (including epigenetic factors) and **the width of the paths** were designed to suggest **the influence of different environmental factors**.
- **Certain individuals** (e.g., C and D) may be **able to greatly influence onset or severity of disease by altering life style** whereas others are **destined for disease** (e.g., F) **or health** (e.g., A) **regardless of life style**.
- **Clinical measurements are taken at discrete time points along this curve and therefore are only a single frame of a movie and *may not accurately predict past physiological processes or future outcomes*.**
- While important, **these snapshot diagnostics need to be supplemented with genetic analyses of susceptibility genes** (at birth) and **a greater understanding of their interactions with diet and the environment**.
- **The majority of individuals may therefore influence health processes** since individuals with extreme susceptibilities are unlikely to be found at high frequencies.

HEALTH DISPARITIES

- The U.S. Secretary's Task Force **Report on *Black* and *Minority* Health**, revealed that **certain minority populations exhibit higher incidence and severity of many chronic diseases including diabetes, obesity, asthma, cardiovascular diseases (CVDs) and certain cancers**.
- Similar health disparities exist in New Zealand, Canada, Australia, and England.
- **Genetic differences alone, however, cannot explain these health disparities since the *incidence of prostate cancer in Africa has increased from 1960 to 1997 and is now approximately as prevalent as in the United States***.
- ***Investigations how individual genetic variation may exacerbate diet as a risk factor for disease and how dietary intervention based on knowledge of nutritional status, nutritional requirements, and genotype can remedy or ameliorate disease symptoms are on course.***
- Nutrigenomics researchers seek to **identify and characterize *genes regulated by naturally occurring chemicals in foods and the subset of those genes that influence balance between healthy and disease states***.

ETHICAL ISSUES IN NUTRITIONAL GENOMICS

PROACTIVE ETHICS AND NUTRITIONAL GENOMICS

- In May 2001, a small start-up company in the United Kingdom launched the world's first nutrigenetic testing service and Sciona Ltd **began offering customized dietary advice to customers on the basis of a life-style questionnaire and genetic test.**
- Within weeks of the products'appearance on store shelves, *Consumers' Association had begun a campaign to raise public awareness about the service and how it was being marketed*.
- They raised fears that **tests might mislead customers about healthy lifestyle choices, customers might learn things about their health which they may not want to know about**, **insurers and employers might gain access to this information i**n the future, and **customer's genetic information might be patented or used for research without their knowledge**.
- Despite the best intentions **to maximize the benefits and minimize the risks** of new science and technology, **not all innovation will prove to be socially acceptable.**

- In other cases, innovations will be taken up but will prompt legal and ethical change.
- In fact, **it appears to be the rare instances when "science" and "society" conjoin in a coordinated effort**.
- More typically, **science advances, ethics and law then react**.
- This point was recognized in the 1990s by the founders of the Human Genome Project, who **set aside funding for the Ethical, Legal and Social Implications (ELSI) program.**
- The mandate of the ELSI program is **to identify and address the ethical, legal, and social implications of human genome research at the same time that the *basic scientific issues* are being studied**.
- In this way, it is hoped that **problem are as will be identified and solutions developed before adverse effects occur**.
- It should be kept in mind that since individuals can benefit or be harmed by nutrigenomics, **the autonomy of individuals is the appropriate initial "object" of analysis for nutrigenomics**.

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1. QUANTITATIVE TRAIT LOCUS ANALYSIS

- A QTL is **a polymorphic locus** containing one or more genes that contribute to the **phenotypic expression of a continuously variable trait**.
- QTLs are typically found by statistical analyses of **how frequently a region of chromosome is associated with a certain measurable phenotype** such as **insulin levels.**
- Since multiple genes are involved in those responses, **a QTL and the gene(s) variant(s) encoded within contribute less than 100% to the phenotype**.
- QTLs mapping is done in humans but **the process is best explained with laboratory animals**.
- **Two parental inbred mouse strains**, selected for differences in some observable or measurable phenotype—such as susceptibility to T2DM—are bred **to produce an F1 generation**.
- F1 mice are backcrossed to each of the parental strains producing an F2 generation **differing in disease susceptibility because of independent assortment of chromosomes and chromosomal rearrangements that occur during meiosis**.

- The incidence or severity of **subphenotypes of the disease** is measured in F2 mice and **statistically associated with chromosomal regions** from each of the original parental strains.
- A given pair of inbred mice may have **10–15 regions** that contribute to the complex phenotype in those strains.
- Different pairs of strains may reveal new QTLs because **inbreeding selects for a subset of genetic variation.**
- **If one or more of the genes within the QTLs regions were mutated**, each parental strain would not develop the particular disease because the specific trait or disease is produced by **the sum** of contributions from alleles of causative genes **within different QTLs.**
- Two hypotheses were proposed to explain **gene variants that contribute to quantitative traits**.
- The **first,** called the **common disease/common variant** hypothesis (i.e., CDCV hypothesis, says that **combinations of normally occurring gene variants produce disease**.
- These gene variants occur in **greater than 1%** of the population.

- Others dismiss this theory, suggesting that **combinations of rare** (<1% in the population) **allelic variants cause common diseases**.
- This theory is called the **multilocus/multiallele hypothesis**.
- Regardless of the outcome of the controversy, the consequences of **the polygenic nature of chronic disease complicate the search for genes involved in disease processes**.
- Jackson Laboratory currently (Kaput & Rodriguez, 2006) lists 58 QTLs for insulin with subclasses for Type **1 diabetes** (28 QTLs), insulin levels (13 QTLs), and T2DM (17 QTLs).
- Since QTL analysis is inherently more **straightforward with inbred animals** and different inbred strains are known to express different subphenotypes of disease, ***it is possible to identify more QTLs with smaller influences on each quantitative trait compared to analyses in outbred animals such as humans***.
- As importantly, **a subset of these murine QTLs map to syntenic regions of T2DM QTLs found in humans.**
- Analyses of **inbred strains with differing disease susceptibilities** are therefore important components of nutrigenomics research because **the genotypes are known and the diet and other environmental factors can be controlled.**

2. T2DM: QTLS IN HUMANS

- A total of **17 QTLs** distributed on chromosomes 1, 2, 4, 5, 7, 8, 9, 10, 11, 12, 20, and X have been identified.

Table: Hypothetical Genotypes of **Six** Individuals at **Seven** Disease Loci

QTL	A	B	C	D	E	F
1	+	+	+	−	−	−
2	+	o	o	−	+	−
3	+	+	−	o	−	−
4	+	+	+	+	o	−
5	+	+	−	−	+	−
6	+	−	+	−	−	−
7	+	+	−	+	−	−

[a] Each individual inherits one of three alleles of each of the seven genes at QTLs 1 through 7. +, −, and o indicate protective allele, allele that contributes to disease, or allele that is neutral, respectively. Genetic susceptibility increases with increasing number of alleles that contribute to disease.

- If there are only three alleles at each locus with one contributing to T2DM (designated -), providing protection (+), or neutral (0), **the number of possible combinations for seven loci is 2187**, but the actual number is constrained because of allele frequencies.
- For purposes of illustration, **individual A** inherited all "protective" alleles and would thus have a low probability of developing T2DM (**this speaks nothing of susceptibility to other diseases**).
- **Individual F** is the other extreme and is genetically prone to develop symptoms.
- **Individuals B, C, D, and E** have intermediate risks and are **likely to benefit from life-style changes and/or drug treatments**.
- Perhaps the most interesting in this example are individuals **D and E**, who each have the same number of protective, neutral, and disease contributing alleles.
- **They may not have the same genetic susceptibility** because one cannot predict a priori whether each QTL contributes the same amount to a given phenotype.

- Added to this complexity are ***epistatic*** **interactions and** ***epigenetic*** **functions of DNA methylation** and chromatin remodeling.
- At least some of these genes are likely to be **regulated directly or indirectly by diet** and other environmental factors, further complicating analyses of their contribution to diseases or subphenotypes of disease.
- Genetic analyses and studies in experimental systems have led to the identification of **candidate genes that contribute to subphenotypes of T2DM** in at least some populations.
- Given the number of QTLs identified in humans (with significant or suggestive linkage) and in rodents, ***it is likely that many other T2DM genes have yet to be identified***.
- **The reason that QTLs are not consistently found in different populations can be explained by epistasis and epigenetics.**

3. EPISTASIS ALTERS STATISTICAL ASSOCIATIONS

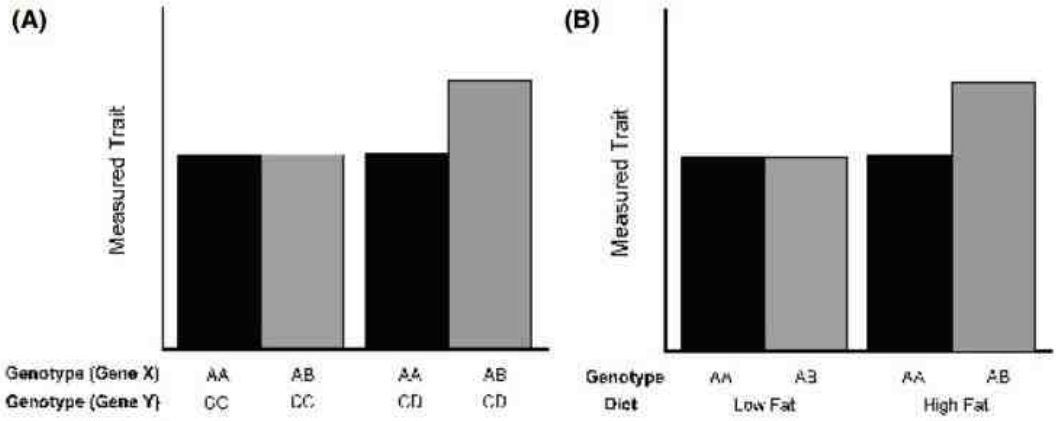
- Epistasis, or gene–gene interactions, provides **the explanation for *how genetic ancestry is so important* in understanding gene–nutrient interactions and, ultimately, health and disease**.
- Gene–gene interactions can occur through protein–protein, protein–gene, RNA–protein, or RNA silencing.
- The molecular explanation for these genetic results is that **proteins or enzymes produced by a gene or its variant *do not act alone***, but are usually part of a pathway, and **many pathways are interconnected**.
- As one example, **a G to A polymorphism in the tyrosine phosphatase 1B gene** interacts statistically with **a polymorphism in the leptin receptor (*LEPR*) gene** in a Finnish study of 257 individuals with T2DM and 285 controls.
- *Genes* may not interact directly but **may be in the same signal transduction pathway and variants in one affect the activity of the other**.
- A decrease in activity of one member of a pathway may be **compensated for** by **another member** of the same pathway, or by **variations in a connected pathway**.

- Compensation in the activity of parts to maintain the overall balance within the system is called "**buffering**".
- Understanding and studying **the impact of epistasis at the genetic and biochemical levels** is a key component of nutrigenomics research of health and chronic diseases.

3.1. EPISTASIS AND GENE–ENVIRONMENT INTERACTIONS IN OBESITY AND DIABETES

- While the example of **coat color** is easy to visualize, **other traits** such as **body fat** percent or **plasma glucose are harder to understand intuitively** because such traits are quantitative **and cannot be put into discrete categories**.
- **However**, the principle of epistasis remains the same.
- The presence of **alleles of one gene that *increase* body fat may *mask* the effects of alleles of a second gene that *decrease* body fat**.
- In figure (**A)** on the following slide, **heterozygous alleles for both gene X and gene Y are needed to increase the phenotype**, whereas each **heterozygous allele alone** is not sufficient to produce an effect.
- Likewise, **diet and/or exercise may interact with genes to produce unexpected outcomes.**

- A certain phenotype depends on both the **alleles one possesses** and **the diet one consumes**, in this case (figure B) **amount of fat in the diet**.
- The effect of the **low-fat diet masks the effect of the B allele to increase body weight**.
- On a low-fat diet, both genotypes **AA and AB respond equally**. However, on a high-fat diet, **the presence of the B allele yields a greater response to the high-fat diet**.



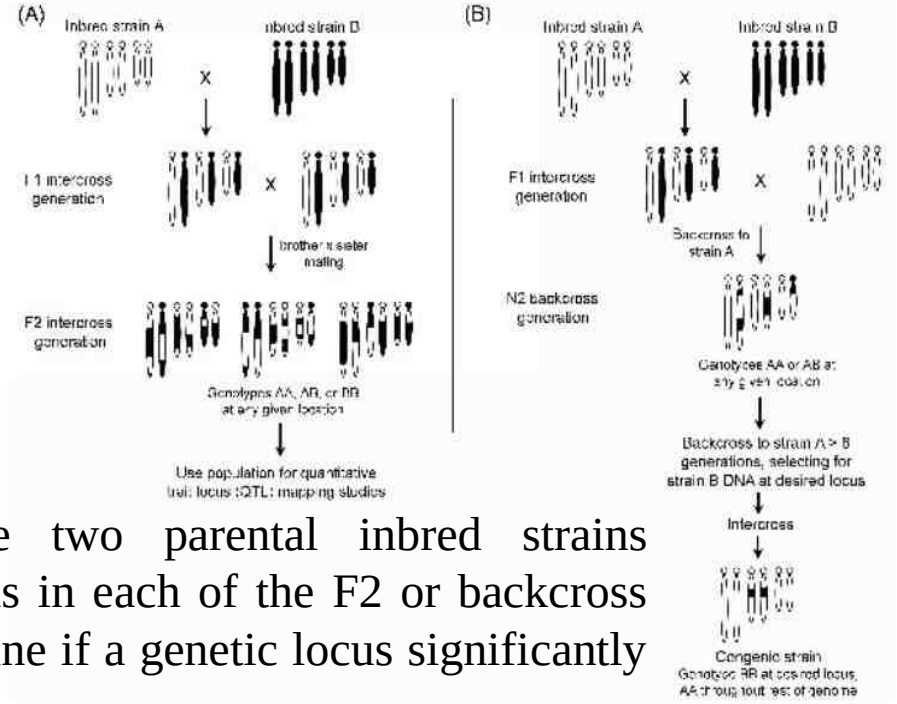
- Both **obesity** and **T2DM** are complex traits and increasing evidence suggests that **both gene–gene interactions and gene–diet interactions contribute to the pathogenesis of both diseases.**
- Gene–diet interactions will allow **better dietary recommendations** to be made to individuals **based on their genetic makeup** and **subsequent response to dietary factors**.

ANIMAL MODELS FOR DETECTING GENE INTERACTIONS

- Since the **homologous regions of mouse and human chromosomes are well-defined**, ***mapping and identification of a gene in mice allows immediate examination in humans***.
- Ability to **manipulate diet** and **obtain any tissues at any time** point are ***critical advantages of animal studies***.
- Use of **experimental crosses in inbred animal** models is an efficient method for **dissection of complex** (quantitative) traits **into discrete genetic factors**.
- QTL analysis identifies the **chromosomal location of a gene** causing the phenotype of interest **without needing any biochemical or physiological knowledge of the gene's function**.
- The gene needs both **to participate in the target pathway** and to **have functionally different alleles in the two inbred strains being studied**, that is, to have **sequence differences** that alter protein function or mRNA expression for that gene.

- To accomplish QTL mapping, **two inbred strains that differ in the trait of interest are bred to produce F1 mice**.
- The F1 mice, which are all genetically identical, are then intercrossed to produce **F2 progeny having three possible genotypes at each marker** (Figure A), or backcrossed to either parent to produce progeny having **two possible genotypes** at each marker (Figu

Strains can be termed inbred if they have been mated brother x sister for 20 or more consecutive generations, and individuals of the strain can be traced to a single ancestral pair at the 20 th or subsequent generation.



Genotyping identifies which of the two parental inbred strains contributed the allele at a specific locus in each of the F2 or backcross progeny and analysis is done to determine if a genetic locus significantly affects a trait

EPIGENESIS AND CHROMOSOME STRUCTURE—ANOTHER LEVEL OF NUTRIENT CONTROL

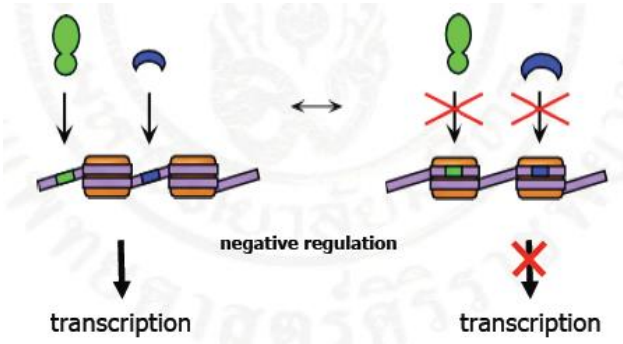
- Epigenetics is the study of **heritable changes in gene function that occur without a change in the sequence of nuclear DNA**.
- X chromosome inactivation and gene silencing are examples of epigenesis.
- Epigenetic mechanisms of altering gene regulation are **DNA methylation** and **chromatin remodeling**.
- Both mechanisms **change the accessibility of DNA** to **regulatory proteins** and **complexes altering transcriptional regulation**.
- Methylated DNA is considered transcriptionally inactive although there are exceptions to this rule.
- DNA **can be methylated at specific sites**, usually at CpG dinucleotides with islands rich in cytosine and guanosine.
- Methylated CpG within these islands binds **methyl-CpG binding proteins** (MBDs).

- MBDs subsequently "**recruit" histone deacetylases** whose activities **alter histone proteins, inducing further changes to chromatin structure**.
- **Nutrient intake affects DNA methylation** status because *DNA methyltransferases catalyze the transfer of a methyl group from **S-adenosylmethionine** (S-AM) to specific sites in DNA.*
- The products of the reaction are DNA methylated at (usually) CpG residues and ***S*-adenosylhomocysteine** (*S*-hcy).
- ***SAM* is generated by the one-carbon metabolic pathway, a network of interconnected biochemical reactions that transfer one-carbon groups from one metabolite to another**.
- Dietary deficiencies of **choline**, methionine, **folate**, **vitamin B12**, vitamin B6, and **riboflavin (B2)** affect one-carbon metabolism and DNA methylation and **increase the risk of neural tube defects, cancer, and cardiovascular diseases**.
- Chromatin remodeling is **regulated in part by the energy balance in a cell**.
- Specifically, changing calorie intake has been shown to alter chromatin remodeling, changing the NADH/NAD+ ratio and the activity of SIR2, an **NAD+-dependent histone deacetylase**.

- Chromatin remodeling may also be affected by **changing the expression of genes involved in chromosome structure or its regulation**.
- Expression of genes involved in chromatin remodeling is **altered by a lunasin**, a peptide isolated from soybean.
- **Long-term exposure** to diets that remodel chromatin structure and DNA methylation could **induce permanent epigenetic changes**.
- **Such changes might explain why *certain individuals* can more easily control symptoms of chronic diseases by changing life style but *many* seem to pass an irreversible threshold**.
- Epigenetic changes may also explain "**developmental windows**"—key times during development, such as in utero, where short-term environmental influences may produce long-lasting changes in gene expression and metabolic potential.
- **Altering the level** of the **proteins**, **enzymes**, and **RNAi** (interfering RNA) involved in chromatin remodeling ***by diet or other environmental factors*** is **another control point for regulating gene expression**.

SREBPs AND ChREBP: *TRANSCRIPTION FACTORS* INFLUENCED BY DIETARY LIPIDS AND GLUCOSE

- Several dietary studies have implicated **dietary polyunsaturated fatty acids** (PUFAs) in the **inhibition of hepatic expression of several genes involved in fatty acid synthesis**.
- PUFAs have dramatic effects on gene expression by regulating the **activity** or **abundance** of several transcription factors.
- ✓ Except for SREBPs and ChREBP, **all other transcription factors are members of the superfamily of nuclear receptors**.



SREBPs

- SREBPs regulate the expression of **over 30 genes connected with endogenous cholesterol, fatty acids, triacylglycerol, and phospholipid synthesis**.
- SREBPs can be separated into three isoforms: SREBP-2, SREBP-1a, and **SREBP-1c** (also known as ADD1), of which **SREBP-1c appears to be the most physiologically relevant**.
- **SREBP-1a** and **SREBP-1c** are derived from a single gene through ***alternative splicing*** **and the use of** ***alternative transcription start sites*** **at their first exon**.
- They are synthesized as ***inactive precursors*** **bound to endoplasmic reticulum**.
- Their release **and activation requires** ***transport from the endoplasmic reticulum*** *to the* ***Golgi complex*** **by** the sterol sensor SREBP cleavage activating protein (**SCAP**) followed by **a two-step** ***cleavage by Golgi located proteases*** (S1P and S2P).

- The formation of mature SREBPs is ***controlled by*** **cellular levels of cholesterol, insulin/glucose, and PUFAs through feedback inhibition of their proteolytic cleavage**.
- SREBPs activate the transcription of their target genes by **binding as *homodimers* at sterol response elements** (SREs) with the **consensus sequence** YCAYnYCAY.
- Although they differ in their potency, SREBP-1a and SREBP-1c induce the **expression of genes facilitating the synthesis of mono- and polyunsaturated fatty acids as well as their incorporation in triglycerides and phospholids**.
- Among those genes are ATP-citrate lyase, acetyl-CoA synthetase, acetyl-CoA carboxylase, fatty acid synthase, **stearoyl CoA desaturase**, and other desaturases.
- SREBP-2 preferentially regulates the expression of genes **involved in uptake of cholesterol from lipoprotein particles** and **the entire cascade of de novo biosynthesis of cholesterol**, among them low-density lipoprotein receptor (**LDLR**) or **farnesyl pyrophosphate synthase**, **HMG-CoA synthase** and **HMG-CoA reductase**.

ChREBP

- The carbohydrate sensitive response element binding protein (ChREBP) is required **for both basal as well as carbohydrate-induced expression of certain lipogenic genes**, which are essential for the **conversion of dietary carbohydrates into triglycerides, thereby promoting long-term storage of carbohydrates as triglycerides**.
- Studies showed that **nutrients themselves appeared to induce the expression of several regulatory *enzymes of glycolysis and lipogenesis without the presence of insulin in primary hepatocytes***.
- These effects appear to be mediated by ChREBP.
- ChREBP was **first isolated from primary hepatocytes and later found to be highly expressed in brown and white adipose tissue, kidney, the intestine, and skeletal muscle as well**.
- Many lipogenic genes that mediate glucose responsiveness **contain glucose/carbohydrate response elements (ChREs) *within their promoters***.

- High carbohydrate diets induce the transcription of more than **15 genes involved in the metabolic conversion of glucose to fat**, including **liver specific pyruvate kinase**, a regulatory enzyme in the pathway of liver glycolysis, **fatty acid synthase**, which uses acetyl-CoA and malonyl-CoA to form long-chain fatty acids, **acetyl-CoA carboxylase**, whose activity provides malonyl CoA, and Spot14, a ***nuclear protein thought to be involved in stimulating lipogenesis***.

CROSSTALK BETWEEN SREBPs AND ChREBP

- ***Fatty acids***, ***cholesterol***, or ***carbohydrates*** apparently ***do not bind directly to SREBPs or ChREBP,*** **which differentiates them from nuclear receptors**.
- Nevertheless, SREBP and ChREBP are **a pair of important transcription factors involved in the *nutrient and hormonal regulation of genes* encoding enzymes of glucose metabolism and lipogenesis**.
- **Key lipogenic genes** are **regulated *synergistically* by both SREBPs and ChREBP**.
- ✓ High carbohydrate diets lead to **insulin secretion from the pancreatic beta cells, which mediate proteolytic cleavage and activation of SREBP**.
- ✓ In addition, **ChREBP is present in the nucleus at high glucose levels but is localized in the cytosolic compartment of the cell during fasting**.

- It appears that **glucose regulates its dephosphorylation**, which enables the activation of the import of ChREBPs into the nucleus, where it **induces the expression of genes containing glucose response elements**.
- Dietary PUFA:

(1) **Can efficiently decrease both the mRNA levels as well as the mature nuclear form of SREBP-1c**, suggesting that in the latter case PUFAs may influence proteolytic processing;

(2) Can inhibit the transcriptional effects of SREBPs and ChREBP and subsequently glucose **utilization in the presence of glucagon**; and

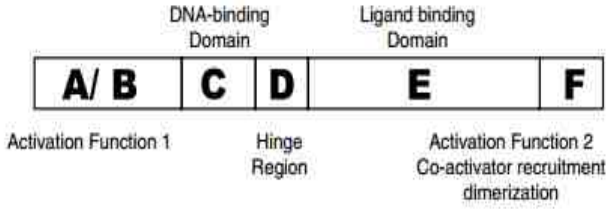
(3) Can inactivate ChREBP by phosphorylation.

SUPERFAMILY OF NUCLEAR RECEPTORS

- **Receptors in the nucleus** can **directly or indirectly regulate gene expression** **in response to lipid-soluble nutrients and their metabolites**.
- The human genome project has revealed **48 members of nuclear transcription factors** that comprise a superfamily of related genes and functions.
- **The classical steroid receptors**, glucocorticoid, estrogen, androgen, progesterone, and mineralocorticoid receptor (GR, ER, AR, PR, and MR) **respond primarily to *endogenous hormonal lipids***.
- **Steroid** hormones bind to their receptors with **high affinity**.
- **Other** nuclear receptors can **mediate the effects of fat-soluble vitamins, fatty acids, or cholesterol metabolites**.
- These nuclear receptors act as **lipid sensor** due to their **ability to bind dietary lipids or lipids that are intermediates in metabolic pathways**.
- **Lipid sensing receptors** were cloned because of their sequence homology to steroid receptors.
- Since their **natural ligands, target genes, and physiological** importance were initially

NUCLEAR RECEPTORS: STRUCTURE AND FUNCTION

- The nuclear receptors **share common structures** (Figure at the end of this slide).
- The **amino terminal A/B domain** is highly variable in **length** and **sequence** in the different family members.
- It contains the **activation function-1** (AF-1), which is responsible for ***ligand-independent transcriptional activation*** **by mediating the coordinated interaction of coregulatory proteins**.
- A highly conserved C domain or **DNA binding domain** (DBD) is found 3′ to the AF-1 domain and structurally consists of **two zinc finger motifs** with affinity to specific target DNA sequences called **responsive elements** (REs, Figure on next slide).
- Adjacent to the DBD is the **short** D domain, also known as **the hinge region**, which harbors a putative **nuclear localization signal** (NLS) and **residues important for** [illegible] **nal corepressor proteins** that mediate the ***tran*** [illegible] nliganded receptors.

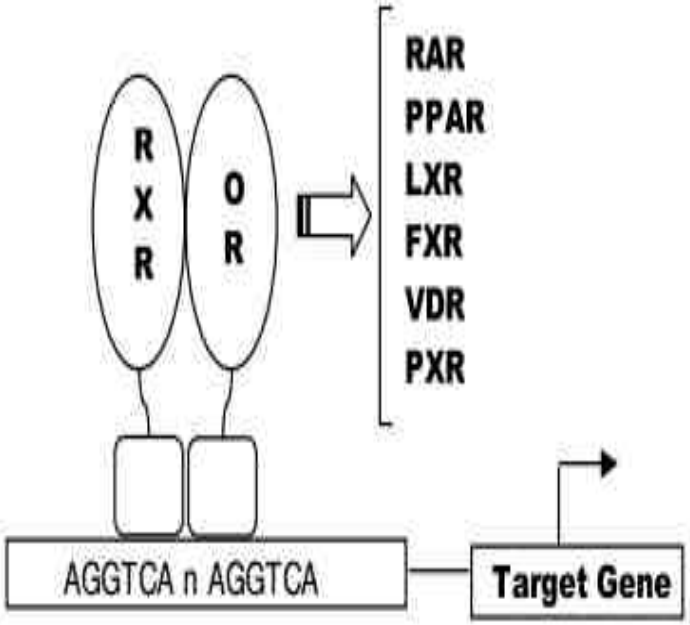


- The E domain includes the **ligand binding domain** (LBD), a NLS, and AF-2.
- This carboxy terminal region **controls ligand binding**, **transactivation**, **nuclear localization**, and **dimerization**.

Responsive element	Half-site orientation	Examples of nuclear receptor
Inverted Repeat	⇨ N_n ⇦	RAR/ RXR (n=0) FXR/ RXR (n=1)
Everted Repeat	⇦ N_n ⇨	RAR/ RXR (n=8) VDR/ RXR (n=9)
Direct repeat	⇨ N_n ⇨	RAR/ RXR (n=2/5) PPAR/RXR (n=1) LXR/ RXR (n=4) VDR/ RXR (n=3)

- The **hinge region** serves as a highly **flexible** link **between the DBD and LBD** that **allows for simultaneous receptor dimerization and DNA binding**.
- This flexibility ensures a high degree of rotational freedom, so that the nuclear receptors ***can bind to a variety of response elements***
- In response to ligand binding, **nuclear receptors undergo transformational changes**, allowing dimerization, protein–DNA interaction, and recruiting of cofactors and other transcription factors.
- **In the absence of ligands**, however, **certain** nuclear receptors, like the **retinoid sensitive RAR/RXR heterodimer**, are located in the nucleus and bound to the response elements in the regulatory region of their target genes.
- Unliganded RAR/RXR is **associated with histone deacetylase-containing** (HDAC) **complexes**, tethered through corepressors, which results in **repression of gene transcription**.
- **Upon ligand binding**, the **corepressor dissociates**, enabling recruitment of **coactivators**, which are associated in complexes with **histone acetylase** (HAT), **methyltransferase**, **kinase**, or **ATP dependent remodeling** (SWI/SNF complex) activities that **decompact chromatin**.

- The group of **adopted *orphan receptors* have low affinities for dietary lipids.**
- (These receptors require **heterodimerization** with RXR in order to mediate DNA binding and regulation of transcription, with **the exception of RXR**, which acts as a homodimer (**Figure on the right**)).
- **Their response elements** are composed of two degenerated hexameric AGGTCA sequences, oriented either as ***direct* repeats in tandem** (DR), ***inverted*** (IR), or ***everted***, and separated by 1 to 8 nucleotides, depending on the receptor.
- Hence, **a direct repeat, spaced by 1 nucleotide, is referred to as DR1**.
- There can be **high variability** in the AGGTCA hexamer sequence.
- The **surrounding** and **intervening** DNA sequences may significantly affect the binding affinity and function of RXR heterodimers.



NUCLEAR RECEPTORS AS METABOLIC SENSOR

The Hepatocyte Nuclear Factor 4

Fatty acids *constitutively bind* to the LBD and are activating the transcriptional activity of HNF4.

In this respect **the fatty acid/HNF4 complex differs significantly from the activation of other nuclear factors by fatty acids**.

HNF4 binds as homodimer to DR1 motifs, regulates the expression of genes encoding **apolipoproteins**, enzymes regulating **carbohydrate and bile acid metabolism**, insulin secretion, and certain cytochrome P450s.

Its transcripts are mainly found **in liver** and **pancreatic islets**, befitting **its pivotal function in glucose and triglyceride metabolism**.

The peroxisome proliferator activated receptors

- The peroxisome proliferator activated receptors (PPARs) were first cloned as nuclear receptors ***mediating the effects of synthetic compounds called peroxisome proliferators on gene expression***.
- **The ligands**, which are activating PPARs and therefore gene expression, are **several PUFAs**, among them α-linoleic (C18:3), γ-linoleic (C18:3), arachidonic (C20:4), and eicosapentaenoic acid (C20:5), **including their derivatives**.
- (PPARα, PPARβ or δ, PPARγ1, and PPARγ2) have **distinct expression patterns and biological functions**.
- **PPARα** is a global **regulator of energy homeostasis** and is expressed in **liver**, **kidney**, **heart**, and **muscle**.
- PPARα directly induces the **expression of genes facilitating *fatty acid uptake* and intracellular transport of fatty acids into peroxisomes and mitochondria for their fatty acid oxidation**, like **fatty acid binding protein** (FATP) in the liver, the major organ of fatty acid metabolism.
- PPARα also regulates the **expression of several catabolic enzymes**, utilizing mitochondrial **β-oxidation** and peroxisome **ω-oxidation**, including **acyl-CoA oxidase** or **cytochrome P450 4A.**

- **Fibrates**, potent synthetic **PPAR*α* agonists**, can **lower serum triglyceride levels and increase HDL cholesterol** in patients with hyperlipidemia.
- **PPARγ**, considered the master switch of adipocyte differentiation, regulates ***adipogenesis*** as well as **diverse physiological processes**, among them ***cellular differentiation*** and ***insulin sensitization***.
- PPARɤ has **two splice variants**, PPARɤ1 and PPARɤ2.
- PPARɤ1 is more widely expressed, but **highest in *adipose tissue*, *macrophages*, and *endothelial cells***.
- PPARγ2 appears to be expressed **exclusively in adipocytes**.
- PPARγ further **promotes the storage of fat** by increasing adipocyte differentiation and **enhancing the transcription of genes** that are important **for lipogenesis**.
- Several in vivo and in vitro studies reported that **PPAR ligands have *pronounced insulin-sensitizing* effects through their influence on glucose and lipid homeostasis in muscle, liver, and adipose tissue.**

- They affect the expression of **adipocyte-secreted hormones** such as **leptin**, **tumor necrosis factor-α** (TNF-α), **plasminogen activator inhibitor-1**, **interleukin-6** (IL-6), and **adiponectin**.
- In addition, **PPARγ ligands reduce the levels of circulating free fatty acids** (FFAs), which are associated with insulin sensitivity, **by increasing the net flux into adipose tissue and by preventing their release**.
- PPARγ stimulates **lipolysis of circulating triglyceride and the subsequent uptake of fatty acids into the adipose cell through induction of the expression of lipoprotein lipase** (LPL), FATP, and the **class B scavenger receptor** that binds long-chain fatty acids and modified LDL.
- Increased expression of **phosphoenolpyruvate carboxykinase** (PEPCK) and glycerol kinase GyK9 enables **triglyceride synthesis** and promotes **the storage of fatty acid**.
- In addition, **other adipogenic factors** involved in **glucose and lipid homeostasis**, like insulin-responsive glucose transporter GLUT4 or **11-β-hydroxysteroid dehydrogenase** 1 (11β-HSD-1)" cortisone reductase" **are modulated by PPARγ**.

weight around their abdomens. It's thought that having a pear-shaped body — that is, carrying more of your weight around your hips and having a narrower waist — doesn't increase your risk of diabetes, heart disease and other complications of metabolic syndrome.

- The prevalent isoform **PPARβ/δ** is **expressed ubiquitously** and is like the other PPAR isoforms **involved in lipid and lipoprotein metabolism.**
- Selective **agonists for PPARβ/δ** appear to have beneficial effect on reverse cholesterol transport from peripheral tissue by ***increasing serum high-density lipoprotein (HDL) cholesterol, while decreasing LDL cholesterol***, and thus can **decrease the risk of cardiovascular disease associated with metabolic syndrome X**
- In addition to metabolic pathways, PPARβ/δ appears to be an essential **transcription regulator for early steps of cell differentiation in various adipocytes, keratinoycytes, and oligodendrocytes**, rather than being involved in terminal differentiation and maintenance of the differentiated state.

THE INFLUENCE OF GENE POLYMORPHISMS ON EVOLUTION OF ATHEROSCLEROSIS (Vana Kolovou and Genovefa Kolovou 2014)

- The evolution of atherosclerosis is **influenced by *environmental risk factors*** such as **dyslipidemia**, **arterial hypertension**, **diabetes mellitus** and **smoking**, **which are significantly related** to cardiovascular (CV) disease **and by genetic factors**.
- Genetic factors may **explain approximately 50%** of the risk for CV disease.
- Atherosclerosis has been widely **evaluated in *apolipoprotein E knockout mouse*** (an animal model where a similar pathological process to that in humans **occurs over a short period of time**) and the results indicate ***a strong genetic component* to atherosclerosis**.
- Numerous studies have used **genes implicated with known risk factors** for atherosclerosis, **for example dyslipidemia**.
- **Genes involved in lipid metabolism** which are frequently studied are for: ***low density lipoprotein receptor***, ***cholesteryl ester transport protein*** (CETP), ***apolipoproteins***, ***hepatic lipase***, ***lipoprotein lipase***, ***peroxisome-proliferator-receptor-α*** and others.

- For example, CETP may have ***pro- or anti*** atherogenic properties depending upon the lipid metabolic setting .
- High density lipoprotein (HDL) particles are influenced by CETP activity; CETP **promotes the exchange of cholesteryl esters for triglycerides (TG) between HDL particles and TG-rich lipoproteins**.
- **Increased HDL-cholesterol levels resulting from lower CETP activity seems to be associated with a *lower risk of coronary heart disease in men*.**
- ATP-binding cassette transporter A1 (ABCA1) mediates the **transport of cholesterol and phospholipids *from cells to lipid-poor apolipoproteins***.
- Animals and human studies documented that ***defects in the ABCA1*** **pathway are significant determinants of CAD (coronary artery disease)**.
- ***Inactivation of ABCA1 gene in macrophages increases atherosclerotic*** **lesions in hyperlipidemic mice, and *overexpressing human ABCA1 in transgenic mice retards atherogenesis*.**

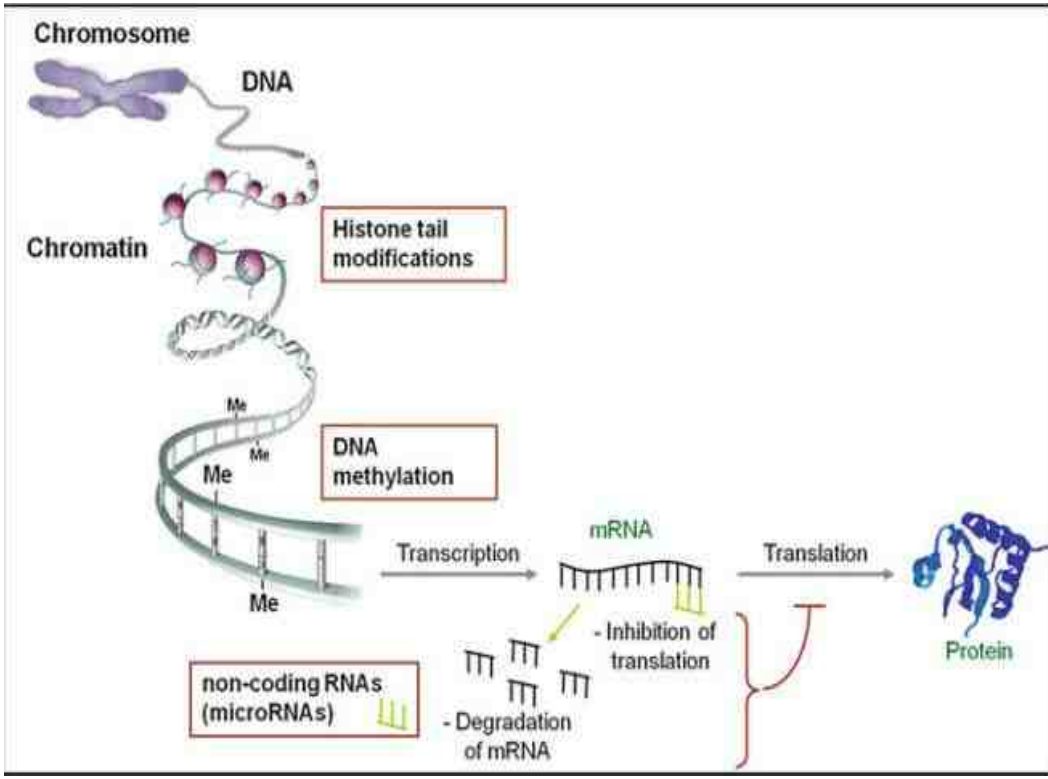
- The ABCA1 gene encodes ABCA1 protein, which is **expressed in liver, macrophages, intestines, lungs** etc.
- Several **ABCA1 Gene polymorphisms were identified** such as R219K, R1587K, and I883M.
- The association between **CAD** and gene polymorphisms involved in lipid metabolism remains **the subject of debate owing to differences in results**.
- The studies **varied markedly by including different populations** (e.g. age, sex and ethnicity), sampling strategies and genotyping procedures.
- Additionally, **lipid profile can be influenced by several environmental factors such as *smoking status, eating habits, associated customs*** (fasting periods), sedentary life style, body composition, gender and others.
- **The most important factors influencing the lipid profile include the *sex hormone status and age*.**
- Evidently, atherosclerosis is a multifaceted disease, and **there is a need for extensive and costly research *before genetics are introduced in every day clinical practice.***

CANCER CHEMOPREVENTION AND NUTRI-EPIGENETICS (Clarissa Gerhauser 2012)

- **During carcinogenesis**, **major cellular functions and pathways**, including drug metabolism, cell cycle regulation, potential to repair DNA damage or to induce apoptosis, response to inflammatory stimuli, cell signalling, and cell growth control and differentiation **become deregulated**.
- Epigenetic alterations contribute to these cellular defects, for example **epigenetic silencing of detoxifying enzymes**, **tumor suppressor genes**, **cell cycle regulators**, a**poptosis-inducing** and **DNA repair genes**, **nuclear receptors**, signal transducers and transcription factors **by *promoter methylation*, and modifications of histones and non-histone proteins** such as p53, NF-kB, **and the chaperone HSP90 by *acetylation or methylation*.**
- So far, data are still **mainly derived from in vitro investigations**, and results of animal models or human intervention studies are limited that demonstrate **the functional relevance of epigenetic mechanisms for *health promoting* or *cancer preventive* efficacy of natural products**

- Given the fact that epigenetic modifications are reversible and **occur early during carcinogenesis** as potentially initiating events for cancer development, **they have been identified as promising new targets for cancer prevention strategies.**

- Major epigenetic mechanisms of gene regulation include **DNA methylation**, modifications of the chromatin structure by histone tail **acetylation and methylation**, and **small non-coding microRNAs**, that affect gene expression by targeted degradation of mRNAs or inhibition of their translation



Overview of epigenetic mechanisms including DNA methylation, histone tail modifications and non-coding (micro) RNAs, targeting DNA, N-terminal histone tails and mRNA.

- ***Distinct patterns* of DNA methylation** regulate tissue specific gene expression and are involved in **X-chromosome inactivation** and **genomic imprinting**.
- Epigenetic profiles can be modified **to adapt to changes** in the environment (e.g., nutrition, chemical exposure, smoking, radiation, etc.) as has been **exemplified in studies with *monozygotic twins and inbred animals***.
- Alterations in **DNA methylation and histone marks** eventually contribute to the development of ***age-related and lifestyle-related diseases***, such as **metabolic syndrome**, Alzheimer's disease, and cancer.

DNA METHYLATION

- DNA methylation is mediated by **DNA methyltransferases** (DNMT) that transfer methyl groups **from S-adenosyl-L-methionine** (SAM) **to the 5'-position of cytosines**.
- This reaction mainly takes place at cytosines **when positioned next to a guanine** (CpG dinucleotides) and creates **5-methylcytosine** (5mC) and **S-adenosyl-L-homocysteine (SAH)**.
- **Three active mammalian DNMTs** have been identified so far, i.e., DNMT1, 3a, and 3b.
- **DNMT1** is a maintenance methyl transferase that **maintains DNA methylation during DNA replication**.
- It preferentially **methylates the newly synthesized, unmethylated DNA strand after replication and thus assures *transmission of DNA methylation patterns to daughter cells***.
- **DNMT3a and DNMT3b are "de novo" methyltransferases** that catalyze methylation of previously unmethylated sequences.
- **DNMT3b** is believed to play an **important role during tumorigenesis**

- **In normal cells, CpG-rich sequences** (so-called CpG islands, CGIs) **in gene promoter regions are generally unmethylated**, with the exception of about 6–8% CGIs methylated in a tissue-specific manner.
- **Conversely**, the majority of CpG sites in repetitive sequences such as ribosomal DNA repeats, satellite repeats, or centromeric repeats are **often heavily methylated, thereby contributing to chromosomal stability by limiting accessibility to the transcription machinery**.
- This controlled pattern of DNA methylation is **disrupted during ageing**, **carcinogenesis**, or **development of chronic diseases**.
- **Increased methylation** (DNA hypermethylation) **of promoter CGIs leads to transcriptional silencing of tumor suppressors** and other genes with important biological functions.

- **In contrast**, global loss of DNA methylation at repetitive genomic sequences (DNA hypomethylation) during carcinogenesis **has been associated with genomic instability and chromosomal aberrations**.
- Different from irreversible gene inactivation by genetic deletions or nonsense mutations, **genes silenced by epigenetic modifications are still intact and can potentially be reactivated by small molecules acting as modifiers of epigenetic mechanisms**.
- Consequently, development of agents or ***food components*** **that prevent or reverse methylation-induced inactivation of gene expression is a new promising approach for cancer prevention**.

HISTONE MODIFICATIONS

- Epigenetic regulation of gene expression is also mediated by **post-translational modifications** at the N-terminal tails of histones.
- These include **acetylation**, **methylation**, **phosphorylation**, **ubiquitinylation**, sumoylation, and ADP ribosylation and contribute to **genomic stability**, **DNA damage response**, and **cell cycle checkpoint integrity**.
- Histones can be **modified through *sequence-specific* transcription factors** or on a more ***global scale* through histone-modifying enzymes**.
- So far, **histone *acetylation* and histone *methylation* have been investigated the most and *disturbance of their balance has been associated with neoplastic transformation***.
- Histone acetylation is **maintained by the interplay of histone acetyltransferases (HATs) and histone deacetylases** (HDACs).
- HATs transfer **acetyl groups *from acetyl-CoA to thee-amino group of lysine*** (K) residues in histone tails, whereas HDACs remove histone acetyl groups by **catalyzing their transfer to Coenzyme A (CoA)**.
- **Acetylation of histone tails opens up the chromatin structure**, allowing transcription factors to access the DNA.

- Consequently, **proteins with HAT catalytic activity are often transcriptional coactivators**.
- So far at least **25 HAT proteins** have been characterized.
- They are organized into **four families based on structure homology** and often **possess distinct histone specificity**.
- In contrast to histone acetylation, histone **deacetylation generally leads to chromatin condensation and transcriptional repression**.
- So far, **18 proteins with HDAC activity have been classified**.
- Interestingly, **HDAC substrates are not limited to histones**.
- Several important regulatory proteins and transcription factors such as **p53, E2F, and nuclear factor-kB (NF-kB)** involved in stress response, inflammation, and apoptosis have been shown to be **regulated by acetylation**.
- **Histone methylation takes place at lysine and arginine residues**.
- Histone lysine methylation has ***activating or repressive*** **effects on gene expression**.

- This is **dependent on *the lysine residue* that is methylated** (e.g., **K4**, K9, K27, **K36**, **K79** in H3) **the *methylation status*** (mono-, di-, or tri-methylation), and ***the location*** (interaction with promoter vs gene coding regions)
- Methylation at H3K4, H3K36, and H3K79 is generally associated with **transcriptional active chromatin** (euchromatin), whereas methylation at H3K9, H3K27, and H4K20 is frequently associated with **transcriptional inactive heterochromatin**.
- Histone lysine methylation is mediated by **histone lysine methyltransferases** (HMTs) that transfer a methyl group **from SAM to the lysine residue**.
- Several types of histone lysine demethylases (HDMs) have been identified so far.
- Similar to lysine acetylation, **lysine methylation is not limited to histone proteins**, and several non-histone protein substrates including p53, **retinoblastoma protein** (RB), the NF-kB subunit Rel A, and estrogen receptor α (ERα) have been identified.

MicroRNAs

- MicroRNAs (miRNAs) are small **non-coding RNAs of 20–22 nucleotides** that ***inhibit gene expression at the post transcriptional level***.
- miRNAs are involved in the regulation of key biological processes, including development, differentiation, apoptosis, and proliferation, and are ***known to be altered in a variety of chronic degenerative diseases including cancer*.**
- miRNAs regulate the transformation of mRNA into proteins, either by ***imperfect base-pairing to the mRNA 3'-untranslated regions*** to repress protein synthesis, or **by *affecting mRNA stability***.
- **Each miRNA is expected to control several hundred genes.**
- They have been implicated in cancer initiation and progression, and ***their expression is often down-regulated during carcinogenesis***.
- Major mechanisms of miRNA deregulation include **genetic** and **epigenetic** alterations as well as **defects in the miRNA processing machinery**

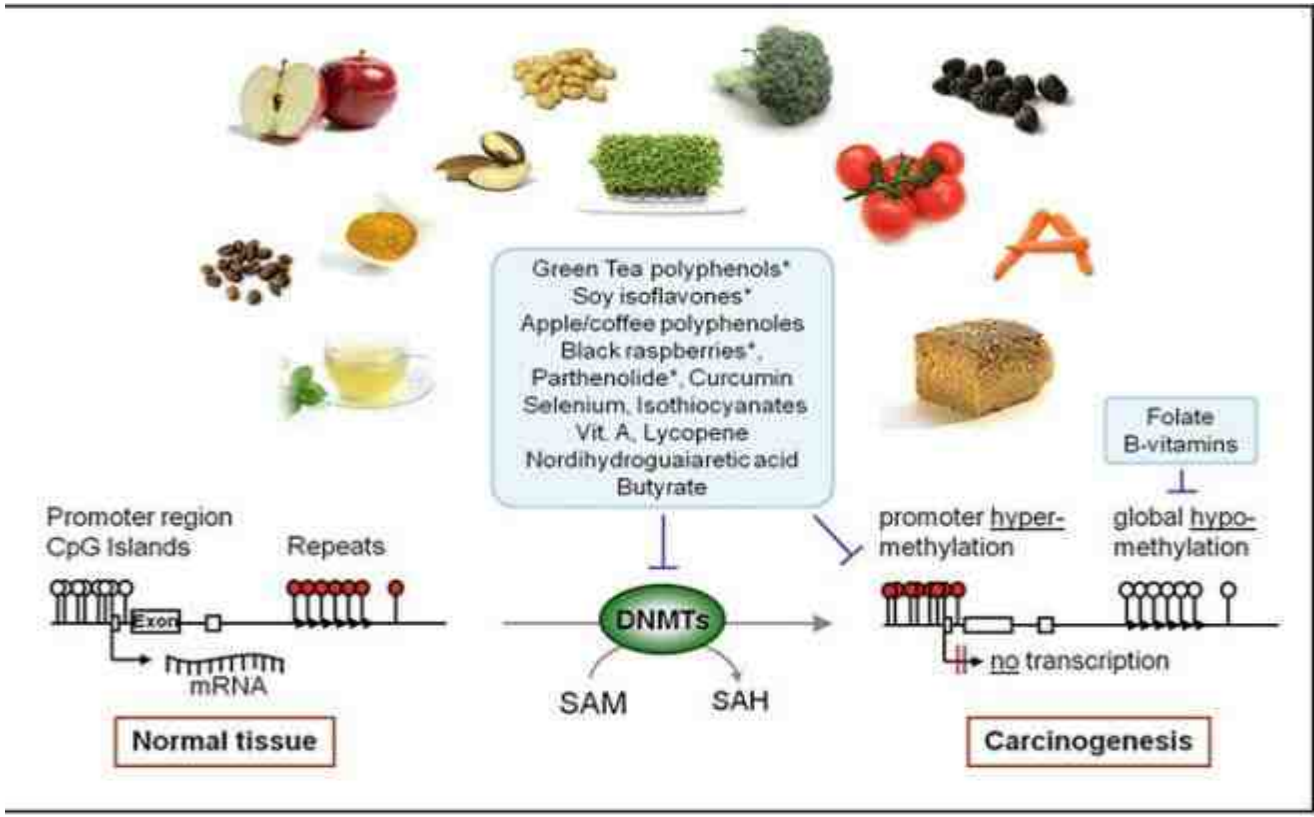
INTERPLAY BETWEEN *CHEMOPREVENTIVE* AND *EPIGENETIC* MECHANISMS AND *NATURAL PRODUCTS EFFECTS*

- **Natural products and dietary** constituents ***with chemopreventive potential*** have an **impact on *DNA methylation, histone modifications* and *miRNA expression***.
- **Folate and B-vitamins have a potential impact on DNA hypomethylation.**
- They **affect the so called "one-carbon metabolism" which *provides methyl groups for methylation reactions***.
- **Folate** is an important factor for the maintenance of **DNA biosynthesis and DNA repair**, and **folate deficiency leads to global DNA *hypomethylation*, *genomic instability*, and chromosomal damage**.
- As an essential micronutrient, folate needs to be taken up from dietary sources, such as **citrus fruits, dark green vegetables, whole grains, and dried beans**.
- Epidemiological studies have indicated that **low folate levels are associated with an increased risk for colorectum, breast, ovary, pancreas, brain, lung, and cervix cancer.**

- Consequently, the relationship between **folate status**, **DNA methylation**, and **cancer risk** has been analyzed in numerous rodent carcinogenesis models and in human intervention studies.
- Overall, the results are inconclusive and depend on various parameters, for example **dose** and **timing of the intervention**, the **severity of folate deficiency**, and **health status**.
- **Excessive intake** of **synthetic folic acid** (from high-dose supplements or fortified foods) may even **increase human cancer risk by *accelerating growth of precancerous lesions***.
- **Therefore folate supplementation cannot be generally recommended, and *deficiencies should be prevented by dietary intake*.**
- Plant compounds which **affect DNA methylation and inhibit DNMT enzymatic activity** (DNMT inhibitors, DNMTi), ***revert*** ***aberrant DNA promoter methylation,*** **or reactivate genes *silenced by promoter hypermethylation.***
- **Natural products** with influence on histone acetylation and methylation **inhibit the activity or modulate the expression of histone-modifying enzymes** including HDACs, HATs, and HMTs.

Overview of DNA methylation **changes** during carcinogenesis and cancer chemopreventive agents inhibiting the activity of expression of DNMTs, thereby **preventing aberrant** (promoter) **hypermethylation** or **genome wide hypomethylation**.

DNA methylation is catalyzed by DNA methyltransferases (DNMTs) using S-adenosylmethionine (SAM) as a substrate.

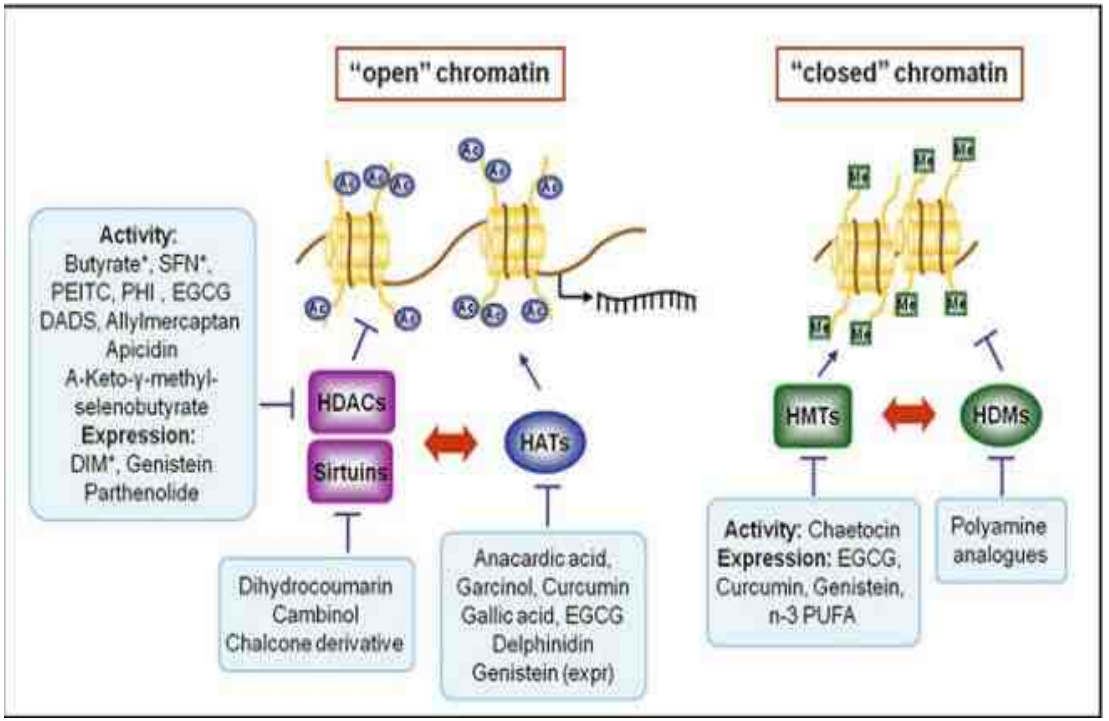


Asterisks indicate epigenetic activity in vivo. Empty circles: unmethylated CpG dinucleotide; red circles: methylated CpG site

Simplified overview of **histone modifying enzymes with a focus on histone deacetylases** (HDACs), histone acetyltransferases (HATs), histone methyl transferases (HMTs), and histone demethylases (HDM), and **their influence on chromatin structure**.

Sirtuins represent a NAD+ dependent subclass of HDACs (class III).

Also indicated is the inhibitory potential of chemopreventive agents.



Asterisks indicate epigenetic activity in vivo

- **Major cellular pathways and cell functions**, including drug metabolism, cell cycle regulation, potential to repair DNA damage or to induce apoptosis, response to inflammatory stimuli, cell signalling, cell growth control and differentiation, **become deregulated during carcinogenesis by defects in epigenetic gene regulation**.
- These include, among others, **silencing by promoter methylation of detoxifying enzymes**, tumor suppressor genes, cell cycle regulators, apoptosis inducing and DNA repair genes, nuclear receptors, signal transducers and transcription factors, **as well as modifications of histones and non-histone proteins** such as p53, NF-kB, and HSP90 **by acetylation or methylation**.
- Accumulating evidence indicates that **dietary chemopreventive agents can prevent or reverse these alterations by 1.affecting global DNA methylation, 2.reexpressing tumor suppressor genes silenced by promoter methylation, and 3.upregulating genes by altering histone and non-histone acetylation and methylation, at least in cell culture systems.**

Bioinformatics

GENE BANK

NCBI

DDBJ

Look for a given gene sequence, recognize its corresponding mRNA sequence and match its corresponding amino acid sequence to its nucleotide sequence

Blast

THANKS