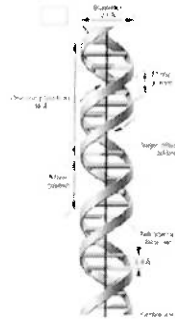


## Molecular Biology: Basic Principles

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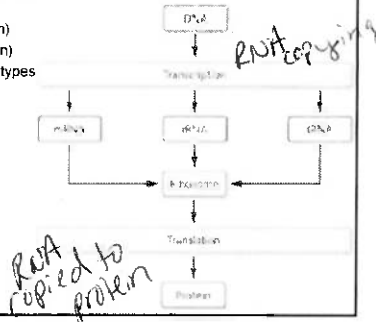


## GMO: Basic Principles

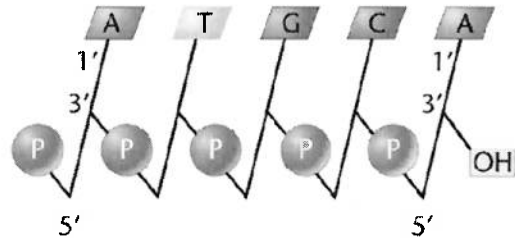
- Basic facts about DNA and proteins
- What is the basic science underlying genetically modified organisms?
  - What is a functioning gene?
- How are genes cloned?
- How do we transfer a functioning gene from one species to another?
- How do we control transferred gene expression?

## The Central Dogma

- Genetic information is stored in DNA
- Gene expression occurs by
  - RNA synthesis (transcription)
  - Protein synthesis (translation)
- Protein actions generate phenotypes

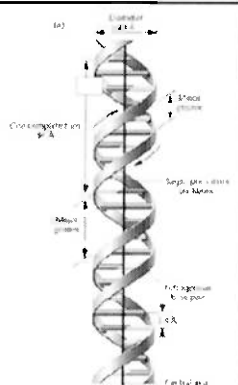


- Genetic information is coded in the sequence of bases in a DNA molecule.



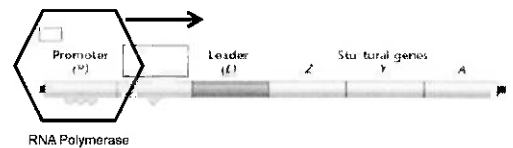
## DNA

- DNA double helix
  - The physical structure in organisms
- Two strands held together by hydrogen bonding between bases.
  - A:T
  - G:C



## Gene action

- Promoter = binding site for RNA polymerase
- For a gene to be expressed it must have a promoter where a polymerase can bind to start transcription



### Transcription and translation

- mRNA is a copy of the DNA coding sequence.
- The ribosome reads the mRNA sequence and generates a protein
- The ribosome adds one amino acid for every three bases

Labels in diagram: Structural gene, mRNA, Ribosome, Amino acid, tRNA, Translation, Proteins, β-Globin, α-Globin, Hemoglobin, Hemoglobin

### THE GENETIC CODE:

		Second position			
		U	C	A	G
First position (5' end)	U	UUU Phe	UUC Phe	UUA Leu	UUG Leu
	C	UCU Leu	UCC Leu	UCA Leu	UCG Leu
	A	AUU Ile	AUC Ile	AUA Ile	AUG Met
	G	GUU Val	GUC Val	GUA Val	GUG Val
		U	C	A	G
Third position (3' end)	U	UUU Phe	UUC Phe	UUA Leu	UUG Leu
	C	UCU Leu	UCC Leu	UCA Leu	UCG Leu
	A	AUU Ile	AUC Ile	AUA Ile	AUG Met
	G	GUU Val	GUC Val	GUA Val	GUG Val

**AUG = START codon (Met)**  
3 STOP codons do not encode amino acids

- All organisms use the same genetic code.
- A human gene can be correctly expressed by a plant.

### Protein structure: processing

- Protein function requires processing.
- Ex. Hemoglobin has four chains.
- Proper folding is essential for function
- Mutations cause amino acid substitutions alter the protein structure, and alter the function.

Labels in diagram: α chain, β chain, heme group

### Molecular Medicine in the News

- Cystic Fibrosis
  - NY Times Jan 29, 2012
  - USDA approves Kalydeco (vaccor) – a drug that treats the cause of cystic fibrosis for individuals with the Gly551Asp mutation.
  - NBD1
- This is a drug that binds to the altered CFTR protein and alters its shape, restoring some normal function.

### GMO

- To make a genetically modified organism:
  - clone the DNA for a gene and transfer it to the host
  - control expression of the transferred gene
- How are genes cloned and transferred?
- How do we control the expression of cloned genes?

### Cloning DNA

- Cloning steps:
  - Cut DNA with a restriction enzyme.
  - Insert into a vector.
  - Insert into a host cell.

### Restriction Enzymes

- Restriction enzymes cut DNA at a particular "recognition sequences"
- Each enzyme recognizes one sequence
- e.g. Eco RI.
- Cutting leaves single stranded ends

- Generating a recombinant DNA molecule:
  - Cut two pieces of DNA with the same enzyme
  - Hybridize: pairing of the single stranded ends
  - Ligate: permanently join the two pieces

### Cloning Vectors

- Cloning vectors replicate DNA in the host cell.
- Cloning vectors
  - Origin of replication
  - Selectable marker
  - Restriction sites
- Insertion of DNA changes the marker phenotype

- Lac Z marker
- Expression gives the blue color
- Expression tells us the cell has a clone

### Vector insertion

- Different techniques are used for different cell host cells.
- Bacteria
- Plant cells
- Mammalian cells
- Oocytes

### Expression patterns

- Expression is controlled by the promoter
- By selecting the promoter we can control where the cloned gene is active
  - Specific promoters for specific tissues

### Bt corn

- 35S promoter - *Expresses in all tissues*
- Cry gene
  - Codes for Bt protein
- Bt protein
  - Kills insects that ingest it
- Plant transformation
  - Insert gene in corn
  - Express clone

35S Cry 1A(b)  
Expresses a Bt Protein in All Tissues

35S Cry 1A  
Expresses a Different Bt Protein in All Tissues

### Bt

Gene	Coding	Promoter	Characteristics	Trade Name
Mon810	CRY 1Ab	CaMV 35S	Bt expressed in all plant tissues, ECB food and livestock feed	YieldGard
BT-11	CRY 1Ab	CaMV 35S	Bt expressed in all plant tissues.	YieldGard
E-176	CRY 1Ab	PEP Carboxylase	Bt expressed in only green plant tissue and pollen.	Knockout or Naturegard
DBT-418	CRY 1Ac	CaMV 35S	Bt expressed at some level in all plant tissues.	BT-K12
TC1507	CRY 1F	Ubiquitin/CaMV	Bt expressed at some level in all plant tissues.	Herculex I
CBH-351	CRY 9C	CaMV 35S	Different protein from 1A, Bt expressed in all plant tissue.	StarLink

### Round-up ready soy beans

- 35s promoter
  - Cauliflower mosaic virus
  - Expressed in all cells
- EPSPS
  - Essential for synthesis of amino acids
  - Roundup => bind/block EPSPS
  - No protein synthesis => no growth
- Transformation with cloned resistance gene
  - Agrobacterial EPSPS protein = resistant to glyphosate
  - Mutation modified, resistant protein = resistant weeds

### Golden rice

- Construct add two enzymes so the plant can make beta carotene in the seed endosperm
  - phytoene synthase
  - phytoene desaturase

### GMO: Basic Principles

- Genetically Modified Organism = organism with an inserted, cloned DNA sequence
- Technology for cloning and transferring genes is well developed
  - Select vector for control of expression
  - Clone gene into vector
  - Insert vector in host
- Potential
  - Industrial production of proteins
  - Resistant crops
  - Human medicine