

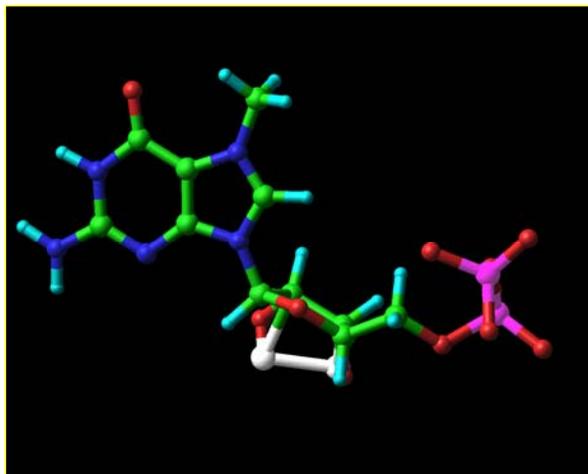
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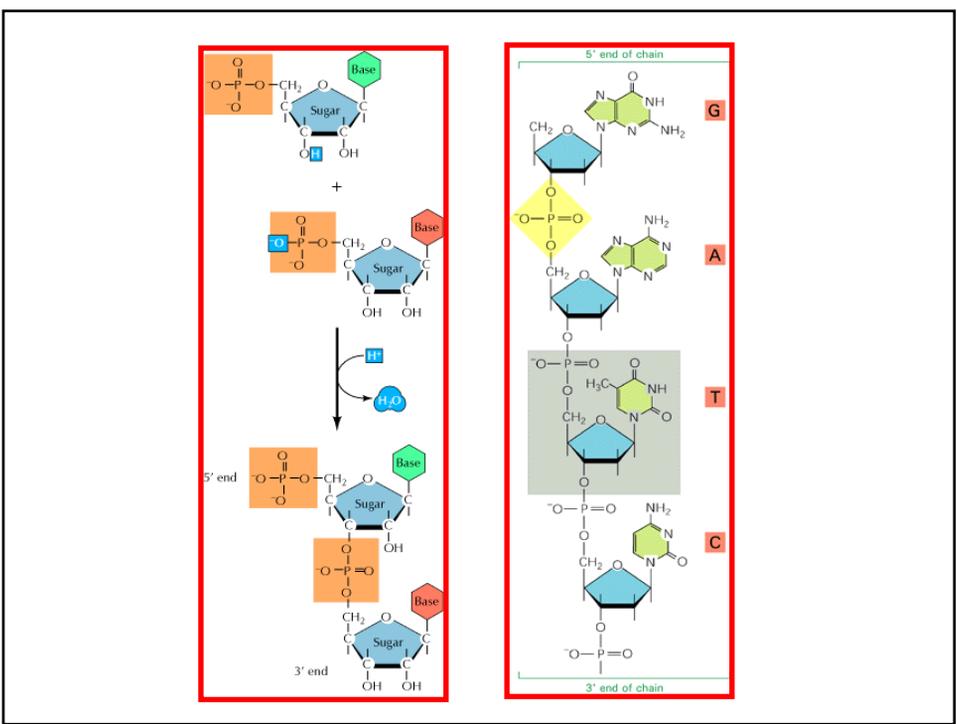
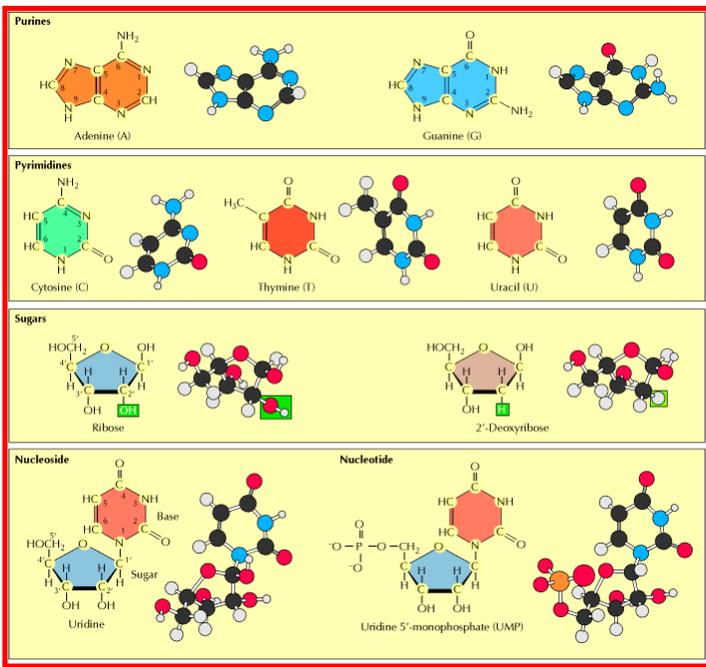


Curso: Biología Celular

Clase : Estructura de Acidos Nucleicos

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The top left diagram shows the Miller-Urey experiment apparatus. It consists of a flask of water heated by a Bunsen burner, connected to a vertical tube containing a mixture of gases (CH₄, NH₃, H₂O, H₂, CH₄). An electric discharge is applied to the gas mixture. The mixture then passes through a condenser and back into the flask. A list of organic molecules produced includes: Alanine, Aspartic acid, Glutamic acid, Glycine, Urea, Lactic acid, Acetic acid, and Formic acid.

The top right diagram illustrates the polymerization of amino acids. On the left, individual amino acids (Ala, Lys, Ser, Gly, Val, Asp, Glu, U) are shown. An arrow labeled H₂O indicates the reaction. On the right, the resulting polypeptide chains are shown: Ala-Lys-Ser, Lys-Gly, Gly-Ser-Asp-Ala-Val, U-G-C-A-U, A-A, and G-G-C-U-U-U-A-C.

The background features a dramatic sky with lightning over a city at night. An inset photo shows Stanley Miller in a laboratory setting.

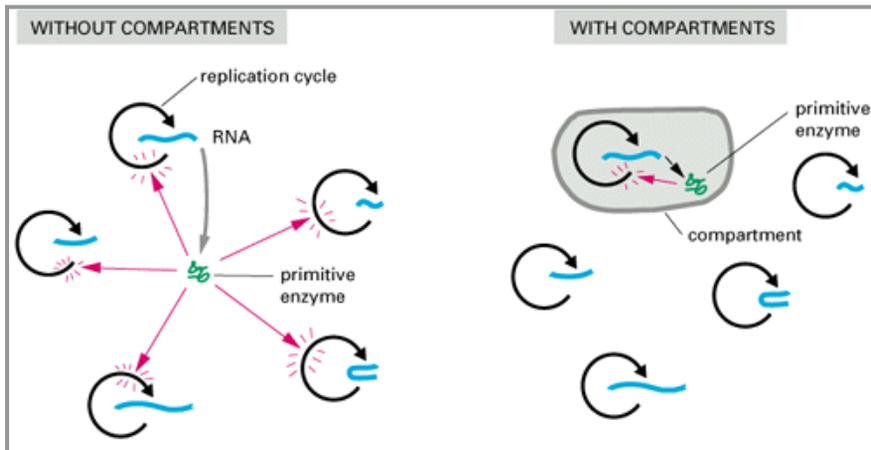
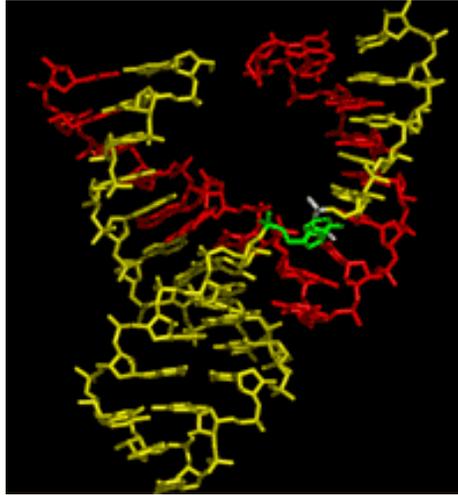
Stanley Miller

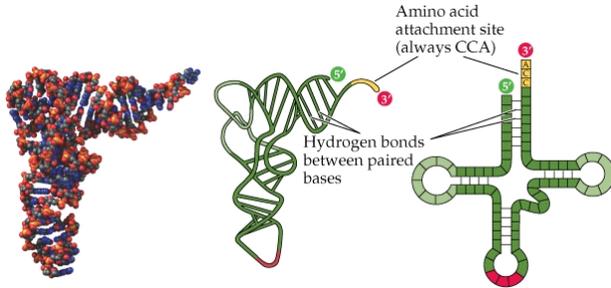
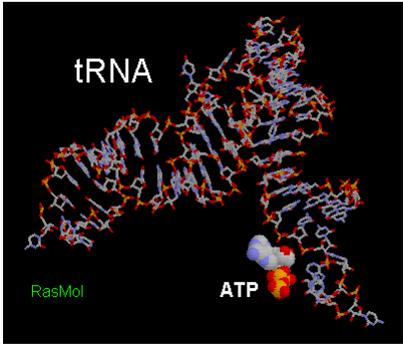
The middle diagram shows the formation of a complementary RNA sequence. On the left, a sequence of C-C-C-C-C-C-C is shown above a collection of nucleotides (A, G, U, C). An arrow labeled H₂O points to the right, where the complementary sequence G-G-G-G-G-G-G has formed, with each G base paired with a C base. A legend indicates the base pairing: U pairs with A, and G pairs with C.

The bottom diagram illustrates the process of complementary base pairing in two steps:

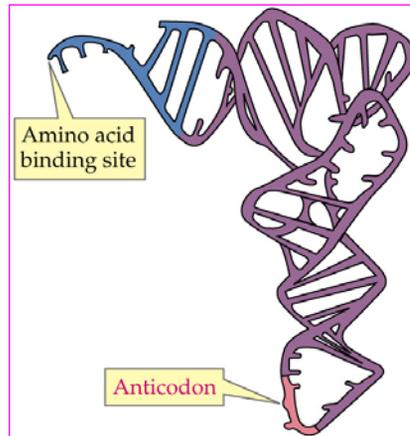
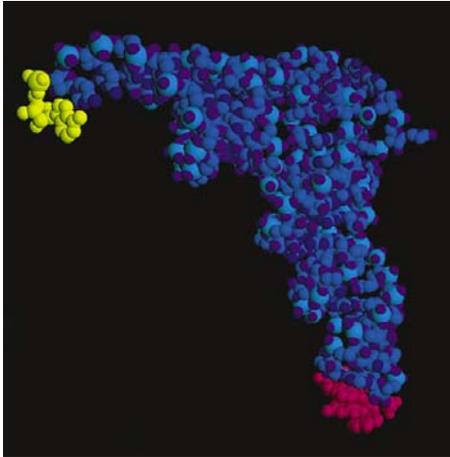
step 1
 ORIGINAL SEQUENCE: A-G-G-U-C-C-A
 FORMS COMPLEMENTARY SEQUENCE: U-C-C-A-G-G-U

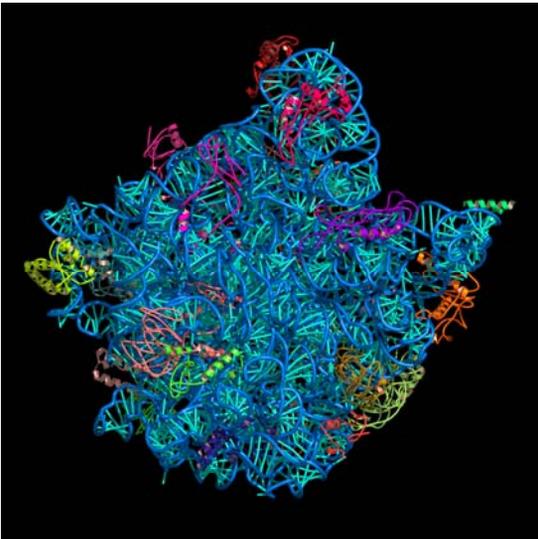
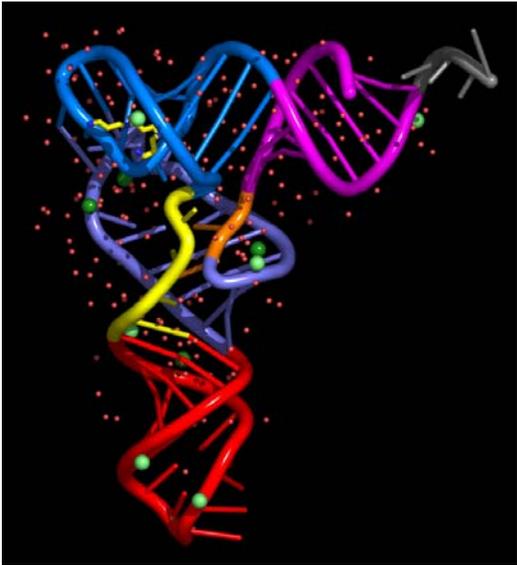
step 2
 COMPLEMENTARY SEQUENCE FORMS ORIGINAL SEQUENCE: U-C-C-A-G-G-U
 ORIGINAL SEQUENCE: A-G-G-U-C-C-A

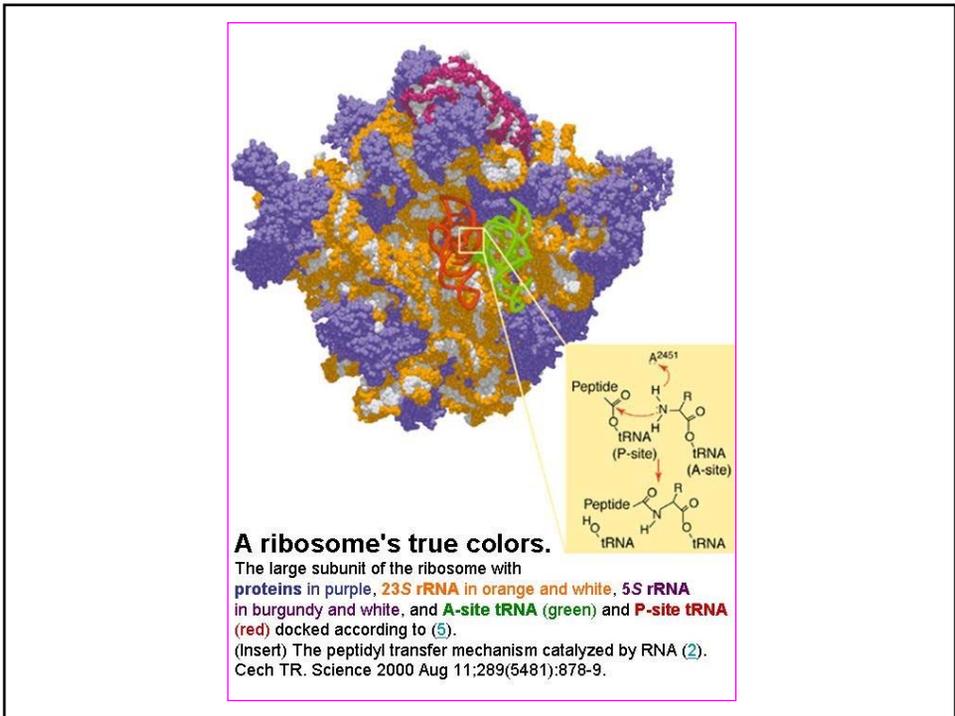
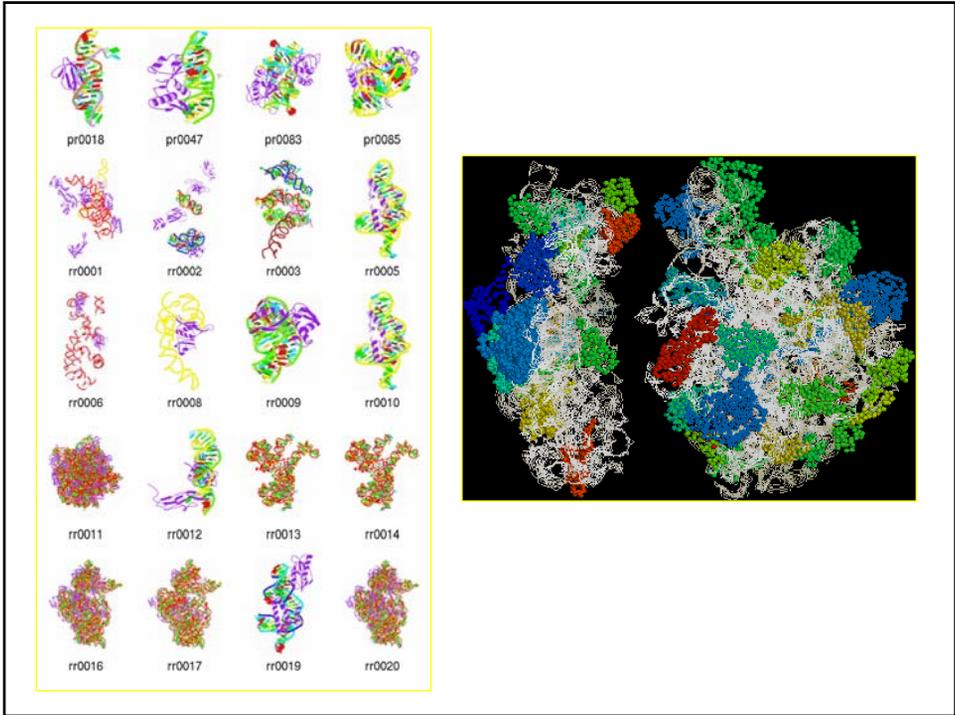


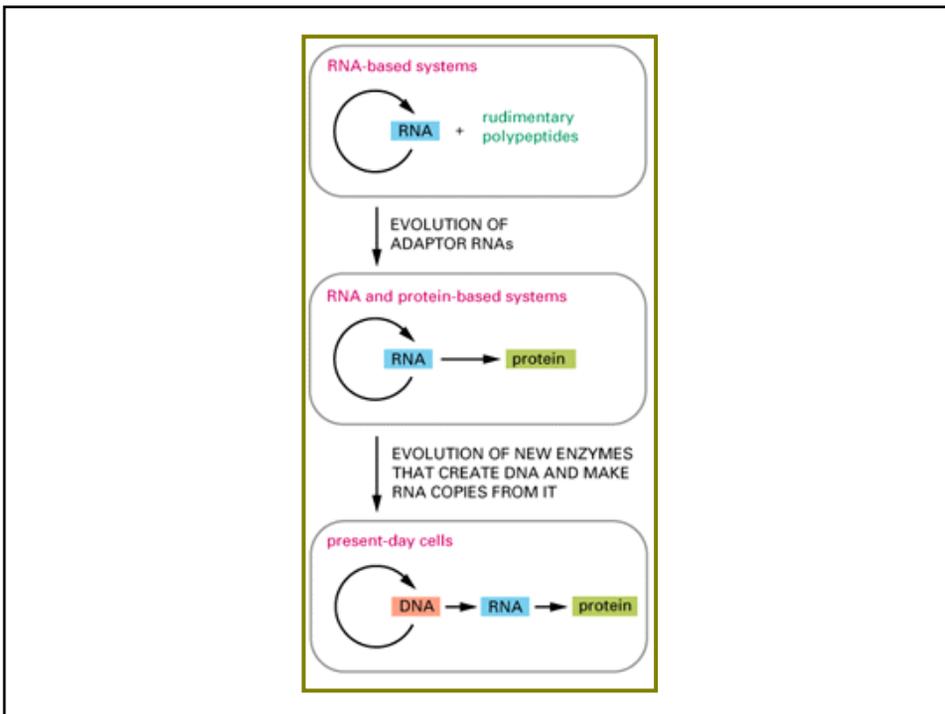
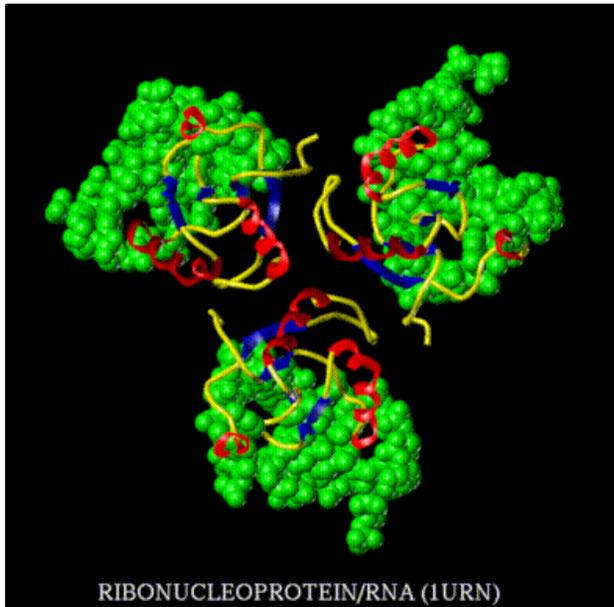


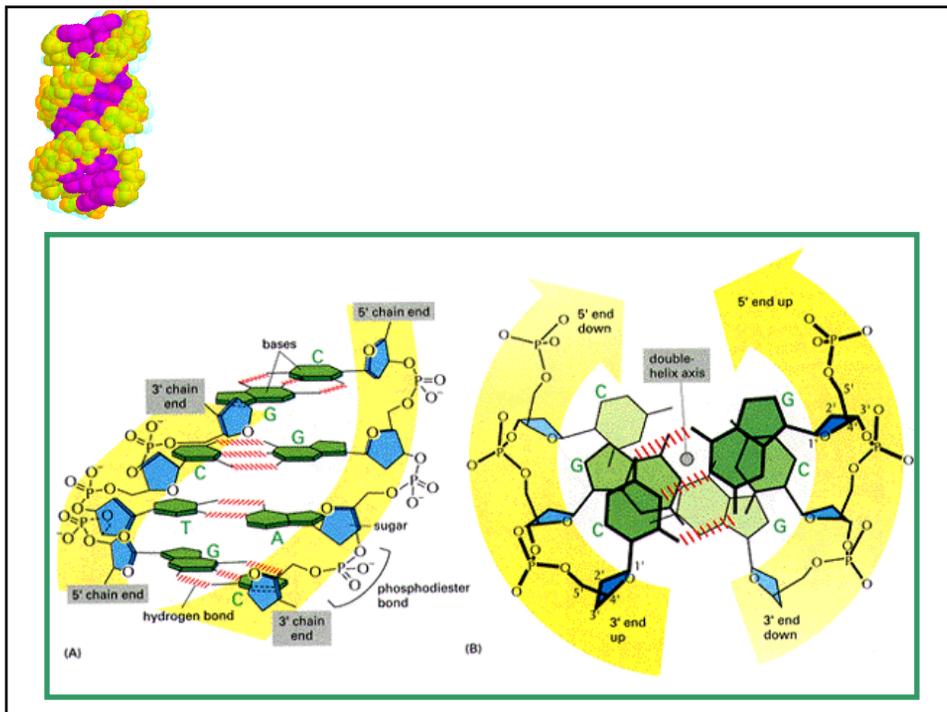
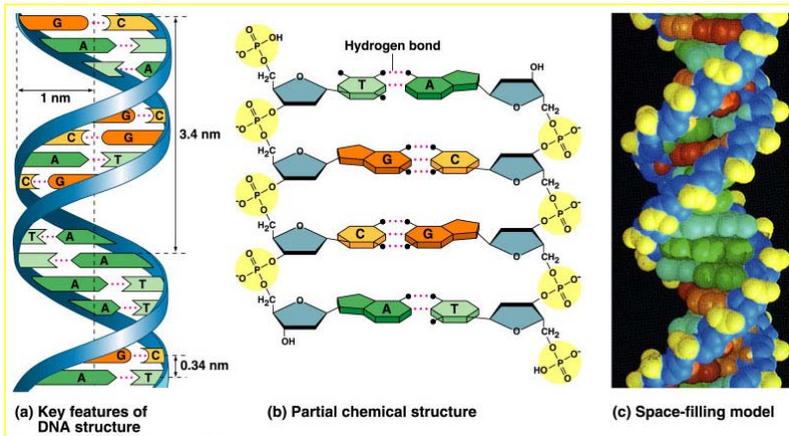
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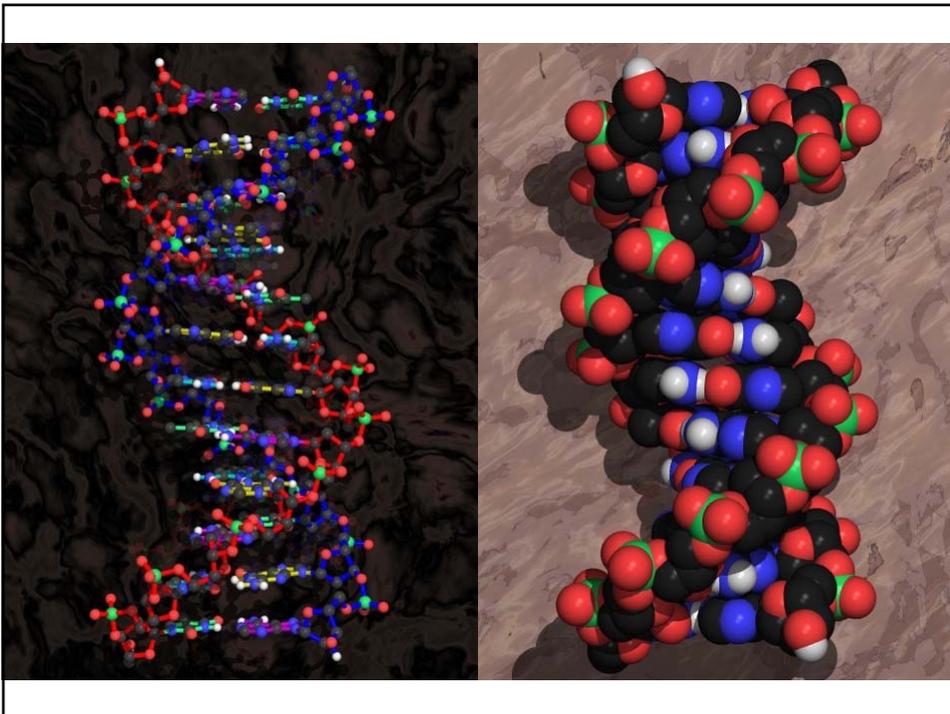




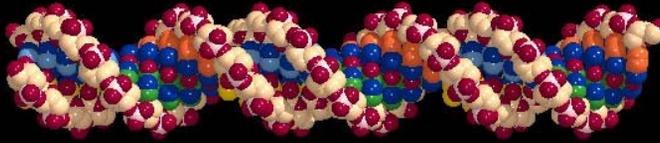




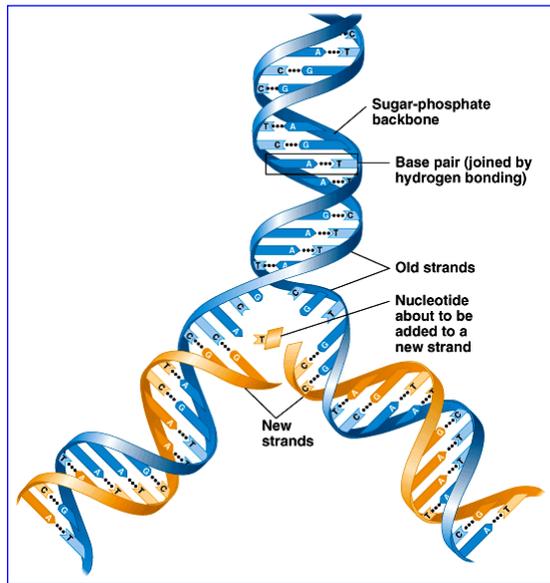


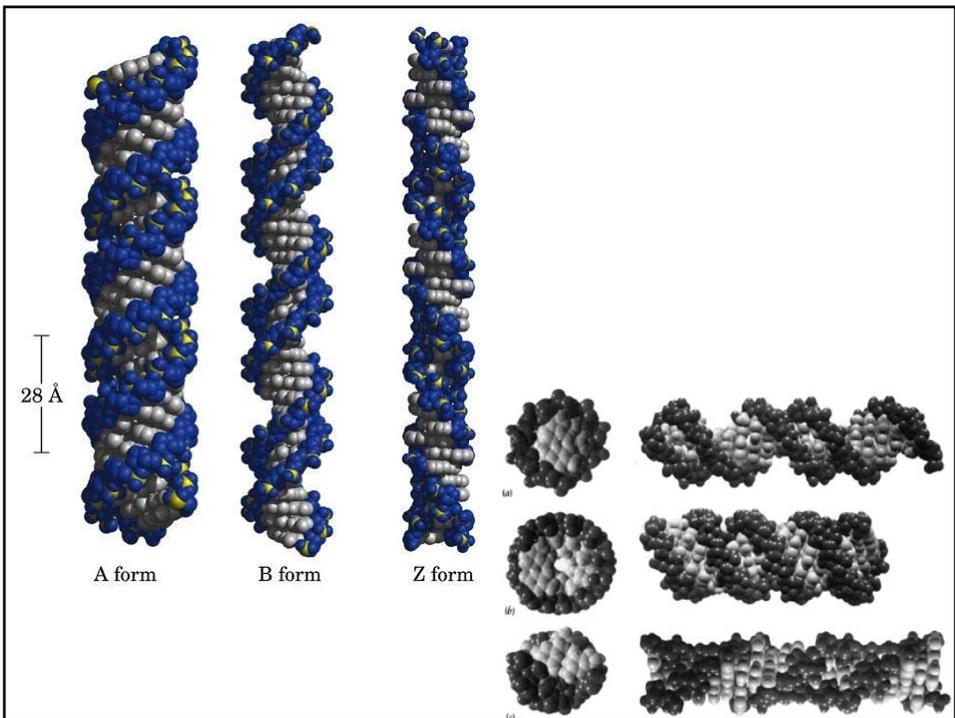
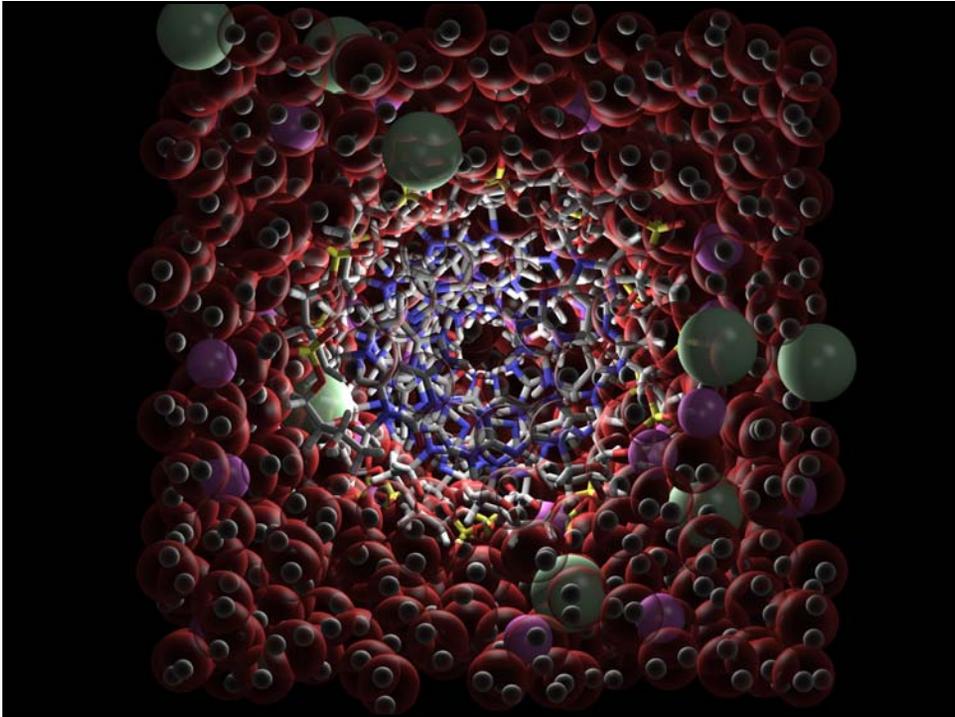


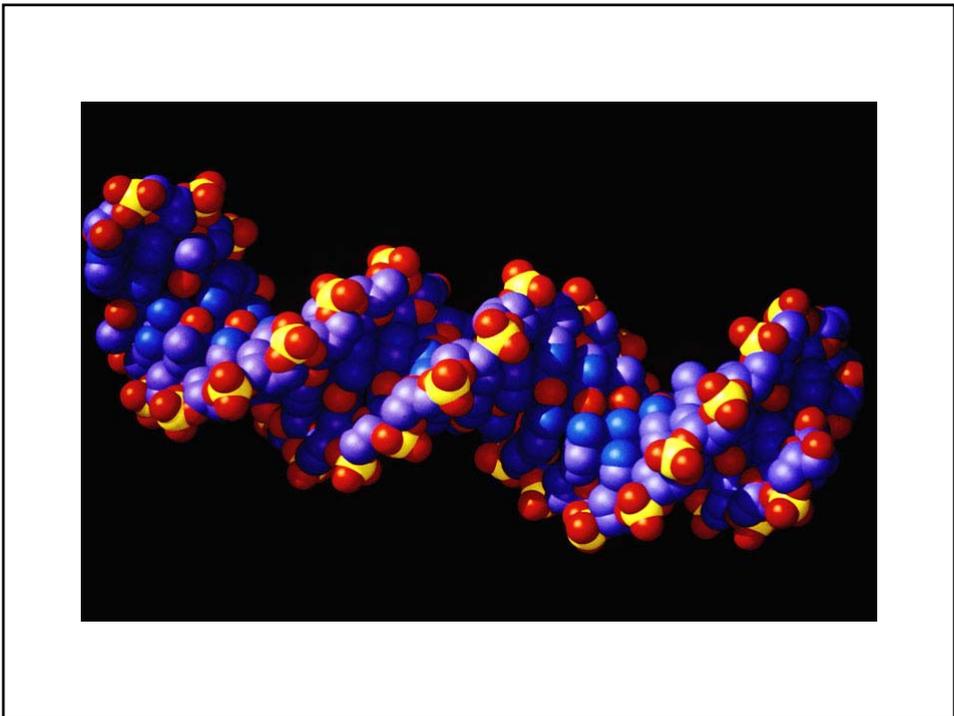
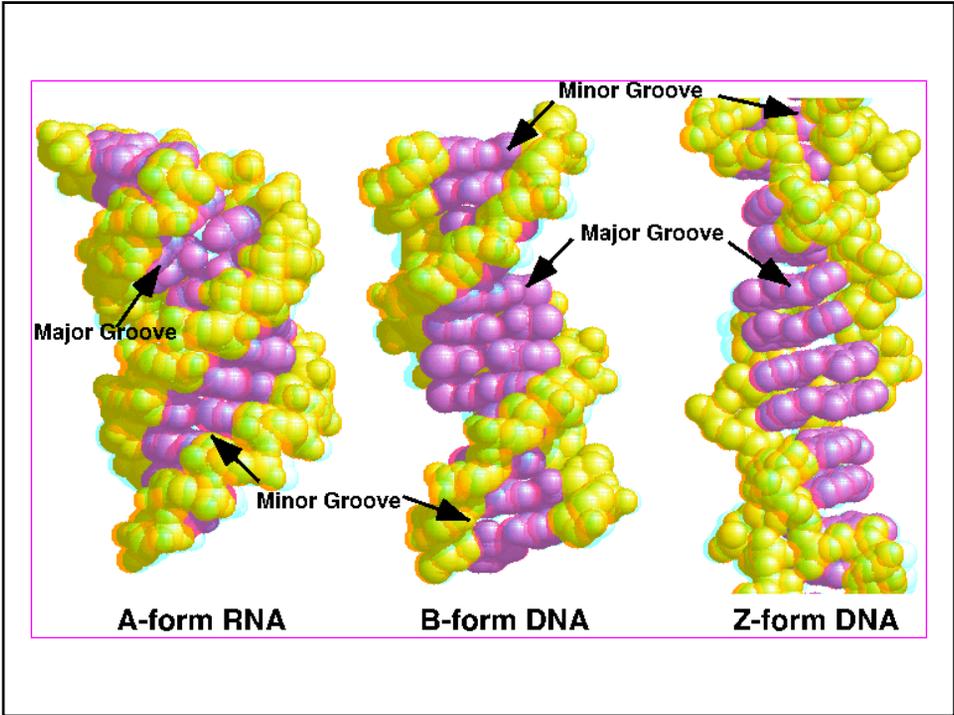
B-form DNA

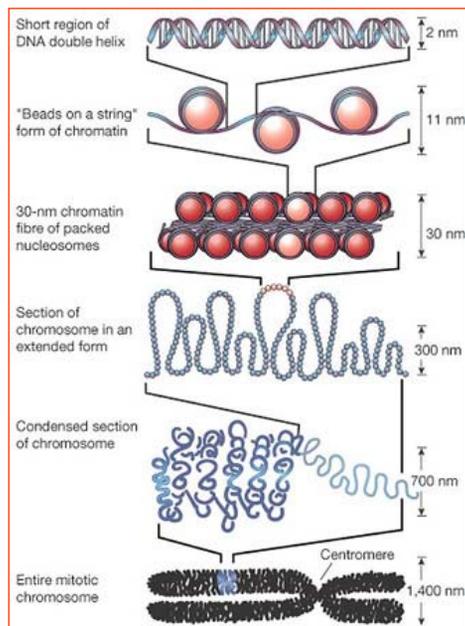


Song Tan, 1999









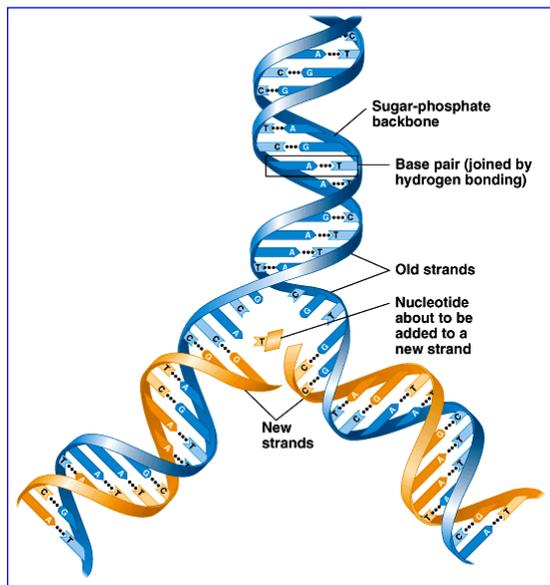
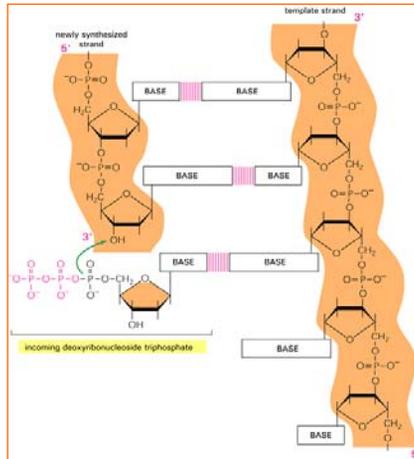
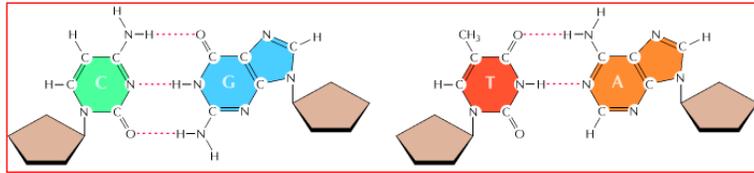
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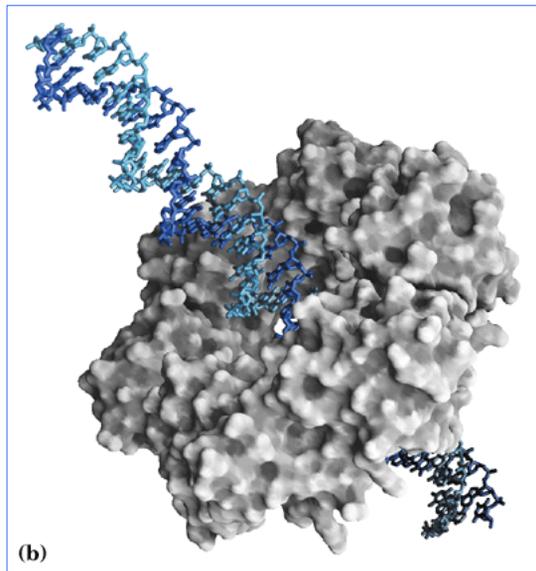
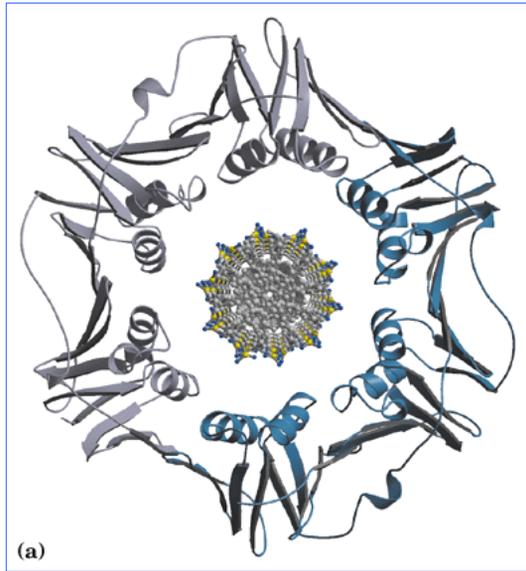


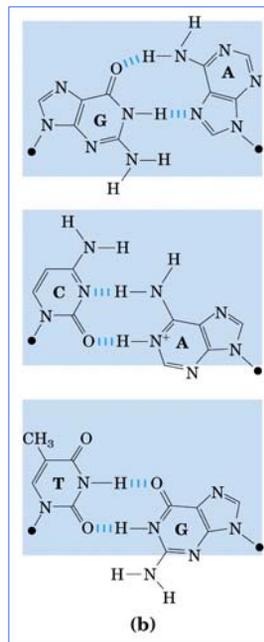
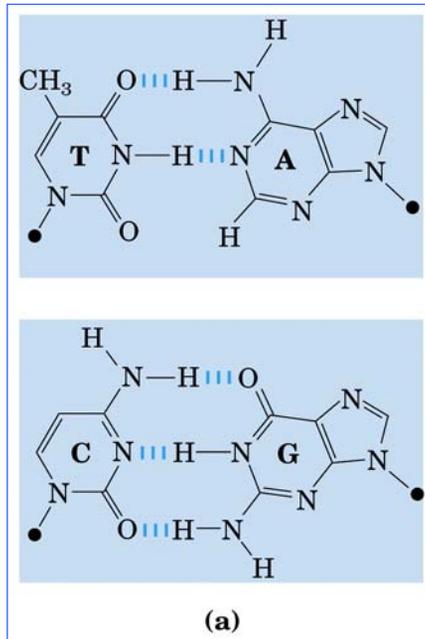
Curso: Biología Celular

Clase : Replicación del DNA

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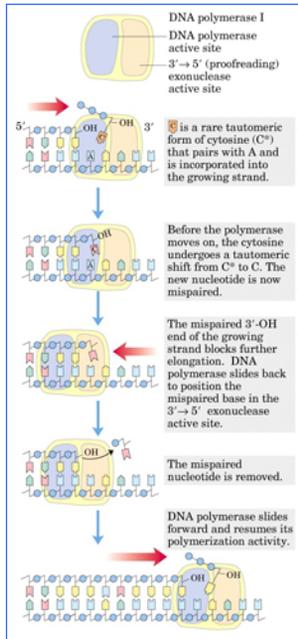


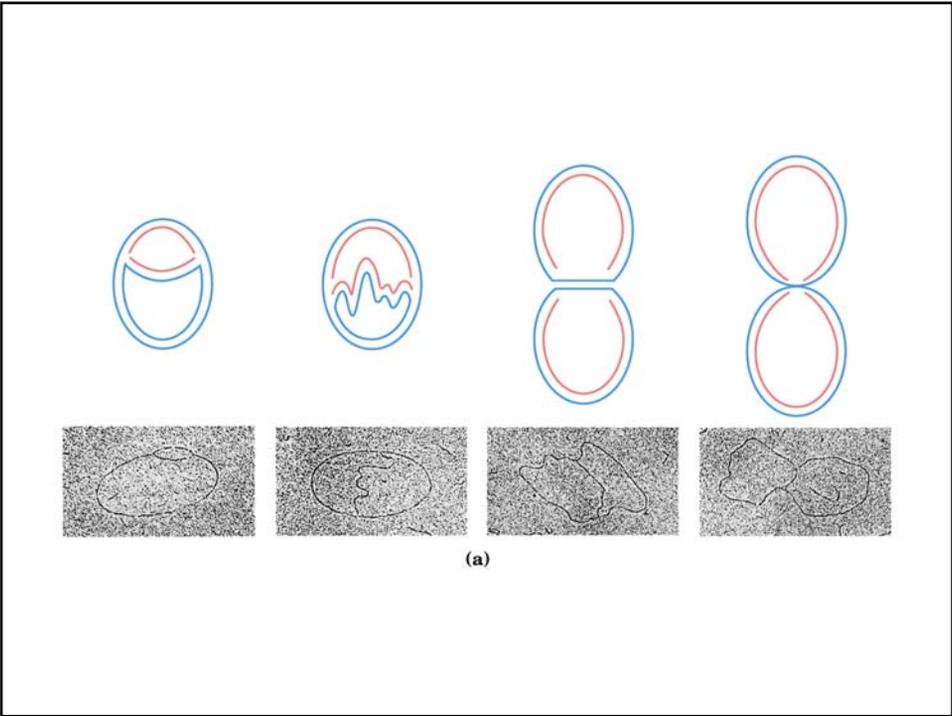
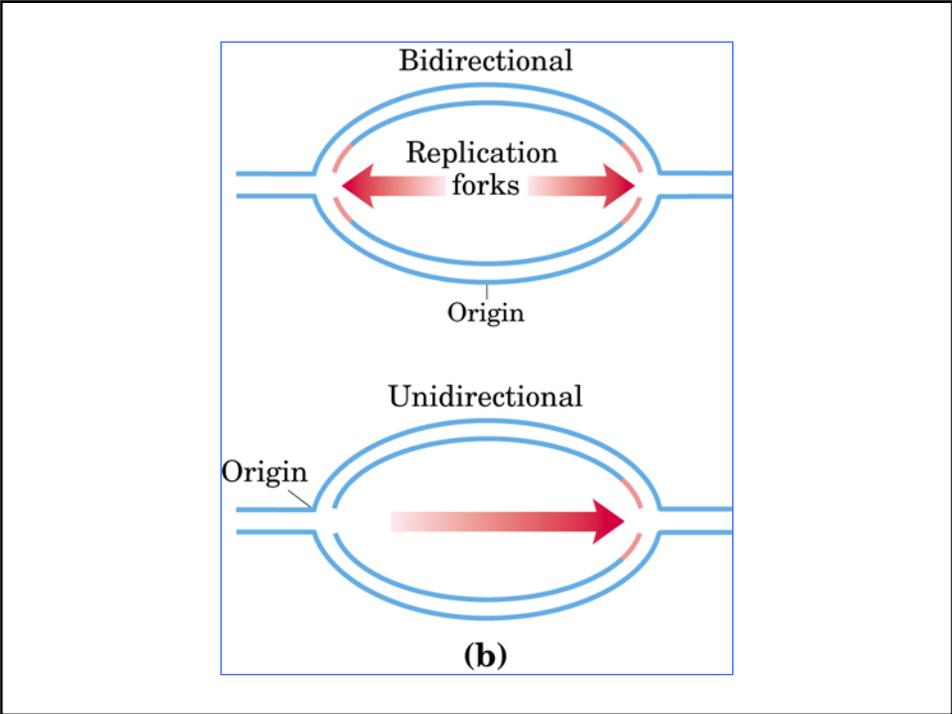
table 25-1

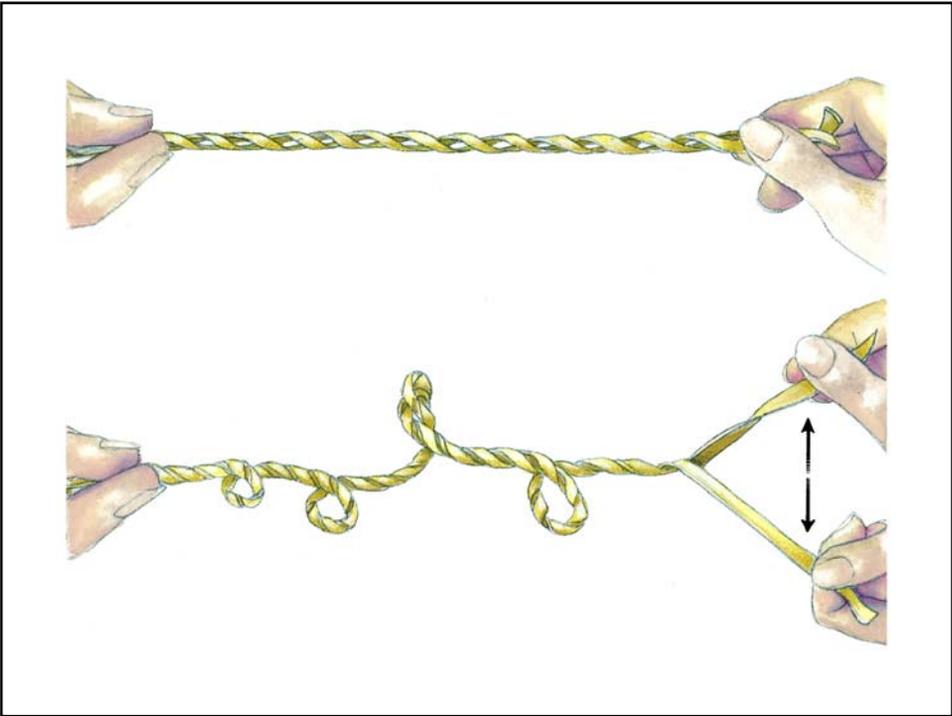
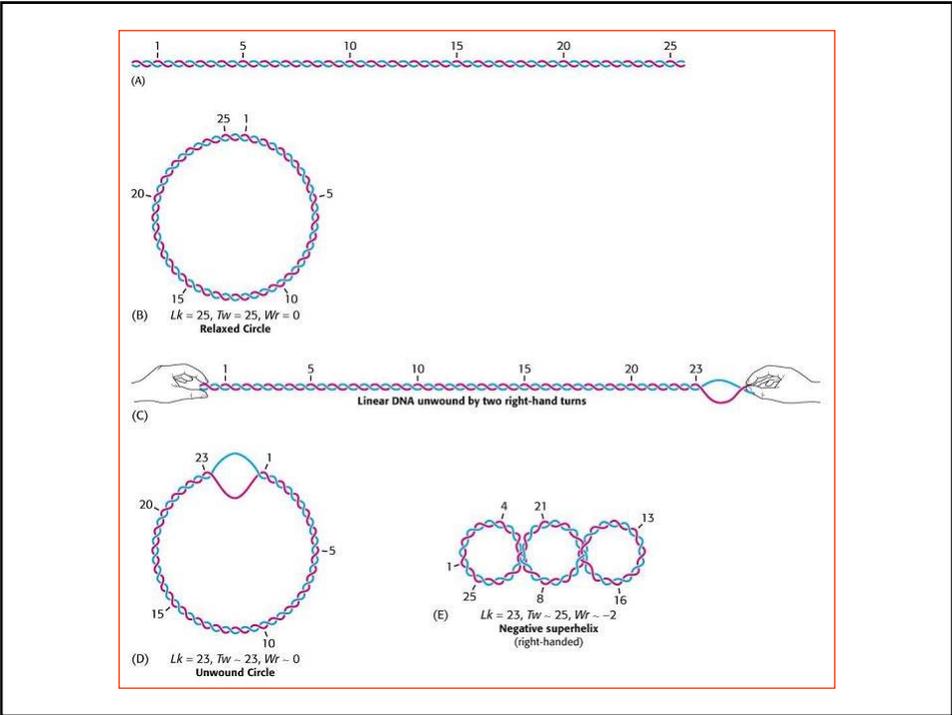
Comparison of DNA Polymerases of *E. coli*

	DNA polymerase		
	I	II	III
Structural gene*	<i>polA</i>	<i>polB</i>	<i>polC (dnaE)</i>
Subunits (number of different types)	1	≥4	≥10
M_r	103,000	88,000 [†]	830,000
3' → 5' Exonuclease (proofreading)	Yes	Yes	Yes
5' → 3' Exonuclease	Yes	No	No
Polymerization rate (nucleotides/sec)	16–20	40	250–1,000
Processivity (nucleotides added before polymerase dissociates)	3–200	1,500	≥500,000

*For enzymes with more than one subunit, the gene listed here encodes the subunit with polymerization activity. Note that *dnaE* is an earlier designation of the gene now referred to as *polC*.

[†]Polymerization subunit only. DNA polymerase II shares several subunits with DNA polymerase III, including the β , γ , δ , δ' , χ , and ψ subunits (see Table 25-2).





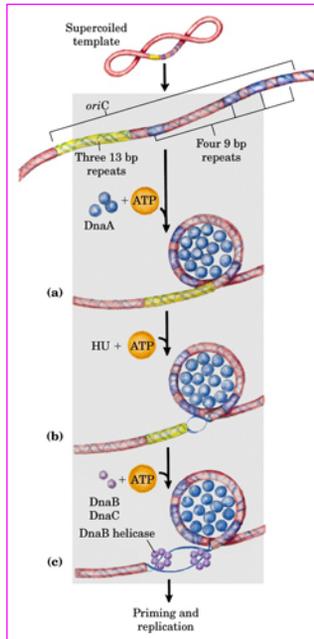
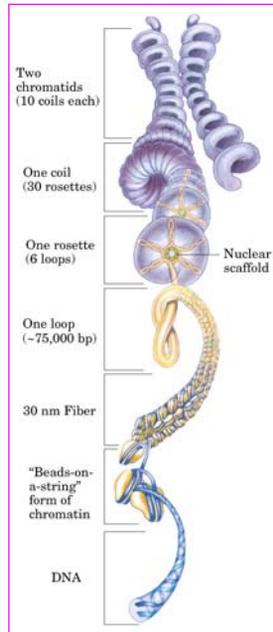
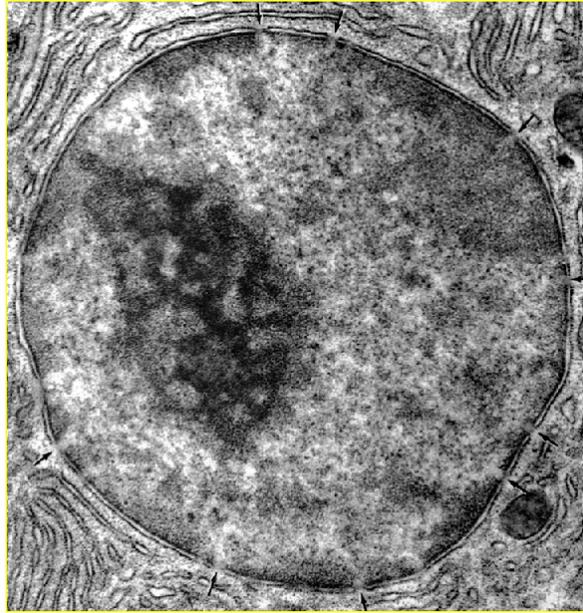


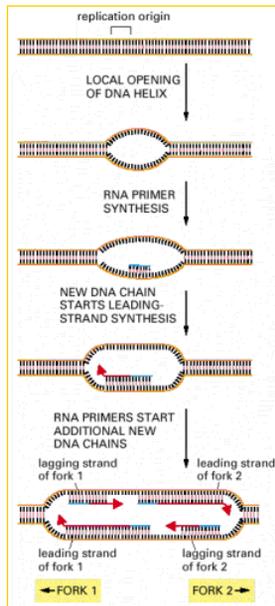
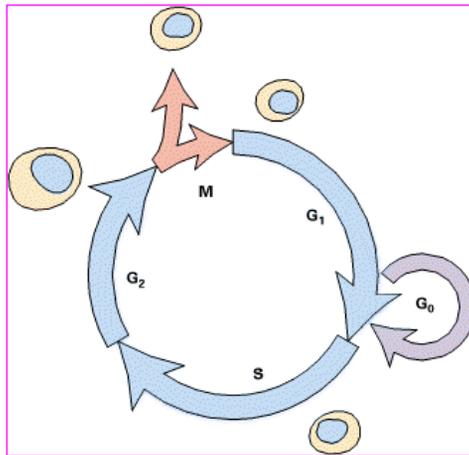
table 25-3

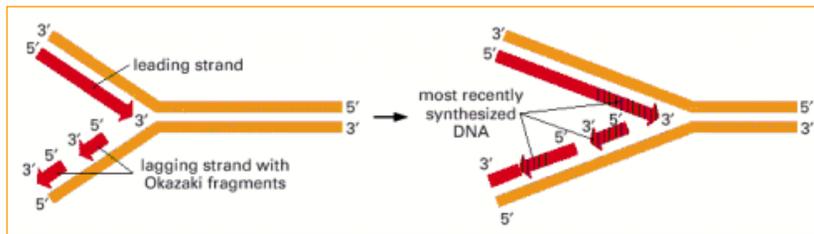
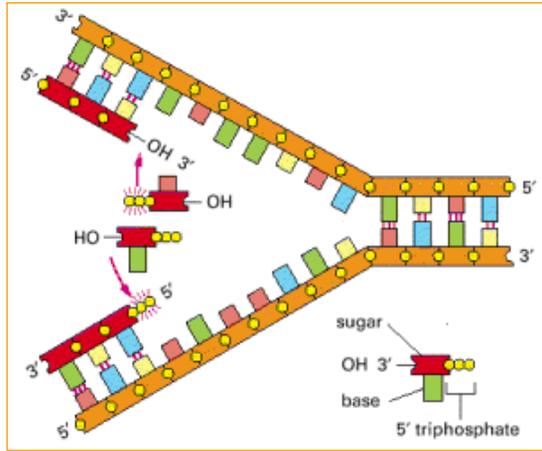
Proteins Required to Initiate Replication at the *E. coli* Origin

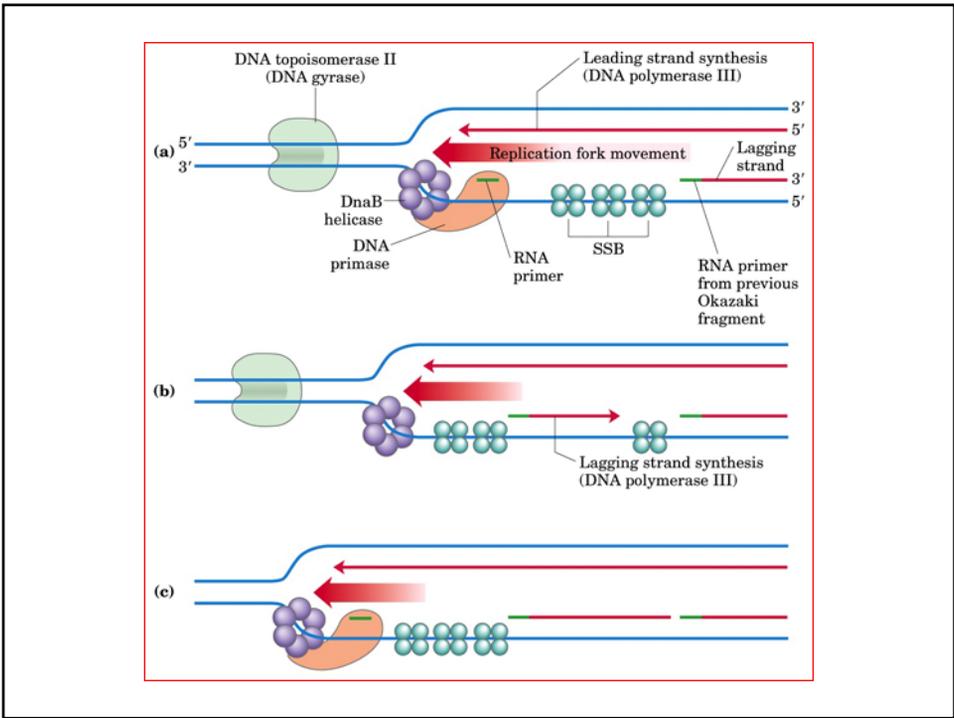
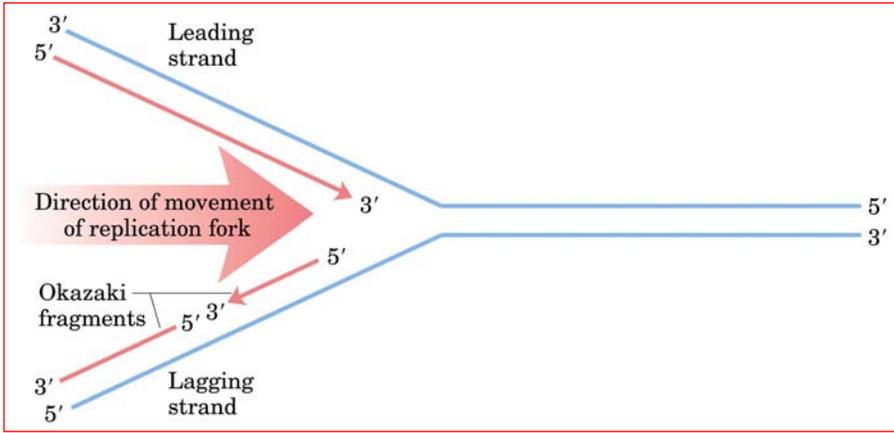
Protein	M_r	Number of subunits	Function
DnaA protein	52,000	1	Recognizes origin sequence; opens duplex at specific sites in origin
DnaB protein (helicase)	300,000	6*	Unwinds DNA
DnaC protein	29,000	1	Required for DnaB binding at origin
HU	19,000	2	Histonelike protein; DNA bending protein; stimulates initiation
Primase (DnaG protein)	60,000	1	Synthesizes RNA primers
Single-stranded DNA-binding protein (SSB)	75,600	4*	Binds single-stranded DNA
RNA polymerase	454,000	5	Facilitates DnaA activity
DNA gyrase (DNA topoisomerase II)	400,000	4	Relieves torsional strain generated by DNA unwinding
Dam methylase	32,000	1	Methylates (5')GATC sequences at oriC

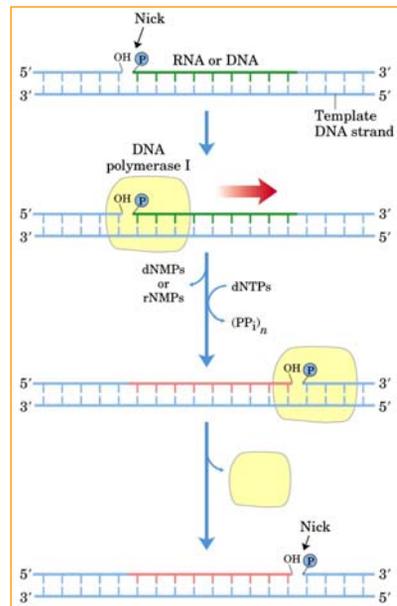
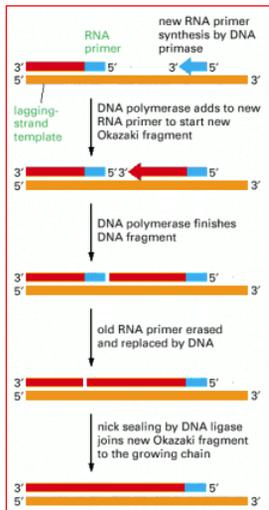
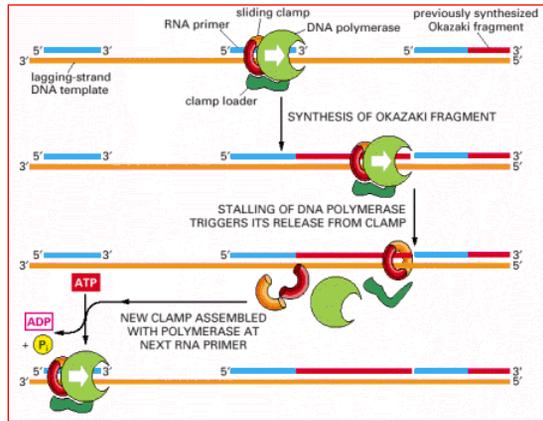
*Subunits in these cases are identical.











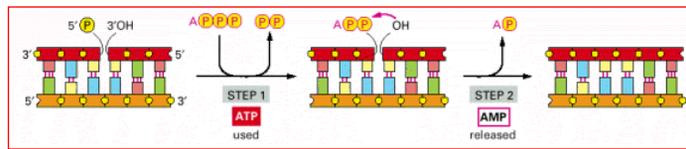
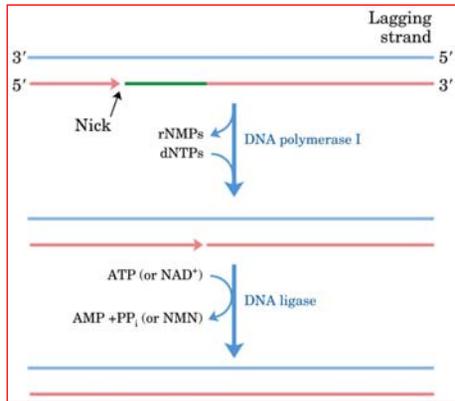
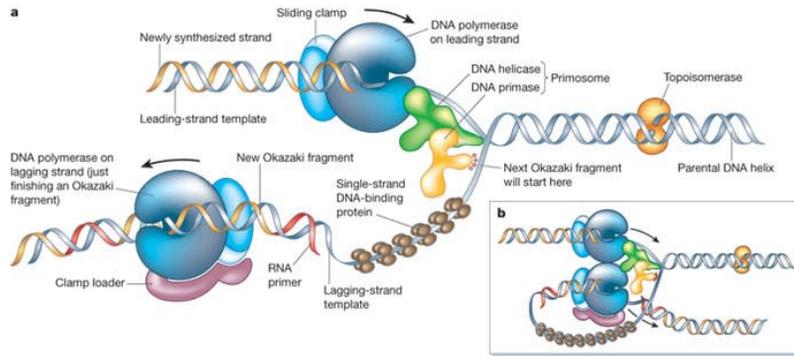


table 25-4

Proteins at the *E. coli* Replication Fork

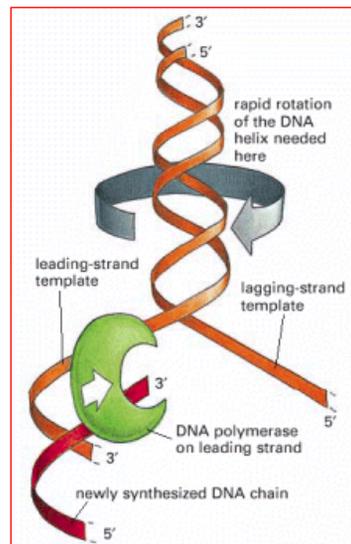
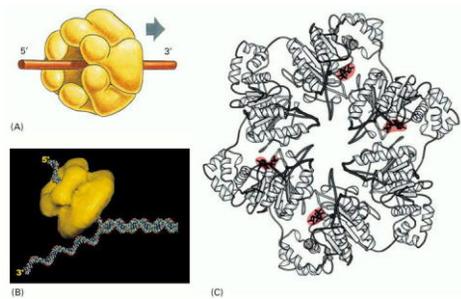
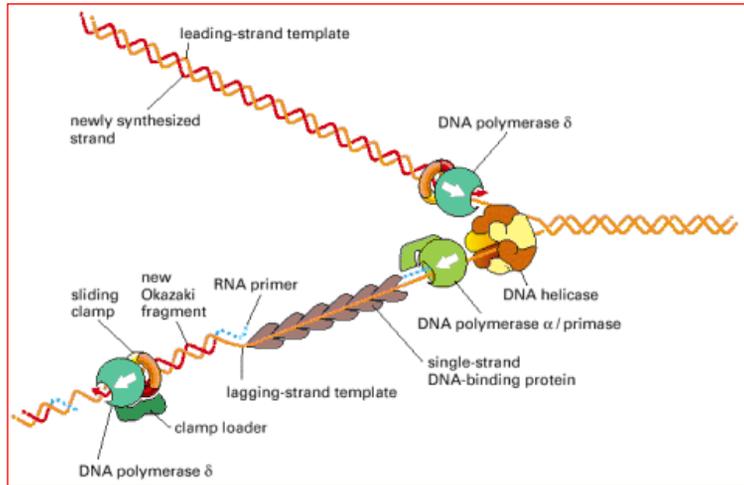
Protein	M_r	Number of subunits	Function
SSB	75,600	4	Binding to single-stranded DNA
DnaB protein (helicase)	300,000	6	DNA unwinding; primosome constituent
Primase (DnaG protein)	60,000	1	RNA primer synthesis; primosome constituent
DNA polymerase III	900,000	18-20	New strand elongation
DNA polymerase I	103,000	1	Filling of gaps, excision of primers
DNA ligase	74,000	1	Ligation
DNA gyrase (DNA topoisomerase II)	400,000	4	Supercoiling

Modified from Kornberg, A. (1982) *Supplement to DNA Replication*, Table S11-2, W.H. Freeman and Company, New York.

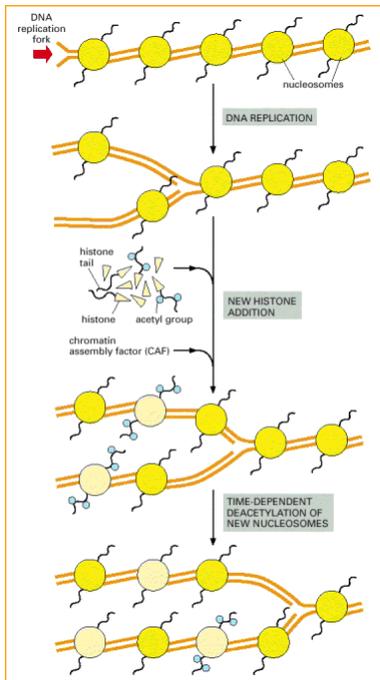
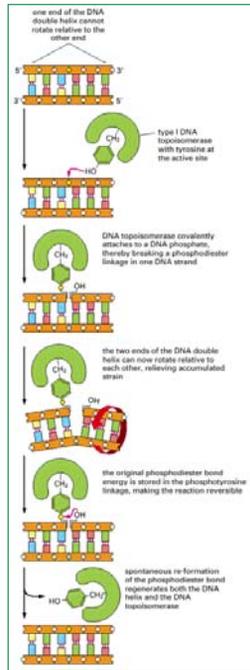


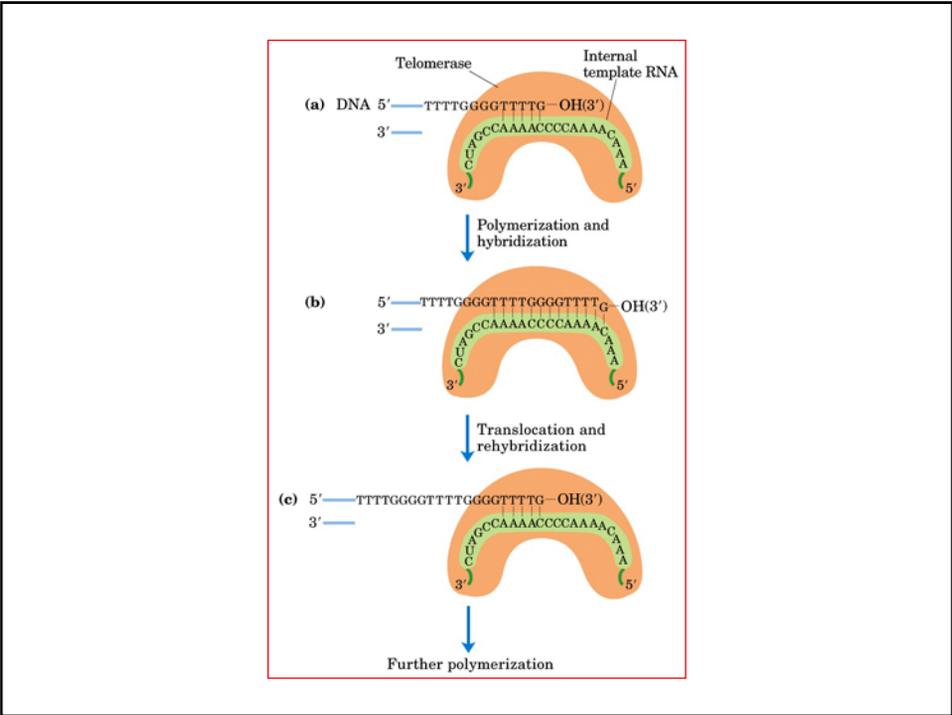
DNA polimerasas eucariontes

Tipo	Función
α	replicación nuclear ; asociada a la hebra retardada y a la primasa
β	reparación nuclear
γ	replicación y reparación mitocondrial
δ	replicación nuclear ; asociada a la hebra lider
ϵ	enzima nuclear



Topoisomerasa





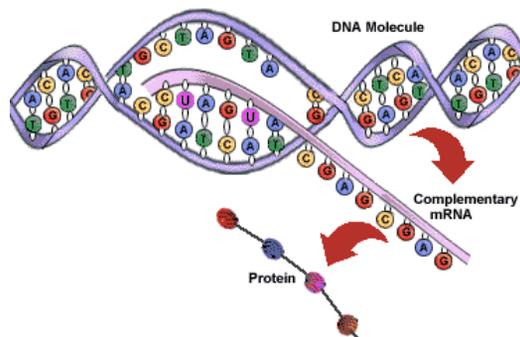
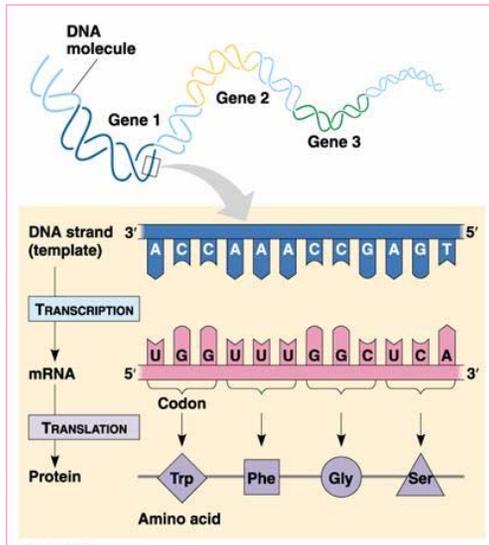
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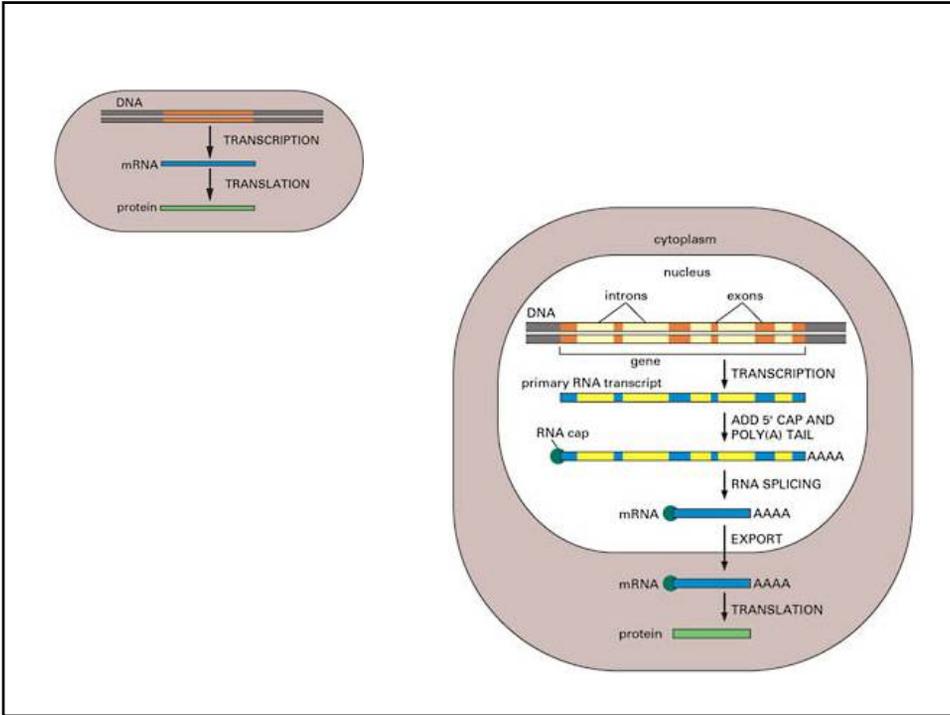


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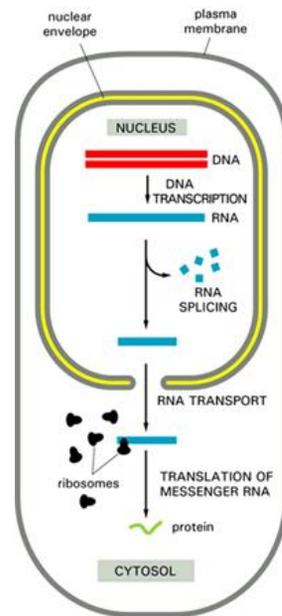
Clase : Transcripción

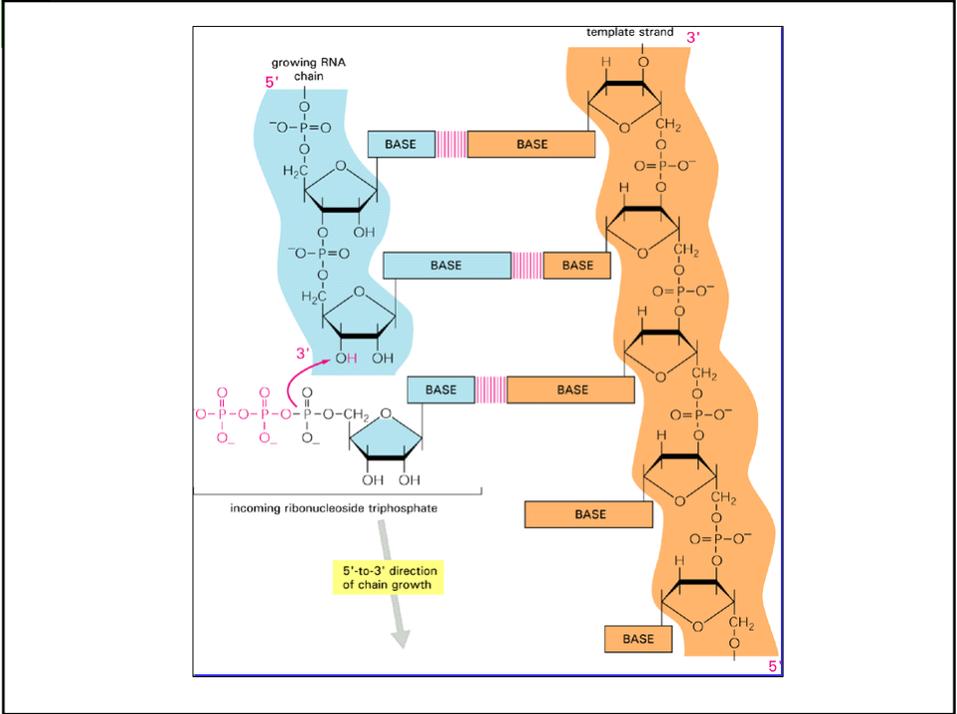
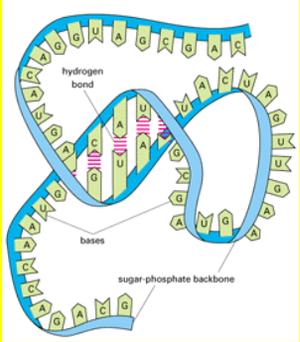
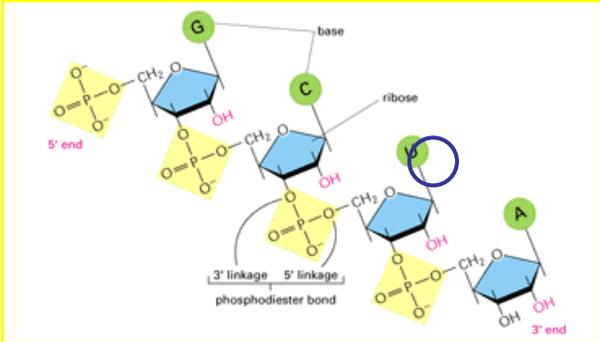
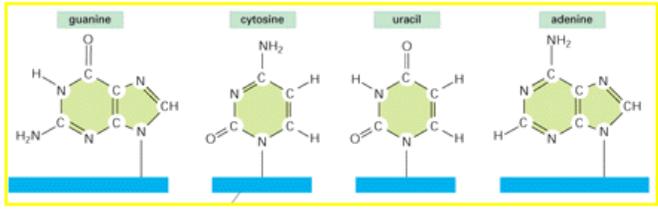
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Facultad de Medicina. Universidad de Chile*



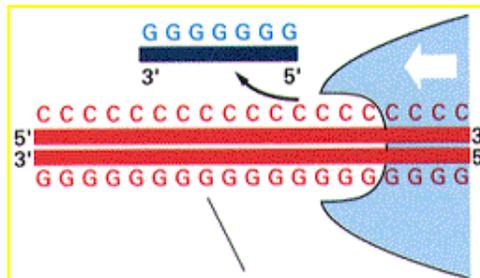
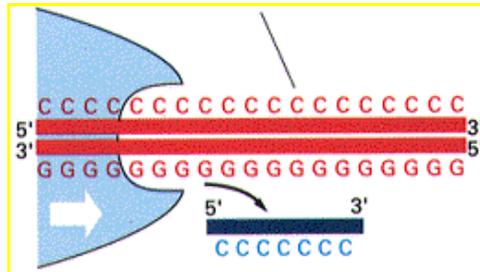
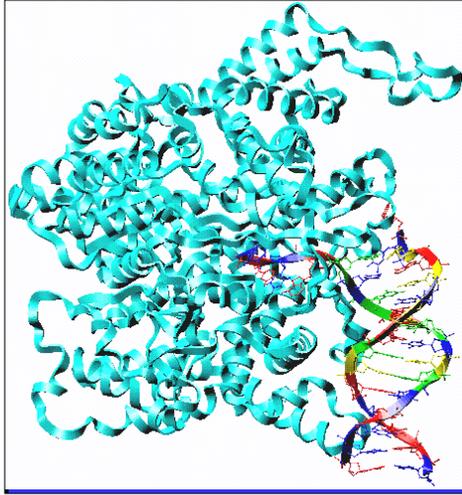


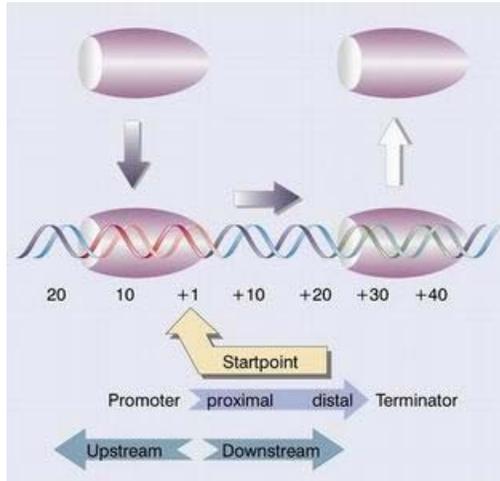
- * tRNA o RNA de transferencia
- * rRNA o RNA ribosomal
- * mRNA o RNA mensajero





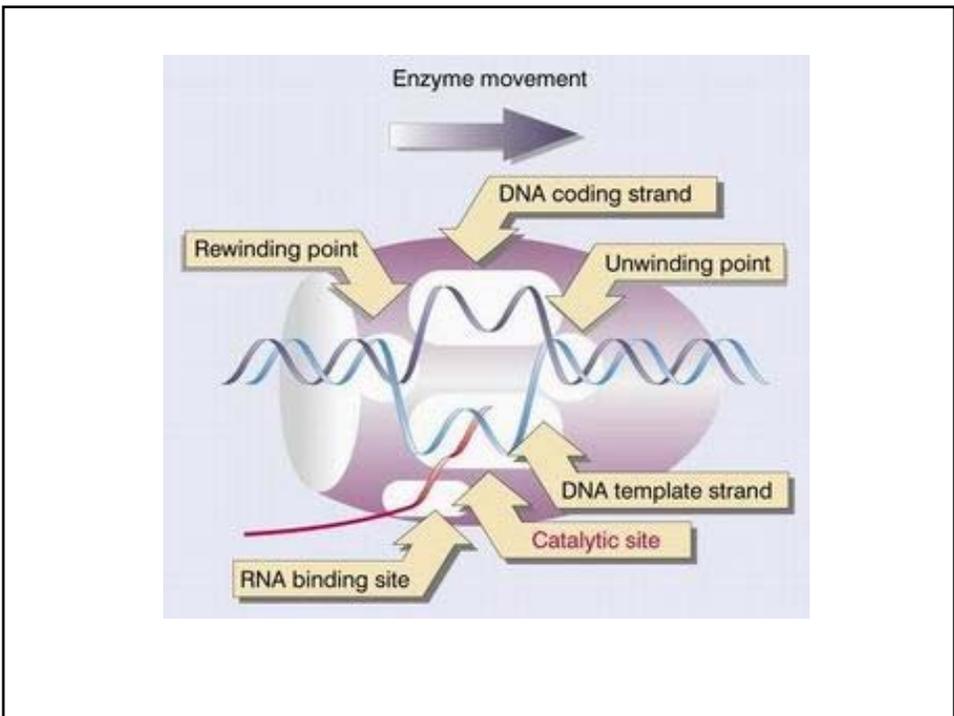
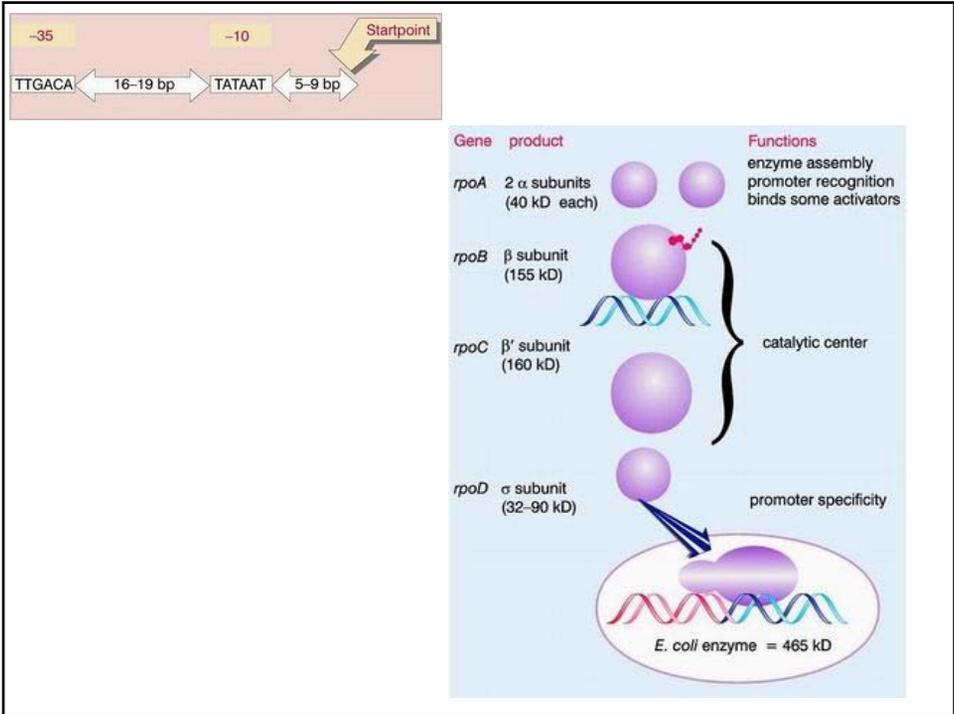
RNA Polimerasa

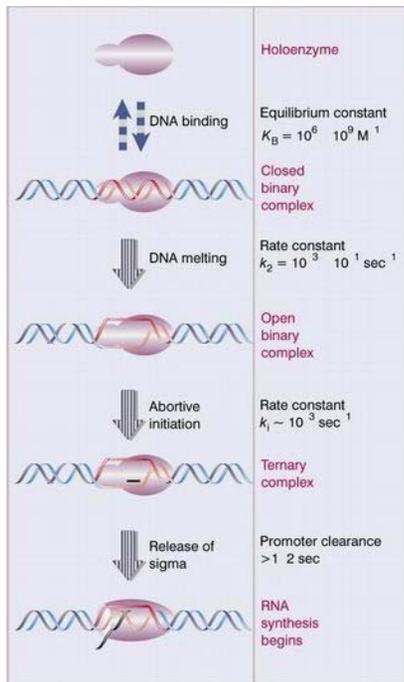
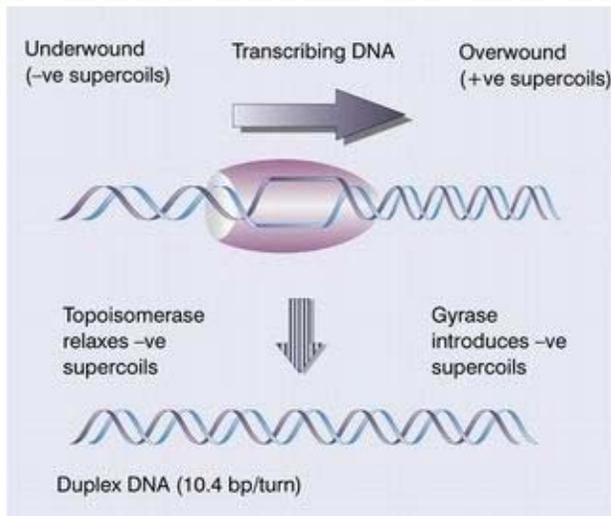


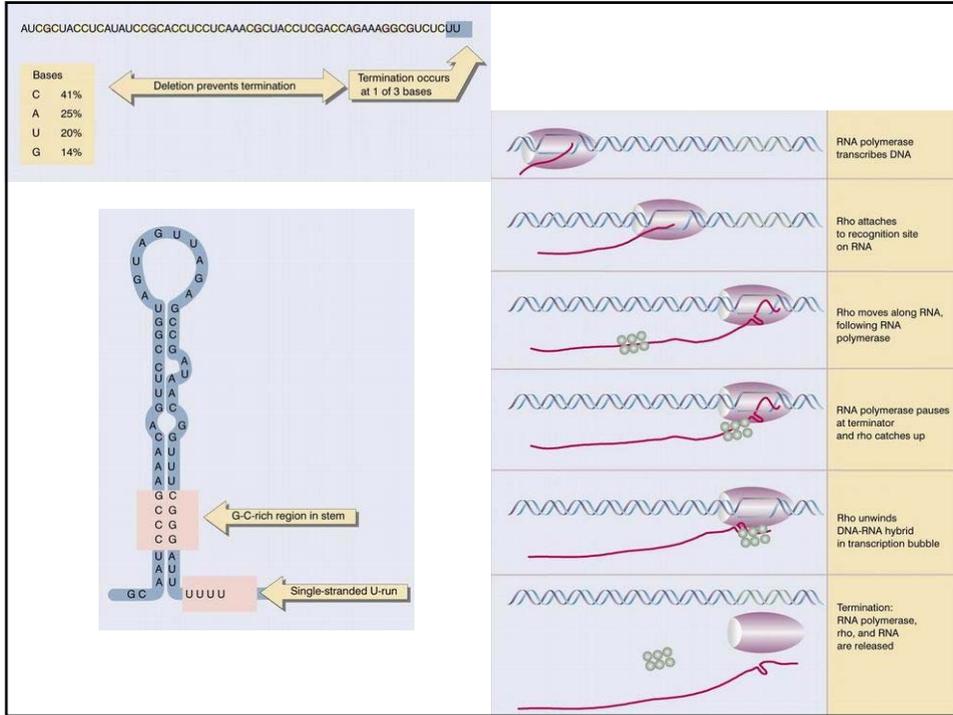


RNA polimerasa Procarionte

Subunidad	Gen	PM (kDa)	Función
β'	rpo C	160	Unión al DNA molde; centro catalítico de la enzima
β	rpo B	155	Formación del enlace fosfodiéster; centro catalítico de la enzima
σ	rpo D	32-90	Confiere especificidad al sitio promotor
α	rpo A	40	Ensamblaje de la enzima; reconoce el promotor; enlaza algunos activadores

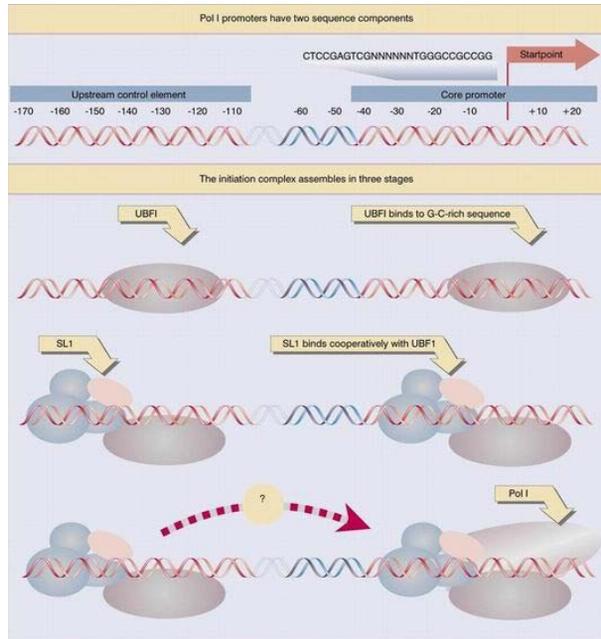
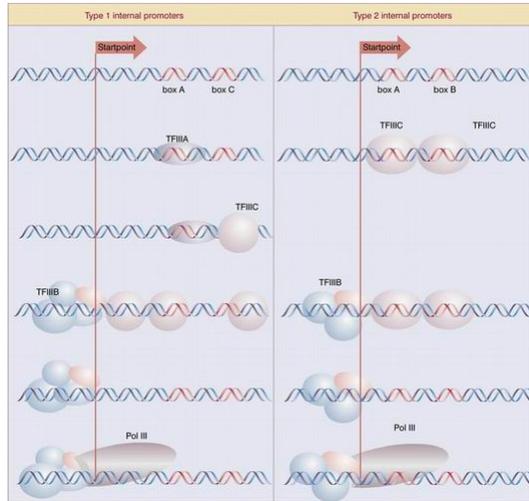
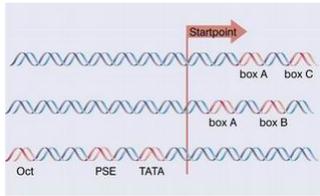


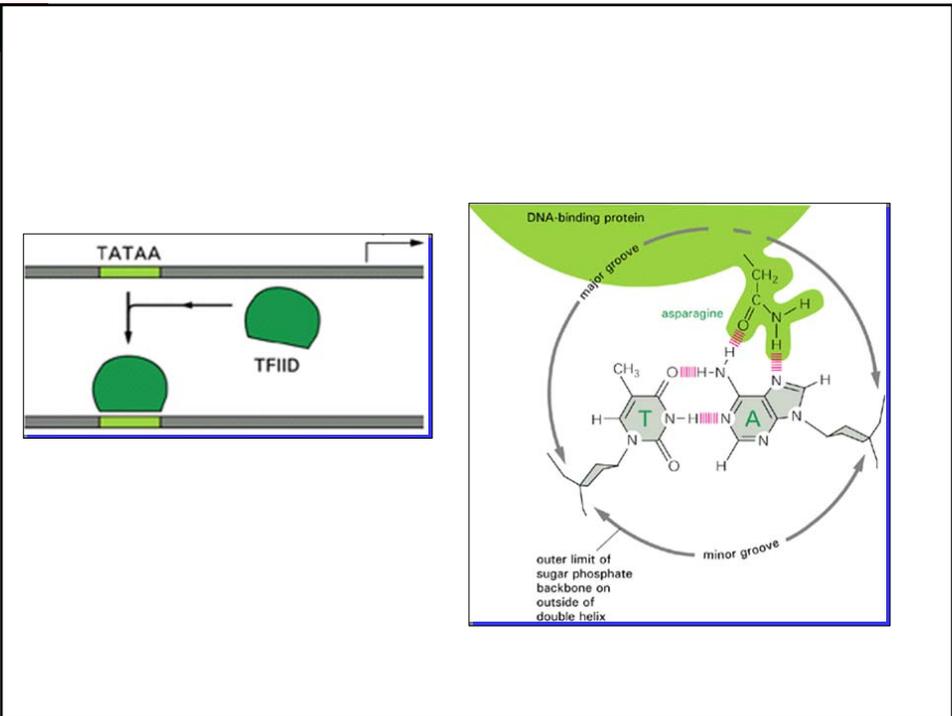
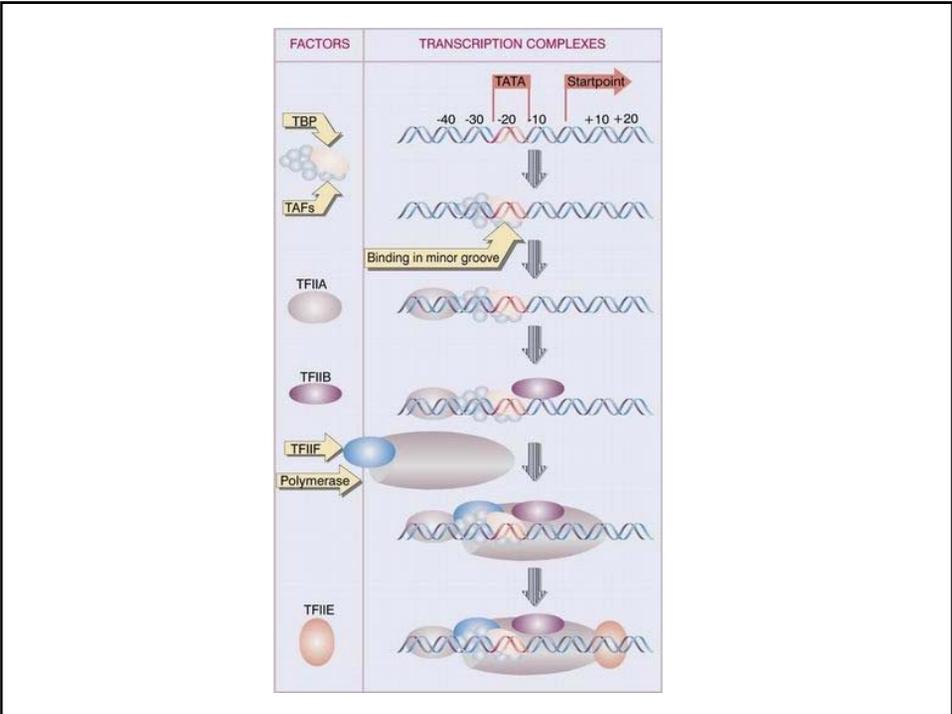


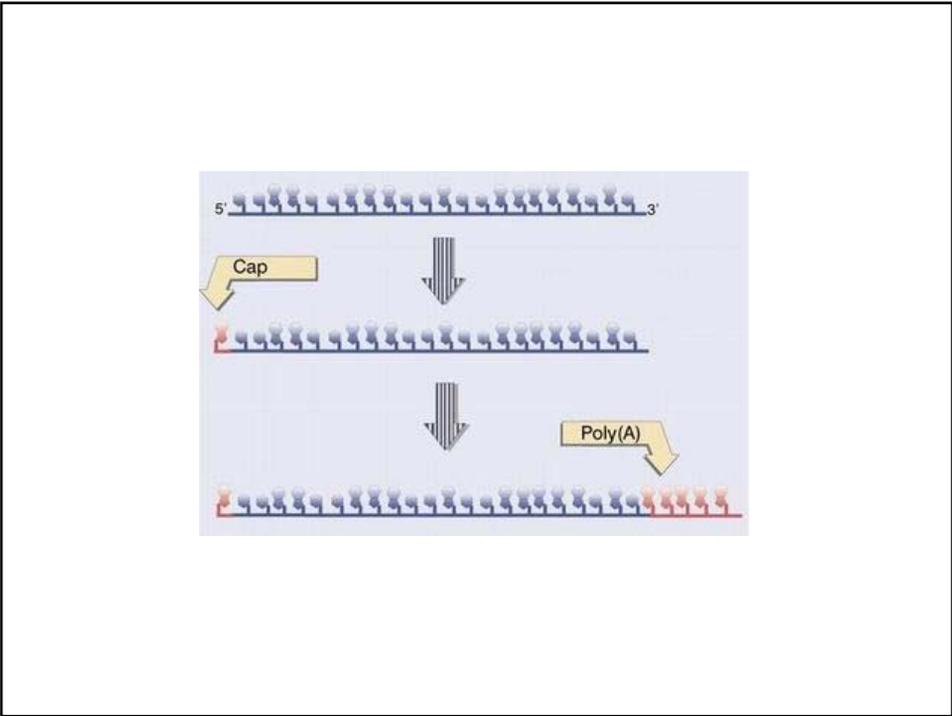
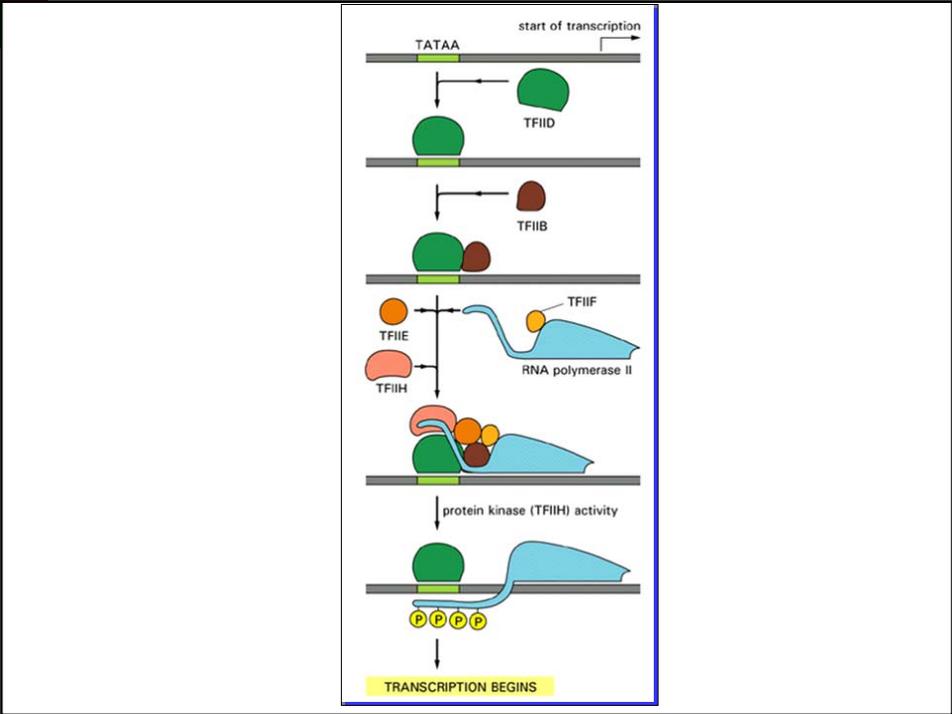


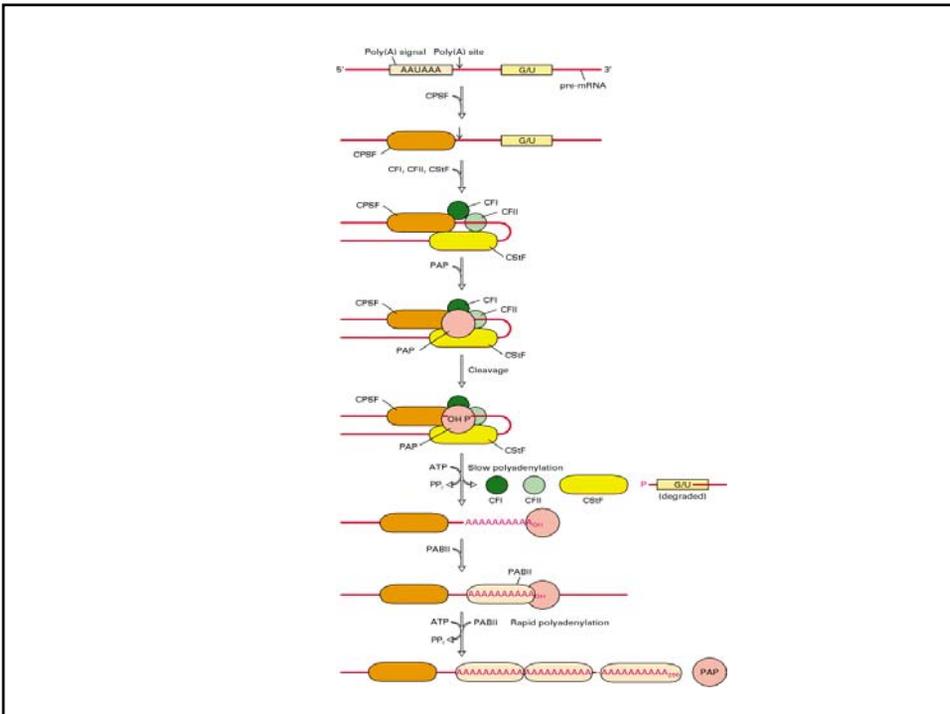
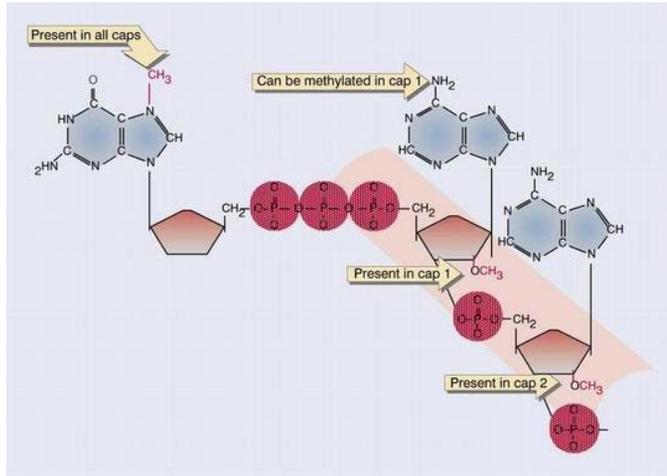
RNA polimerasas eucariontes

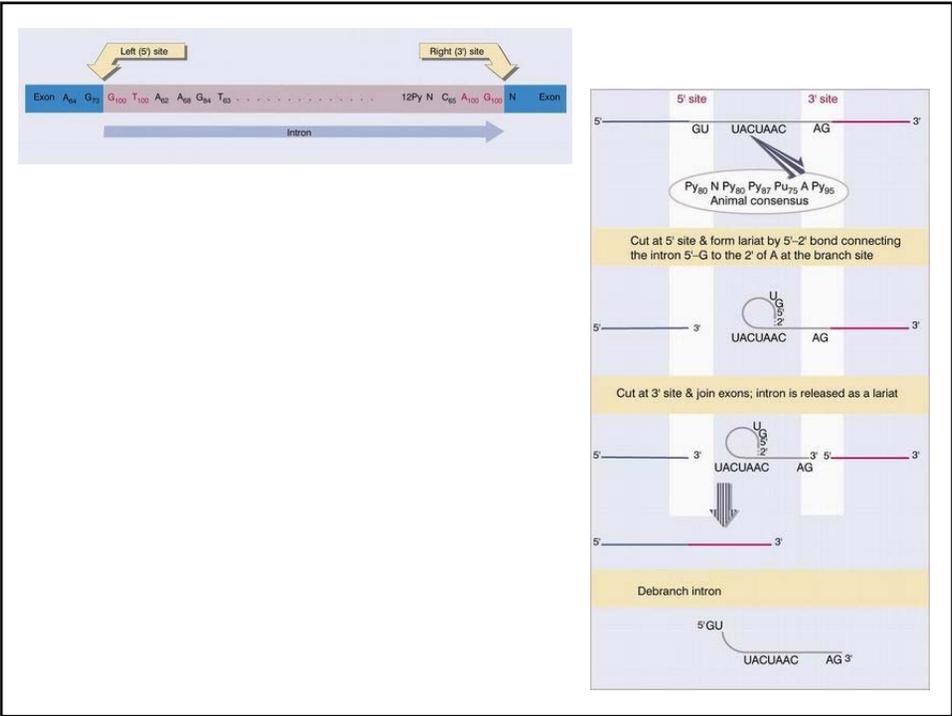
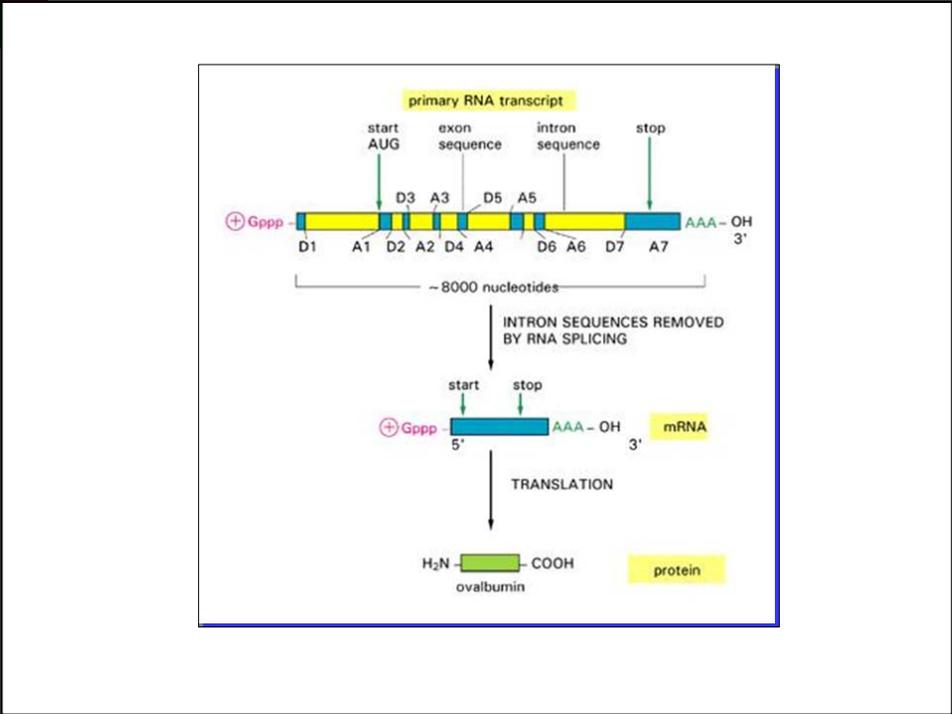
Enzima	Localización	Copias/cél.	Producto	Actividad polimerásica	Sensibilidad a la α -amanitina
RNA Pol I	Nucleolo	40,000	pre-rRNA (35-47s)	50-70%	Ninguna
RNA Pol II	Nucleoplasma	40,000	hnRNA snRNA (U1,U2,U4,U5)	20-40%	Alta
RNA Pol III	Nucleoplasma	20,000	rRNA 5S tRNA snRNA U6 RNA 7S Otros RNA de < 300 pb	~10%	Específica de especie

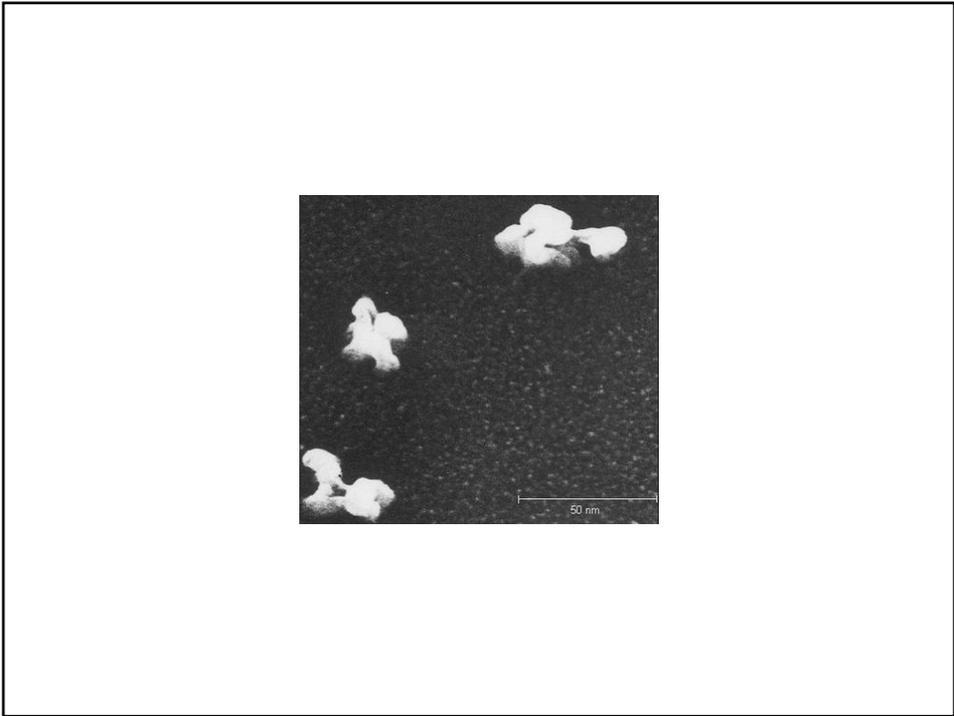
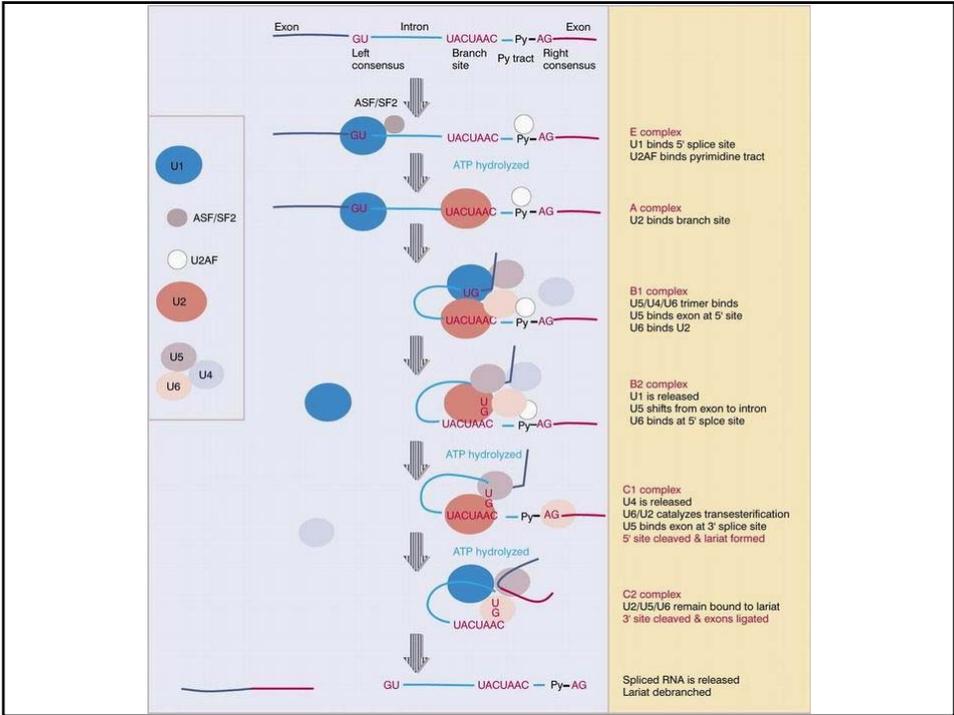


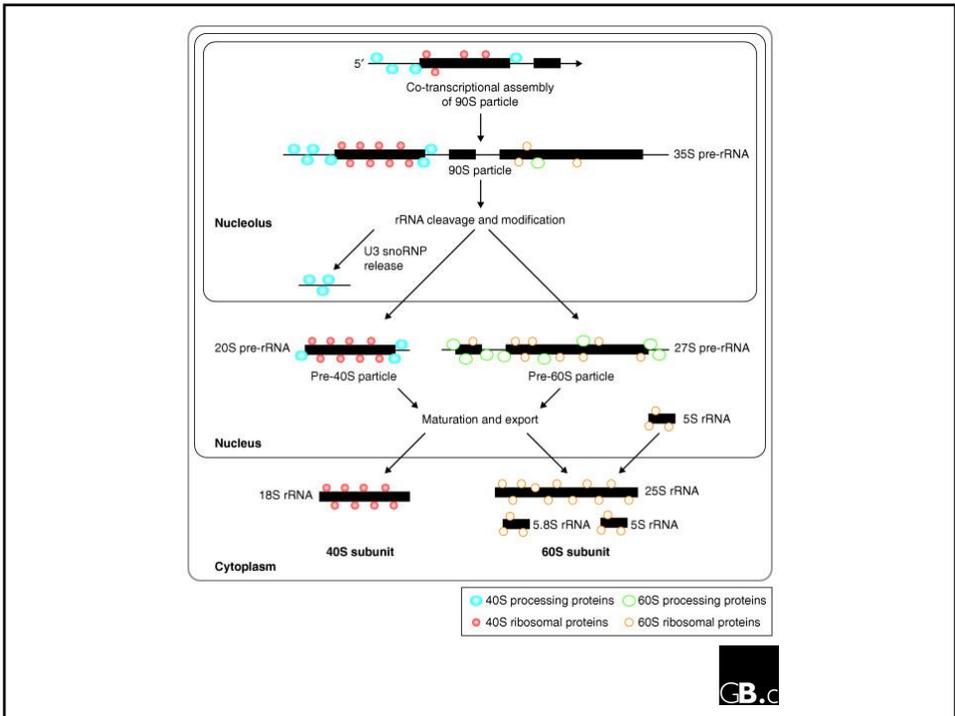
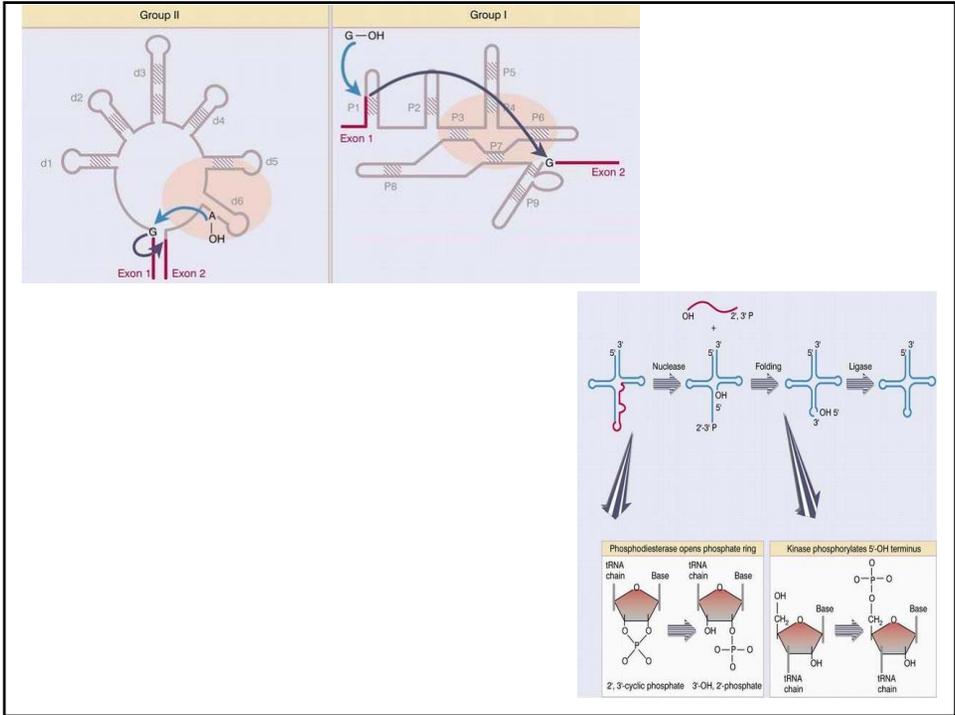












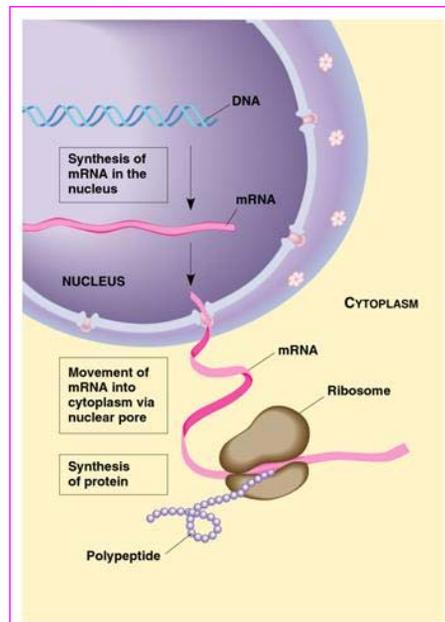
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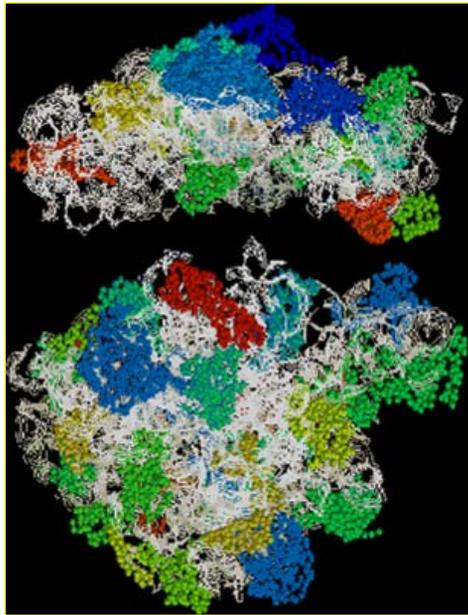
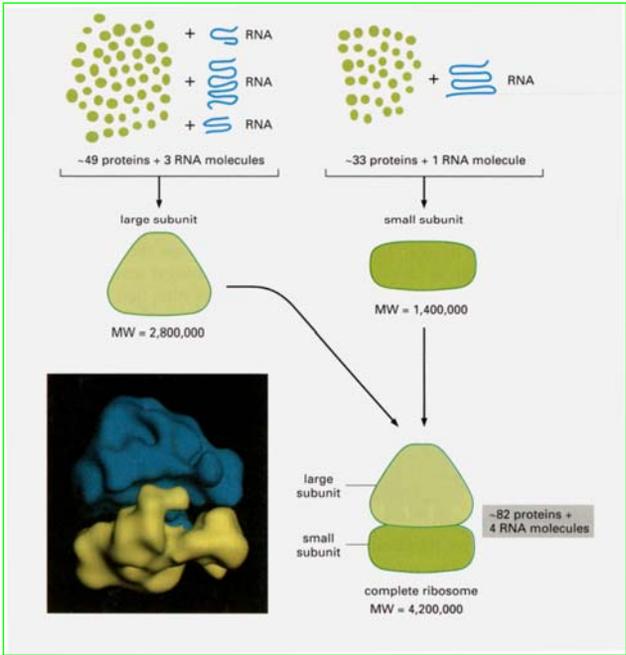


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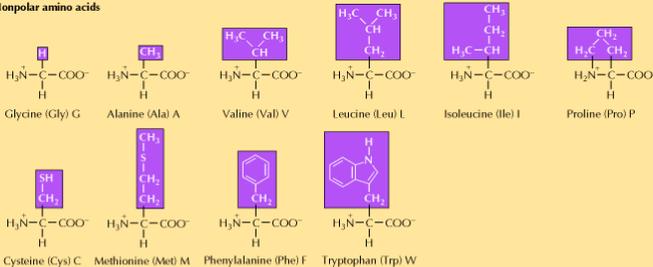
Clase: Traducción de la información genética

*Dr. Enrique Castellón
Programa de Fisiología y Biofísica
Instituto de Ciencias Biomédicas
Facultad de Medicina. Universidad de Chile*

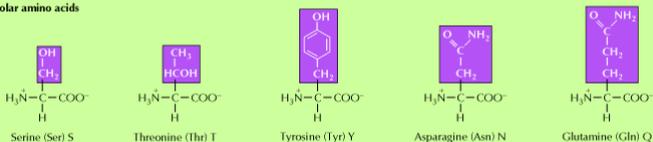




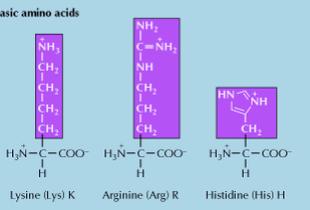
Nonpolar amino acids



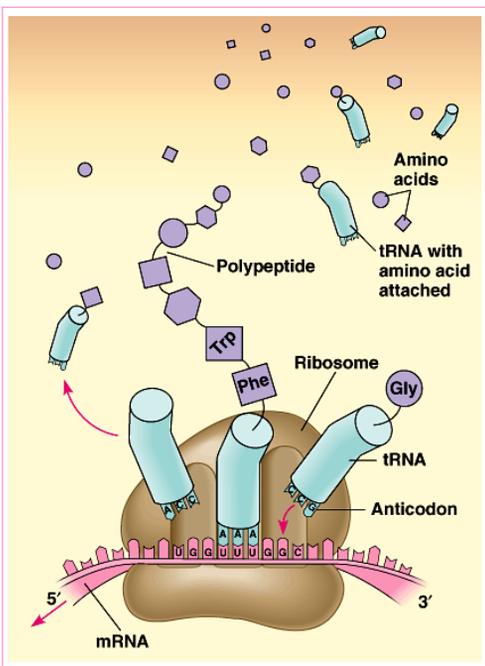
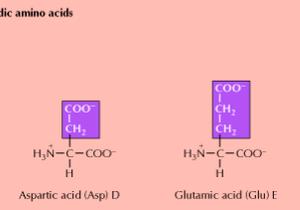
Polar amino acids

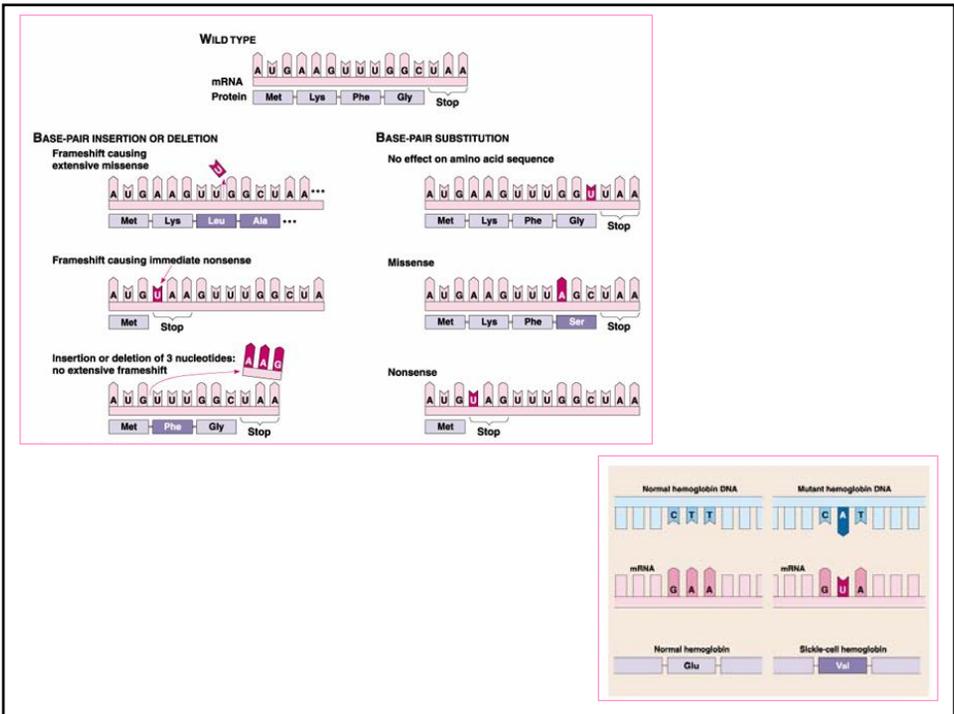
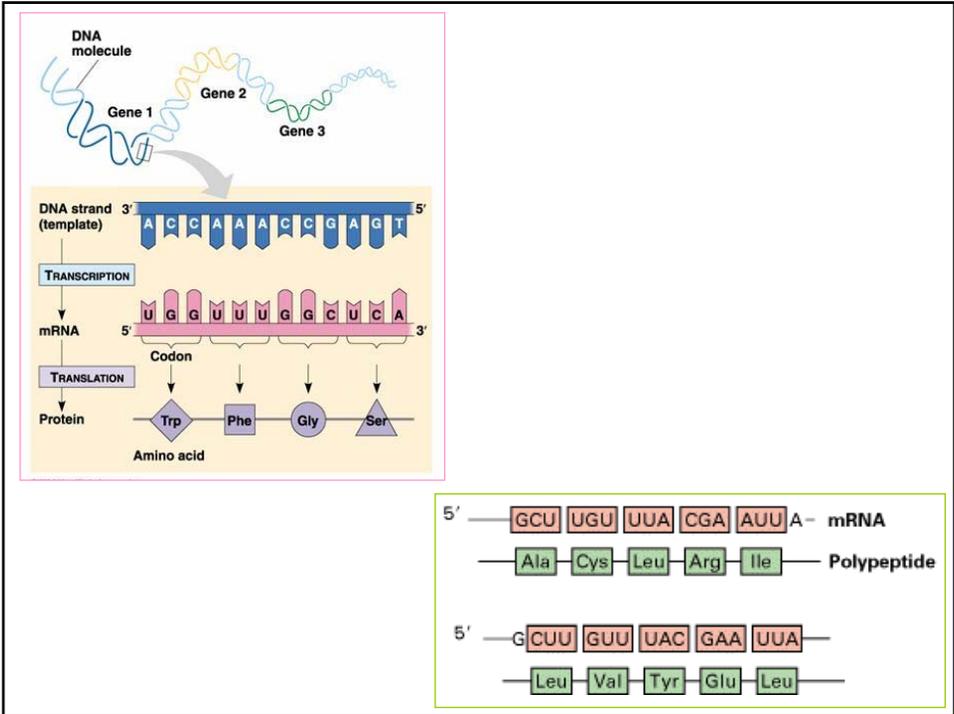


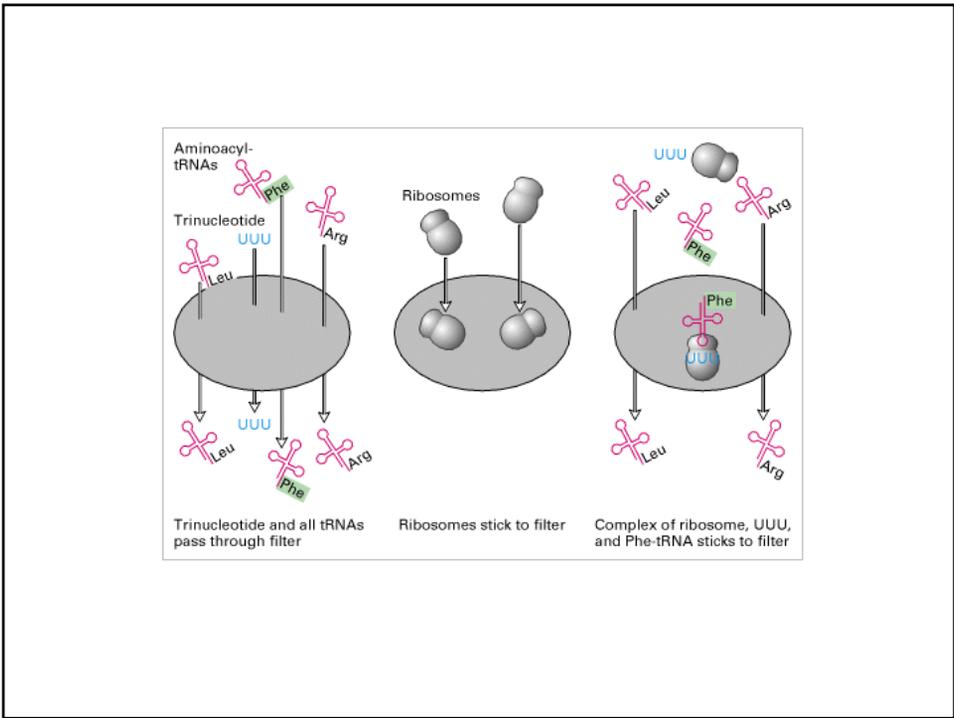
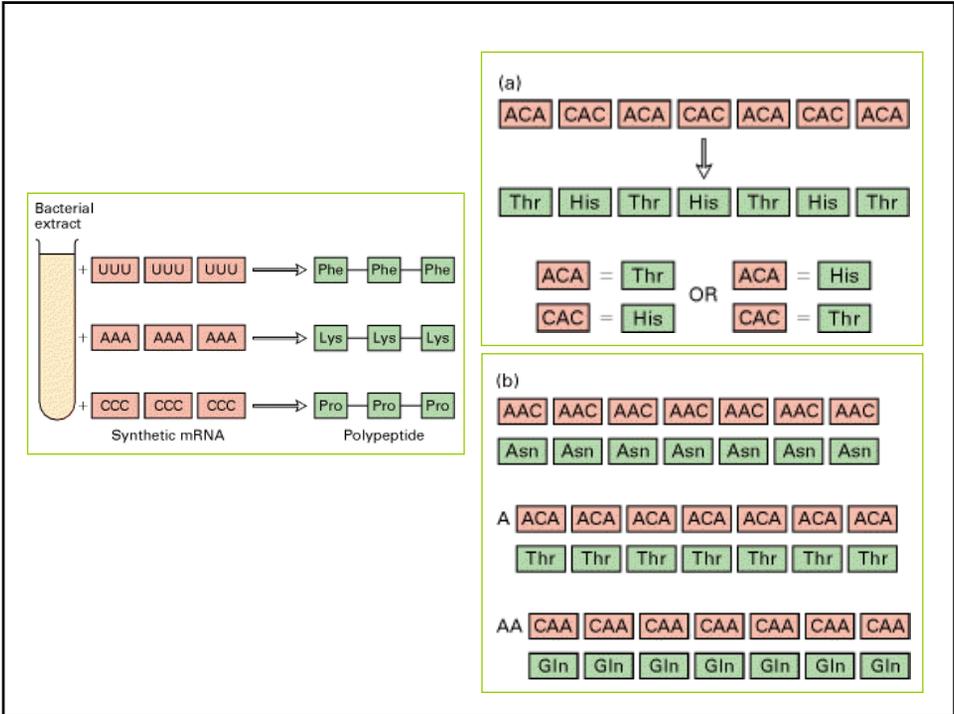
Basic amino acids

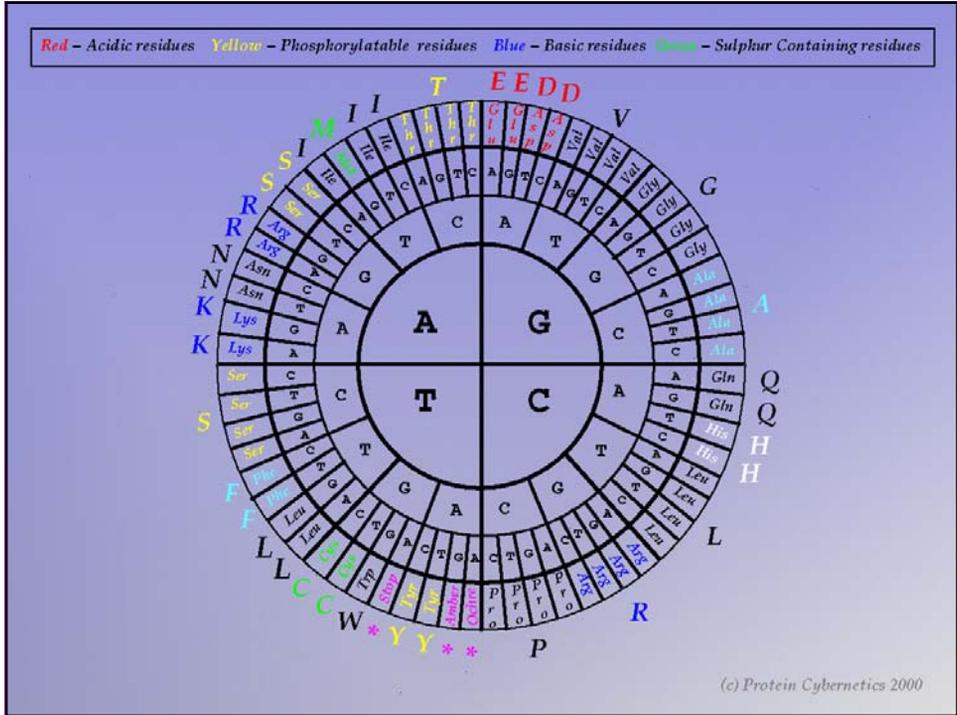


Acidic amino acids









Unusual Codon Usage in Nuclear and Mitochondrial Genes

	Universal	Unusual	
Codon	Code	Code	Occurrence
UGA	Stop	Trp	<i>Mycoplasma</i> , <i>Spiroplasma</i> , mitochondria many species
CUG	Leu	Thr	Mitochondria in yeasts
UAA, UAG	Stop	Gln	<i>Acetabularia</i> , <i>Tetrahymena</i> , <i>Paramecium</i> ,
UGA	Stop	Cys	<i>Euplotes</i>

tRNA

3' tRNA
5' mRNA 3'

If these bases are in **first**, or wobble, position of anticodon

C	A	G	U	I
G	U	C	A	U

then the tRNA may recognize codons in mRNA having these bases in **third** position

5' mRNA 3'

3' tRNA
5' mRNA 3'

If these bases are in **third**, or wobble, position of codon of an mRNA

C	A	G	U
G	U	C	A

then the codon may be recognized by a tRNA having these bases in **first** position of anticodon

Uridine

Adenosine

Guanine

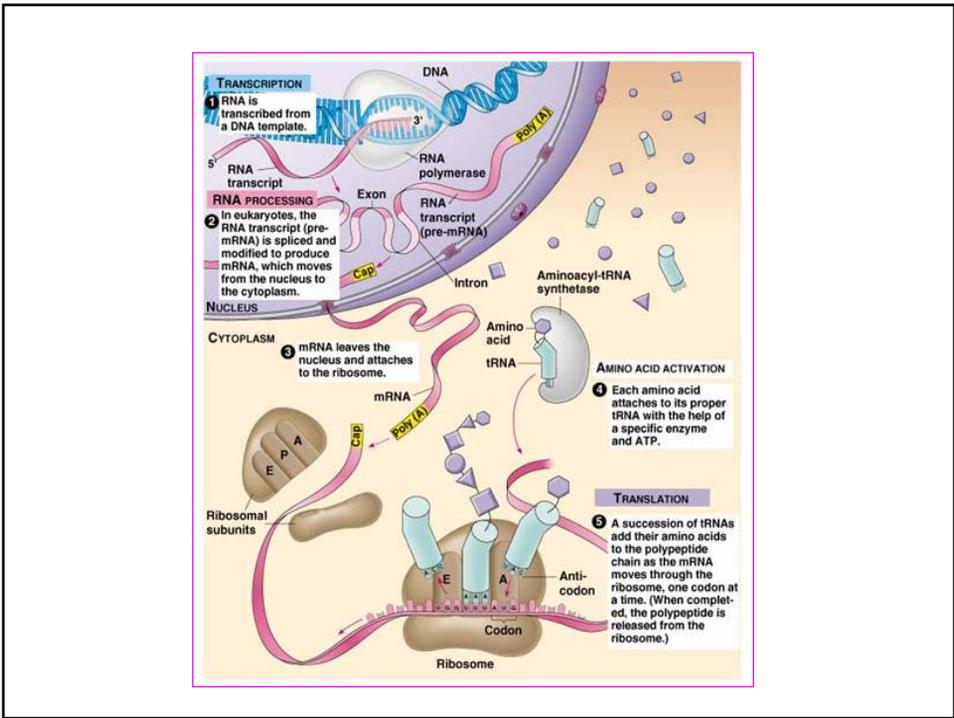
Inosine

Cytidine

Uridine

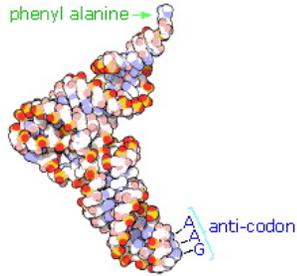
Inosine

Inosine

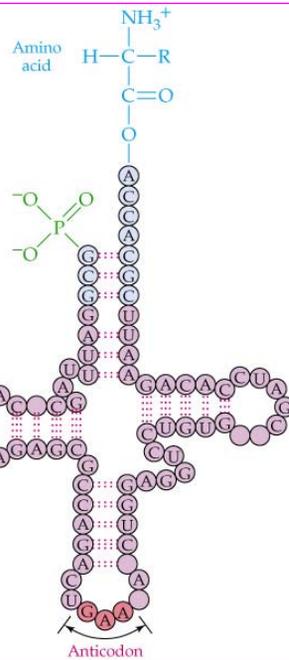
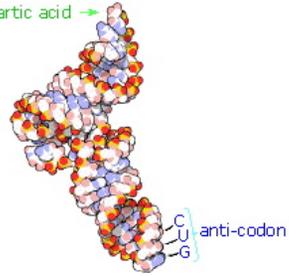


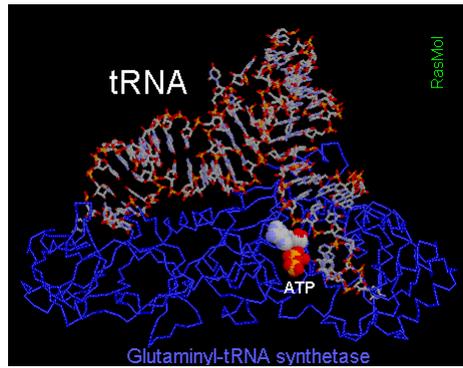
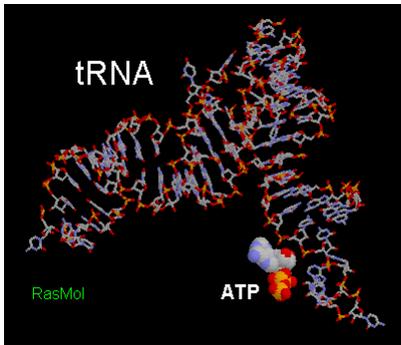
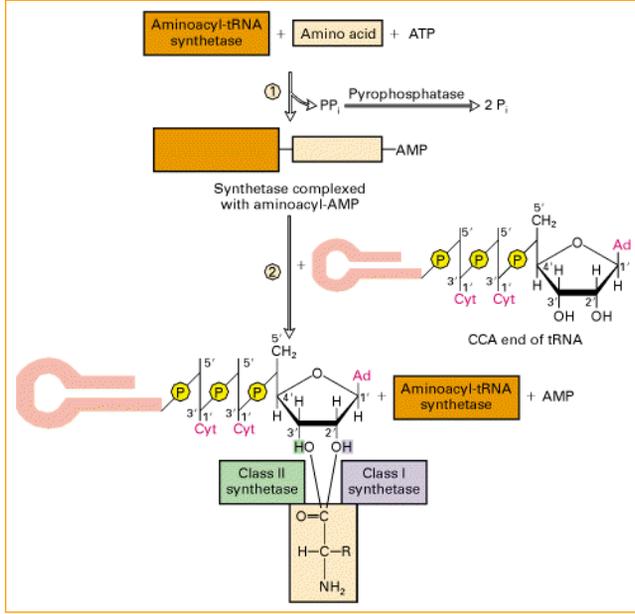
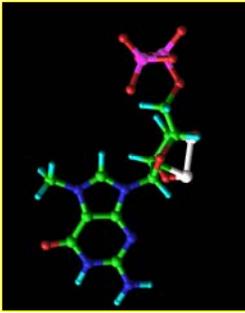
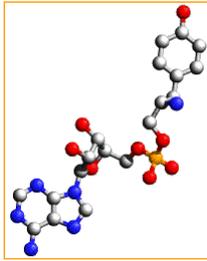
Transfer RNA Molecules

phenyl alanine →

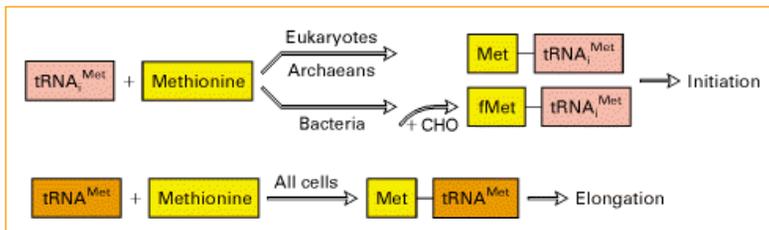
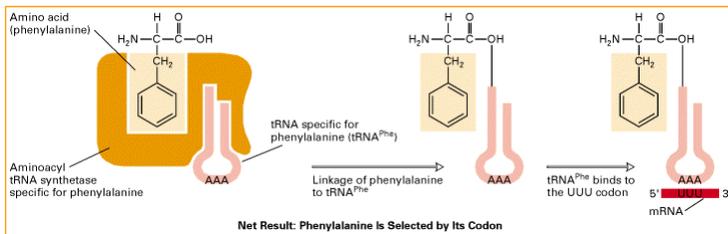
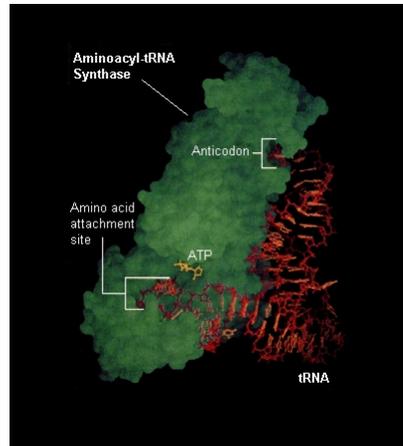


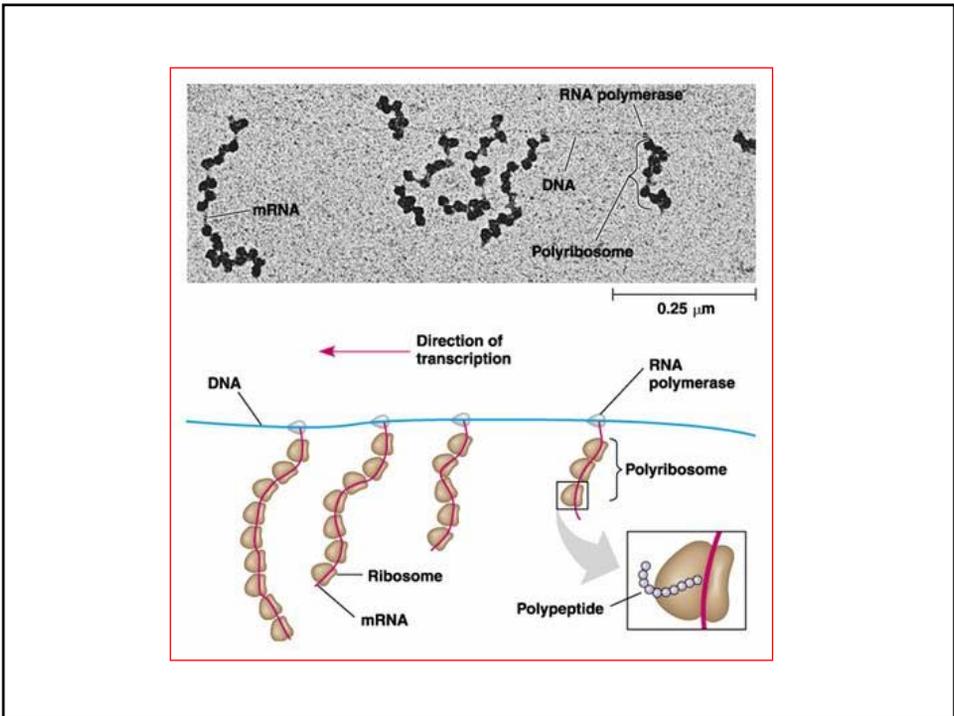
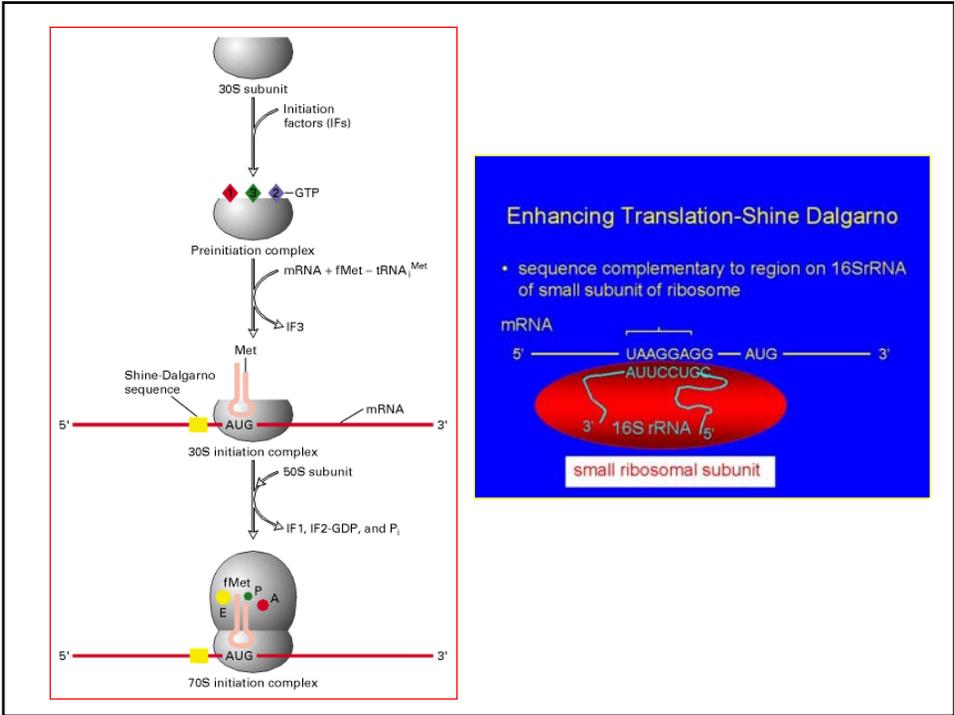
aspartic acid →

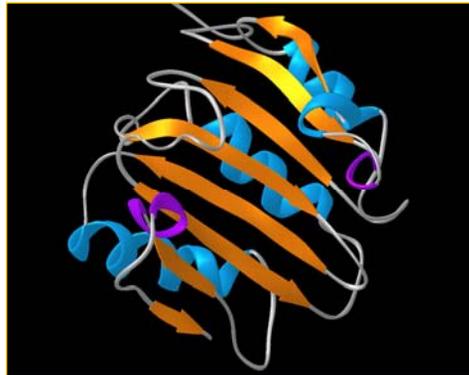
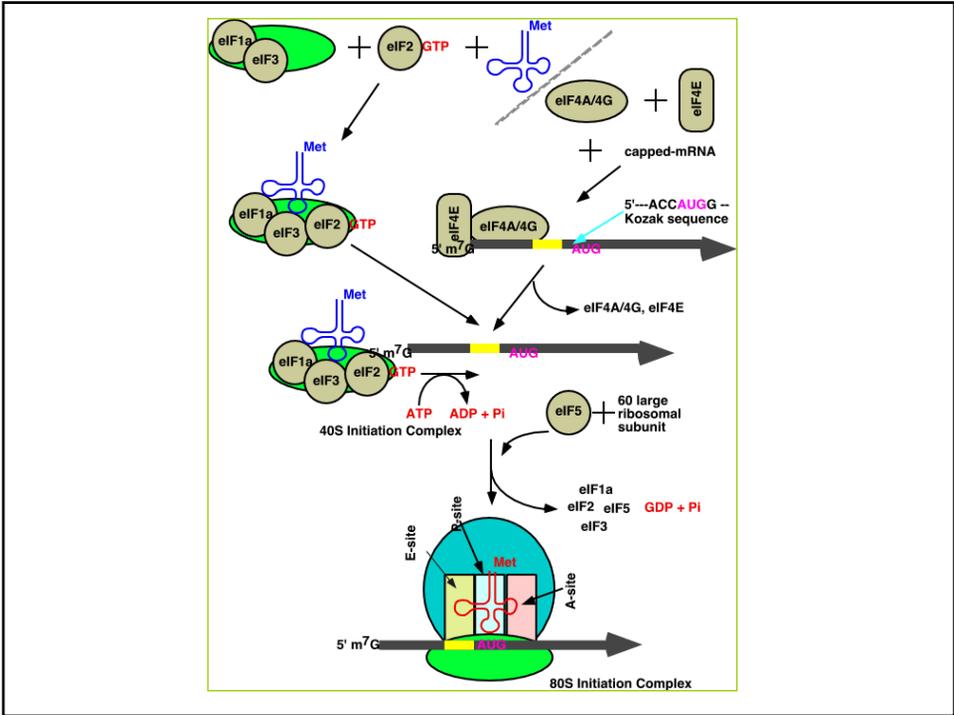




Class I		Class II	
Glu	α	Gly	$\alpha 2\beta 2$
Gln	α	Ala	$\alpha 4$
Arg	α	Pro	$\alpha 2$
Cys	$\alpha 2$	Ser	$\alpha 2$
Met	$\alpha 2$	Thr	$\alpha 2$
Val	α	His	$\alpha 2$
Ile	α	Asp	$\alpha 2$
Leu	α	Asn	$\alpha 2$
Tyr	$\alpha 2$	Lys	$\alpha 2$
Trp	$\alpha 2$	Phe	$\alpha 2\beta 2$

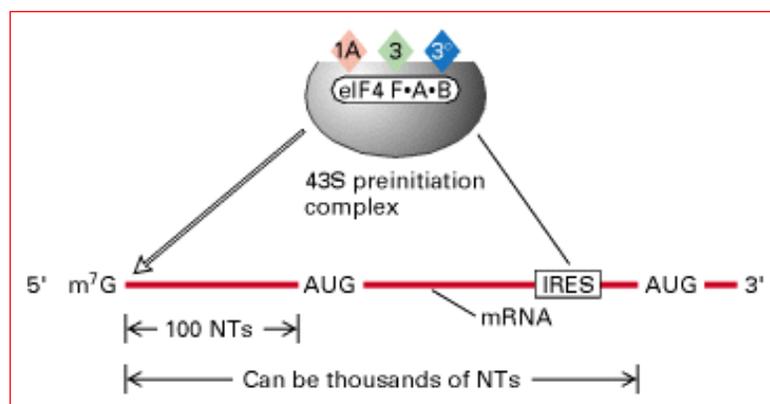


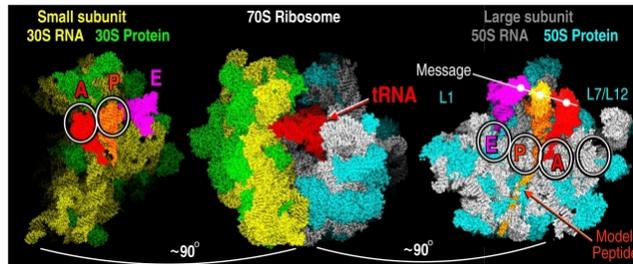
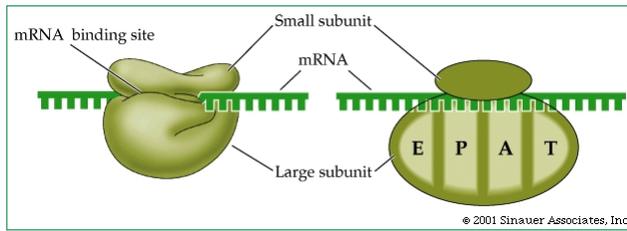




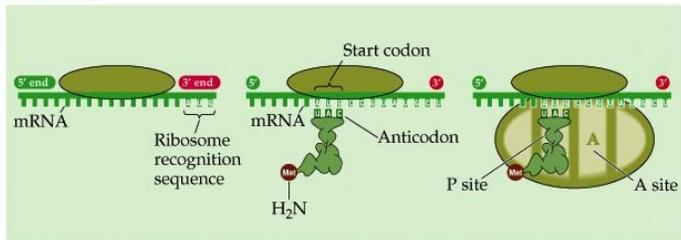
eIF-4E binds 7-methylguanosine, the post-transcriptional cap of mRNA, as its ligand. Eleven amino acid residues come into contact with this ligand and are as follows: Ala51, Asp53, Trp58, Lys90, Asp92, His94, Glu103, Trp104, Glu105, Ile156, and Arg157.

Factor	Earlier name(s)	Function(s)	Mass	Subunits (kD)
eIF1		Stimulation of Met-RNAi and mRNA binding to 40S ribosomes	15 kD	
eIF1A	eIF4C	Stimulation of Met-RNAi and mRNA binding to 40S ribosomes	17 kD	
eIF2		Met-RNAi binding to 40S ribosomes	130 kD	α (36) β (38) γ (55)
eIF2B	GEF	GDP:GTP exchange on eIF2	270 kD	α (26) β (39) γ (58) δ (67) ϵ (82)
eIF2C	Co-eIF2A	Stabilization of ternary complex	94 kD	
eIF3		Ribosome dissociation, Stabilization of ternary complex, Stimulation of mRNA binding.	737 kD	a (170) b (116) c (110) d (66) e (48) f (47) g (44) h (40) i (36) j (35) k (25)
eIF3A	eIF6	Ribosome dissociation	25 kD	
eIF4A		mRNA binding, RNA helicase	44 kD	
Dea1		mRNA binding, RNA helicase	65 kD	
eIF4B		mRNA binding, RNA helicase	70 kD	
eIF4E		mRNA binding, Cap recognition	25 kD	
eIF4F	CBP II	mRNA binding, Cap recognition, RNA helicase	270 kD	α : eIF4E(25) β : eIF4A(44) γ : eIF4G(154)
eIF4G		mRNA binding, Anchor protein	154 kD	
eIF4H		mRNA binding		
eIF5		Ribosomal subunit joining	48 kD	
eIF5B	eIF4D	Ribosomal subunit joining	112 kD	

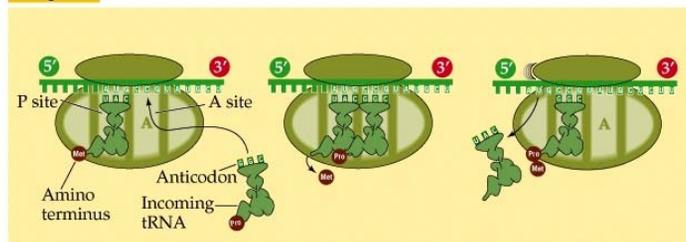




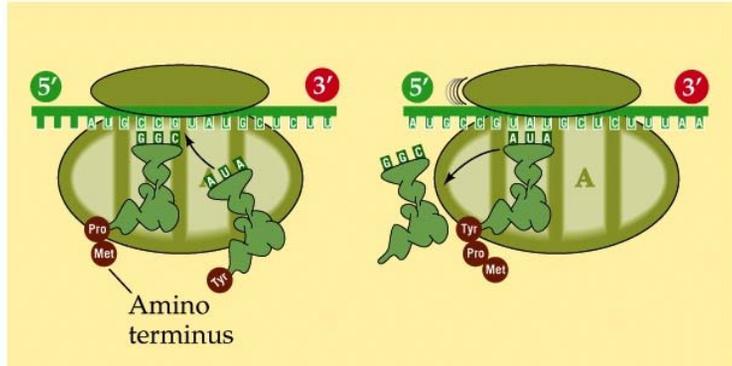
Initiation



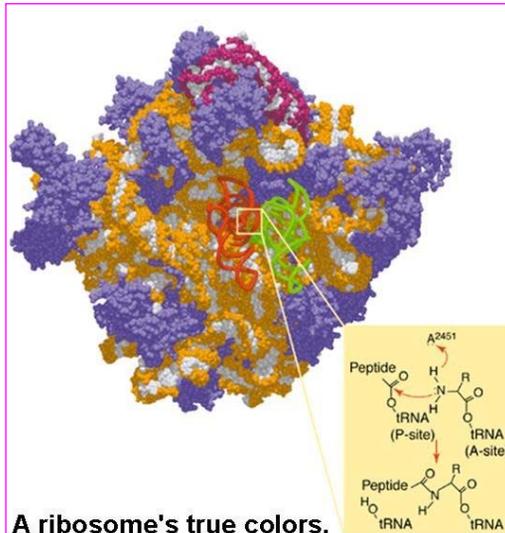
Elongation



Elongation



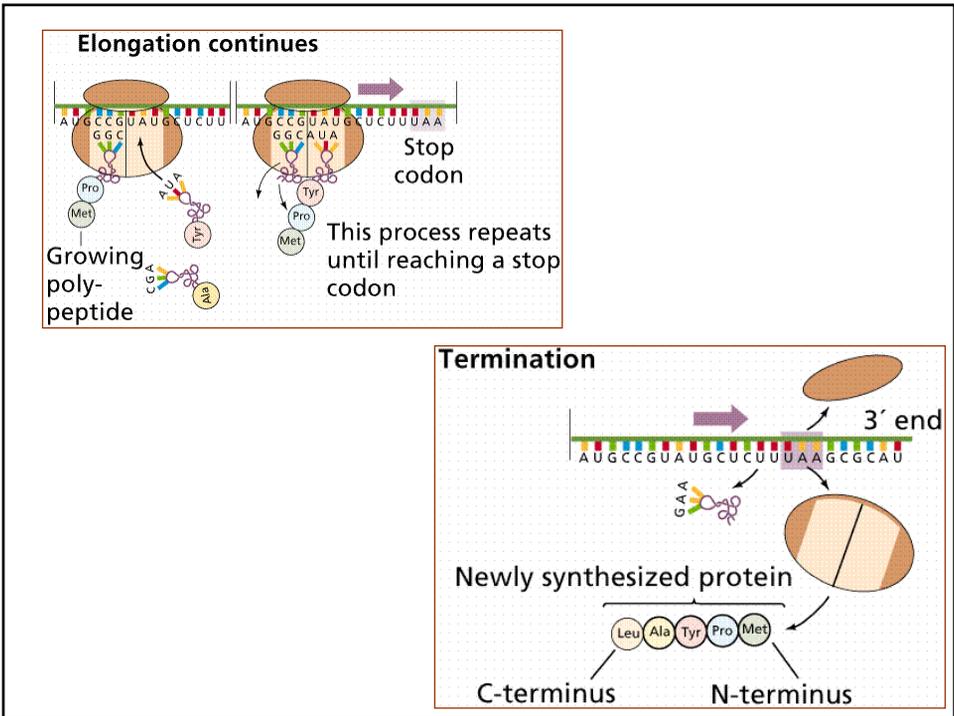
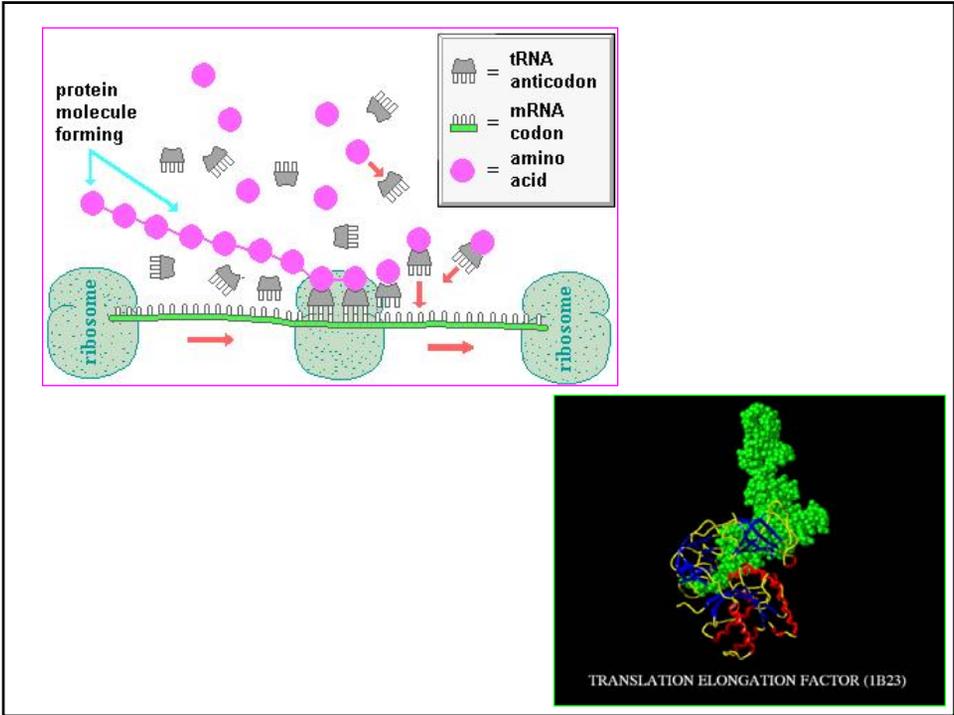
© 2001 Sinauer Associates, Inc.

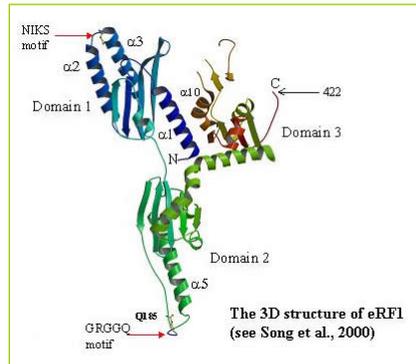
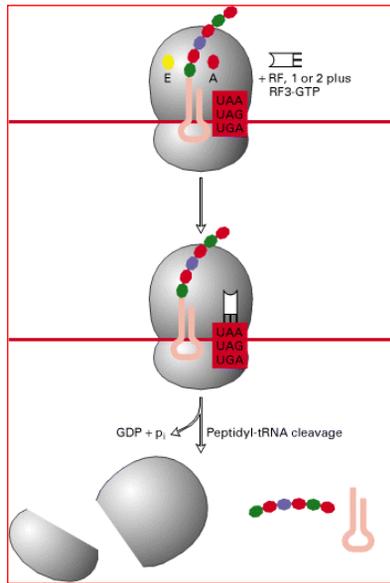


A ribosome's true colors.

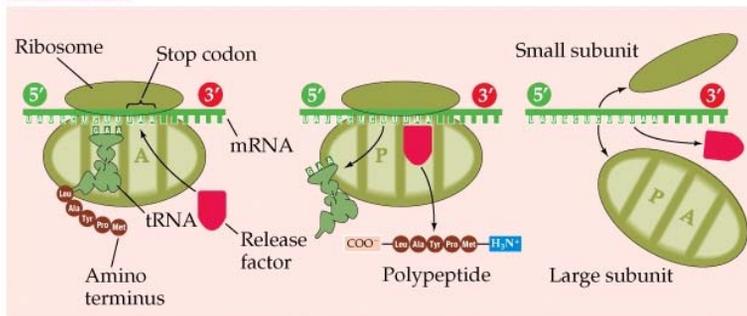
The large subunit of the ribosome with proteins in purple, 23S rRNA in orange and white, 5S rRNA in burgundy and white, and A-site tRNA (green) and P-site tRNA (red) docked according to (5).

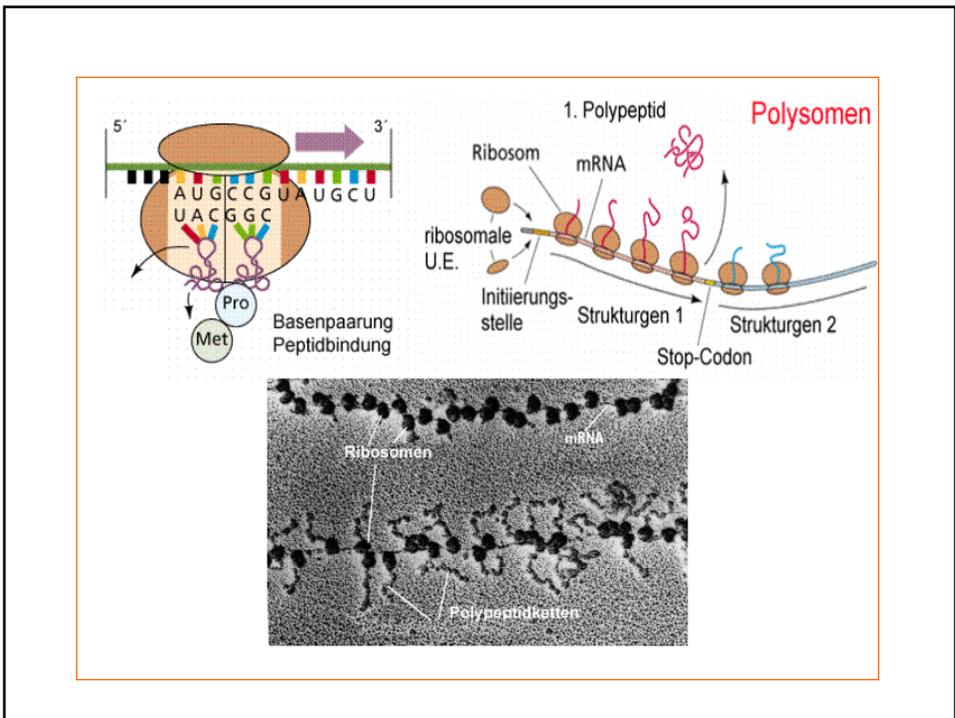
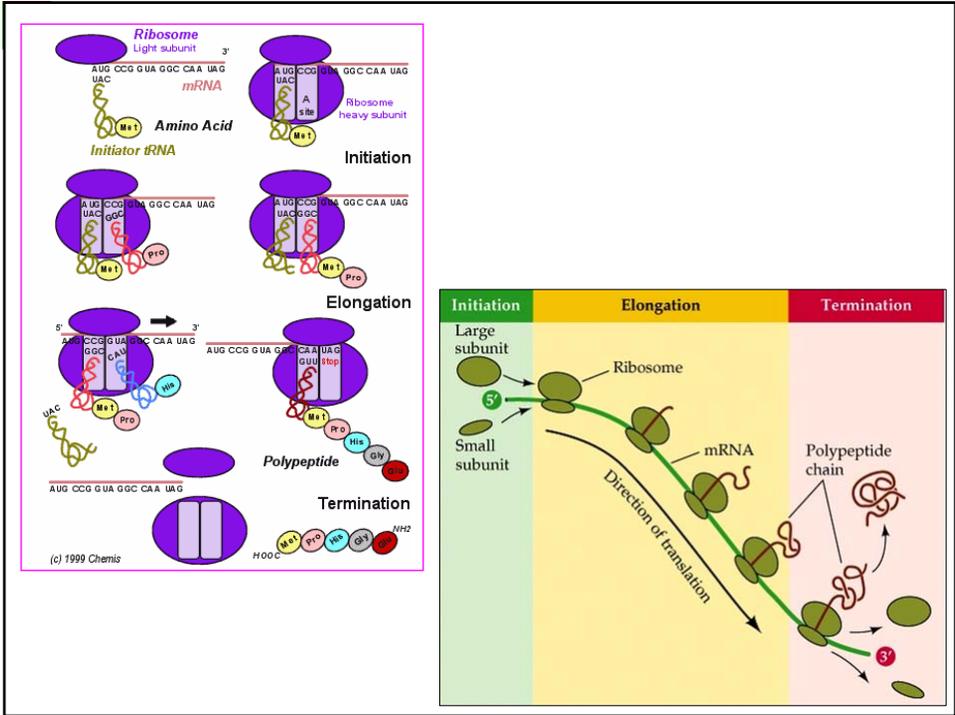
(Insert) The peptidyl transfer mechanism catalyzed by RNA (2).
Cech TR. Science 2000 Aug 11;289(5481):878-9.

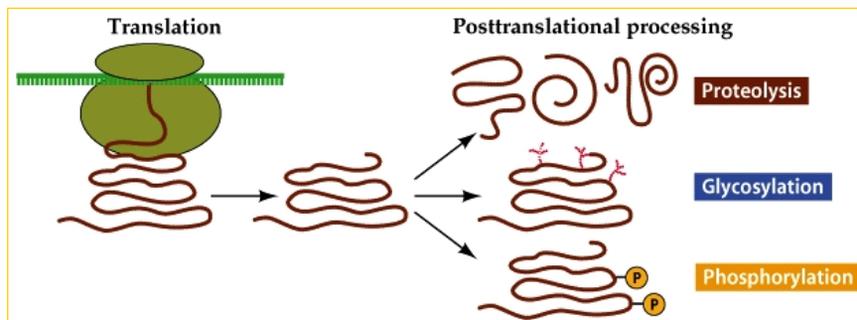
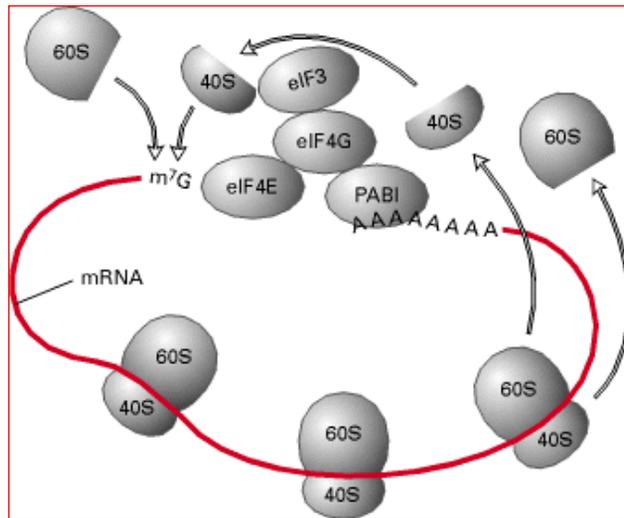


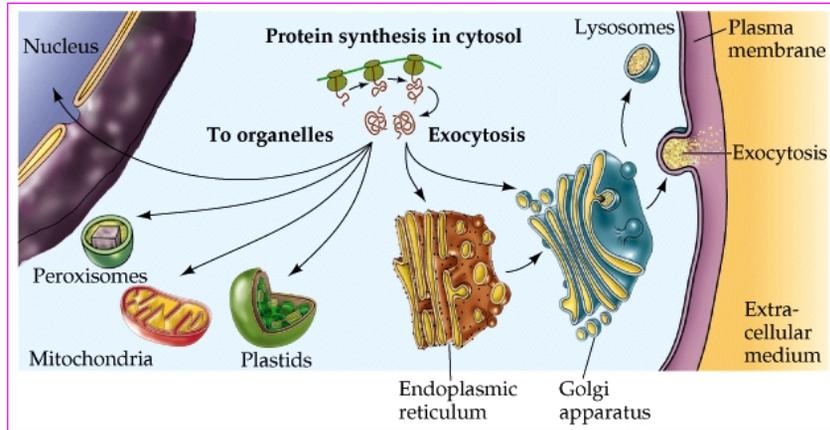


Termination









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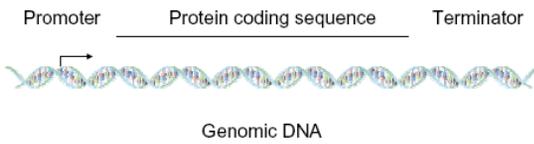


Curso: Biología Celular

**Clase: Regulación de la expresión génica.
Metodología del DNA**

*Dr. Enrique Castellón
Programa de Fisiología y Biofísica
Instituto de Ciencias Biomédicas
Facultad de Medicina. Universidad de Chile*

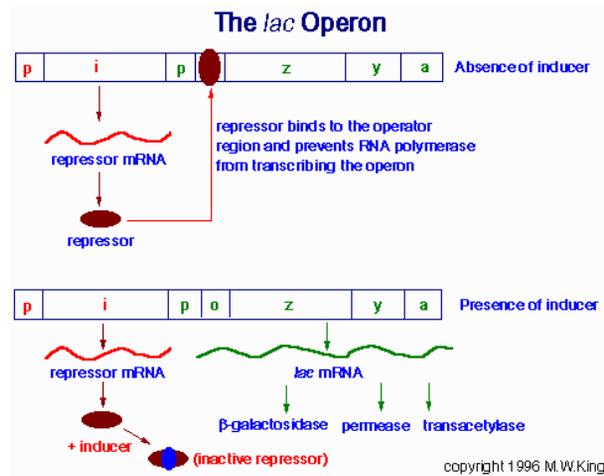
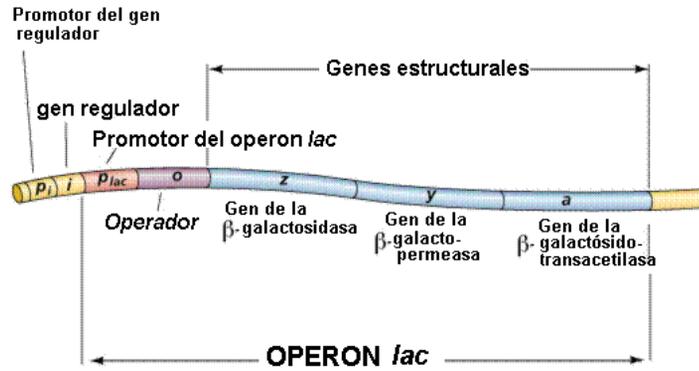
Procariontes

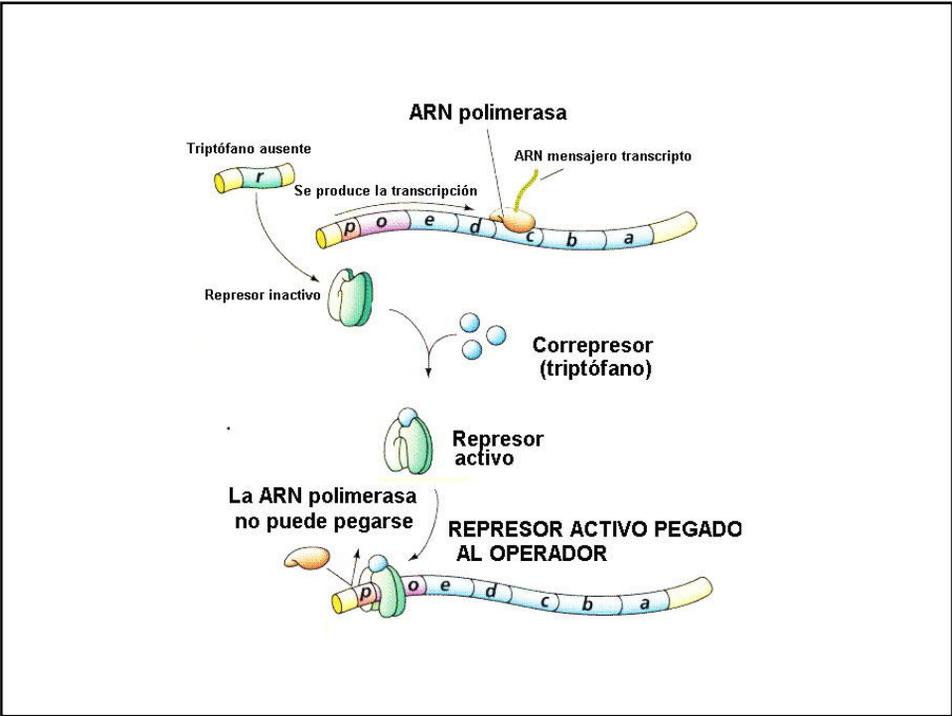
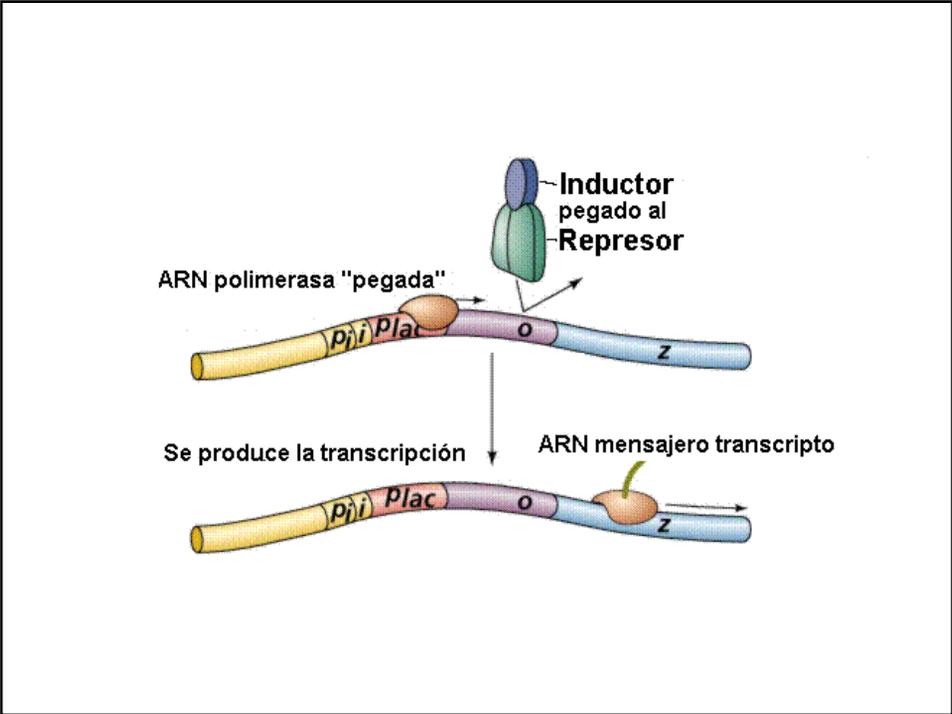


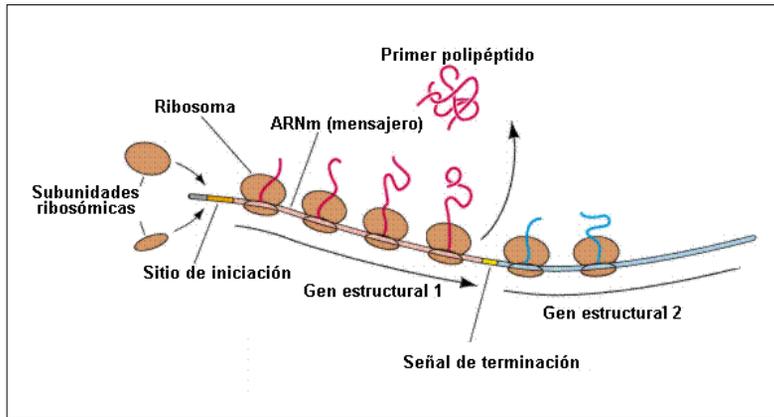
Types of genes

rRNA
tRNA
mRNA
snRNA
sRNA



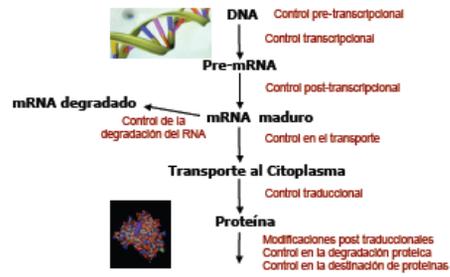






Eucariontes

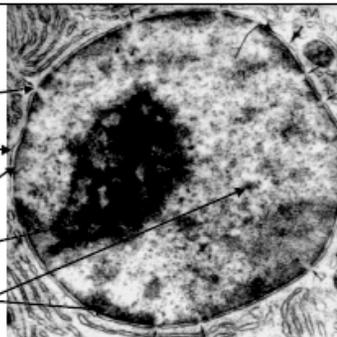
Posibles niveles de control de la expresión genica



- Selección de los genes que se transcriben
- Regulación de la tasa de expresión
- Regulación de la naturaleza del producto

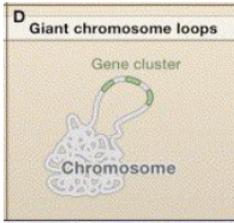
El núcleo y el control de expresión génica

Poros nucleares
Envoltura nuclear
Lámina
Nucleolo
Cromatina condensada



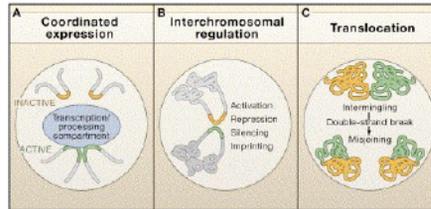
- Organización de la cromatina y estructuras cromosómicas de orden superior: heterocromatina, eucromatina, formación de nucleolo.

Formación de loops de cromatina



major histocompatibility complex II
epidermal differentiation complex
mouse Hox cluster

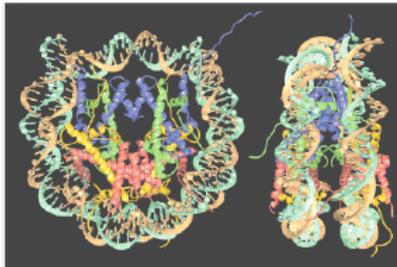
Consecuencias funcionales de la organización de la cromatina



genes de α - y β -globina localizados en los cromosomas 16 y 11

Control a nivel de la organización de la estructura de la cromatina

Modificación covalente de histonas.



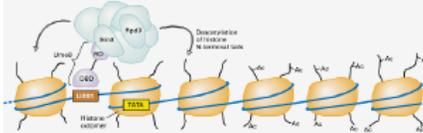
-Acetilación / metilación.
-H3 H4

Hiperacetilación: cromatina activa
Hipermetilación: heterocromatinización

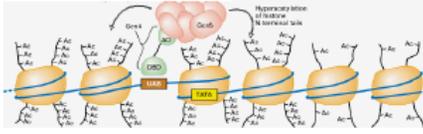
Control a nivel de la organización de la estructura de la cromatina

Acetilación y desacetilación de histonas

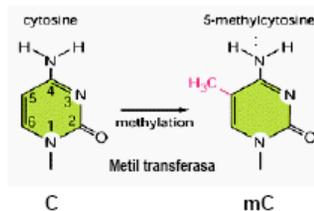
El represor dirige la desacetilación



El activador dirige la hiperacetilación



Regulación de la actividad transcripcional. Metilación del DNA. DNA Metil transferasas (DNMT)



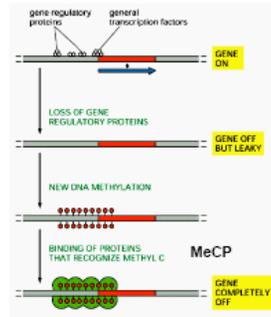
SAM: S-Adenosilmetionina
(Donador del grupo metilo).
SAH: S-AdenosilHomocisteina

- Regiones repetitivas predominantemente metiladas
- Islas CpG no metiladas

Las funciones de la metilación son: Regulación de expresión génica y evitar retrotransposición

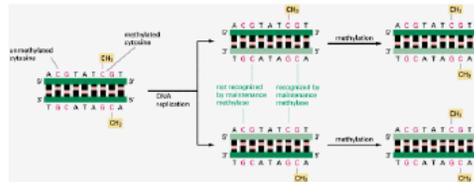
Metilación bloquea los sitios de unión para TF

- MeCP:**
- Bloquea los sitios de unión para factores de transcripción
 - Actuarían como sitios de reconocimiento para histonas desacetilasas (HDAC) o de complejos de remodelamiento de cromatina.



Control a nivel de la organización de la estructura de la cromatina

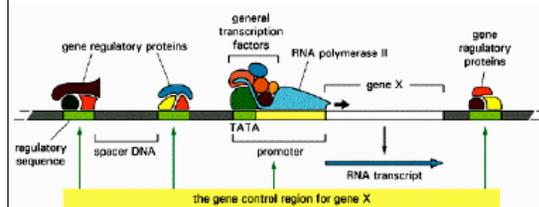
Mantenimiento de la metilación del DNA



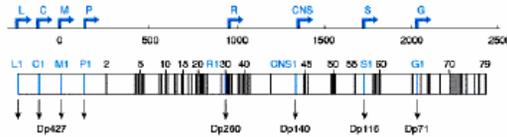
Características asociadas con cromatina transcripcionalmente activa e inactiva

Característica	Cromatina transcripcionalmente activa	Cromatina transcripcionalmente inactiva
conformación de la cromatina	conformación abierta y extendida	Conformación altamente condensada, particularmente en heterocromatina (facultativa y constitutiva)
metilación del DNA	Relativamente no metilada, especialmente en regiones promotoras	Metilada, incluyendo en regiones promotoras
metilación de histonas	Histonas no metiladas	Histonas metiladas
acetilación histonas	Histonas acetiladas	Histonas desacetiladas

Regulación a nivel transcripcional...
Selección del gen que se transcribe -Tasa de expresión.
Estructura típica de un promotor eucarionte



Regulación a nivel transcripcional...
 Selección del gen que se transcribe -Tasa de expresión.
**Uso de promotores
 alternativos.**



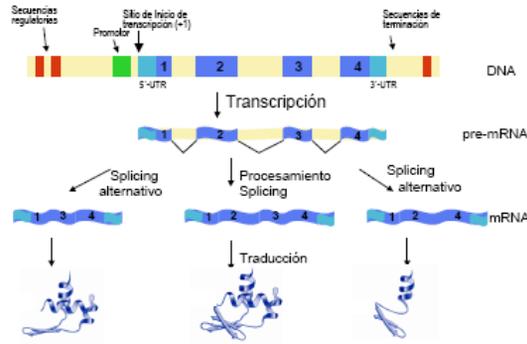
Gen Distrofina:
 Xp21
 79 exones
 2,4 MB

L, lymphocyte
 C, cortical
 M, muscle
 P, Puntaje
 R, retinal
 CNS, central nervous system
 S, Schwann cell
 G, general

Músculo: serum response factor y dystrophin promoter bending factor (DPBF) en un elemento CArG presente en el promotor.
 YY1 también une a CArG pero inhibiendo la transcripción.

Regulación a nivel post-transcripcional...

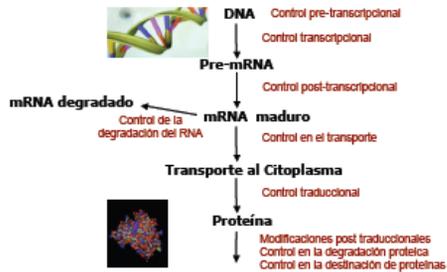
Splicing alternativo



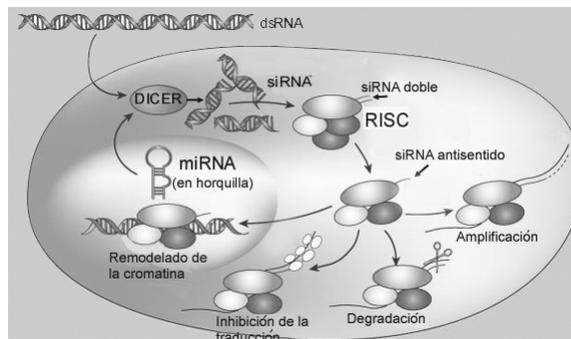
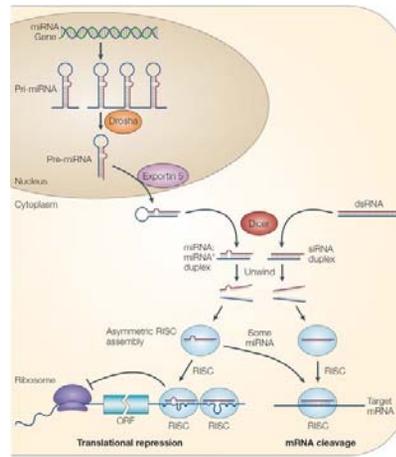
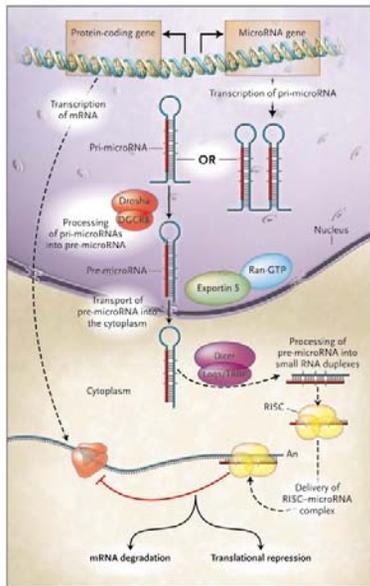
Regulación post transcripcional. Edición del RNA

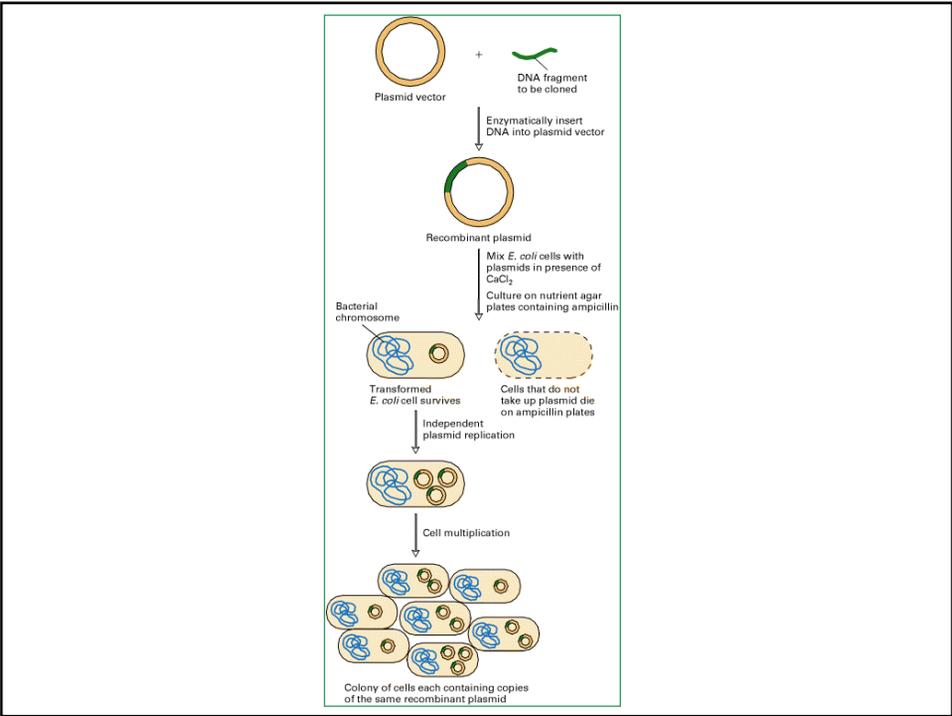
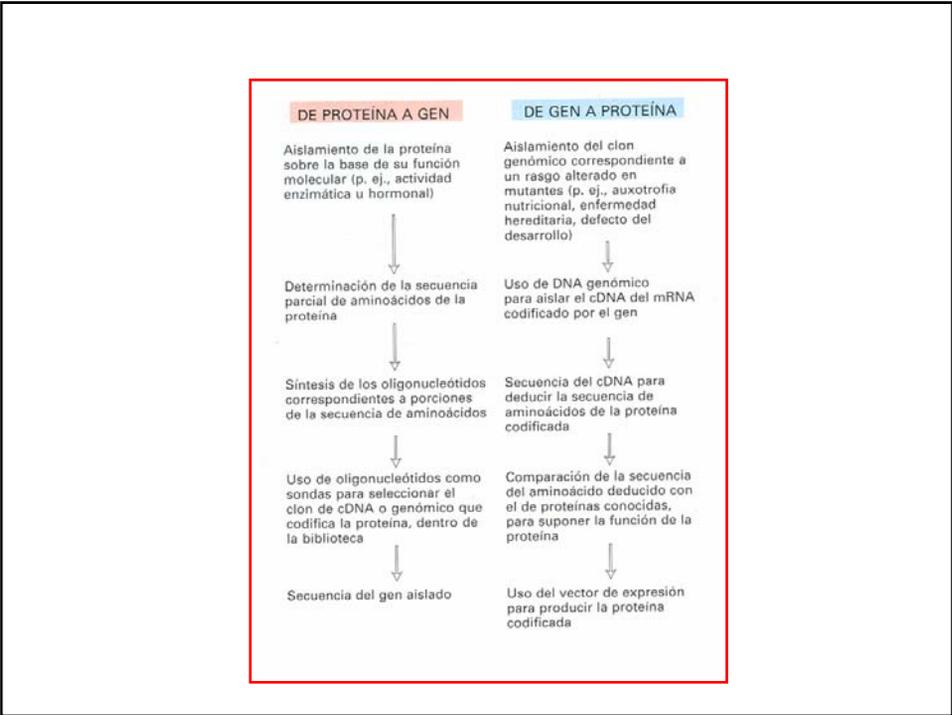
- Modificación del RNA que genera RNAm funcional diferente a la secuencia codificante.
- Hay dos tipos de edición de RNA: por sustitución y por inserción/delección.
- Descubierta a fines de 1980.
- La edición por sustitución hay cambios puntuales de bases y ocurre en transcritos nucleares, mitocondriales y en cloroplastos.
- La edición por inserción/delección se ha observado en Trypanosoma. Se ha descubierto un gRNA ó RNA guía que actúa de templado en esta edición.

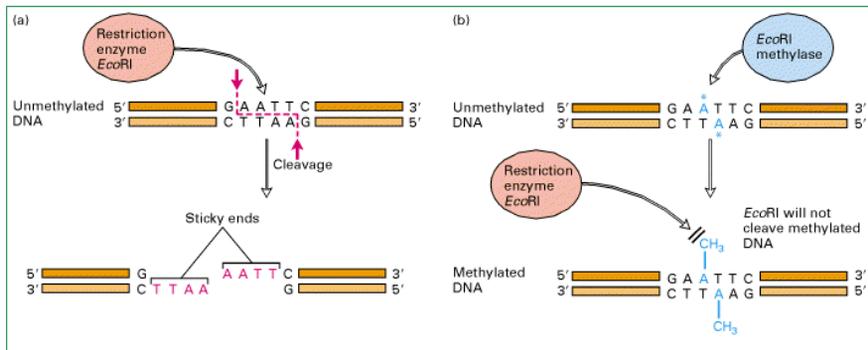
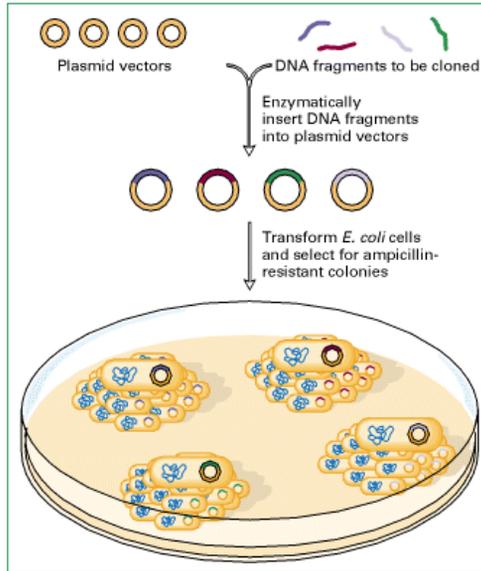
Posibles niveles de control de la expresión genica

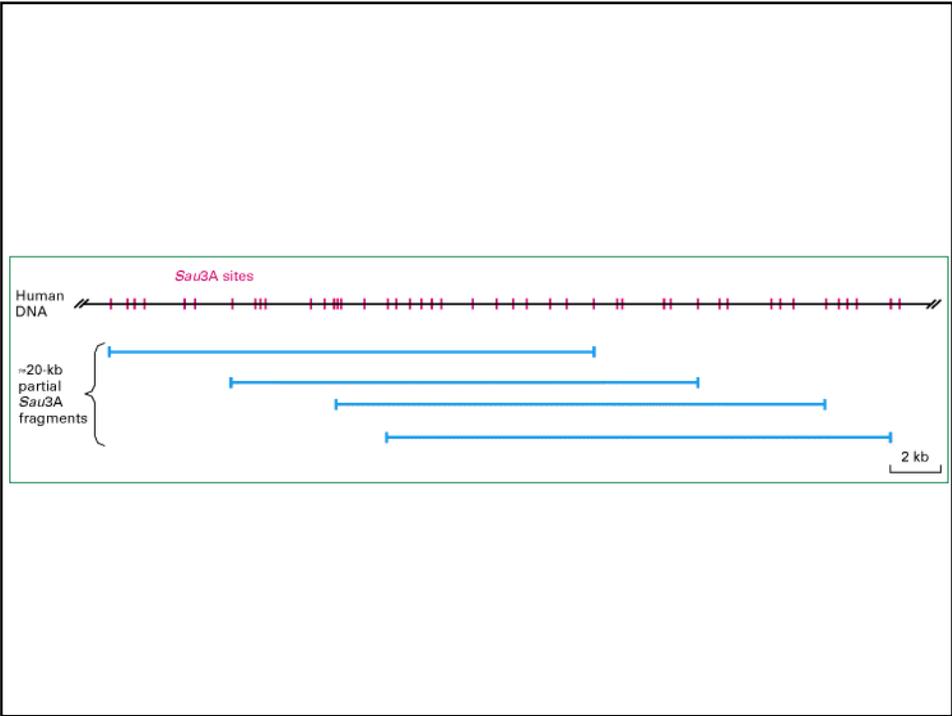
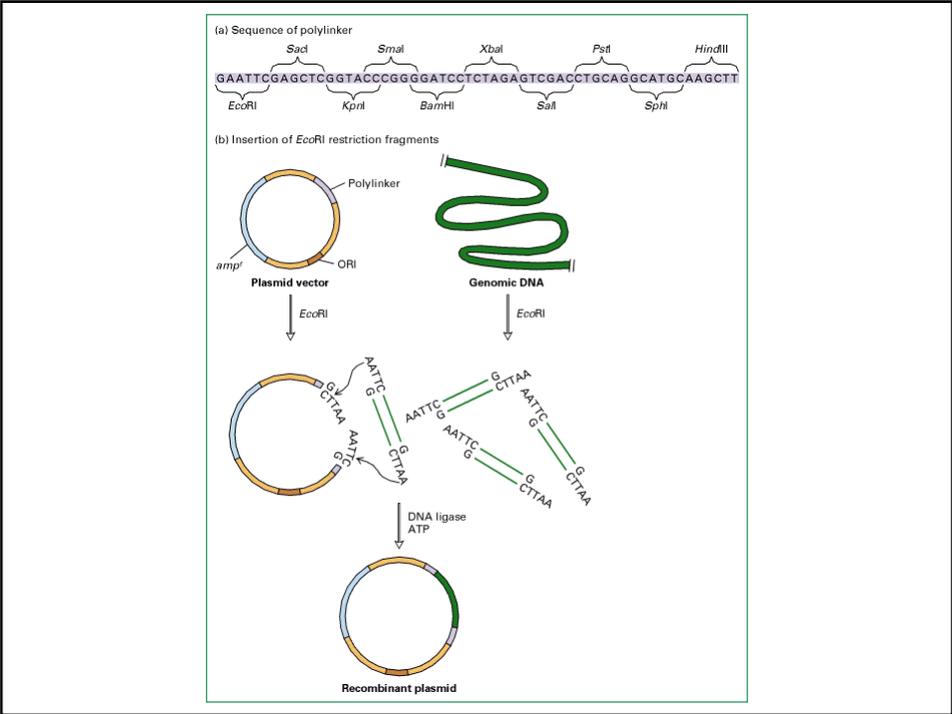


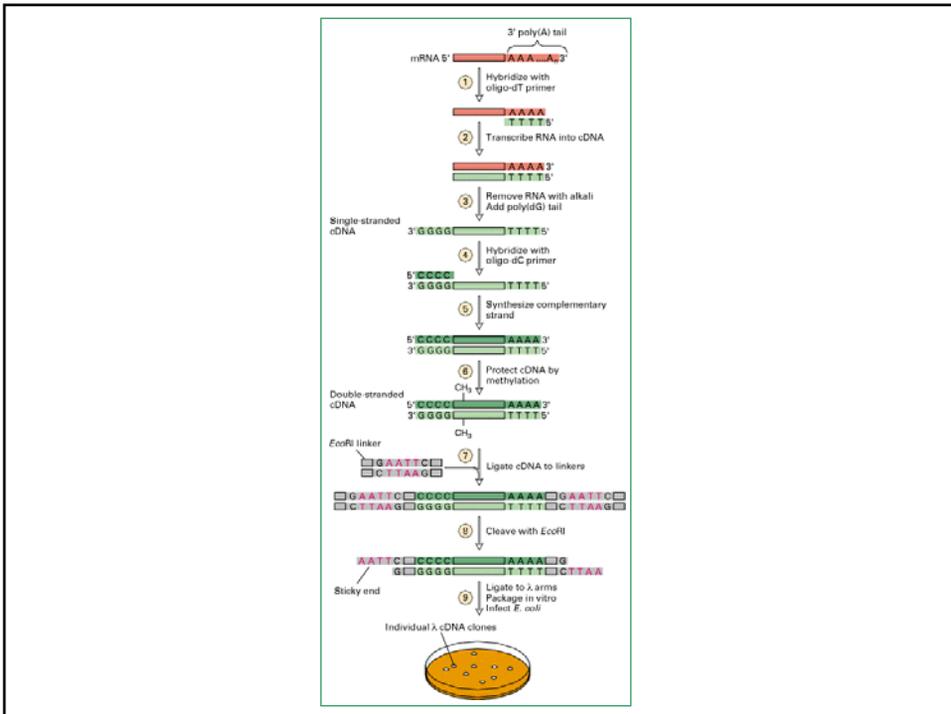
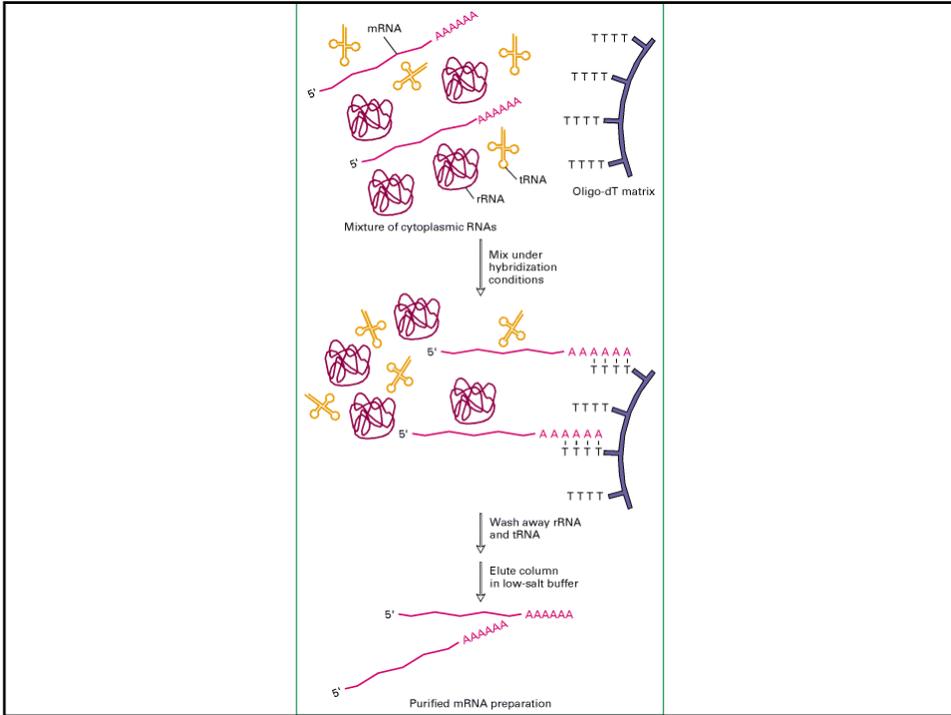
- Selección de los genes que se transcriben
- Regulación de la tasa de expresión
- Regulación de la naturaleza del producto

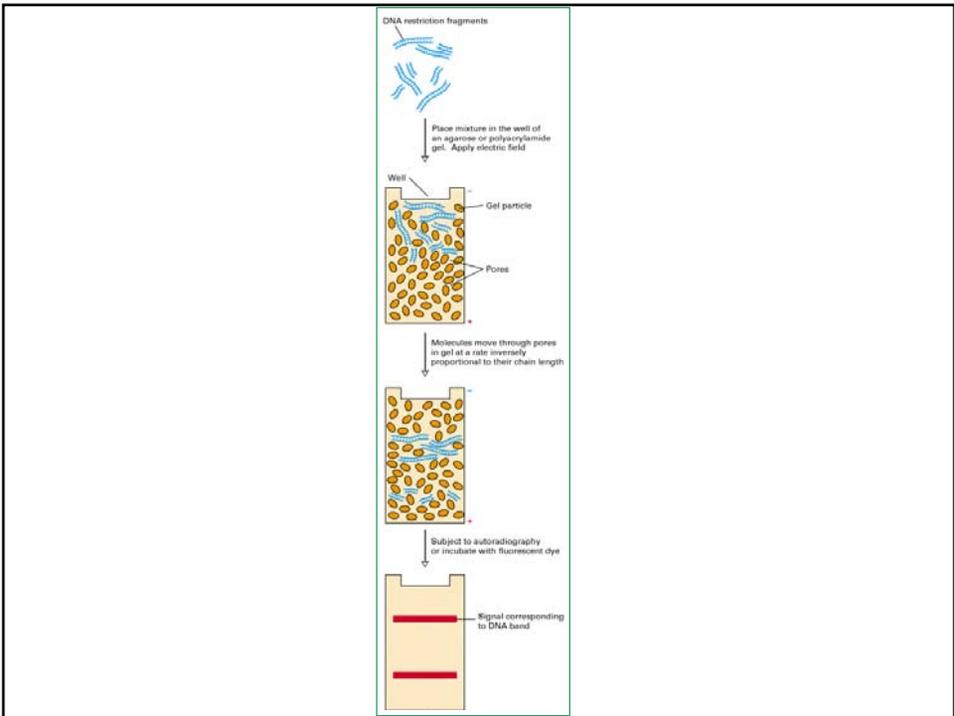
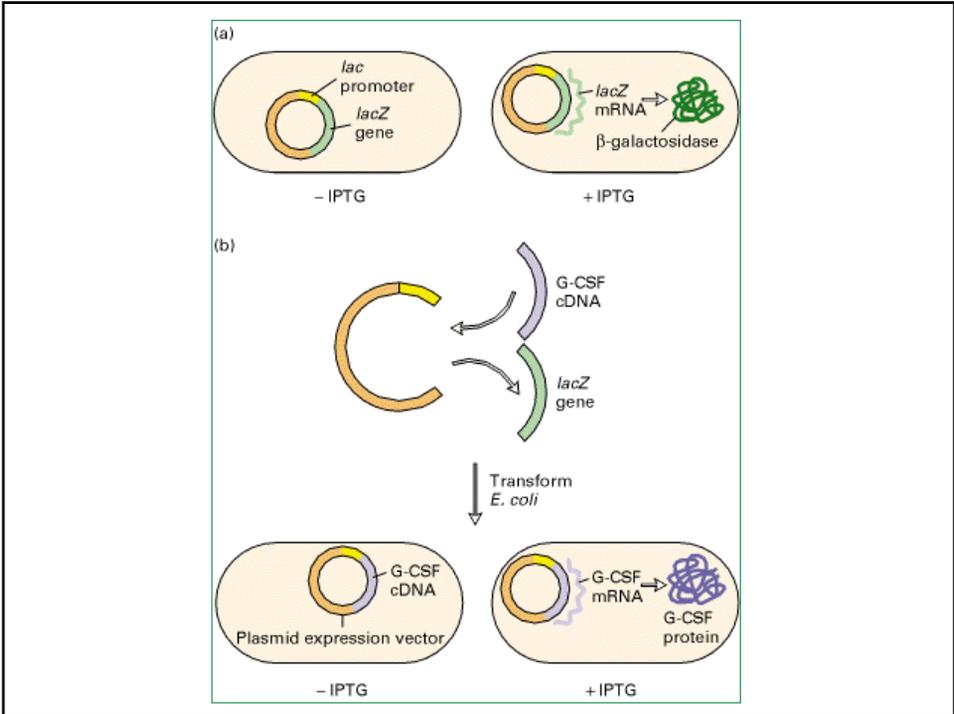


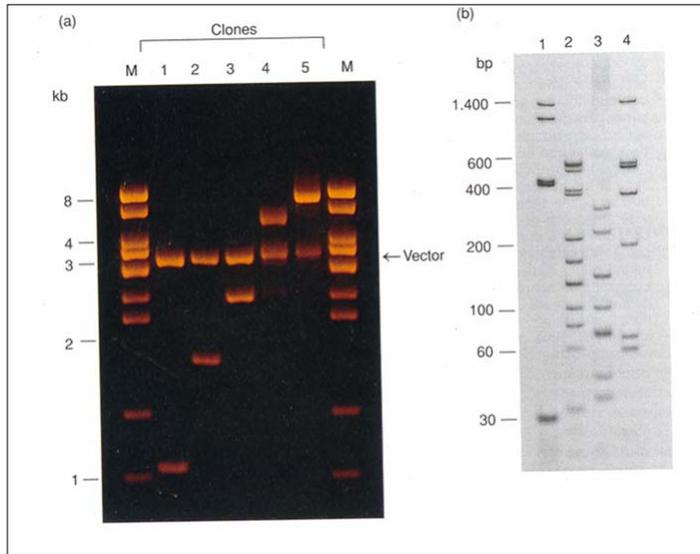












The Polymerase Chain Reaction (PCR)

Method:



FIG. 1. DNA template. DNA is composed of two polynucleotide chains that associate to one another in an antiparallel arrangement by forming hydrogen bonds between complementary base pairs. Prior to initiating the PCR reaction, the template DNA must be isolated from the sample to be tested.



FIG. 2. Denaturation of the DNA template. The template DNA is heated to 95°C disrupting the hydrogen bonds between the base pairs resulting in two single strands of DNA from the one double stranded template DNA.

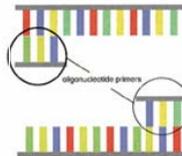


FIG. 3. Annealing of the oligonucleotide primers. Oligonucleotide primers are short, 17–24 base pair, single stranded DNA that define the boundaries of the sequence of template DNA to be amplified and are required to initiate DNA polymerase activity. After denaturation, the temperature is reduced to 50–60°C to allow primers to bind with high specificity to their complementary bases on the template DNA. Annealing temperature varies depending on the nucleotide composition of the primer selected.

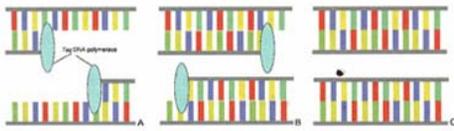


FIG. 4. Synthesis of new DNA. After annealing the primers, the temperature is increased to 72°C, the optimal temperature for *Taq* DNA polymerase activity. Beginning at the primer annealing site (A), *Taq* DNA polymerase synthesizes new DNA by adding complementary nucleotide bases to the denatured single stranded template DNA (B). The end result of synthesis is two double stranded DNA molecules identical to the initial template DNA. *Taq* DNA polymerase is derived from *Thermus aquaticus*, a heat stable bacterium.

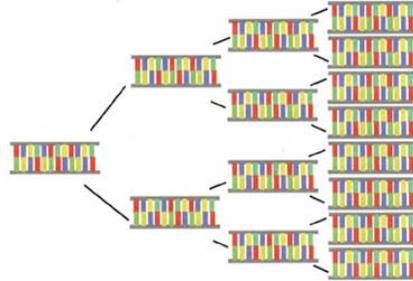


FIG. 5. The chain reaction. The three step process of denaturation, annealing, and synthesis is typically repeated for 25–30 cycles. At the end of each cycle the template DNA is amplified 2ⁿ. After 30 cycles, there will be 2³⁰ (over 1 billion) double stranded DNA molecules identical to the initial template DNA fragment.



FIG. 6. Product analysis. The PCR product has an expected size determined by the region of DNA that is flanked by the primer annealing sites. The product can be visualized by gel electrophoresis and ethidium bromide staining. In this example, lane 1 is the molecular weight marker; lanes 2, 3 and 5 are PCR negative; lanes 4, 6–9 are PCR positive with a product size of 280 base pairs.

