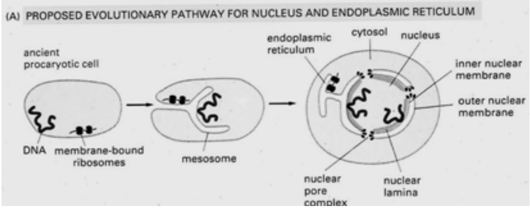
PROGRAMA DE BACHILLERATO

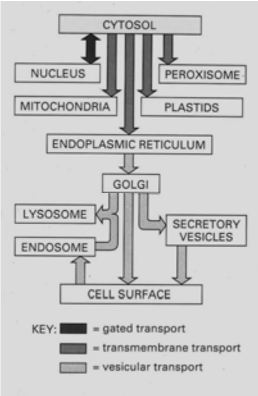
Núcleo interfásico. Cromatina. Transcripción.

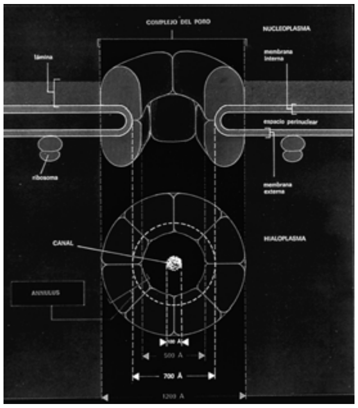
- ***Prof. Héctor R. Contreras M.***

NÚCLEO

-Envoltura y poros
-Cromatina y cromosomas
- Sistema eucarionte
- Expresión génica diferencial
- Nucléolo y ribosomas







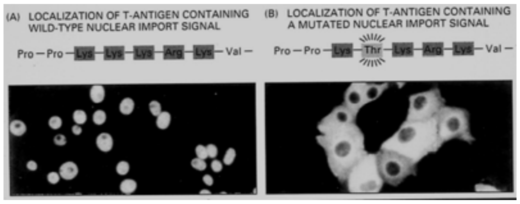
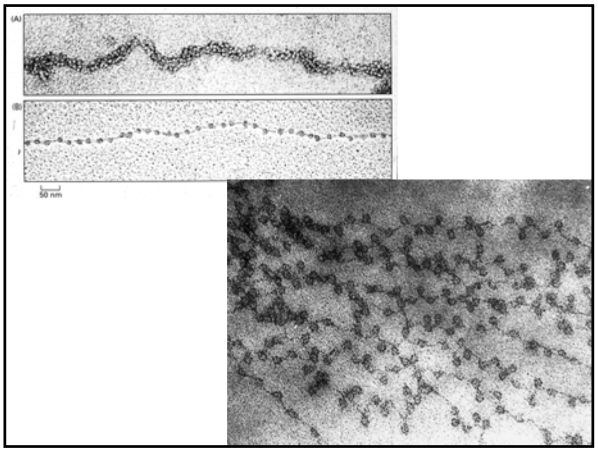
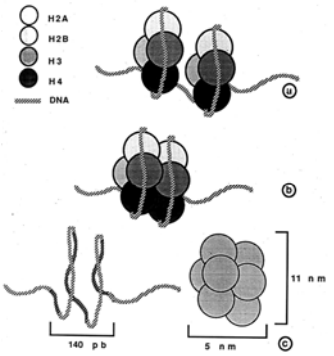
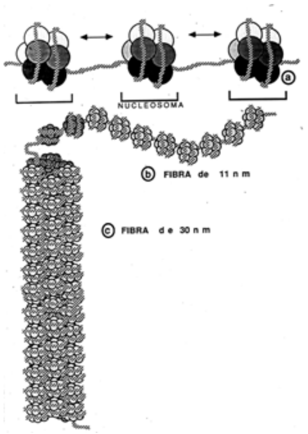
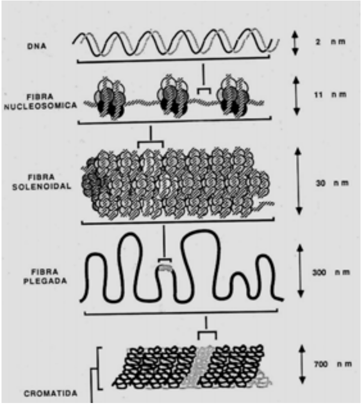


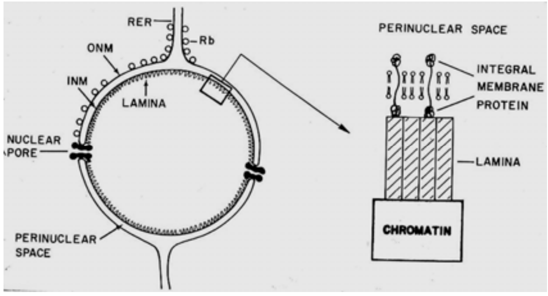
Figure 1. An electron micrograph clearly demonstrating close-packed ν bodies in 20- to 30-nm chromatin fibers spilling out of a chicken erythrocyte nucleus. Isolated nuclei were washed in 0.2 M KCl and diluted 1:100 in 0.02 M KCl, 0.5 mM $MgCl_2$.

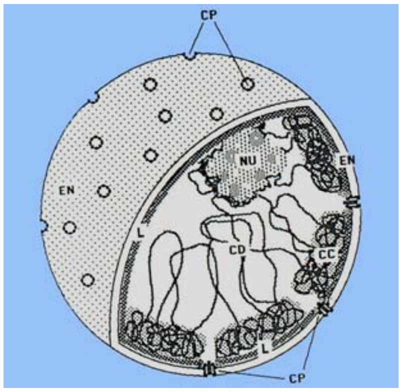




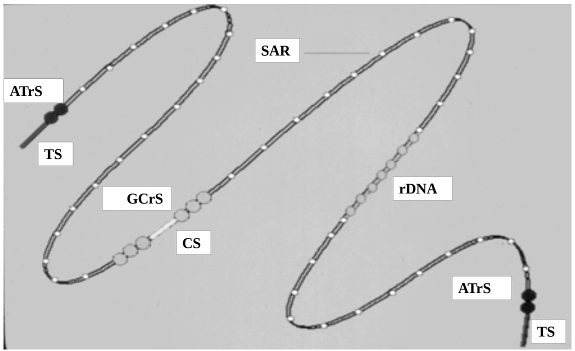


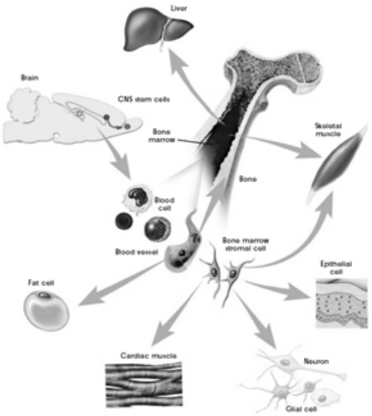


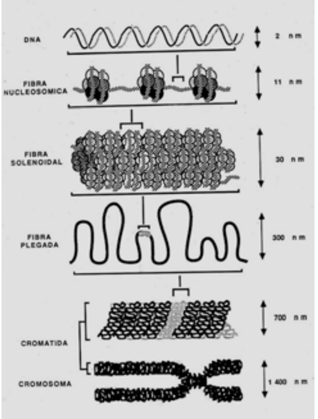


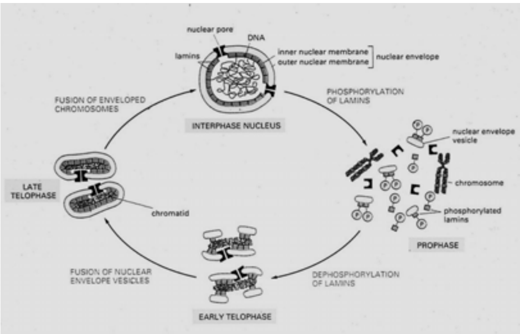


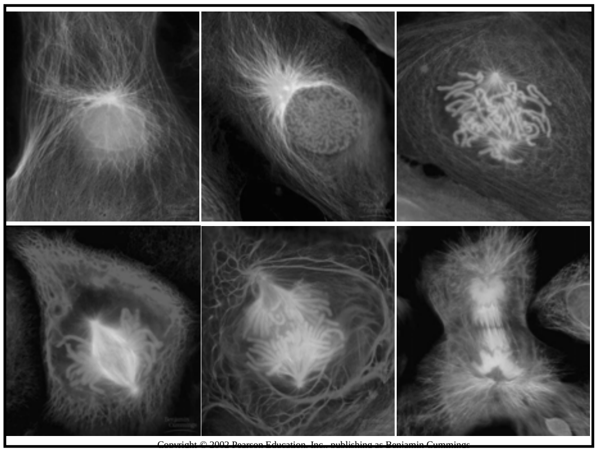
EN CADA CROMOSOMA SE ENCUENTRA UNA MOLÉCULA DE DNA CON DISTINTOS TIPOS DE SECUENCIAS NUCLEOTÍDICAS

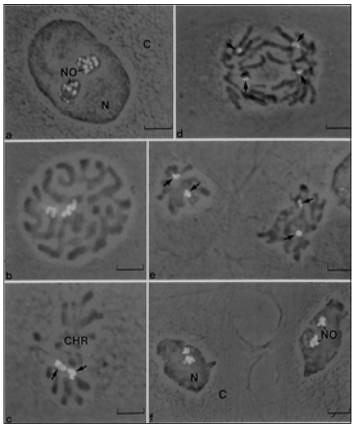


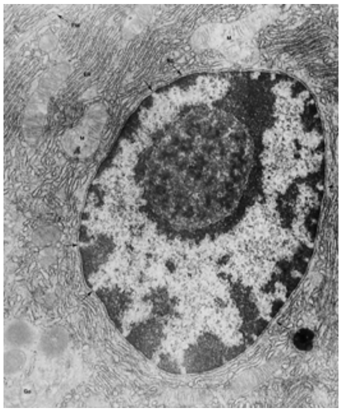


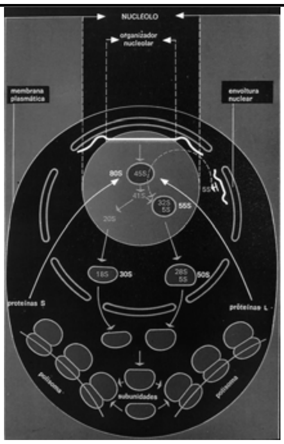


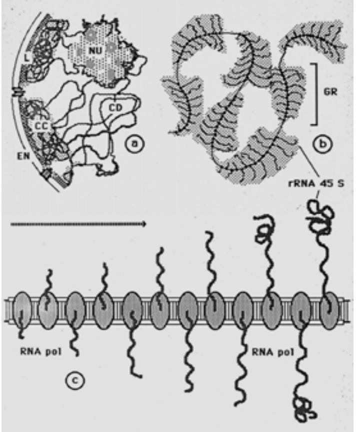


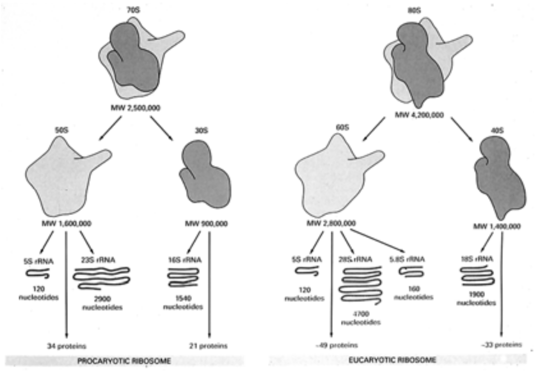




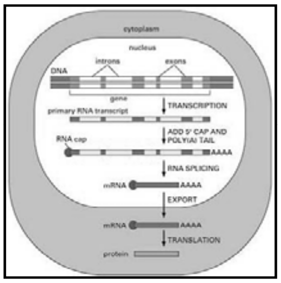








Dónde



célula procarionte

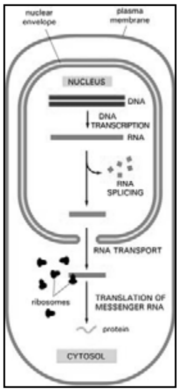
Núcleo de la célula eucarionte

Síntesis de RNA

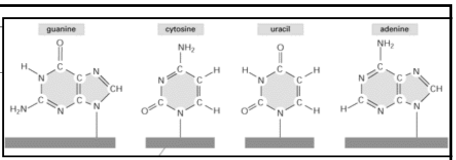
* tRNA o RNA de transferencia

* rRNA o RNA ribosomal

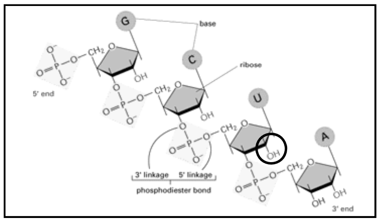
* mRNA o RNA mensajero

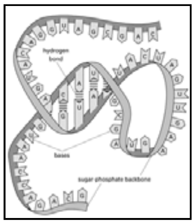


RNA



Bases





Azúcar Polaridad Estructura secundaria

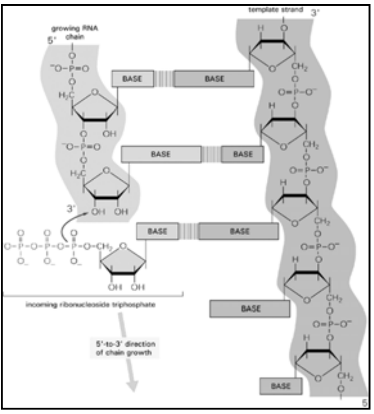
Síntesis de RNA

* Formación de enlace fosfodiester

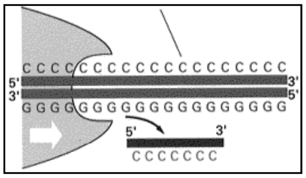
* RNA polimerasa

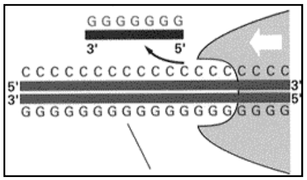
* Cadena crece desde 5’ a 3’

* Cadena crece antiparalela y complementaria a DNA templado o molde



Acerca de la dirección de transcripción





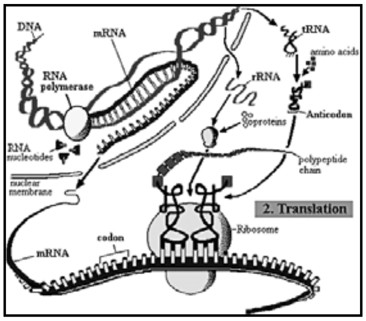
* ¿Están todos los genes en sólo una de las hebras del DNA?

NO !

RNA polimerasa

* Procariontes : 1tipo

* Eucariontes: 3 tipos
  * Pol I → rRNA
  * Pol II → mRNA
  * Pol III → tRNA y otros RNAs pequeños



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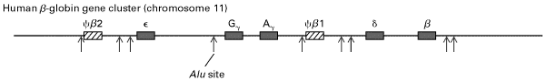


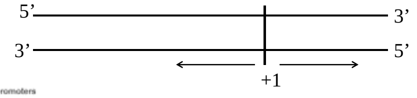
* ¿Cómo sabe la RNApol dónde comenza a transcribir?

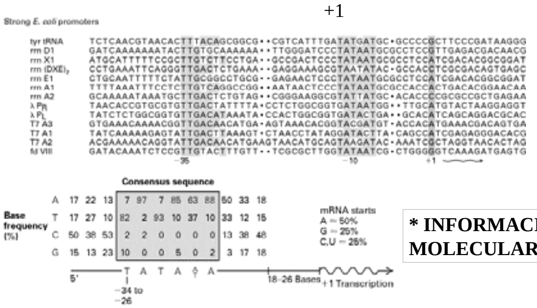
* NO sabe. La RNApol es una molécula, y las moléculas NO SABEN ! !

* **INFORMACIÓN MOLECULAR**

Inicio transcripción

DNA



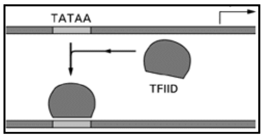


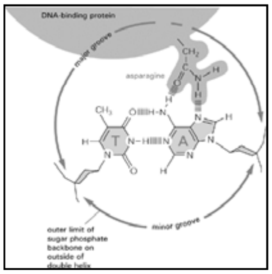
A	17	22	13	7	97	7	85	63	88	50	33	18
T	17	27	10	82	2	93	10	37	10	33	12	15
C	50	38	53	2	2	0	0	0	0	13	38	48
G	15	13	23	10	0	0	5	0	2	3	17	18

*** INFORMACIÓN MOLECULAR**

Inicio transcripción:

TBP (TATA-box Binding Protein)





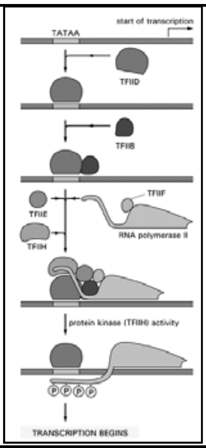
TBP-TATA

Inicio transcripción:

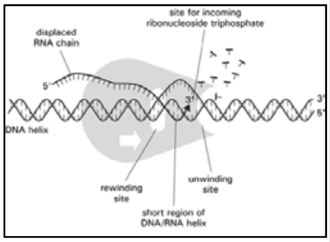
TF (Factores de

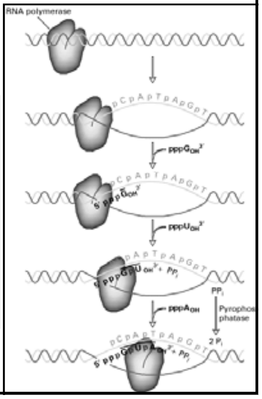
transcripción)

(A+B)+C ≠ A+(B+C)

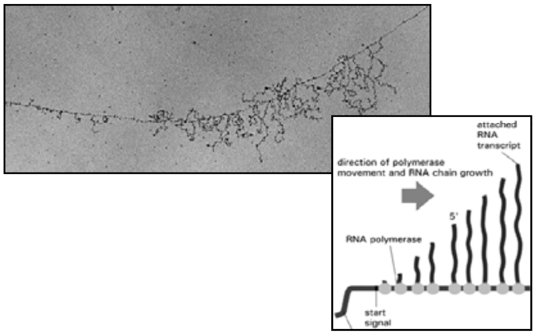


Elongación:

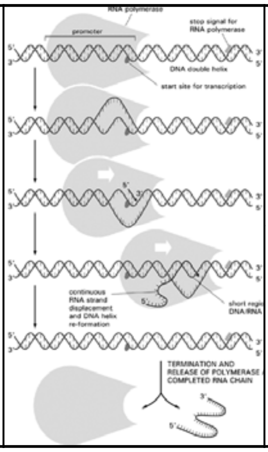




Transcripción

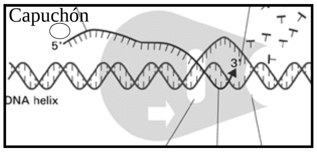


Término:



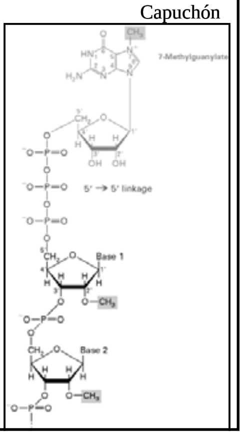
Procesamiento de mRNAs

* Modificación co-transcripcional del extremo 5’ = capping



Función ?

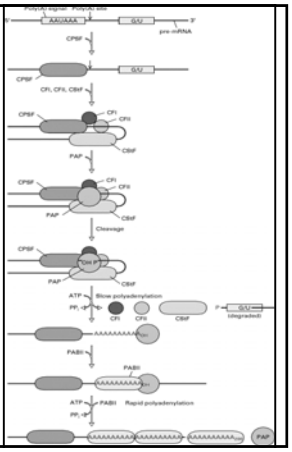
Capuchón



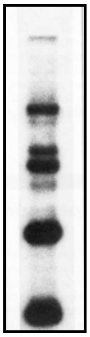
Procesamiento de mRNAs

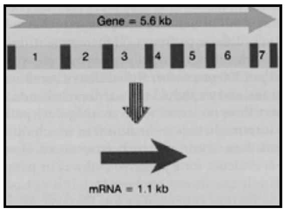
* Modificación del extremo 3’ con una cola de Poli A = Poliadenilación

Función ?



Procesamiento de mRNAs:

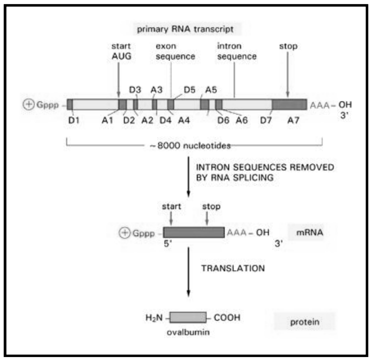




Procesamiento de mRNAs:

* Remoción de

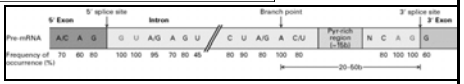
intrones: splicing



Procesamiento de mRNAs:

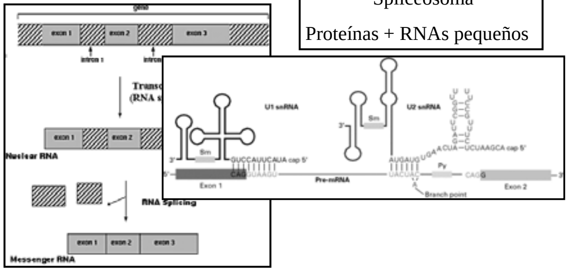
* Remoción de

intrones: splicing

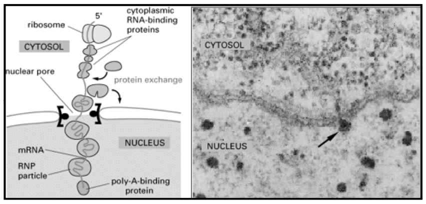


Spliceosoma

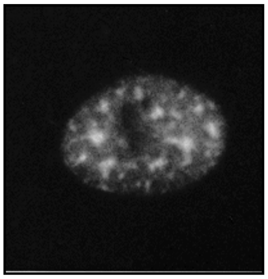
Proteínas + RNAs pequeños



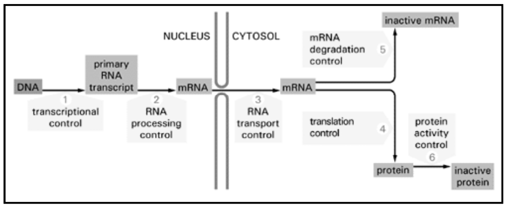
Transporte hacia el citoplasma:



Arquitectura Nuclear y transcripción

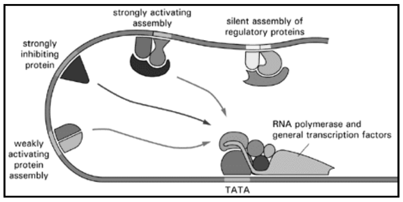


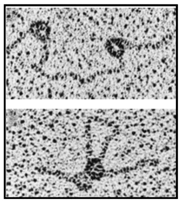
Control de la expresión génica

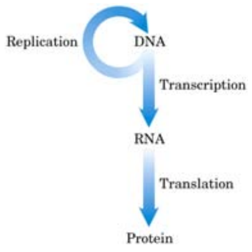


Control de la expresión génica

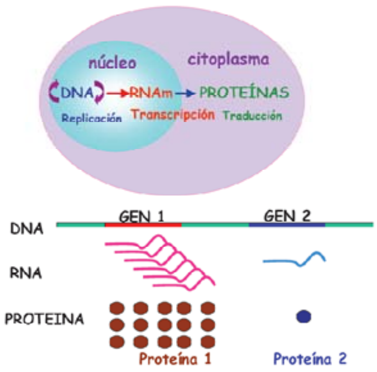
Nivel transcripcional





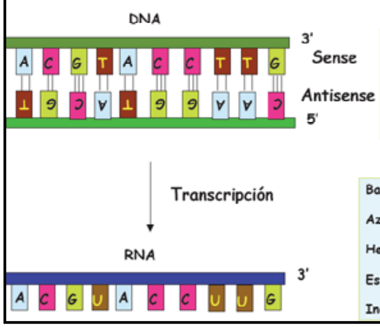


DOGMA CENTRAL DE LA BIOLOGIA MOLECULAR



Reconocimiento Complementario de los pares de Watson-Crick o Complementaridad de bases

A=T	A=U	G=C
DNA	RNA	DNA y RNA



Bases: A, G, C y T

Azúcar: desoxiribosa

Doble hebra antiparalela

Estable

Bases: A, G, C y U

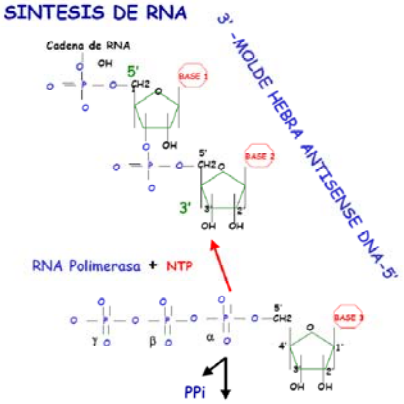
Azúcar: ribosa

Hebra única

Estructuras secundarias y terciaria

Inestable

SINTESIS DE RNA



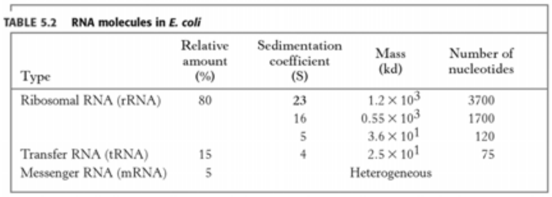
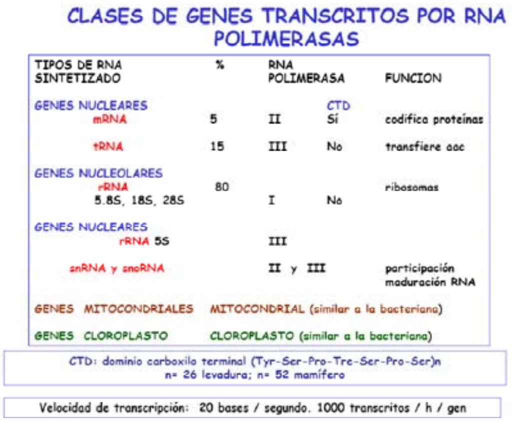


TABLE 5.2 RNA molecules in *E. coli*

Type	Relative amount (%)	Sedimentation coefficient (S)	Mass (kd)	Number of nucleotides
Ribosomal RNA (rRNA)	80	23	1.2×10^3	3700
		16	0.55×10^3	1700
		5	3.6×10^1	120
Transfer RNA (tRNA)	15	4	2.5×10^1	75
Messenger RNA (mRNA)	5		Heterogeneous	

Tipos de RNA para células Eucariontes

Type of RNA	Function
mRNAs	mesenger RNAs, code for proteins
rRNAs	ribosomal RNAs, form the basic structure of the ribosome and catalyze protein synthesis
tRNAs	transfer RNAs, central to protein synthesis as adaptators between mRNA and amino acids
snRNAs	small nuclear RNAs, function in a variety of nuclear processes, including the splicing of pre-mRNA
snoRNAs	small nucleolar RNAs, used to process and chemically modify r RNAs
other noncoding RNAs	function in diverse cellular processes, including telomere synthesis, X-chromosome inactivation, and the transport of proteins into the endoplasmic reticulum

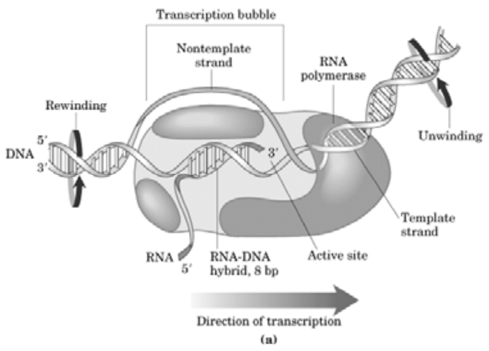


CLASES DE GENES TRANSCRITOS POR RNA POLIMERASAS

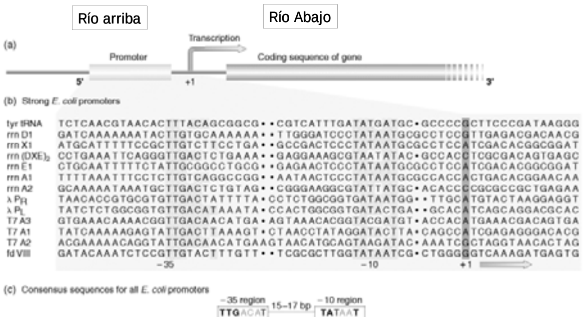
TIPOS DE RNA SINTETIZADO	%	RNA POLIMERASA	CTD	FUNCION
GENES NUCLEARES				
mRNA	5	II	Sí	codifica proteínas
tRNA	15	III	No	transfiere aac
GENES NUCLEOLARES				
rRNA	80			ribosomas
5.8S, 18S, 28S		I	No	
GENES NUCLEARES				
rRNA 5S		III		
snRNA y snoRNA		II y III		participación maduración RNA
GENES MITOCONDRIALES	MITOCONDRIAL (similar a la bacteriana)			
GENES CLOROPLASTO	CLOROPLASTO (similar a la bacteriana)			

CTD: dominio carboxilo terminal (Tyr-Ser-Pro-Tre-Ser-Pro-Ser)n
n= 26 levadura; n= 52 mamífero

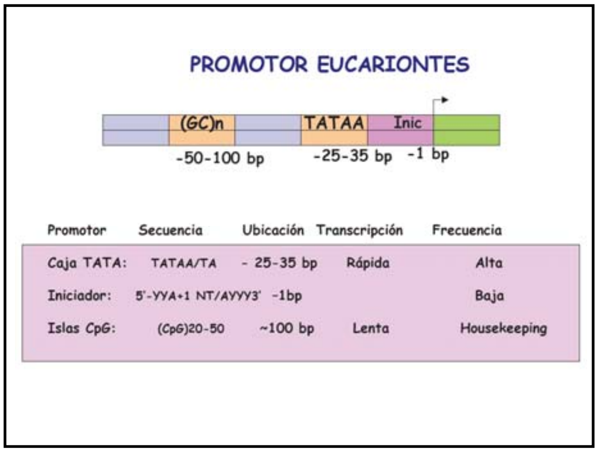
Velocidad de transcripción: 20 bases / segundo. 1000 transcritos / h / gen



Secuencia Promotora

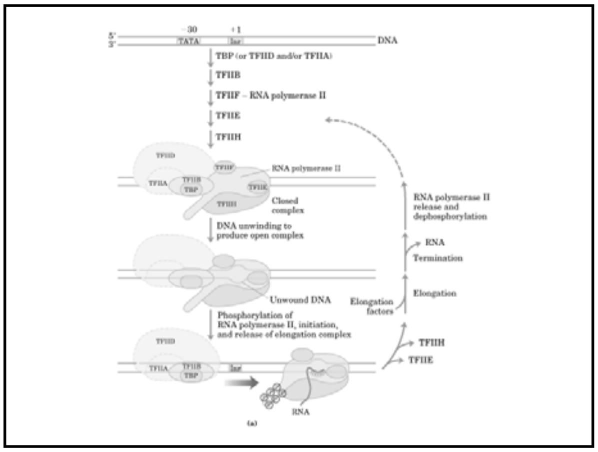


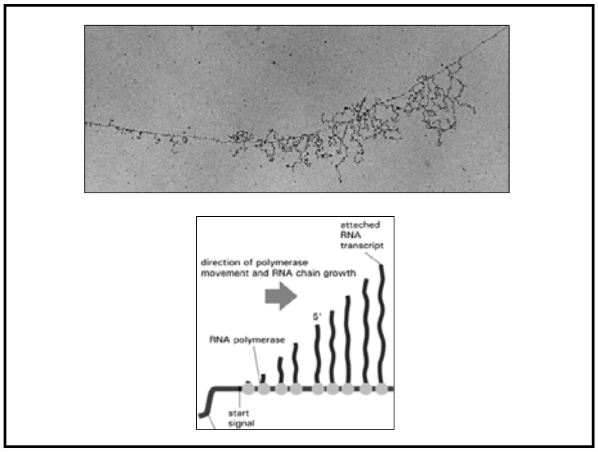
```
tyr tRNA   TCTCAACGTAACACTTTACAGCGGCG••CGTCATTTGATATGATGC•GCCCCGCTTCCCGATAAGGG
rrn D1     GATCAAAAAAATACTTGTGCAAAAAA••TTGGGATCCCTATAATGCGCCTCCGTTGAGACGACAACG
rrn X1     ATGCATTTTTCCGCTTGTCTTCCTGA••GCCGACTCCCTATAATGCGCCTCCATCGACACGGCGGAT
rrn (DXE)2 CCTGAAATTCAGGGTTGACTCTGAAA••GAGGAAAGCGTAATATAC•GCCACCTCGCGACAGTGAGC
rrn E1     CTGCAATTTTTCTATTGCGGCCTGCG••GAGAACTCCCTATAATGCGCCTCCATCGACACGGCGGAT
rrn A1     TTTTAAATTTCCTCTTGTCAGGCCGG••AATAACTCCCTATAATGCGCCACCACTGACACGGAACAA
rrn A2     GCAAAAATAAATGCTTGACTCTGTAG••CGGGAAGGCGTATTATGC•ACACCCCGCGCCGCTGAGAA
λ PR       TAACACCGTGCGTGTTGACTATTTTA•CCTCTGGCGGTGATAATGG••TTGCATGTACTAAGGAGGT
λ PL       TATCTCTGGCGGTGTTGACATAAATA•CCACTGGCGGTGATACTGA••GCACATCAGCAGGACGCAC
T7 A3      GTGAAACAAAACGGTTGACAACATGA•AGTAAACACGGTACGATGT•ACCACATGAAACGACAGTGA
T7 A1      TATCAAAAAGAGTATTGACTTAAAGT•CTAACCTATAGGATACTTA•CAGCCATCGAGAGGGACACG
T7 A2      ACGAAAAACAGGTATTGACAACATGAAGTAACATGCAGTAAGATAC•AAATCGCTAGGTAACACTAG
fd VIII    GATACAAATCTCCGTTGTACTTTGTT••TCGCGCTTGGTATAATCG•CTGGGGGTCAAAGATGAGTG
```

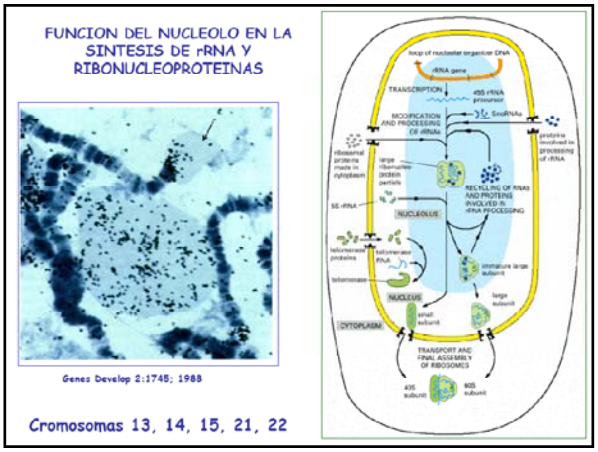


PROMOTOR EUCARIONTES

Promotor	Secuencia	Ubicación	Transcripción	Frecuencia
Caja TATA:	TATAA/TA	- 25-35 bp	Rápida	Alta
Iniciador:	5'-YYA+1 NT/AYYY3'	-1bp		Baja
Islas CpG:	(CpG)20-50	~100 bp	Lenta	Housekeeping



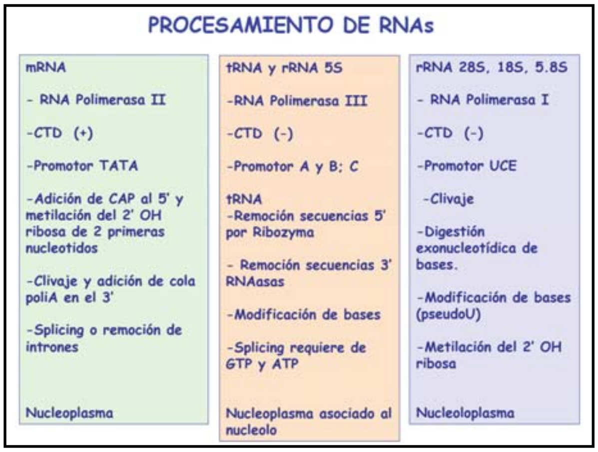




FUNCION DEL NUCLEOLO EN LA SINTESIS DE rRNA Y RIBONUCLEOPROTEINAS

Genes Develop 2:1745; 1988

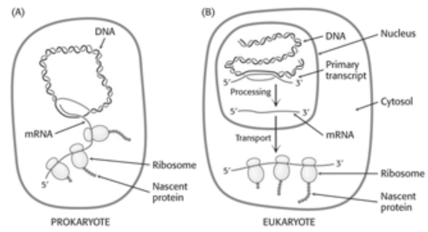
Cromosomas 13, 14, 15, 21, 22

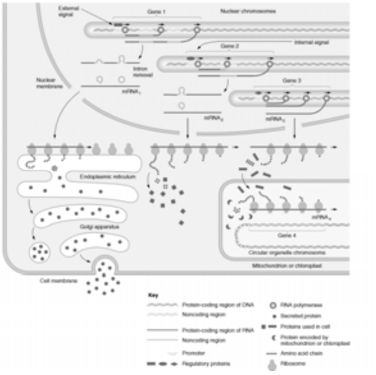


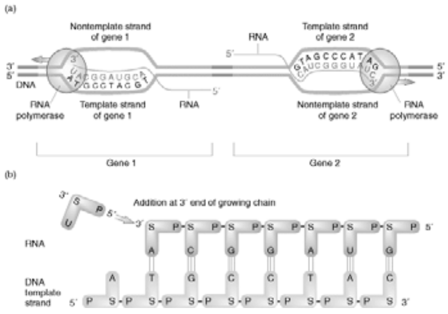
PROCESAMIENTO DE RNAs

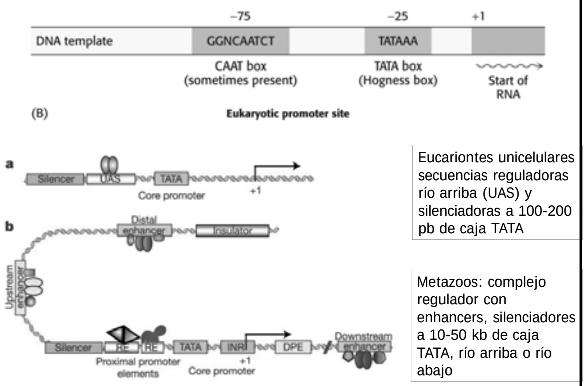
mRNA	tRNA y rRNA 5S	rRNA 28S, 18S, 5.8S
- RNA Polimerasa II	-RNA Polimerasa III	- RNA Polimerasa I
-CTD (+)	-CTD (-)	-CTD (-)
-Promotor TATA	-Promotor A y B; C	-Promotor UCE
-Adición de CAP al 5' y metilación del 2' OH ribosa de 2 primeras nucleotidos	tRNA -Remoción secuencias 5' por Ribozyma	-Clivaje
-Clivaje y adición de cola poliA en el 3'	- Remoción secuencias 3' RNAasas	-Digestión exonucleotídica de bases.
-Splicing o remoción de intrones	-Modificación de bases	-Modificación de bases (pseudoU)
	-Splicing requiere de GTP y ATP	-Metilación del 2' OH ribosa
Nucleoplasma	Nucleoplasma asociado al nucleolo	Nucleoloplasma

SINTESIS DE PROTEINAS



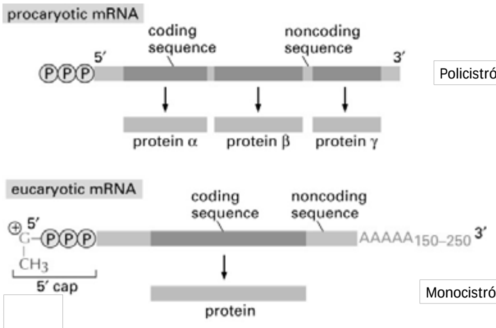






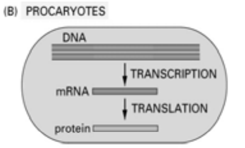
Eucariontes unicelulares secuencias reguladoras río arriba (UAS) y silenciadoras a 100-200 pb de caja TATA

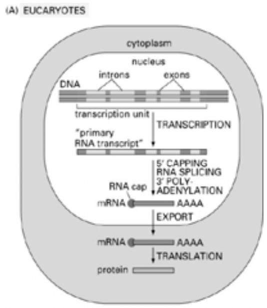
Metazoos: complejo regulador con enhancers, silenciadores a 10-50 kb de caja TATA, río arriba o río abajo

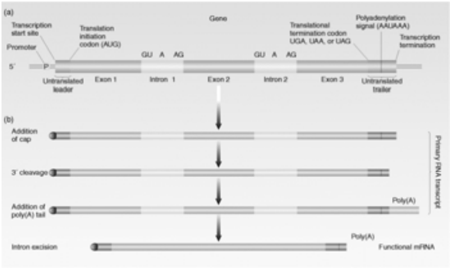


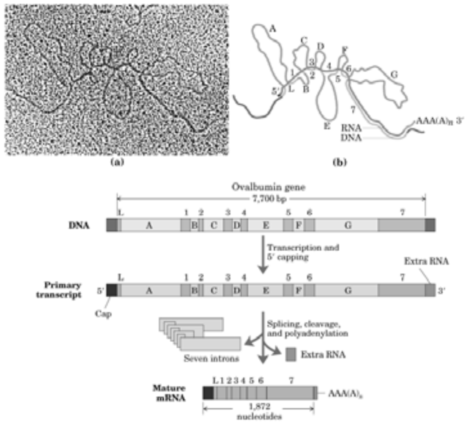
Policistrónico

Monocistrónico









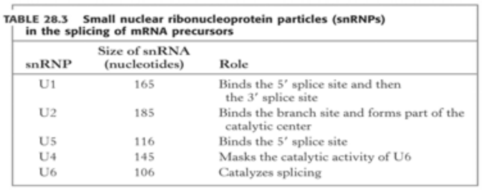
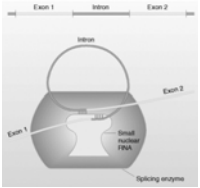


TABLE 28.3 Small nuclear ribonucleoprotein particles (snRNPs) in the splicing of mRNA precursors

snRNP	Size of snRNA (nucleotides)	Role
U1	165	Binds the 5′ splice site and then the 3′ splice site
U2	185	Binds the branch site and forms part of the catalytic center
U5	116	Binds the 5′ splice site
U4	145	Masks the catalytic activity of U6
U6	106	Catalyzes splicing



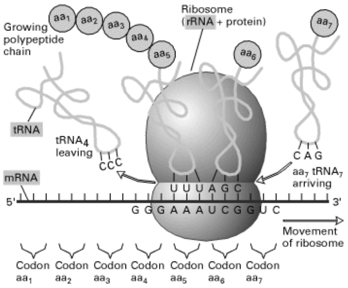
Nontemplate strand 5′ — CTGCCATTGTCAGACATGTATACCCCGTACGTCTTCCCGAGCGAAAACGATCTGCGCTGC — 3′

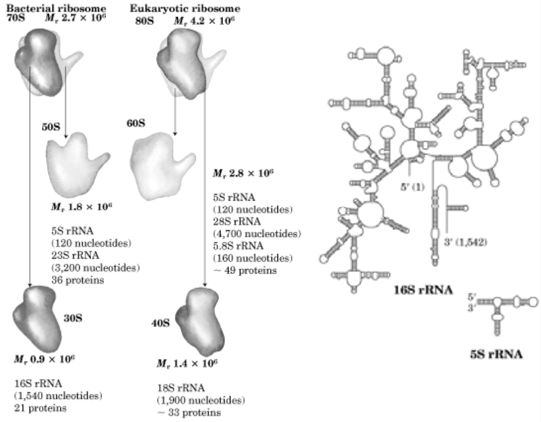
Template strand 3′ — GACGGTAACAGTCTGTACATATGGGGCATGCAGAAGGGCTCGCTTTTGCTAGACGCGACG — 5′

DNA

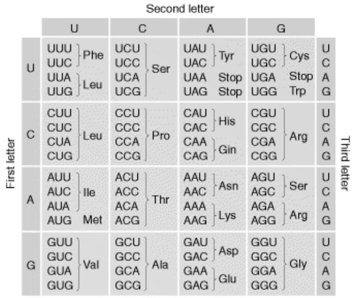
5′ — CUGCCAUUGUCAGACAUGUAUACCCCGUACGUCUUCCCGAGCGAAAACGAUCUGCGCUGC — 3′ mRNA

NH2- aa1-aa2-aa3-aa4-aa5-aa6-aa7-aa8- **COOH**





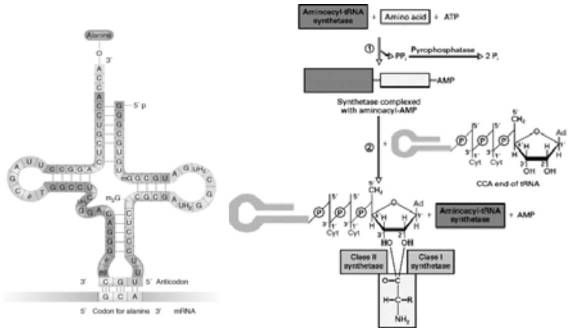
Código Genético

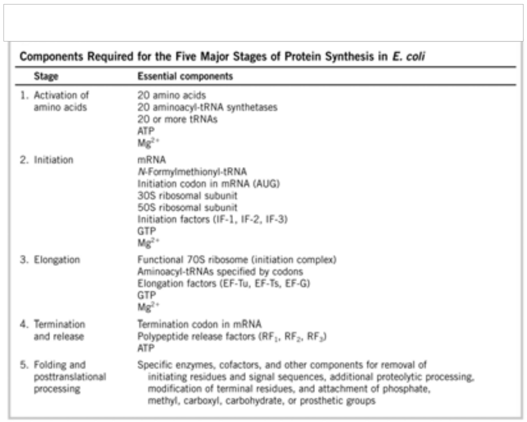


Second letter

First letter	U	C	A	G	Third letter
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA Stop UAG Stop	UGU Cys UGC Cys UGA Stop UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
A	AUU Ile AUC Ile AUA Ile AUG Met	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A G
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G

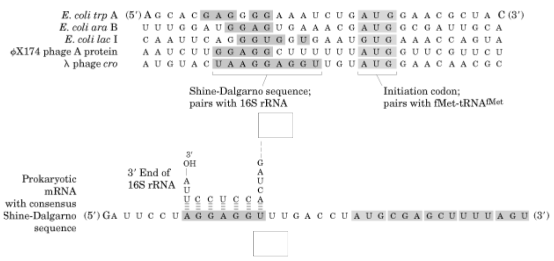
RNA de transferencia

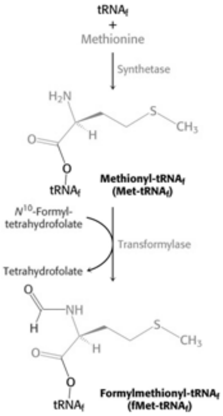


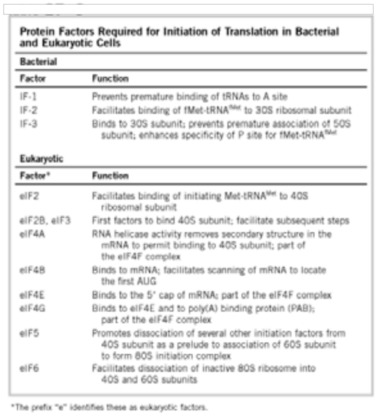


Components Required for the Five Major Stages of Protein Synthesis in *E. coli*

Stage	Essential components
1. Activation of amino acids	20 amino acids 20 aminoacyl-tRNA synthetases 20 or more tRNAs ATP Mg^{2+}
2. Initiation	mRNA *N*-Formylmethionyl-tRNA Initiation codon in mRNA (AUG) 30S ribosomal subunit 50S ribosomal subunit Initiation factors (IF-1, IF-2, IF-3) GTP Mg^{2+}
3. Elongation	Functional 70S ribosome (initiation complex) Aminoacyl-tRNAs specified by codons Elongation factors (EF-Tu, EF-Ts, EF-G) GTP Mg^{2+}
4. Termination and release	Termination codon in mRNA Polypeptide release factors (RF_1, RF_2, RF_3) ATP
5. Folding and posttranslational processing	Specific enzymes, cofactors, and other components for removal of initiating residues and signal sequences, additional proteolytic processing, modification of terminal residues, and attachment of phosphate, methyl, carboxyl, carbohydrate, or prosthetic groups







Protein Factors Required for Initiation of Translation in Bacterial and Eukaryotic Cells

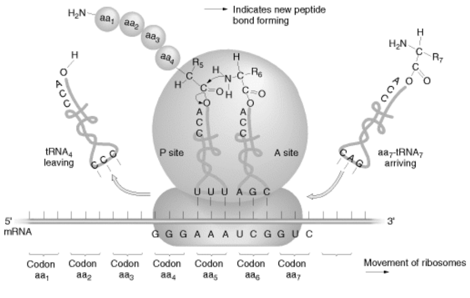
Bacterial

Factor	Function
IF-1	Prevents premature binding of tRNAs to A site
IF-2	Facilitates binding of fMet-tRNA^{fMet} to 30S ribosomal subunit
IF-3	Binds to 30S subunit; prevents premature association of 50S subunit; enhances specificity of P site for fMet-tRNA^{fMet}

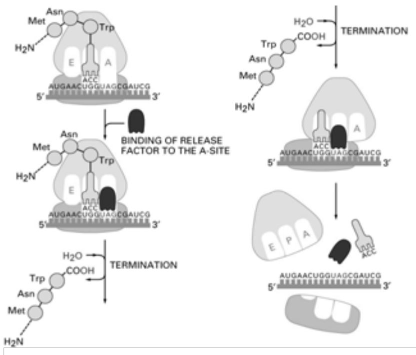
Eukaryotic

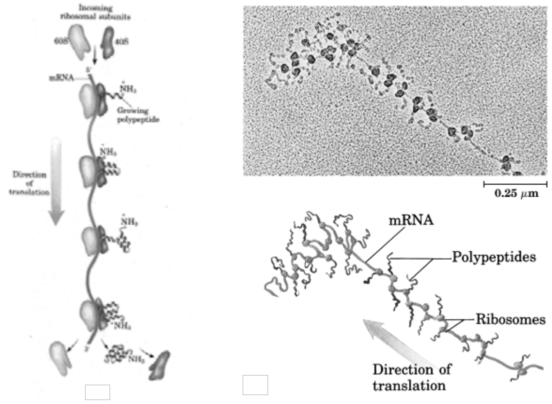
Factor*	Function
eIF2	Facilitates binding of initiating Met-tRNA^{Met} to 40S ribosomal subunit
eIF2B, eIF3	First factors to bind 40S subunit; facilitate subsequent steps
eIF4A	RNA helicase activity removes secondary structure in the mRNA to permit binding to 40S subunit; part of the eIF4F complex
eIF4B	Binds to mRNA; facilitates scanning of mRNA to locate the first AUG
eIF4E	Binds to the 5′ cap of mRNA; part of the eIF4F complex
eIF4G	Binds to eIF4E and to poly(A) binding protein (PAB); part of the eIF4F complex
eIF5	Promotes dissociation of several other initiation factors from 40S subunit as a prelude to association of 60S subunit to form 80S initiation complex
eIF6	Facilitates dissociation of inactive 80S ribosome into 40S and 60S subunits

*The prefix "e" identifies these as eukaryotic factors.



Sitio A : aminoacil, se une el tRNA
Sitio P : peptidil, se unen aminoácidos por enlace peptídico (peptidil transferasa)





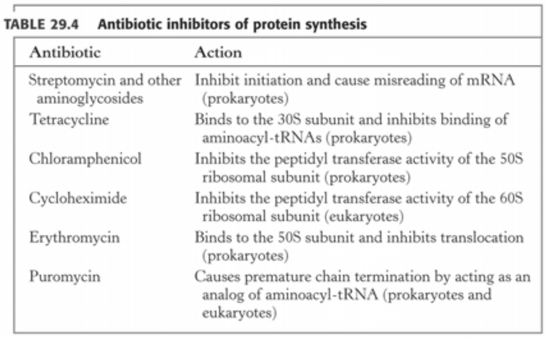


TABLE 29.4 Antibiotic inhibitors of protein synthesis

Antibiotic	Action
Streptomycin and other aminoglycosides	Inhibit initiation and cause misreading of mRNA (prokaryotes)
Tetracycline	Binds to the 30S subunit and inhibits binding of aminoacyl-tRNAs (prokaryotes)
Chloramphenicol	Inhibits the peptidyl transferase activity of the 50S ribosomal subunit (prokaryotes)
Cycloheximide	Inhibits the peptidyl transferase activity of the 60S ribosomal subunit (eukaryotes)
Erythromycin	Binds to the 50S subunit and inhibits translocation (prokaryotes)
Puromycin	Causes premature chain termination by acting as an analog of aminoacyl-tRNA (prokaryotes and eukaryotes)