traducción

síntesis de proteínas (3)

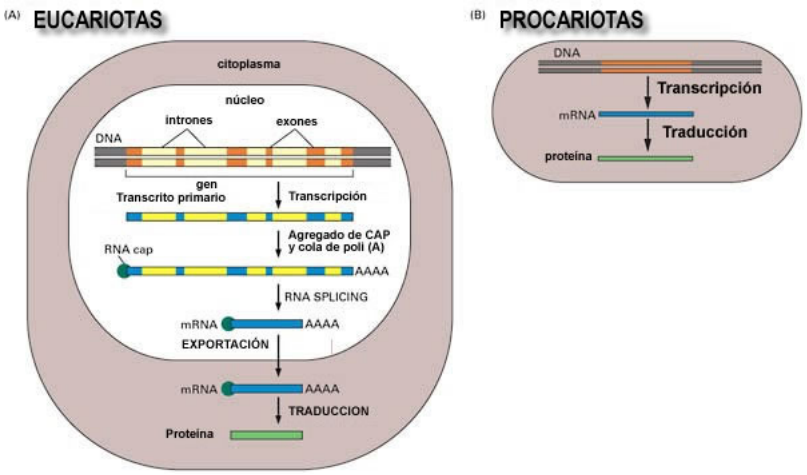
***splicing*, *splicing* alternativo, *frameshifting*, edición, inteínas, procesamiento proteolítico**

regulación

Dr. Víctor Romanowski, 2013

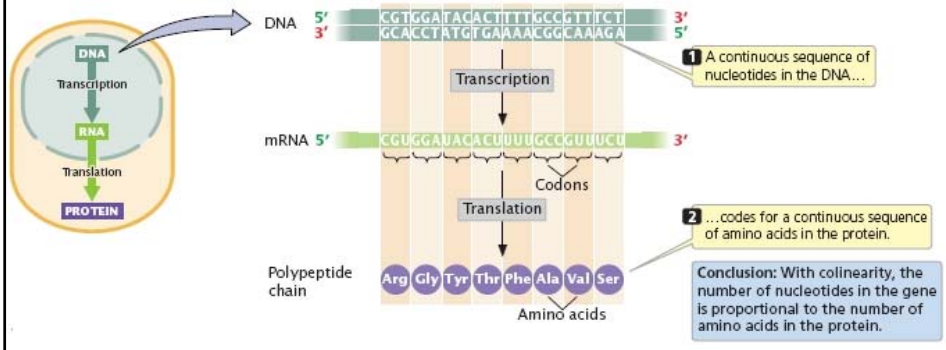
transcripción

y procesamiento de RNA



Transcripción + traducción

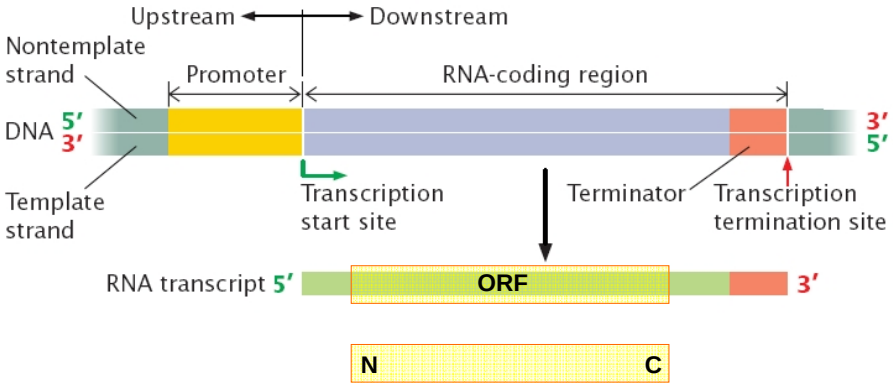
colinealidad se secuencia de nucleótidos en el DNA y de aminoácidos en la proteína



The concept of colinearity.
Colinearity suggests that a continuous sequence of nucleotides in DNA encodes a continuous sequence of amino acids in a protein.
http://www.nature.com/scitable/topicpage/what-is-a-gene-colinearity-and-transcription-430 3

Transcripción + traducción

colinealidad se secuencia de nucleótidos en el DNA y de aminoácidos en la proteína



A transcription unit includes a promoter, an RNA-coding region, and a terminator.
http://www.nature.com/scitable/topicpage/what-is-a-gene-colinearity-and-transcription-430 4

transcripción y procesamiento de RNA

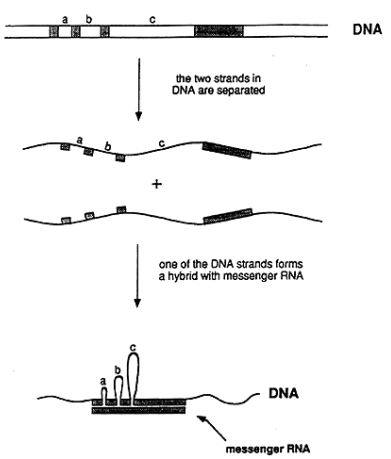
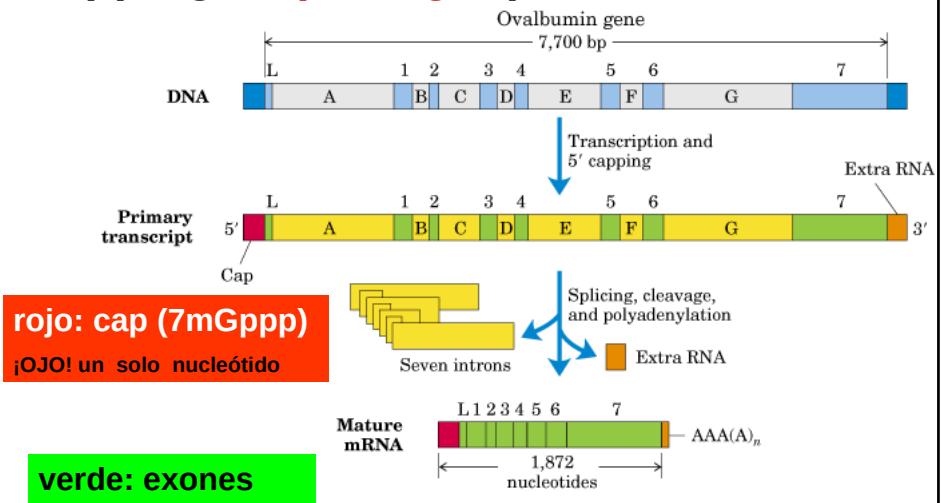


Figure 2 : Schematic representation of the experiment that demonstrated that adenovirus DNA contains split genes. The genetic information in the messenger RNA resides in the DNA as four segments, which are separated by three intervening regions (a, b, and c). In the experimentally produced hybrid between one of the DNA strands and the RNA, the intervening sequences in the DNA strand appear as loops, i.e., the corresponding segments lack counterparts in the RNA. The hybrid could be directly visualized in the electron microscope.

5

Capping + splicing + poliadenilación



rojo: cap (7mGppp)

¡OJO! un solo nucleótido

verde: exones

amarillo: intrones

6

la secuencia de aminoácidos de la proteína
no siempre refleja
la secuencia continua de nucleótidos en el genoma

splicing de pre-mRNA (eliminación de intrones)

splicing alternativo
(varios polipéptidos a partir de la misma secuencia de DNA)

edición del mRNA

corrimiento del marco de lectura (*translational frameshifting*)

procesamiento proteolítico de polipéptidos
(en algunos casos se obtienen productos alternativos en diferentes tipos celulares)

splicing de proteínas (eliminación de inteínas)

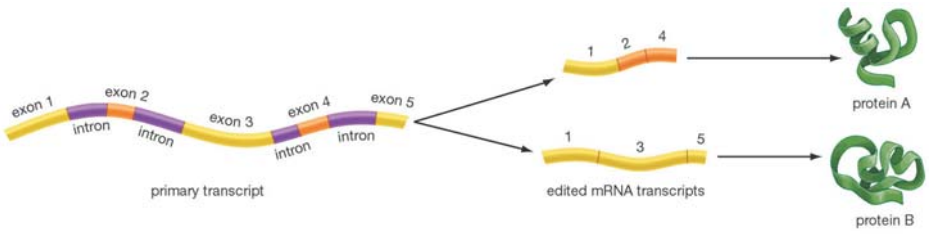
Glicosilación, fosforilación y otras modificaciones covalentes de proteínas

7

***splicing* alternativo**
y edición de mRNA

8

Alternative splicing – More bang for the buck

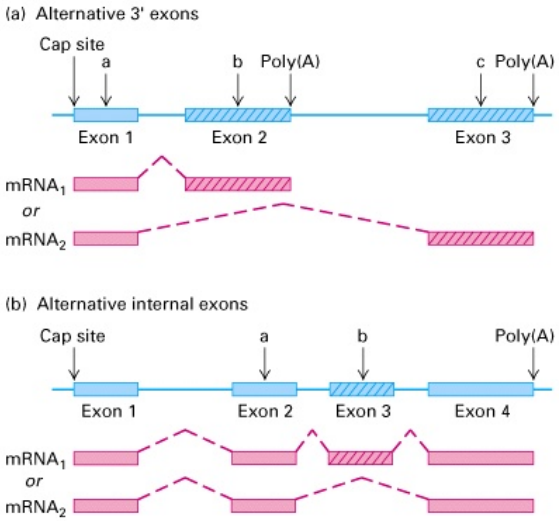


This has the consequence that the count of our genes (~20,000) seriously underestimates the count of our different proteins.

9

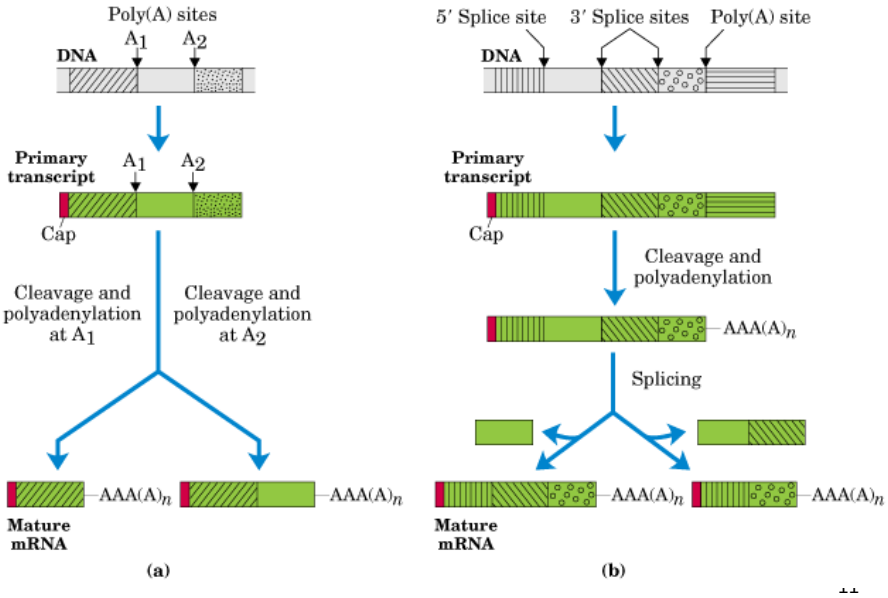
splicing alternativo

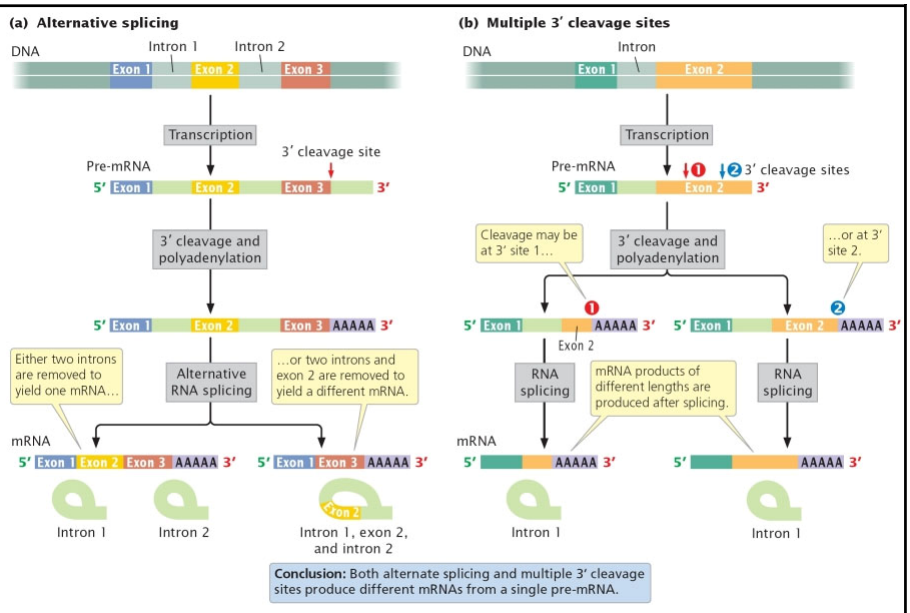
los RNAs pueden procesarse de diferente manera eliminando parte del producto de transcripción original



10

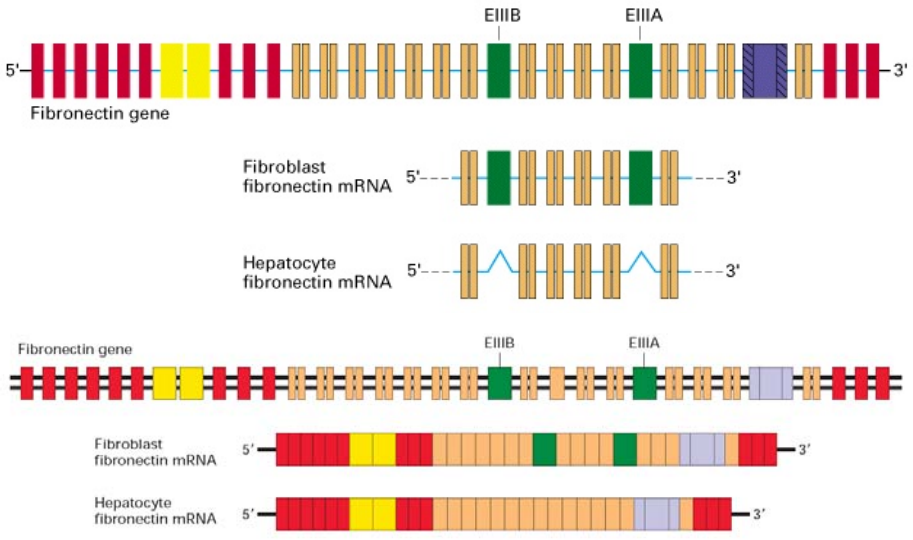
splicing alternativo



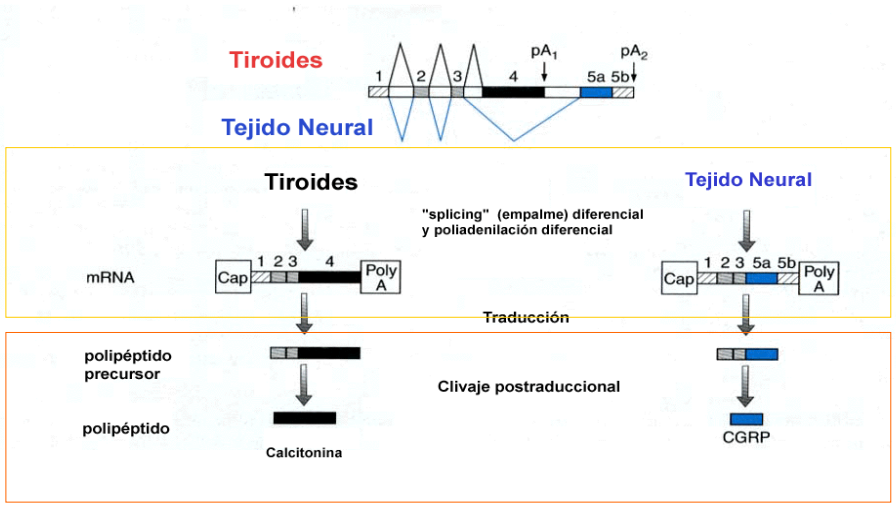


Eukaryotic cells have alternative pathways for processing pre-mRNA.
(a) With alternative splicing; pre-mRNA can be spliced in different ways to produce different mRNAs. (b) With multiple 3 cleavage sites, there are two or more potential sites for cleavage and polyadenylation; use of the different sites produces mRNAs of different lengths.

Tissue-specific RNA splicing controls expression of alternative fibronectins



13

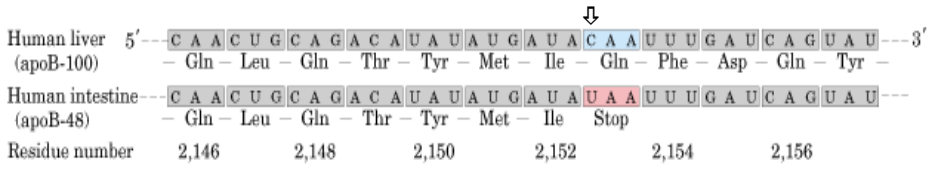


splicing alternativo del gen de calcitonina

Traducción de un mRNA editado
translation of edited mRNA

mRNA **original** no editado

C → U



mRNA **editado**

15

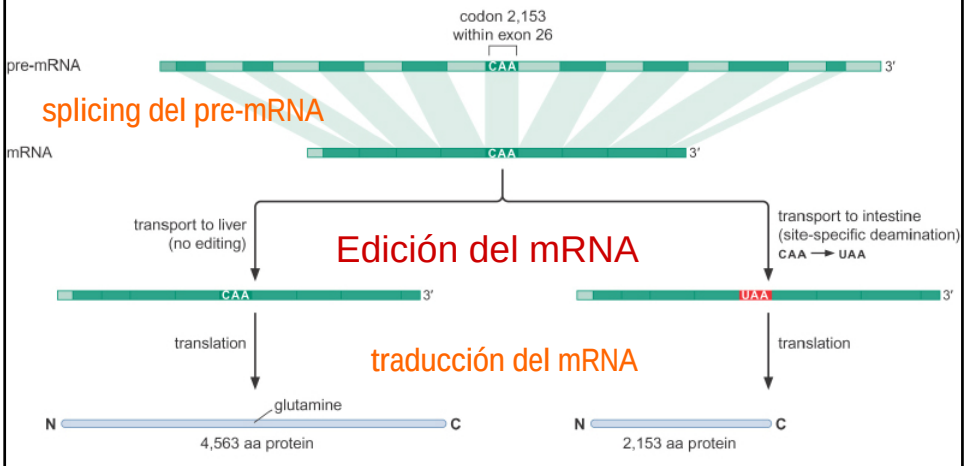


FIGURE 13-24 RNA editing by deamination. The RNA made from the human apolipoprotein gene is edited in a tissue-specific manner by deamination of a specific cytodine to generate a uridine. This event occurs in RNAs destined for the intestine, but not those for the liver. The result, as described in the text, is that a stop codon introduced into the intestinal mRNA generates a shorter protein than that produced in the liver. The figure is not drawn to scale: thus the edited exon is exon 26; and the codon marked as filling it is in reality only a very short part of that exon.

RNA editing alters the sequences of pre-mRNAs

A mammalian example

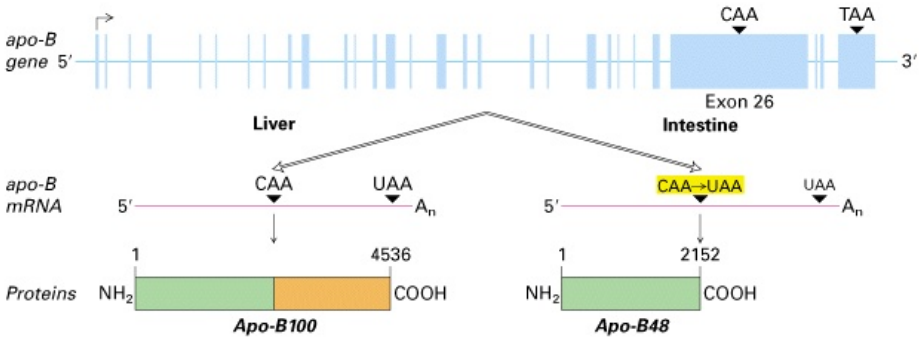


Figure 11-39

17

mRNA editing can regulate the function of protein products – *e.g.,* AMPA receptor gene in mammals

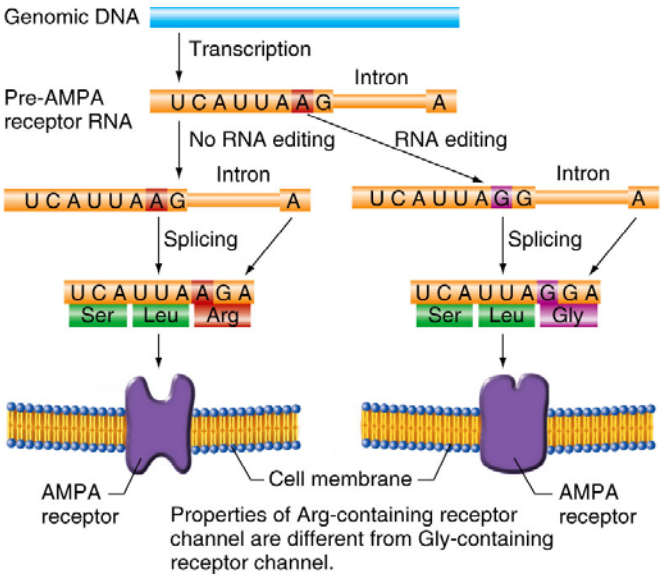


Fig. 17-18

18

RNA editing in protozoans

Editing in the kinetoplast of trypanosomes

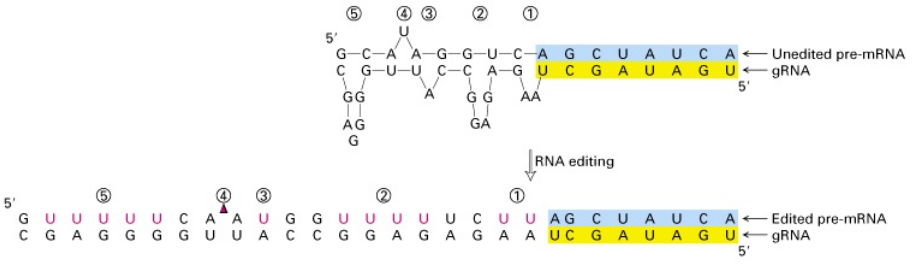
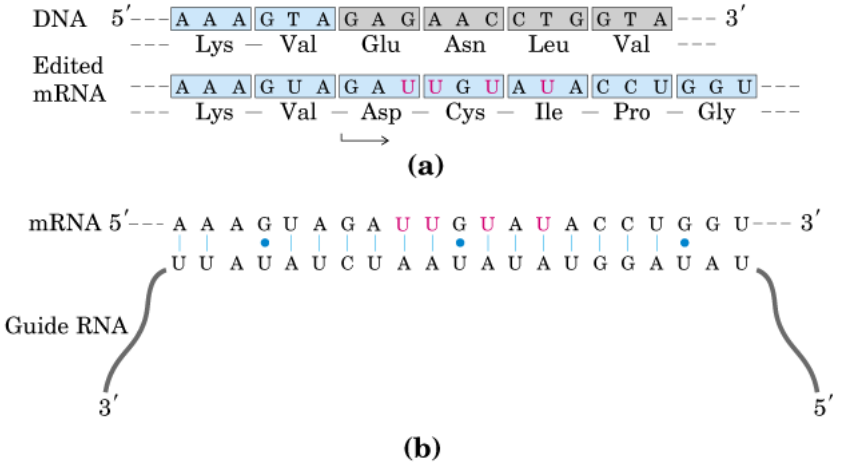


Figure 11-40

translation of edited mRNA



RNA editing

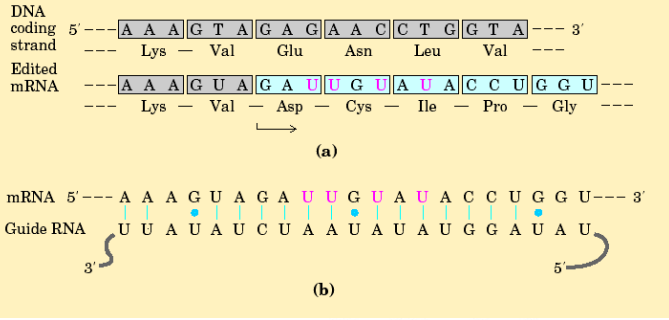
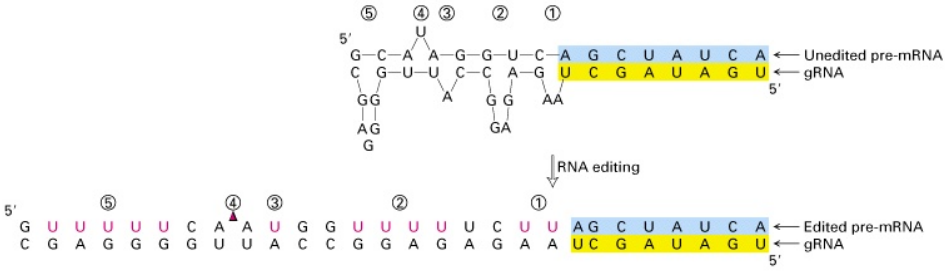
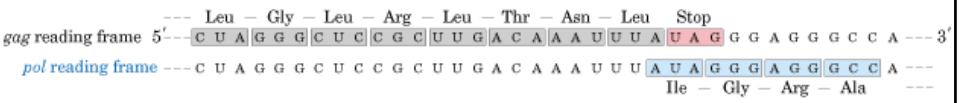


FIGURE 2 RNA editing of the transcript of the cytochrome oxidase subunit II gene from *Trypanosoma brucei* mitochondria. **(a)** Insertion of four U residues (pink) produces a revised reading frame. **(b)** A special class of guide RNAs, complementary to the edited product, may act as templates for the editing process.



Corrimiento del marco de lectura
Translational frameshifting



Ejemplo: retrovirus

22

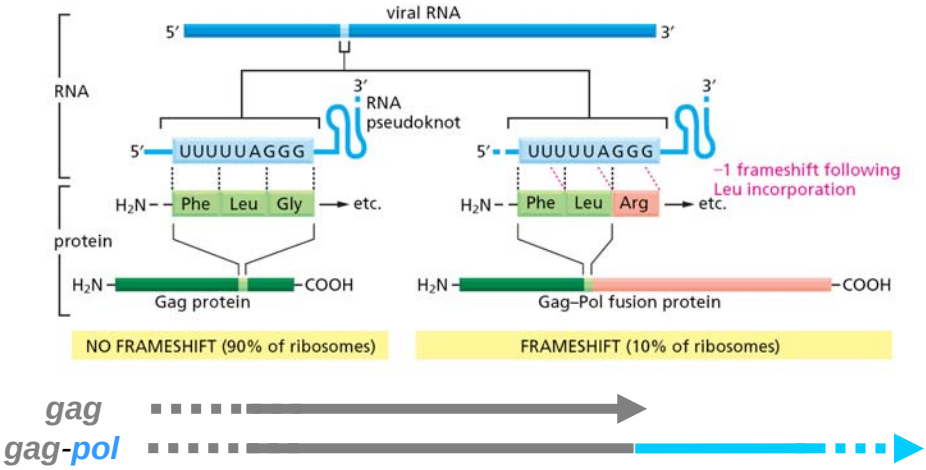


Figure 6–78 The translational frameshifting that produces the reverse transcriptase and integrase of a retrovirus. The viral reverse transcriptase and integrase are produced by proteolytic processing of a large protein (the Gag–Pol fusion protein) consisting of both the Gag and Pol amino acid sequences. Proteolytic processing of the more abundant Gag protein produces the viral capsid proteins. Both the Gag and the Gag–Pol fusion proteins start with identical mRNA, but whereas the Gag protein terminates at a stop codon downstream of the sequence shown, translation of the Gag–Pol fusion protein bypasses this stop codon, allowing the synthesis of the longer Gag–Pol fusion protein. The stop-codon-bypass is made possible by a controlled translational frameshift, as illustrated. Features in the local RNA structure (including the RNA loop shown) cause the tRNALeu attached to the C-terminus of the growing polypeptide chain occasionally to slip backward by one nucleotide on the ribosome, so that it pairs with a UUU codon instead of the UUA codon that had initially specified its incorporation; the next codon (AGG) in the new reading frame specifies an arginine rather than a glycine. This controlled slippage is due in part to a pseudoknot that forms in the viral mRNA (see Figure 6–102). The sequence shown is from the human AIDS virus, HIV. (Adapted from T. Jacks et al., Nature 331:280–283, 1988. With permission from Macmillan Publishers Ltd.)

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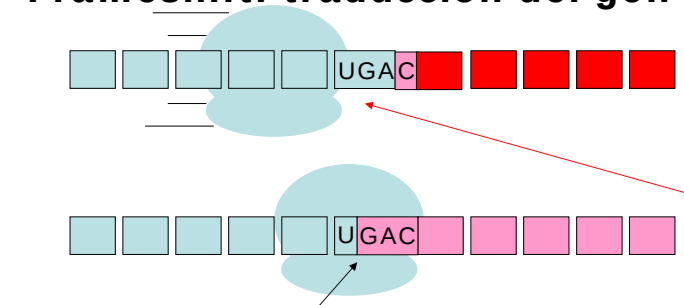
Frameshift: traducción del gen RF2



RF2

Unión de RF2
terminación de la traducción

RF2 (bajo)
Pausa

Frameshift: **UGAC** se lee como **GAC** y sigue en el marco de lectura **+1**

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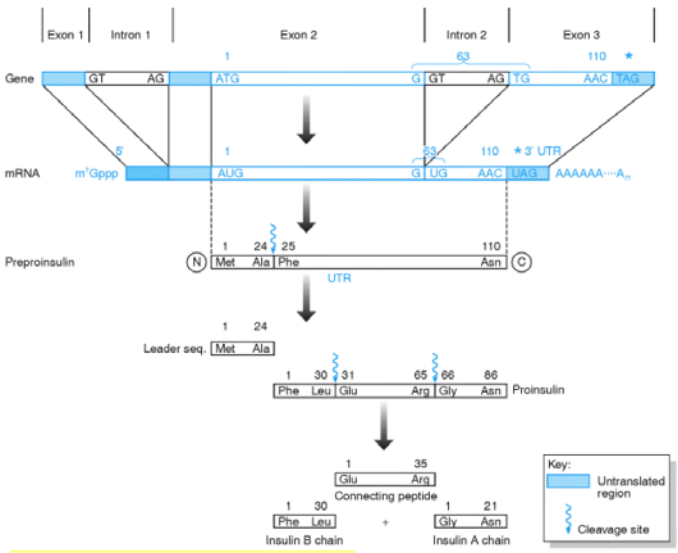
Modificación de proteínas

la secuencia de aminoácidos de la proteína no siempre refleja la secuencia continua de nucleótidos en el genoma

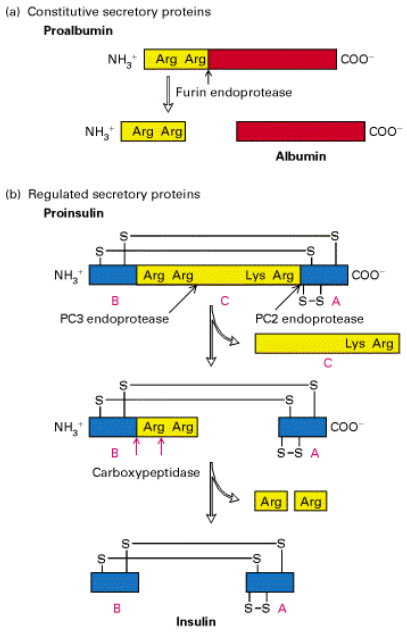


Ejemplo: insulina
del gen a la proteína activa

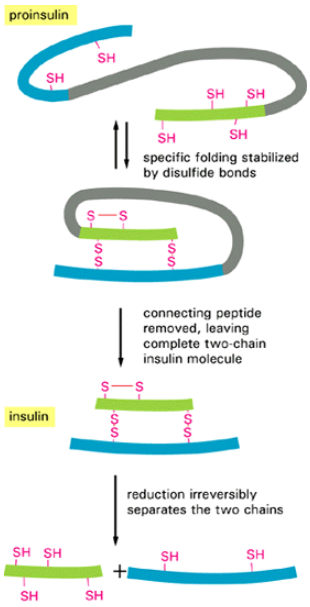
Gen (DNA)…
transcripción...
Pre mRNA
Procesamiento
(remoción de intrones, cap, poliA)
OJO: esto ocurre de manera co-transcripcional
mRNA (maduro)…
traducción…
Pre-pro-proteína
modificaciones co- y pos-traduccionales
(direccionamiento, remoción del péptido señal, cortes protelíticos en sitios específicos; ver puentes disulfuro en la figura siguiente)
polipéptido maduro

Figure 1.23. Insulin synthesis involves multiple post-translational cleavages of polypeptide precursors. The first intron interrupts the 5 untranslated region; the second intron interrupts base positions 1 and 2 of codon 63 and is classified as a phase I intron (see *Box 14.3*, and also *Figure 1.19* for other intron phases). The primary translation product, preproinsulin, has a leader sequence of 24 amino acids which is required for the protein to cross the cell membrane, and is thereafter discarded. The proinsulin precursor contains a central segment, the connecting peptide, which is thought to be important in maintaining the conformation of the A and B chain segments so that they can form disulfide bridges (see *Figure 1.25*). Strachan: Human Genetics

procesamiento proteolítico



insulina: sobre-simplificación



Genentech

Genentech Inc., a composite of **Genetic Engineering Technology, Inc.**, is a leading biotechnology corporation, which was founded in 1976 by venture capitalist Robert A. Swanson and biochemist Dr. Herbert W. Boyer.

It is considered to have founded the biotechnology industry

1982 - Synthetic "human“ insulin

expressed in *E. coli*; approved by the U.S. Food and Drug Administration (FDA), thanks largely to its partnership with insulin manufacturer Eli Lilly and Company, who shepherded the product through the FDA approval process. The product (Humulin) was licensed to and manufactured by Lilly, and was the first-ever approved genetically engineered human therapeutic.

1985 - Protropin (somatrem) - Supplementary growth hormone for children with growth hormone deficiency (ceased manufacturing December 2002).
1987 - Activase (alteplase) - A recombinant tissue plasminogen activator (tPa) used to dissolve blood clots in patients with acute myocardial infarction. Also used to treat non-hemorrhagic stroke.
1990 - Actimmune (interferon gamma 1b) - Treatment of chronic granulomatous disease (licensed to Intermune).
1993 - Nutropin (recombinant somatropin) - Growth hormone for children and adults for treatment before kidney transplant due to chronic renal insufficiency.
1993 - Pulmozyme (dornase alfa) - Inhalation treatment for children and young adults with cystic fibrosis - recombinant DNAse.
1997 - Rituxan (rituximab)- Treatment for specific kinds of non-Hodgkins lymphomas. In 2006, also approved for rheumatoid arthritis.
1998 - Herceptin (trastuzumab) - Treatment for metastatic breast cancer patients with tumors that overexpress the HER2 gene. Recently approved for adjuvant therapy for breast cancer.
2000 - TNKase (tenecteplase) - "Clot-busting" drug to treat acute myocardial infarction.
2003 - Xolair (omalizumab) - Subcutaneous injection for moderate to severe persistent asthma.
2003 - Raptiva (efalizumab) - Antibody designed to block the activation and reactivation of T cells that lead to the development of psoriasis. Developed in partnership with XOMA. In 2009, voluntary U.S. market withdrawal after reports of progressive multifocal leukoencephalopathy.
2004 - Avastin (bevacizumab) - Anti-VEGF monoclonal antibody for the treatment of metastatic cancer of the colon or rectum. In 2006, also approved for locally advanced, recurrent or metastatic non-small cell lung cancer. In 2008, accelerated approval was granted for Avastin in combination with chemotherapy for previously untreated advanced HER2-negative breast cancer. Additional filings have been made for Avastin in previously treated glioblastoma and kidney cancer.
2004 - Tarceva (erlotinib) - Treatment for patients with locally advanced or metastatic non-small cell lung cancer, and pancreatic cancer.
2006 - Lucentis (ranibizumab injection) - The U.S. Food and Drug Administration (FDA) has approved LUCENTIS(TM) (ranibizumab injection) for the treatment of neovascular (wet) age-related macular degeneration (AMD). The FDA approved LUCENTIS after a Priority Review (six-month). Genentech started shipping product on June 30, 2006, the day the product was approved.

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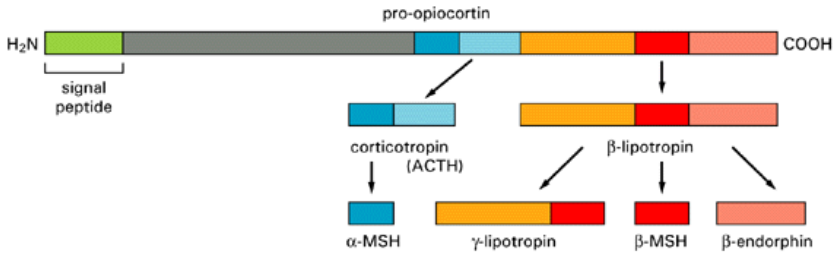
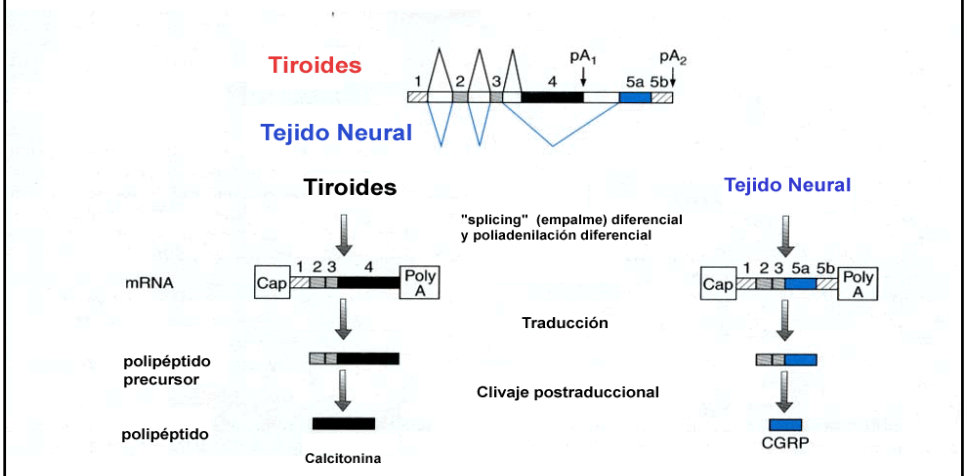
Rutas de procesamiento alternativas de la prohormona pro-opiocortina

POMC: pro-opio-melano-cortin precursor



Alternative processing pathways of the prohormone pro-opiocortin. The initial cleavages are made by membrane-bound proteases that cut next to pairs of positively charged amino acid residues (Lys-Arg, Lys-Lys, Arg-Lys, or Arg-Arg pairs), and trimming reactions then produce the final secreted products. Different cell types contain different processing enzymes, so that the same prohormone precursor can be used to produce different peptide hormones. In the anterior lobe of the pituitary gland, for example, only corticotropin (ACTH) and b-lipotropin are produced from pro-opiocortin, whereas in the intermediate lobe of the pituitary, mainly a-MSH, g-lipotropin, b-MSH, and b-endorphin are produced.

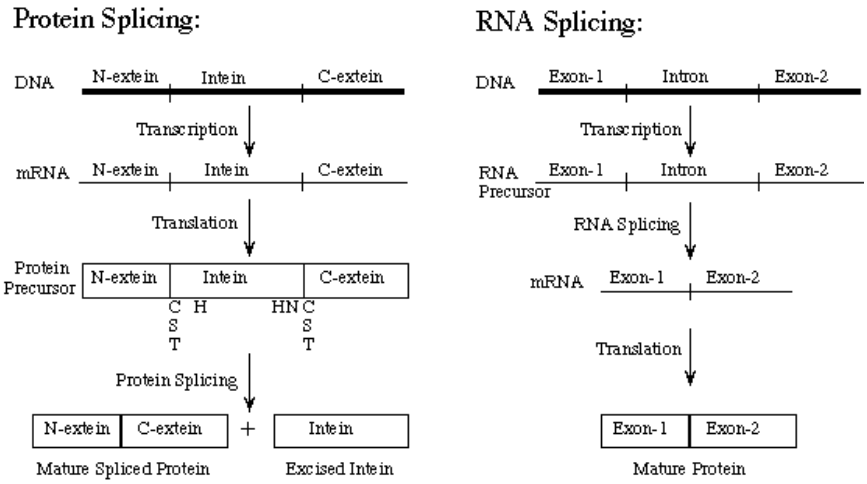
30



splicing alternativo del gen de calcitonina y procesamiento proteolítico

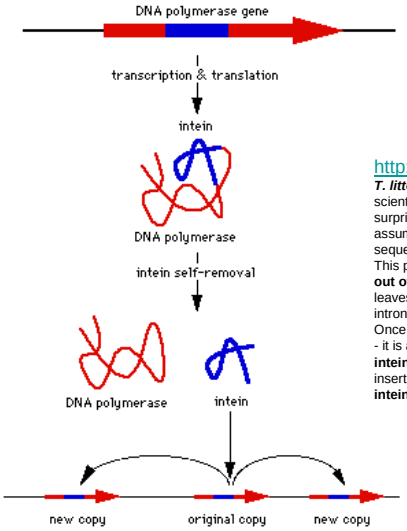
Inteínas (splicing de proteínas)

RNA *vs.* protein splicing



32

Protein Splicing

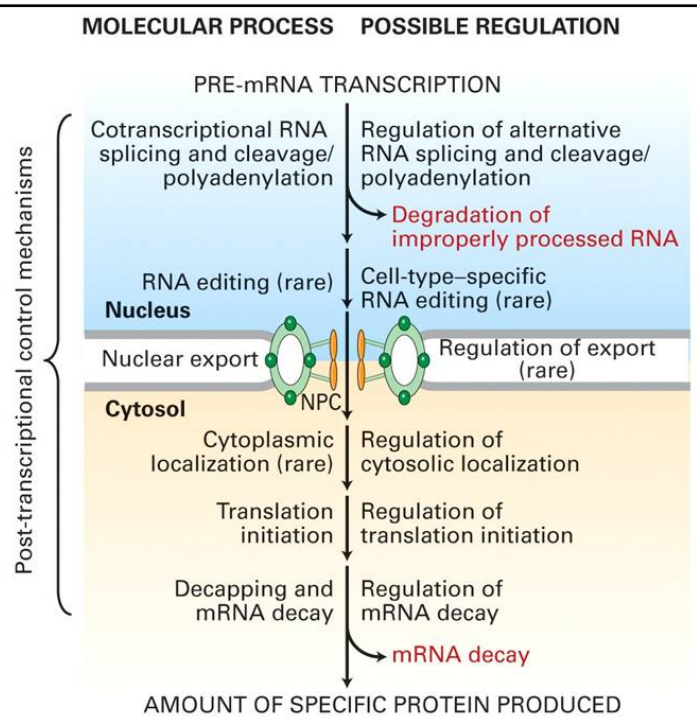


http://soils1.cses.vt.edu/ch/biol_4684/Microbes/inteins.gif

T. littoralis is the source of vent **DNA polymerase**, used extensively in PCR. When scientists at New England Biolabs cloned the gene for the enzyme, they were surprised to find that it contains an extra sequence of non-DNA-polymerase. They assumed it was an intron, but found that the mRNA isn't spliced and the extra sequence is translated into a novel domain in the enzyme.
This polypeptide domain is a **peptidyltransferase** that specifically **splices itself out of the DNA polymerase**, rejoining the 2 parts of the DNA polymerase as it leaves. This protein-splicing reaction is remarkably analogous to RNA-splicing by introns, and so it's called an 'intein' (intervening protein).
Once the **intein** has removed itself from the DNA polymerase, it has another activity - it is a **transposase**. This enzyme cleaves DNA specifically at the **ends of the intein-encoding sequence** and directs a DNA repair process that results in the insertion of the intein DNA into other protein-encoding genes - in other words, the **intein** DNA is also a **transposon.**

¡OJO! esta inteína es una transposasa que cataliza la inserción del DNA en nuevos lugares

33



34

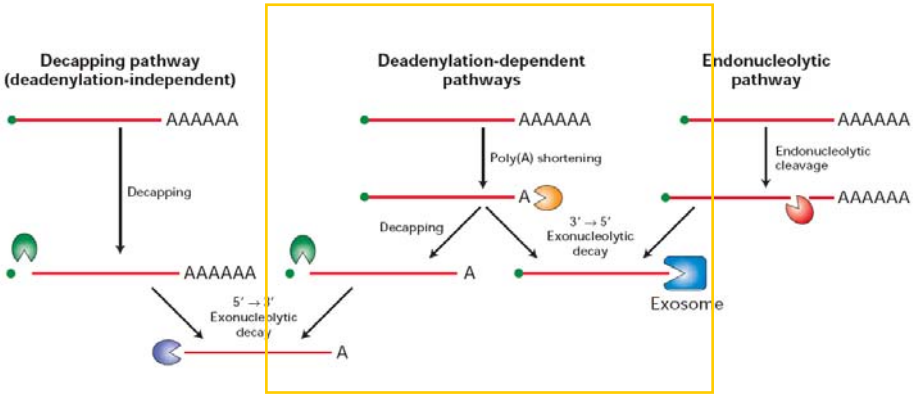
Niveles de traducción

- Disponibilidad de mRNA:

- Niveles de transcripción y degradación
- Transporte del mRNA al citosol (RNP)
- Estabilización – desestabilización del mRNA
- Accesibilidad a la maquinaria de traducción

35

Degradación de mRNA

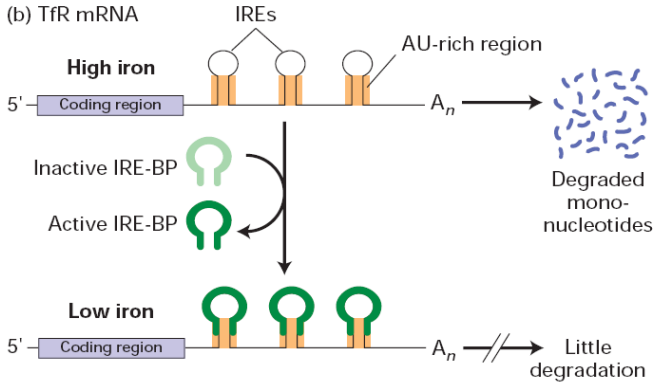


36

Untranslated regions control **mRNA stability** *e.g.* transferrin receptor

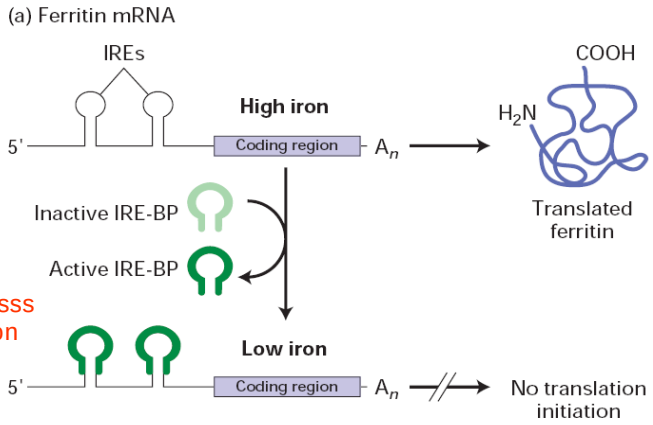
Transferrin receptor – role to **get iron into cells**. Important that it is expressed in low iron conditions to get as much iron (essential to cell) into the cytoplasm but also important to reduce iron uptake when too much iron around

At high iron concentrations, IRE-BP undergoes a conformational change and cannot bind mRNA



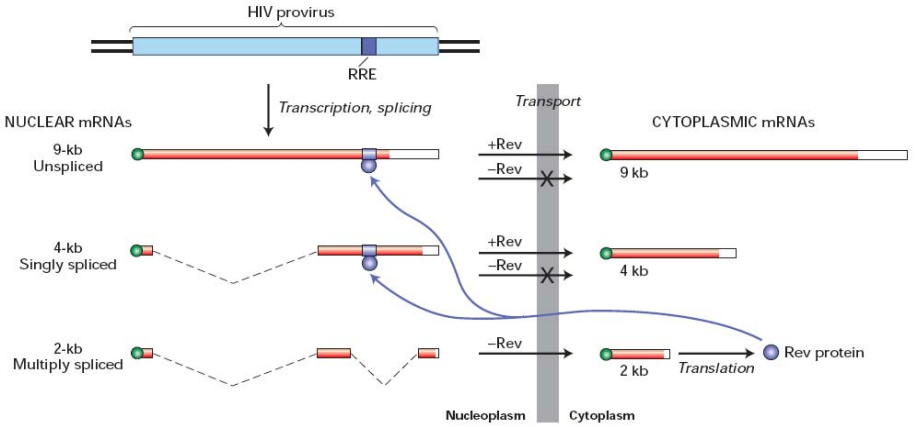
37

Untranslated regions control **mRNA accessibility** *e.g.* ferritin



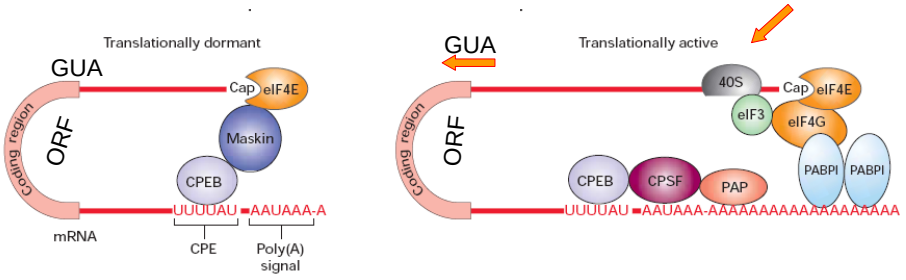
38

Regulación del transporte de mRNAs de HIV



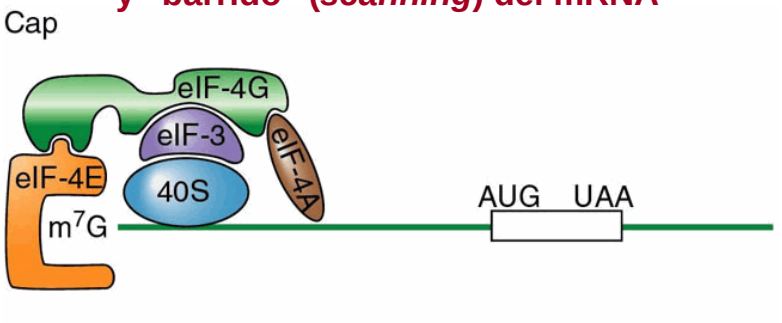
▲ FIGURE 12-26 Role of Rev protein in transport of HIV mRNAs from the nucleus to the cytoplasm. The HIV genome, which contains several coding regions, is transcribed into a single 9-kb primary transcript. Several ≈4-kb mRNAs result from alternative splicing out of any one of several introns (dashed lines), and several ≈2-kb mRNAs from splicing out of two or more alternative introns. After transport to the cytoplasm, the various RNA species are translated into different viral proteins. Rev protein, encoded by a 2-kb mRNA, interacts with the Rev-response element (RRE) in the unspliced and singly spliced mRNAs, stimulating their transport to the cytoplasm. [Adapted from B. R. Cullen and M. H. Malim, 1991, *Trends Biochem. Sci.* **16**:346.]

control of cytoplasmic polyadenylation and translation initiation



▲ FIGURE 12-28 Model for control of cytoplasmic polyadenylation and translation initiation. *Left:* In immature oocytes, mRNAs containing the U-rich cytoplasmic polyadenylation element (CPE) have short poly(A) tails. CPE-binding protein (CPEB) mediates repression of translation through the interactions depicted, which prevent assembly of an initiation complex at the 5′ end of the mRNA. *Right:* Hormone stimulation of oocytes activates a protein kinase that phosphorylates CPEB, causing it to release Maskin. The cleavage/polyadenylation specificity factor (CPSF) then binds to the poly(A) site, interacting with both bound CPEB and the cytoplasmic form of poly(A) polymerase (PAP). After the poly(A) tail is lengthened, multiple copies of the cytoplasmic poly(A)-binding protein I (PABPI) can bind to it and interact with eIF4G, which functions with other initiation factors to bind the 40S ribosome subunit and initiate translation. [Adapted from R. Mendez and J. D. Richter, 2001, *Nature Rev. Mol. Cell Biol.* **2**:521.]

Reconocimiento del *cap* y "barrido" (*scanning*) del mRNA



Para que se produzca el *scanning* de la subunidad 40S es necesaria la estructura del *cap*. El factor responsable de este reconocimiento es eIF-4F que está formado por:

- eIF-4E: con actividad de cap binding protein
- eIF-4A
- eIF-4G

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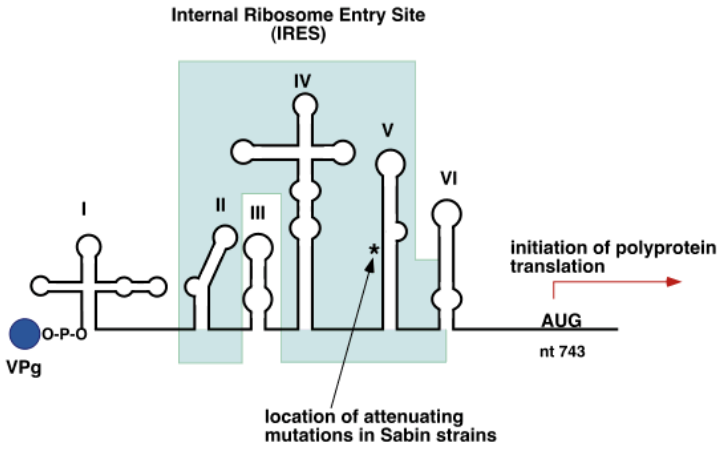
IRES: sitio interno de entrada al ribosoma

Internal Ribosome Entry Site

Secondary Structure of the 5' nontranslated region of picornavirus genomes (rhinovirus and enterovirus type)



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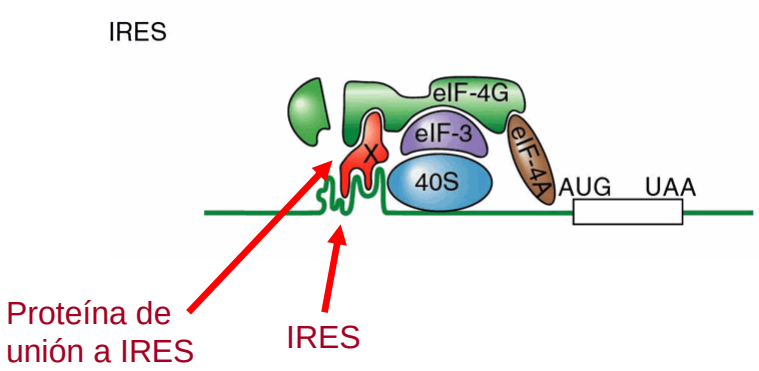
Caso: célula infectada por poliovirus

Los picornavirus estimulan la **proteólisis de eIF-4G** (proteína que estabiliza la unión del complejo eIF-4F con el cap).
En definitiva el virus disminuye la capacidad de la célula de traducir mensajeros "cappeados" (propios).



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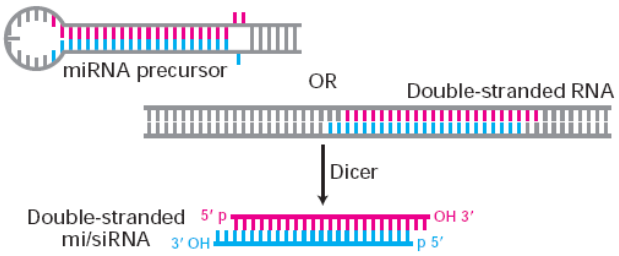
miRNA/siRNA

RNA interference



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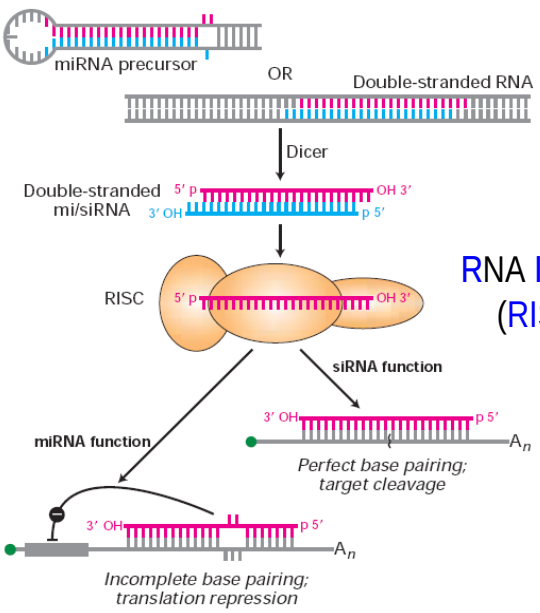
miRNA/siRNA



RNA Induced Silencing Complex (RISC)

45

miRNA in the human genome

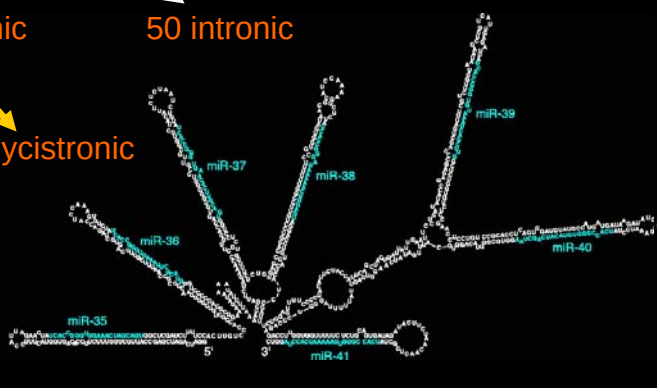
180 known miRNAs in human

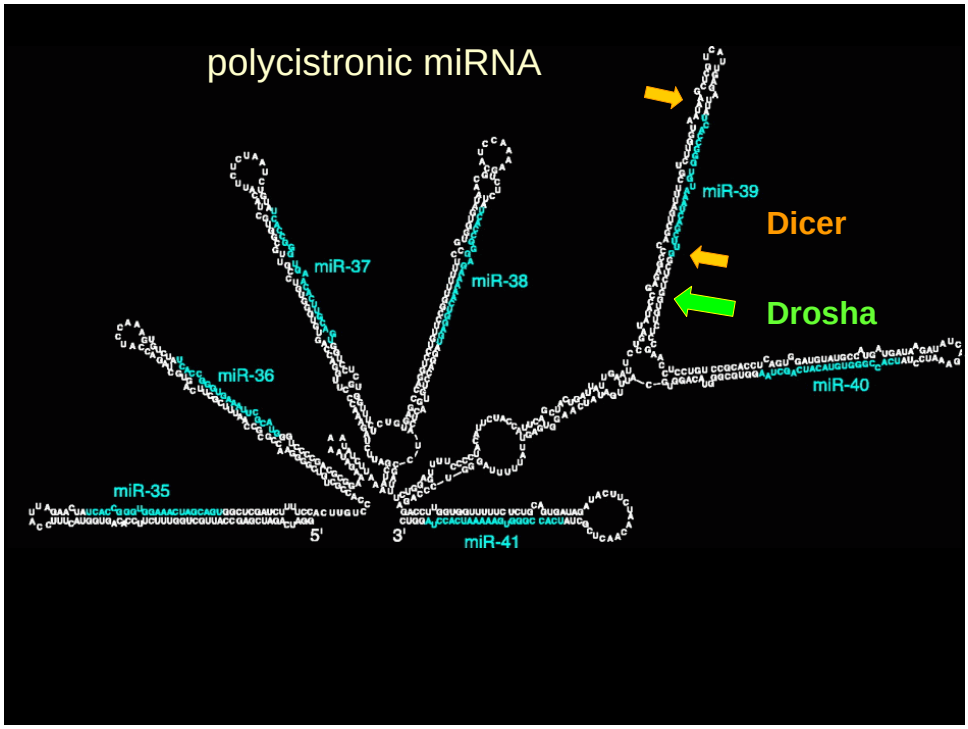
130 intergenic

50 intronic

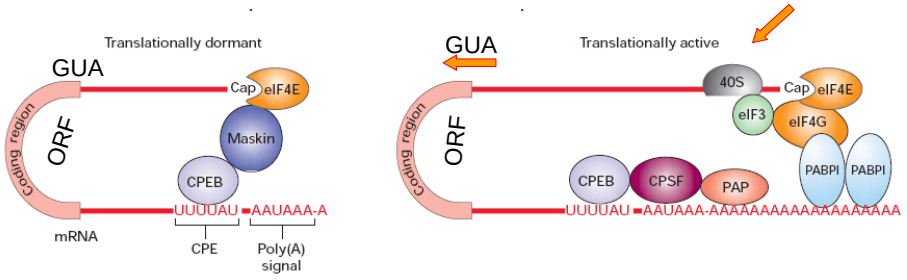
60 polycistronic

70 monocistronic





control of cytoplasmic polyadenylation and translation initiation



▲ FIGURE 12-28 Model for control of cytoplasmic polyadenylation and translation initiation. *Left:* In immature oocytes, mRNAs containing the U-rich cytoplasmic polyadenylation element (CPE) have short poly(A) tails. CPE-binding protein (CPEB) mediates repression of translation through the interactions depicted, which prevent assembly of an initiation complex at the 5′ end of the mRNA. *Right:* Hormone stimulation of oocytes activates a protein kinase that phosphorylates CPEB, causing it to release Maskin. The cleavage/polyadenylation specificity factor (CPSF) then binds to the poly(A) site, interacting with both bound CPEB and the cytoplasmic form of poly(A) polymerase (PAP). After the poly(A) tail is lengthened, multiple copies of the cytoplasmic poly(A)-binding protein I (PABPI) can bind to it and interact with eIF4G, which functions with other initiation factors to bind the 40S ribosome subunit and initiate translation. [Adapted from R. Mendez and J. D. Richter, 2001, *Nature Rev. Mol. Cell Biol.* **2**:521.]

Niveles de proteína activa

- **síntesis** (niveles y accesibilidad del mRNA)
- **modificación** (activación o inactivación)
- **degradación**

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Proteins have variable life-spans

EnzymeHalf-life	Hours
Ornithine decarboxylase	0.2
RNA polymerase I	1.3
Serine dehydratase	4.0
PEPcarboxylase	5.0
Aldolase	118
GAPDH	130
cytochrome *c*	150

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Relación entre la vida media de las proteínas y el residuo del extremo N-terminal

TABLE 27–9 Relationship between Protein Half-Life and Amino-Terminal Amino Acid Residue

Amino-terminal residue	*Half-life**
Stabilizing	
Met, Gly, Ala, Ser, Thr, Val	>20 h
Destabilizing	
Ile, Gln	~30 min
Tyr, Glu	~10 min
Pro	~7 min
Leu, Phe, Asp, Lys	~3 min
Arg	~2 min

Source: Modified from Bachmair, A., Finley, D., & Varshavsky, A. (1986) In vivo half-life of a protein is a function of its amino-terminal residue. *Science* **234,** 179–186.

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Two routes to digest proteins

- Lysosomes
  - Receptor mediated endocytosis & phagocytosis
- Proteasomes: for endogenous proteins
  - transcription factors
  - cell cycle cyclins
  - virus coded proteins
  - improperly folded proteins
  - damaged proteins

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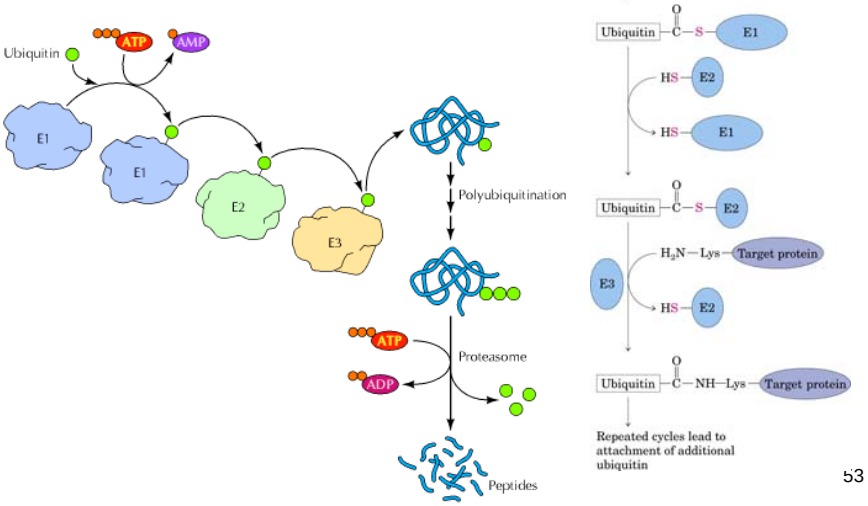
poliubiquitinilación de proteínas:

marcado para la degradación



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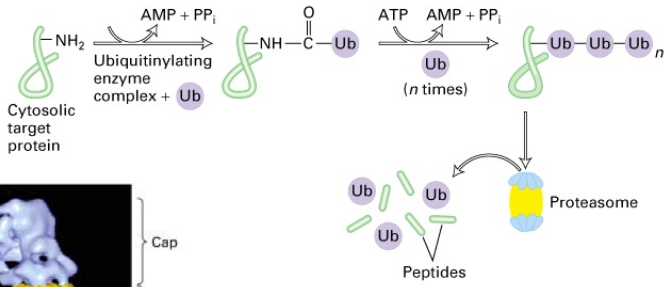
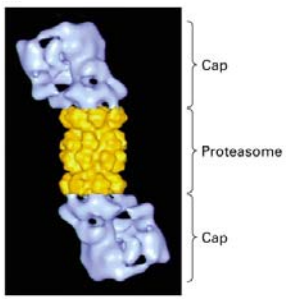
Proteasome: protein degradation via the ubiquitin-mediated pathway





Cells contain several other pathways for protein degradation in addition to this pathway

example: lisosomes

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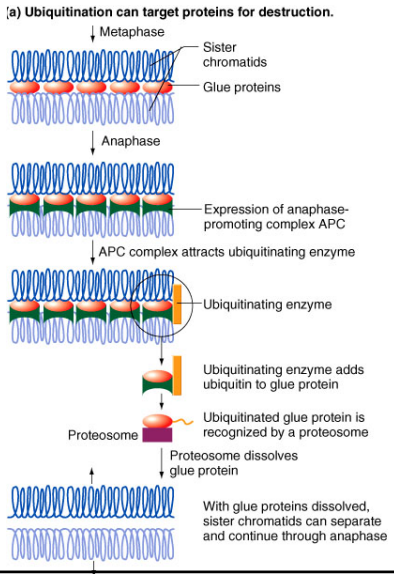
http://www.metabolic-database.com/html/normal_flash_proteasome.html

Protein modifications after translation provide a final level of control over gene function



- **Phosphorylation** (deactivation)
- **Ubiquitin** (protein) targets proteins for degradation
  - Covalently attaches to other proteins
  - Ubiquitinized proteins are marked for **degradation** by proteosomes

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Other Proteases

- Cell cycle control/stress response proteases
  - Proteasome
  - HtrA
- Calcium activated proteases (Calpains)
- Apoptotic proteases
  - ICE family (caspases)
- Autocatalytic proteases
- Nutrient regulated proteolysis (lysosome)
- Intramembrane cleave protease (ICLiPs)

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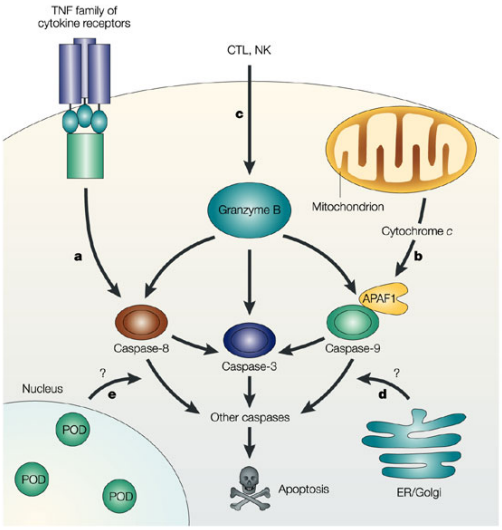
Proteolysis regulates cell death



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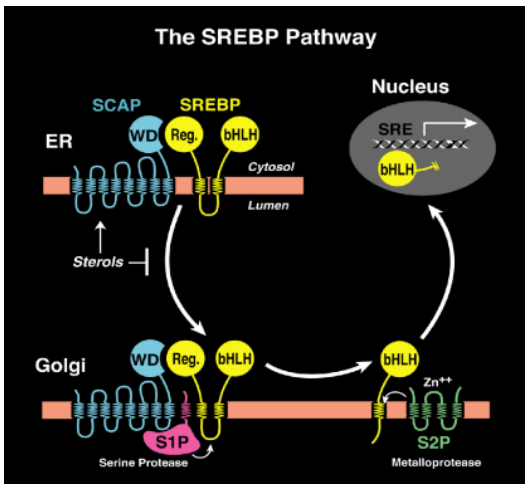
Proteolysis as a regulatory mechanism

(sequestration of sterol response element transcription factor)



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