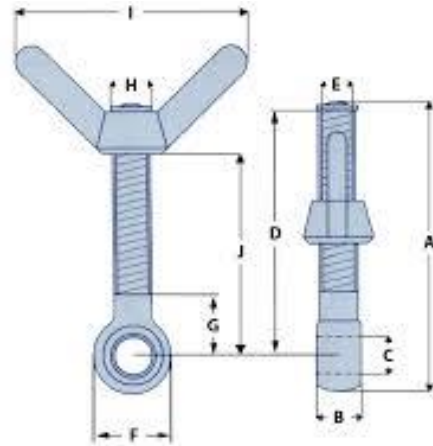
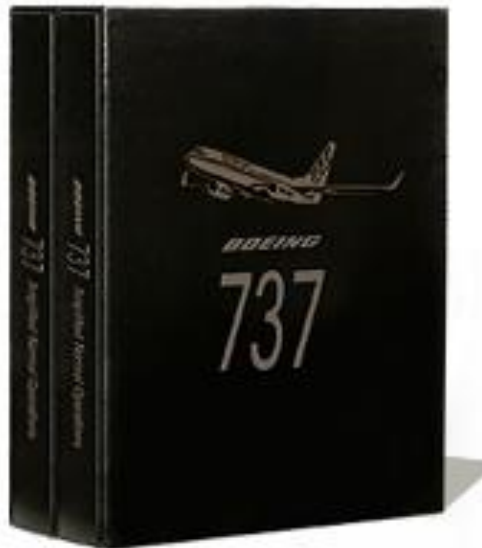
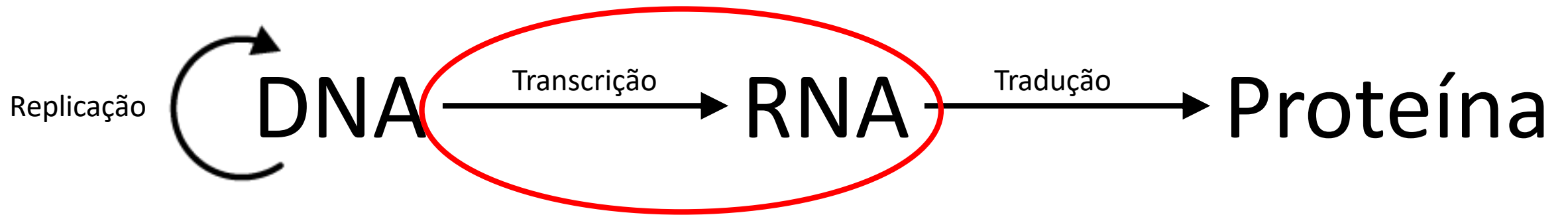


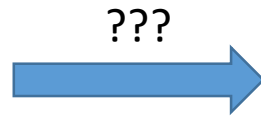
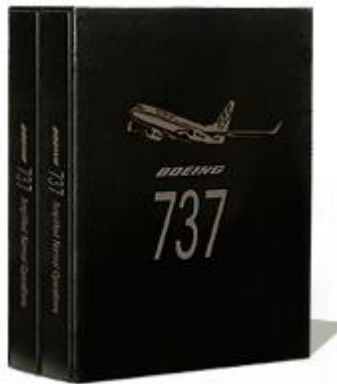
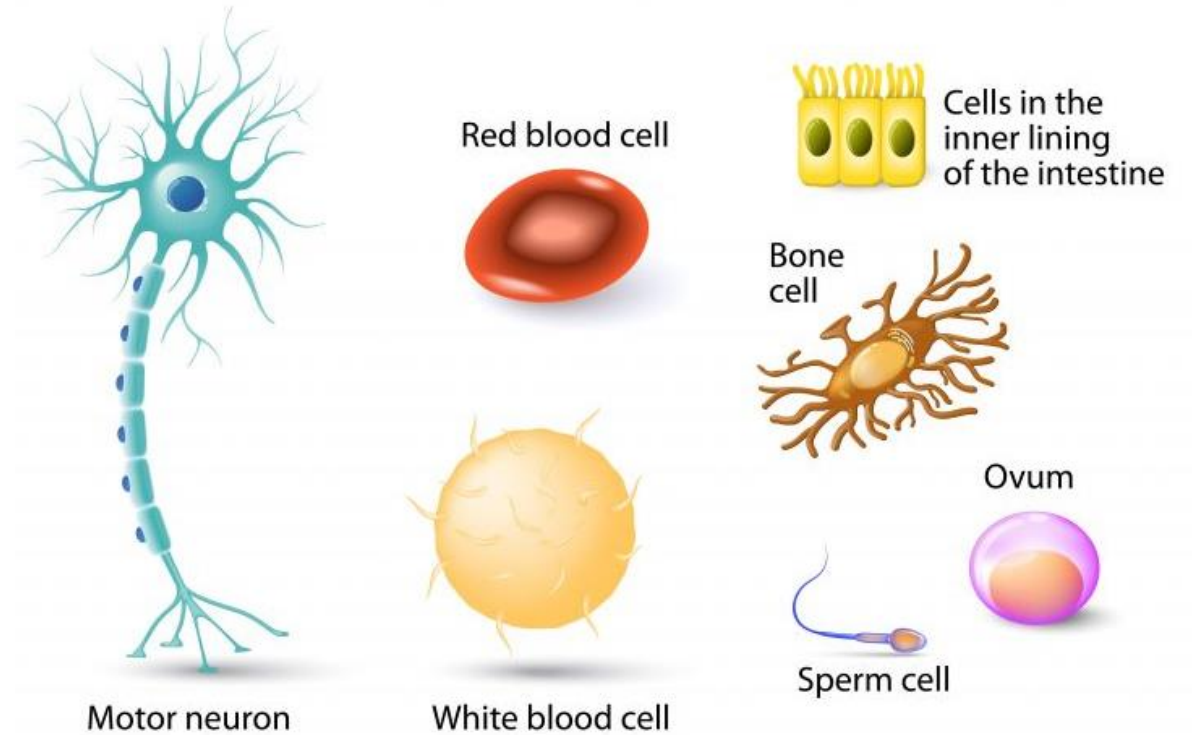
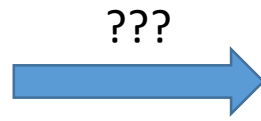
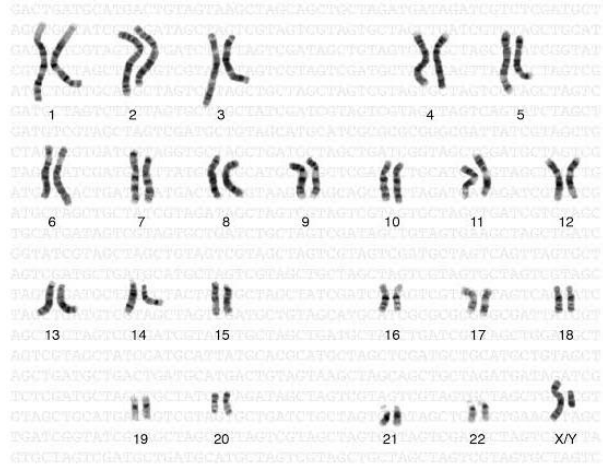
Transcrição
Processamento de RNA
Tradução
Regulação da expressão gênica

QBQ0313 – Bioquímica (2023)

Nícolas Hoch



Como pode haver diferenciação de função em organismos pluricelulares?



Regulação da Expressão Gênica

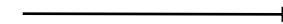
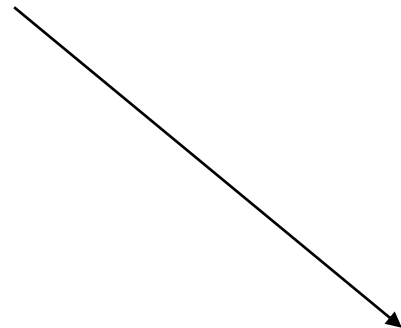
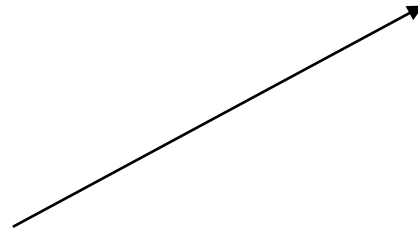
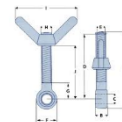
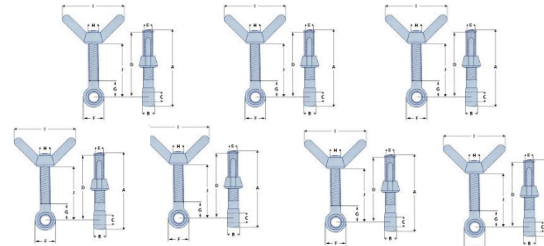
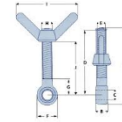
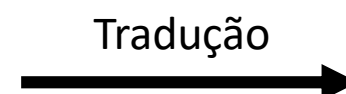
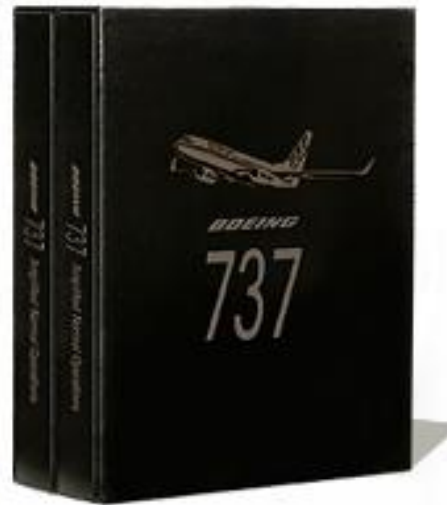
DNA

Transcrição

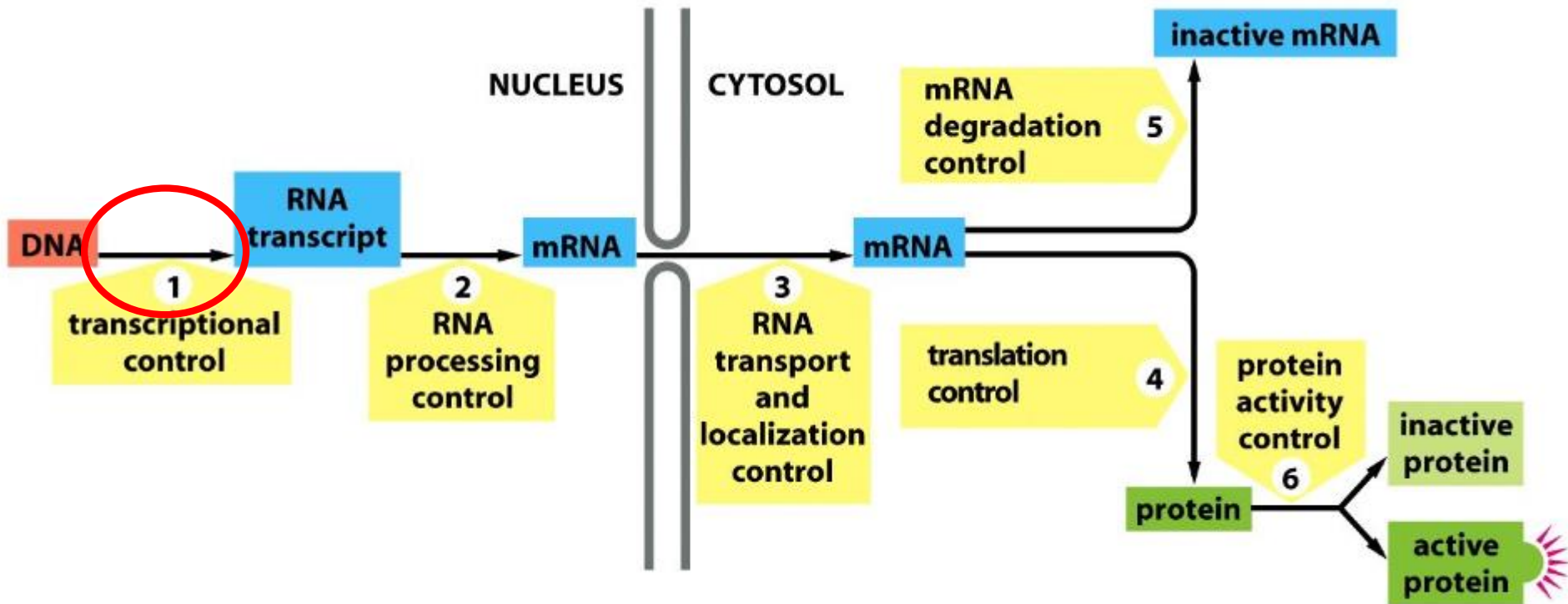
RNA

Tradução

proteína



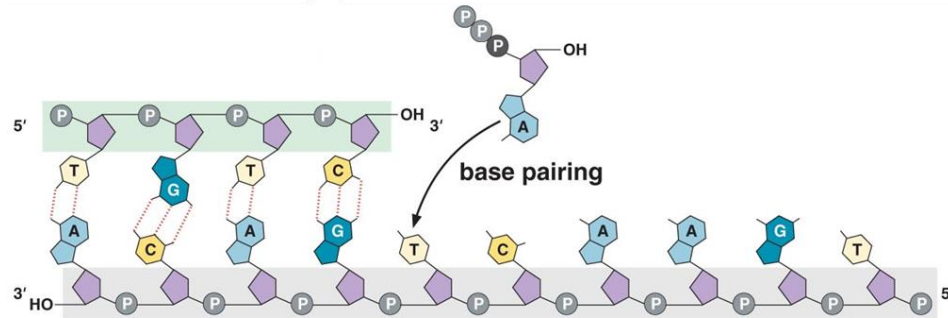
Regulação da Expressão Gênica



Tempo até resposta biológica

Custo energético

Replicação vs Transcrição



Ambas copiam informação do DNA a partir de pareamento de novos nucleotídeos

| Replicação | Transcrição |
|--------------------------------|---------------------------|
| Utiliza desoxiribonucleotídeos | Utiliza ribonucleotídeos |
| Pareia A com T (timidina) | Pareia A com U (uracila) |
| Gera DNA dupla fita | Gera RNA fita simples |
| Copia ambas as fitas | Copia apenas a fita molde |
| Copia o genoma inteiro | Copia apenas os genes |

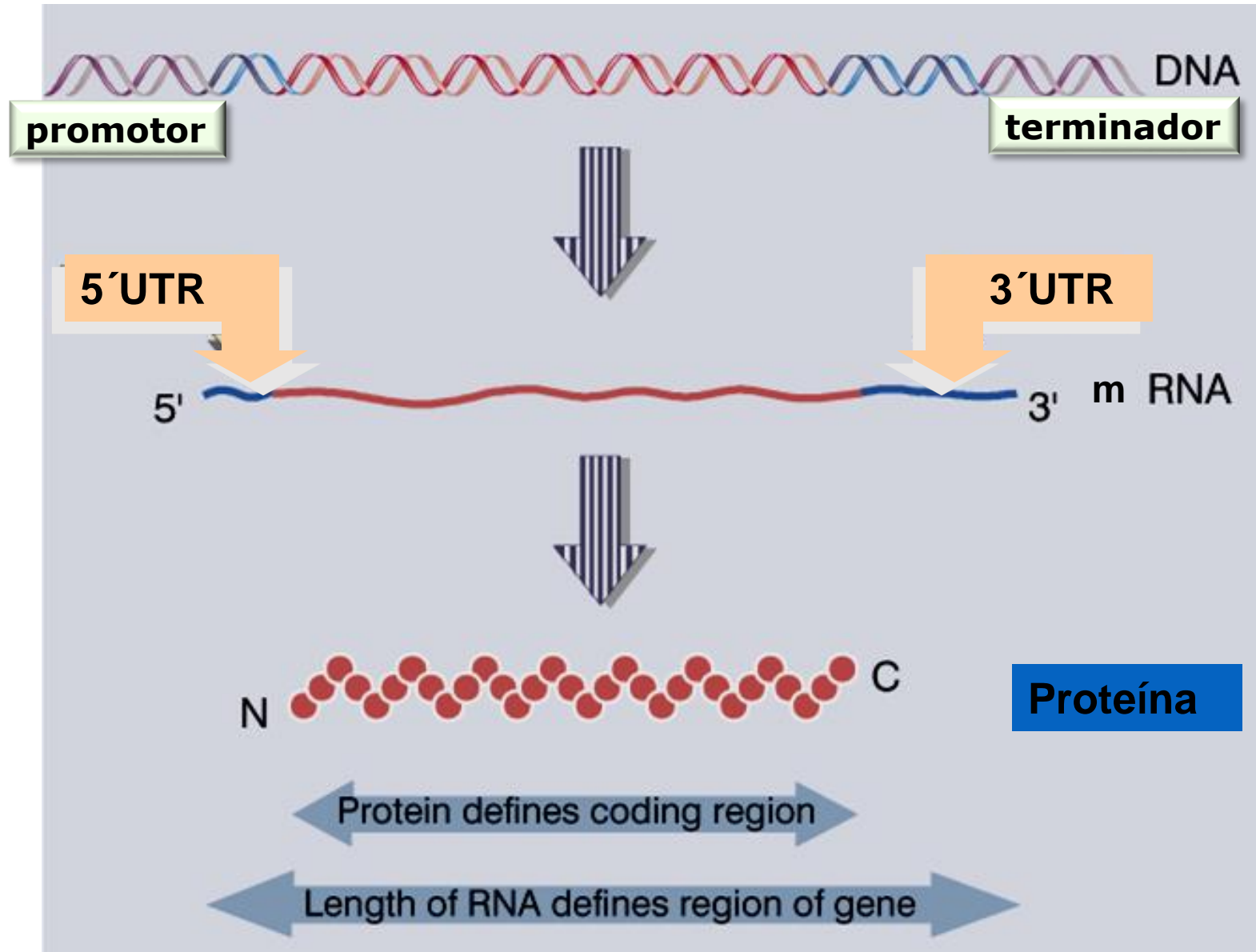
Número de genes (que codificam proteínas)

| organismo | Número de genes (aprox) |
|------------------------------|-------------------------|
| <i>Mycoplasma genitalium</i> | 500 |
| <i>Escherichia coli</i> | 4.000 |
| Levedura | 6.000 |
| <i>C. elegans</i> (verme) | 13.000 |
| Mosca | 20.000 |
| Camundongo | 20.000 |
| Humanos | 20.000 |
| Tomate | 36.000 |
| Arroz | 46.000 |



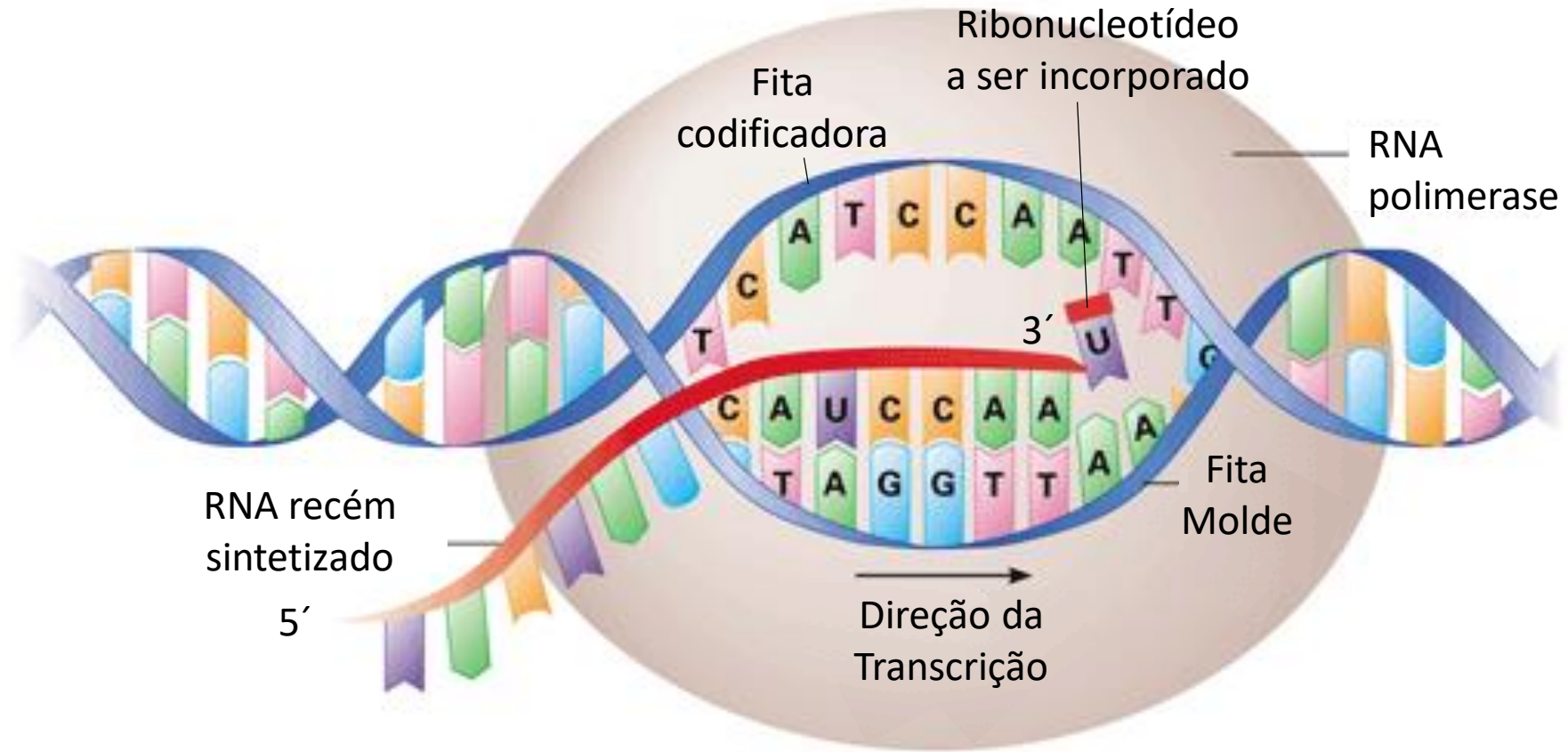
1,5% do genoma codifica proteína
Resto: íntrons (RNA não-traduzido)
regulação genes
função estrutural
50% DNA repetitivo (lixo???)

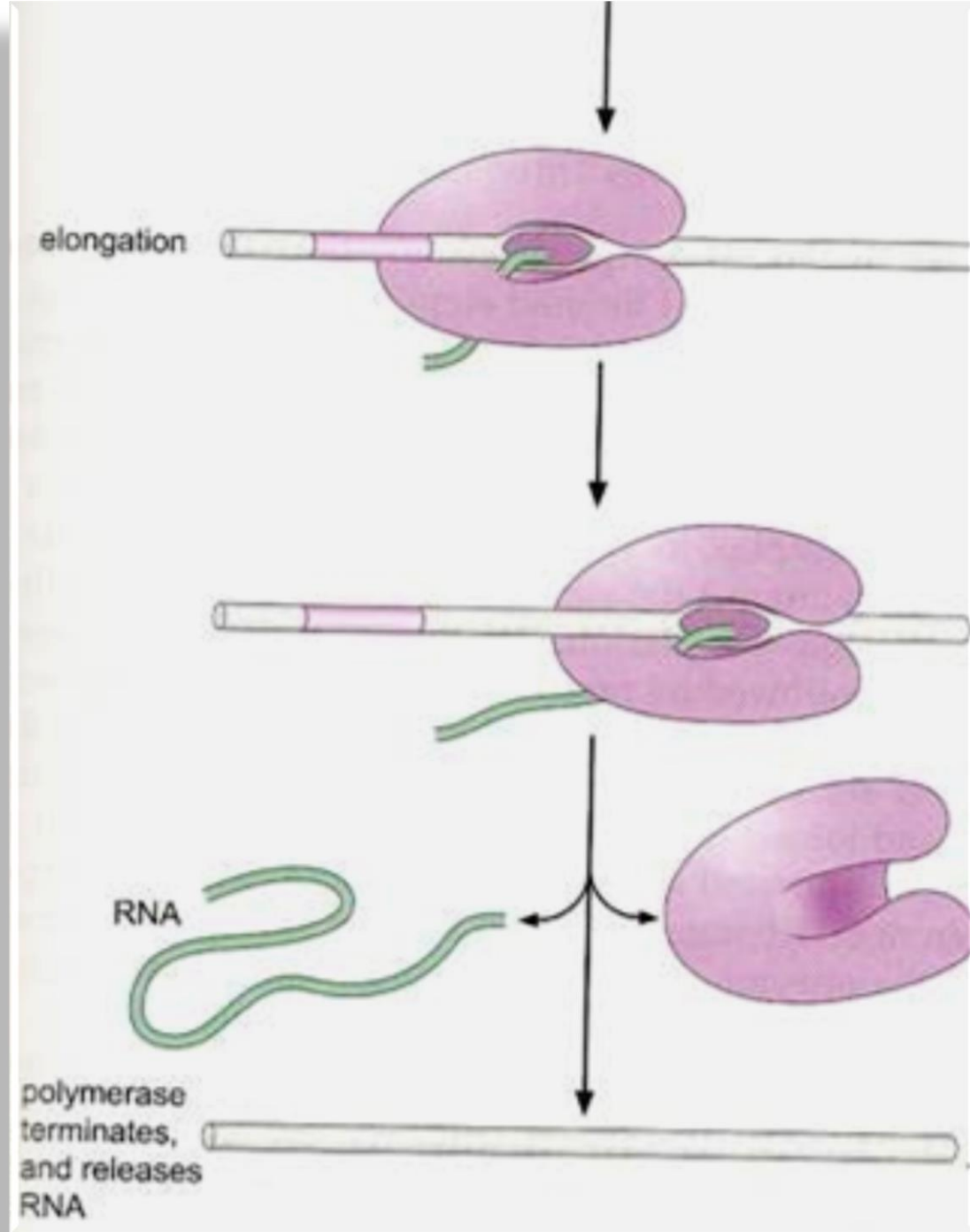
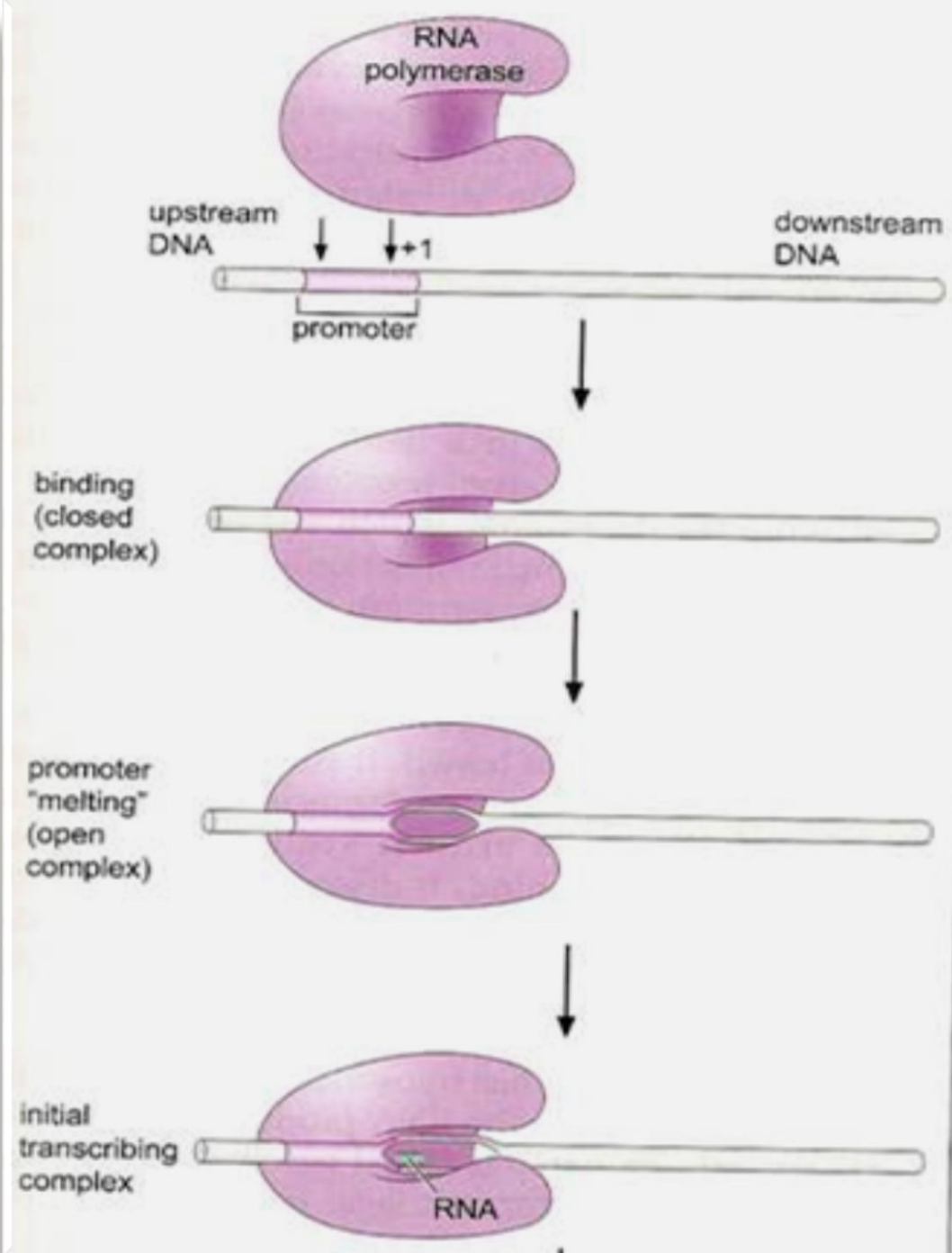
Um gene (simplificado)



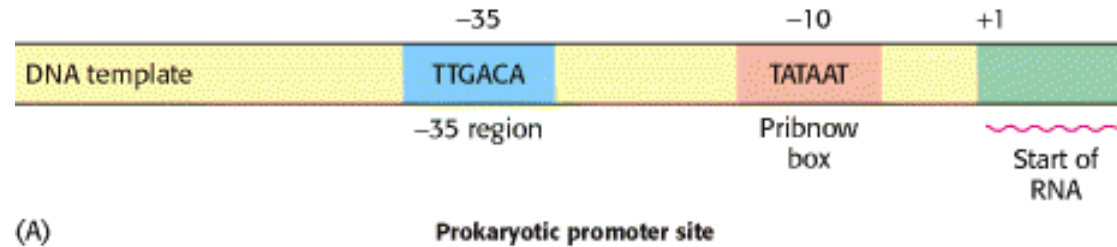
UTR: Região não traduzida (Untranslated region)

RNA polimerase





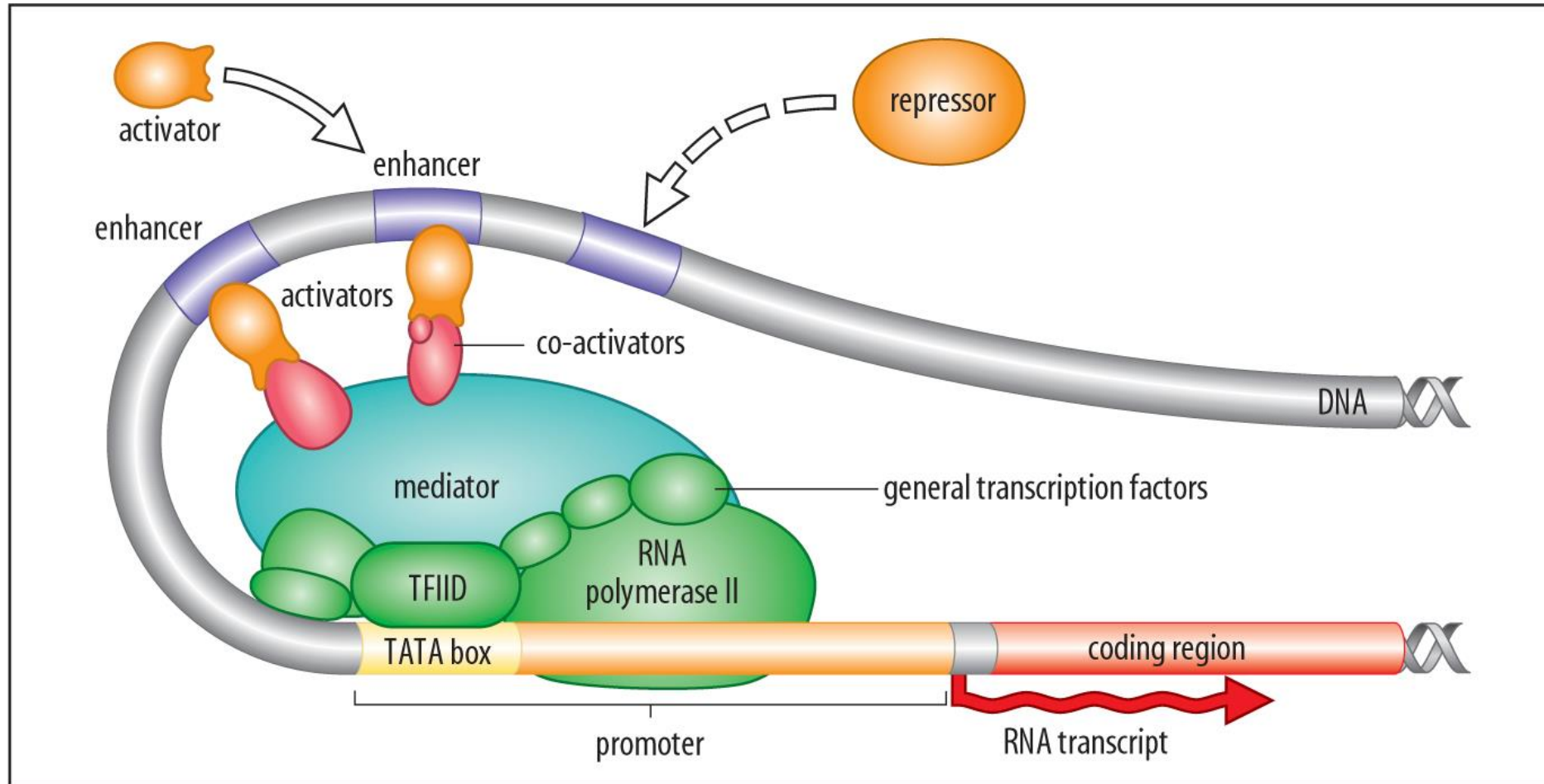
Promotores indicam para RNA polimerase onde começar



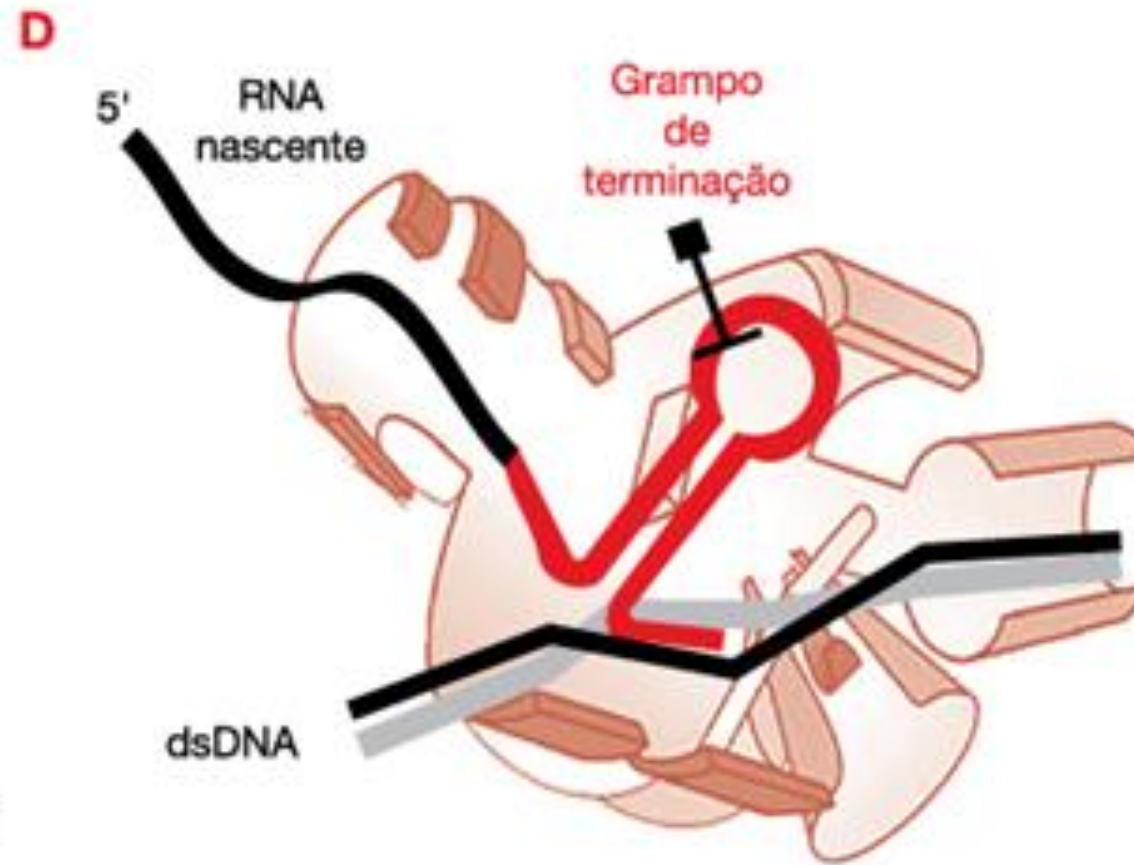
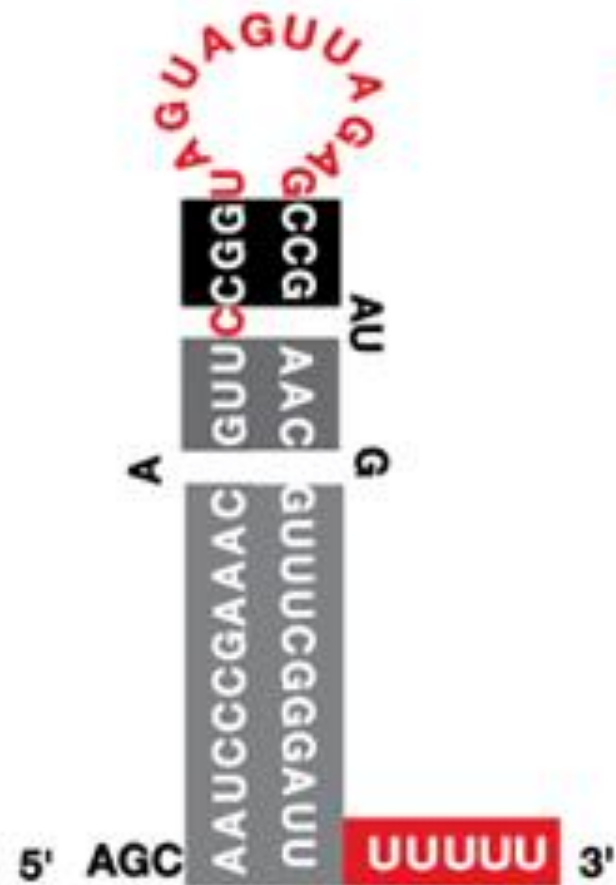
(a) Strong *E. coli* promoters

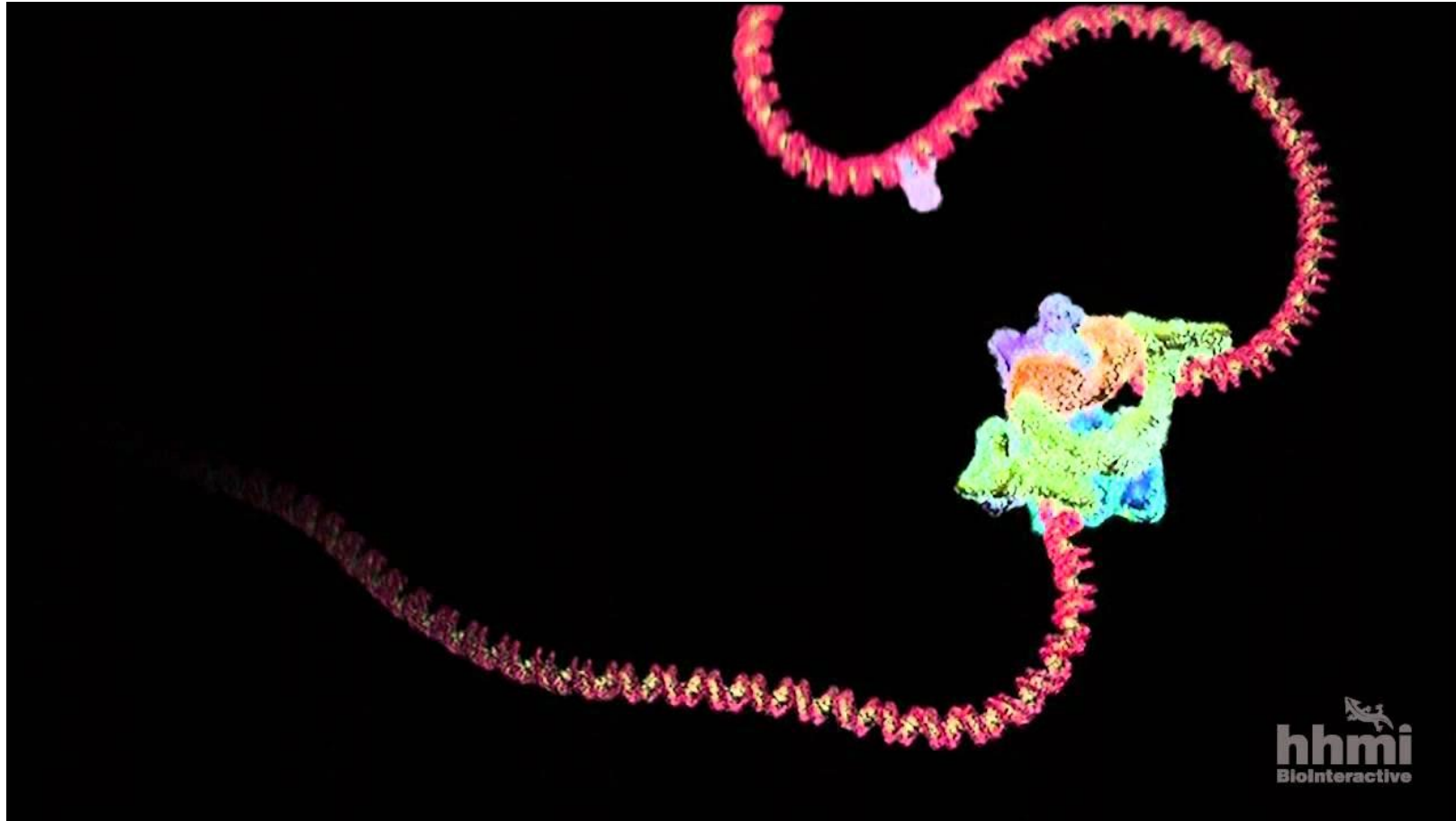
| | | | | | |
|------------------------|----------------|--------------------------|-------------------|----------------------|----------------------|
| tyr tRNA | TCTCAACGTAACAC | TTACAGCGGCG | CGTCATTTGA | TATGATGC | GCCCCGCTTCCCGATAAGGG |
| rrn D1 | GATCAAAAAAATAC | TTGTGCAAAAAA | TTGGGATCCC | TATAATGCGCCTCCG | TTGAGACGACAACG |
| rrn X1 | ATGCATTTTTCCGC | TTGTCTTCTCTGA | GCCGACTCCC | TATAATGCGCCTCCA | TCGACACGGCGGAT |
| rrn (DXE) ₂ | CCTGAAATTCAGGG | TTGACTCTGAAA | GAGGAAAGCG | TAATATAC | GCCACCTCGCGACAGTGAGC |
| rrn E1 | CTGCAATTTTTCTA | TTGCGGCCTGCG | GAGAACTCCC | TATAATGCGCCTCCA | TCGACACGGCGGAT |
| rrn A1 | TTTTAAATTTCTCT | TTGTCAAGCCGG | AATAACTCCC | TATAATGCGCCACCA | CTGACACGGAACAA |
| rrn A2 | GCAAAAATAAATGC | TTGACTCTGTAG | CGGGAAGGCG | TATTATGC | ACACCCGCGCCGCTGAGAA |
| λ P _R | TAACACCGTGCGTG | TTGACTATTTTA | CCTCTGGCGGTGATAAT | GG | TTGCATGTAATAAGGAGGT |
| λ P _L | TATCTCTGGCGGTG | TTGACATAAATA | CCACTGGCGGTGATACT | GA | GCACATCAGCAGGACGCAC |
| T7 A3 | GTGAAACAAAACGG | TTGACAACATGA | AGTAAACACGG | TACGATGT | ACCACATGAAACGACAGTGA |
| T7 A1 | TATCAAAAAGAGTA | TTGACTTAAAGT | CTAACCTATAGGATACT | TA | CAGCCATCGAGAGGGACACG |
| T7 A2 | ACGAAAACAGGTA | TTGACAACATGAAGTAACATGCAG | TAAGATAC | AAATCGCTAGGTAACACTAG | |
| fd VIII | GATACAAATCTCCG | TTGTACTTTTGT | TCGCGCTTGG | TATAATCG | CTGGGGTCAAAGATGAGTG |
| | | -35 | | -10 | +1 |

Fatores de transcrição regulam a ativação da RNA polimerase



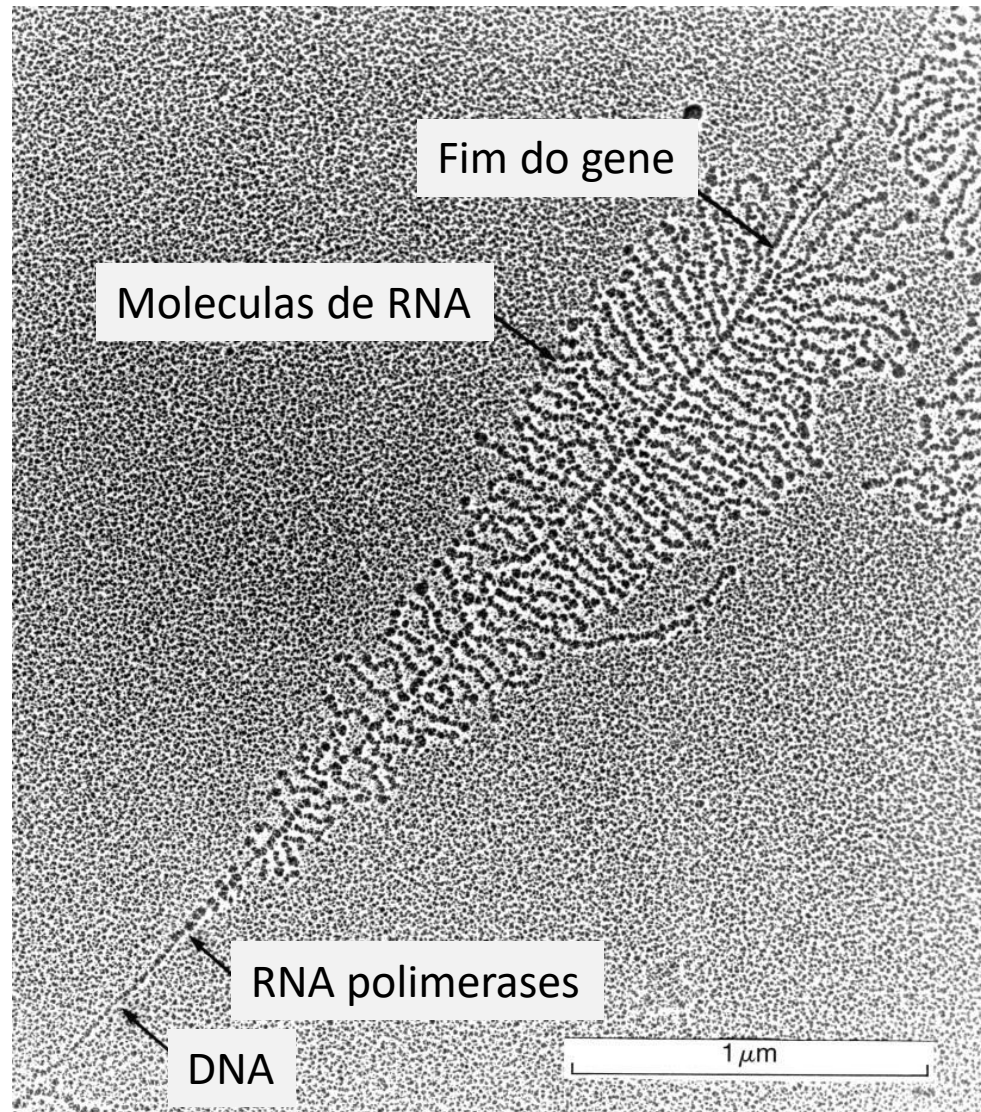
Locais de terminação da transcrição também envolvem sequências específicas





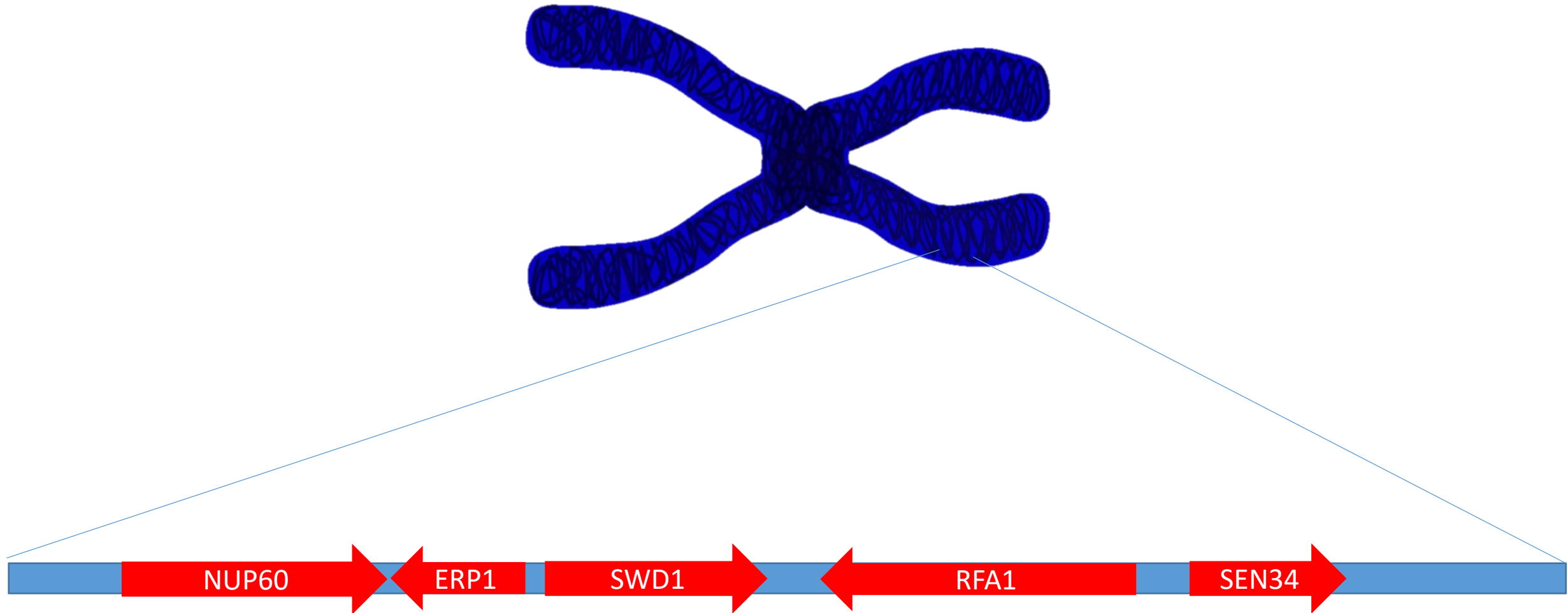
https://www.youtube.com/watch?v=8M198uHJd_8

Diversas RNA polimerases podem transcrever o mesmo gene ao mesmo tempo



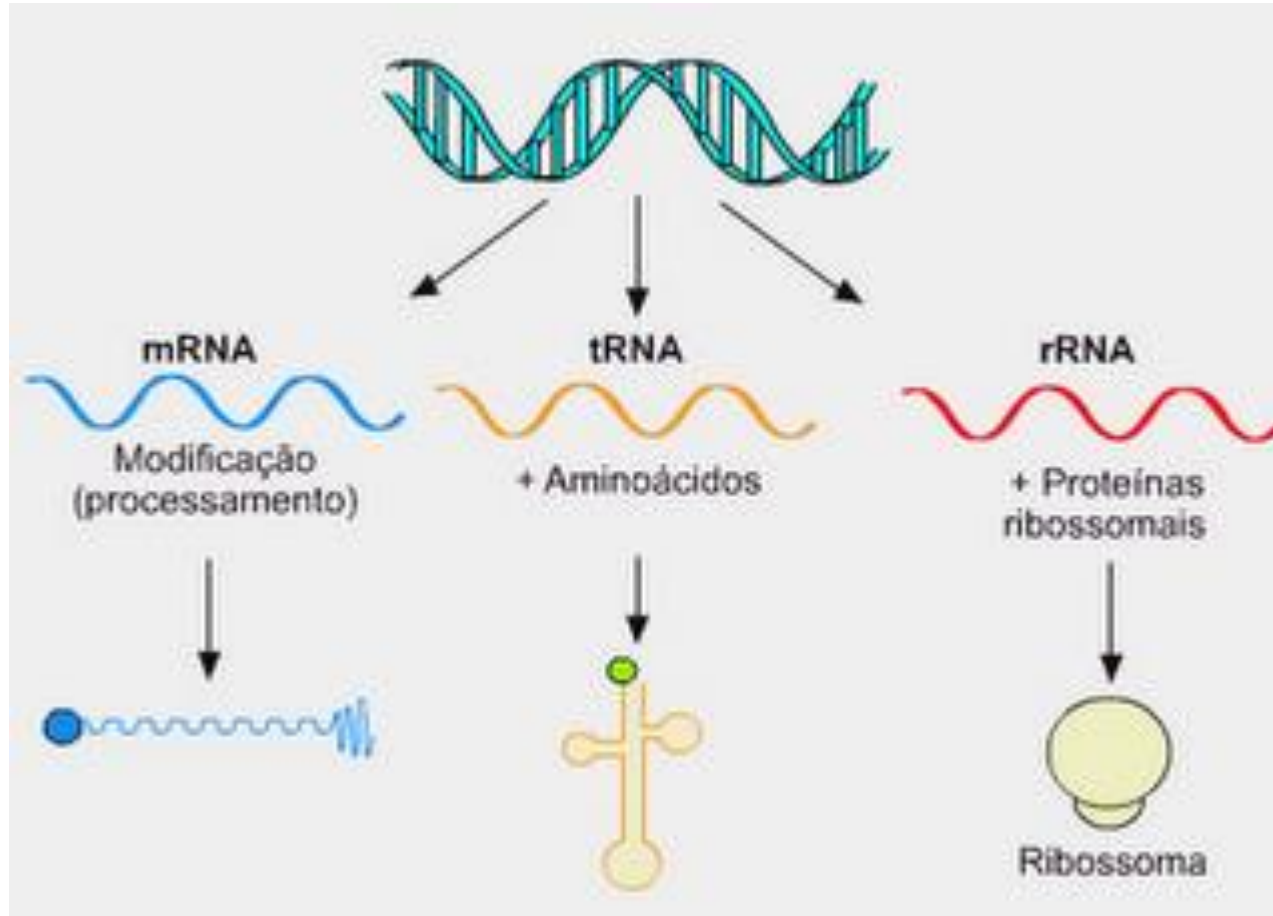
Miller, 1969

Genes podem estar ser transcritos de qualquer uma das duas fitas de DNA



20kb do Cromossomo 1 de levedura

Nem todo RNA codifica proteínas



RNAs não-codificadores (siRNA, miRNA, lncRNA, snRNA, snoRNA, piRNA...)

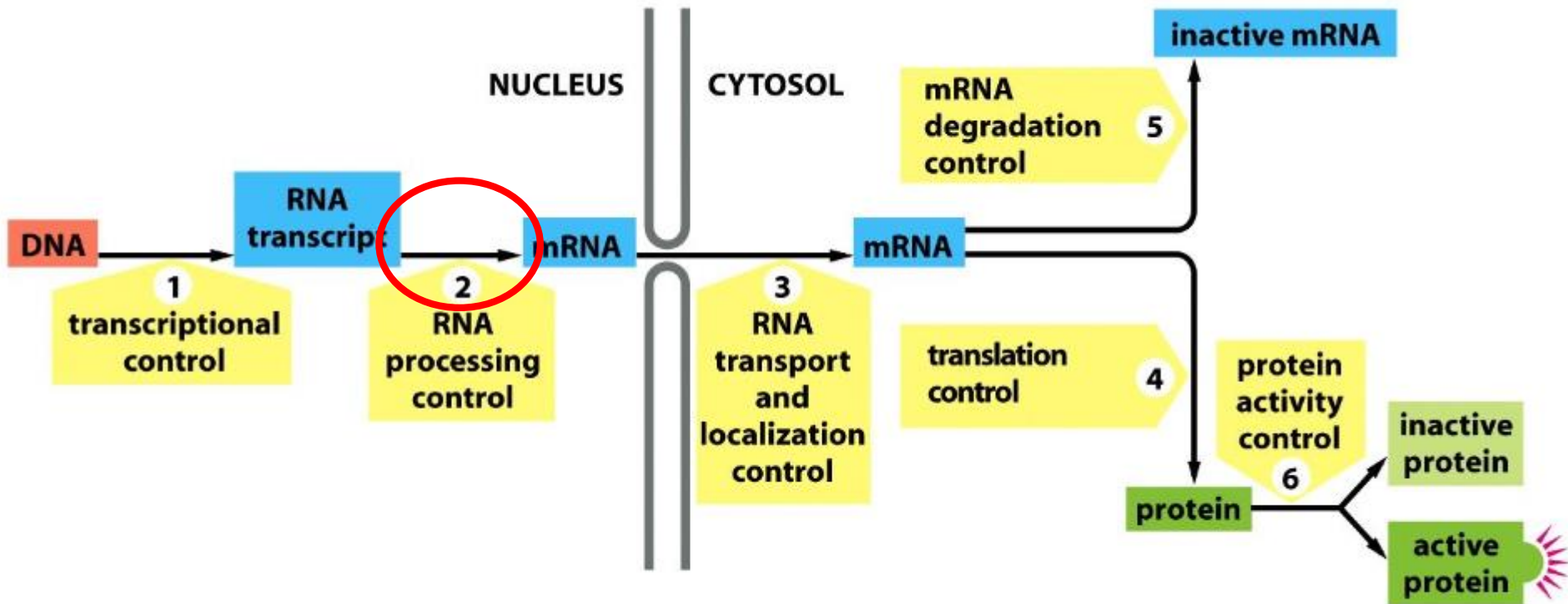
Em eucariotos, há três RNA polimerases diferentes

RNA Polimerase I transcreve RNA ribossomal (rRNA)

RNA Polimerase II transcreve RNA mensageiro (mRNA)

RNA Polimerase III transcreve RNA de transferência (tRNA)

Regulação da Expressão Gênica

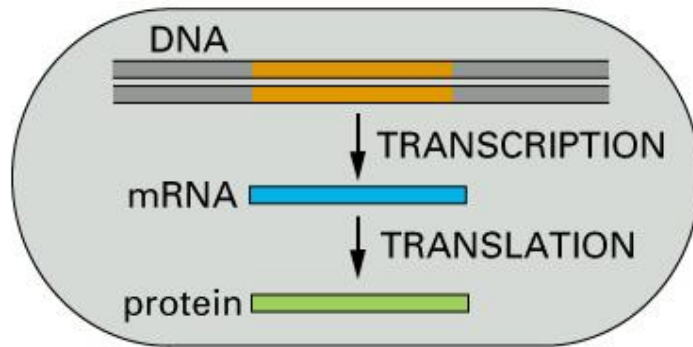


Tempo até resposta biológica

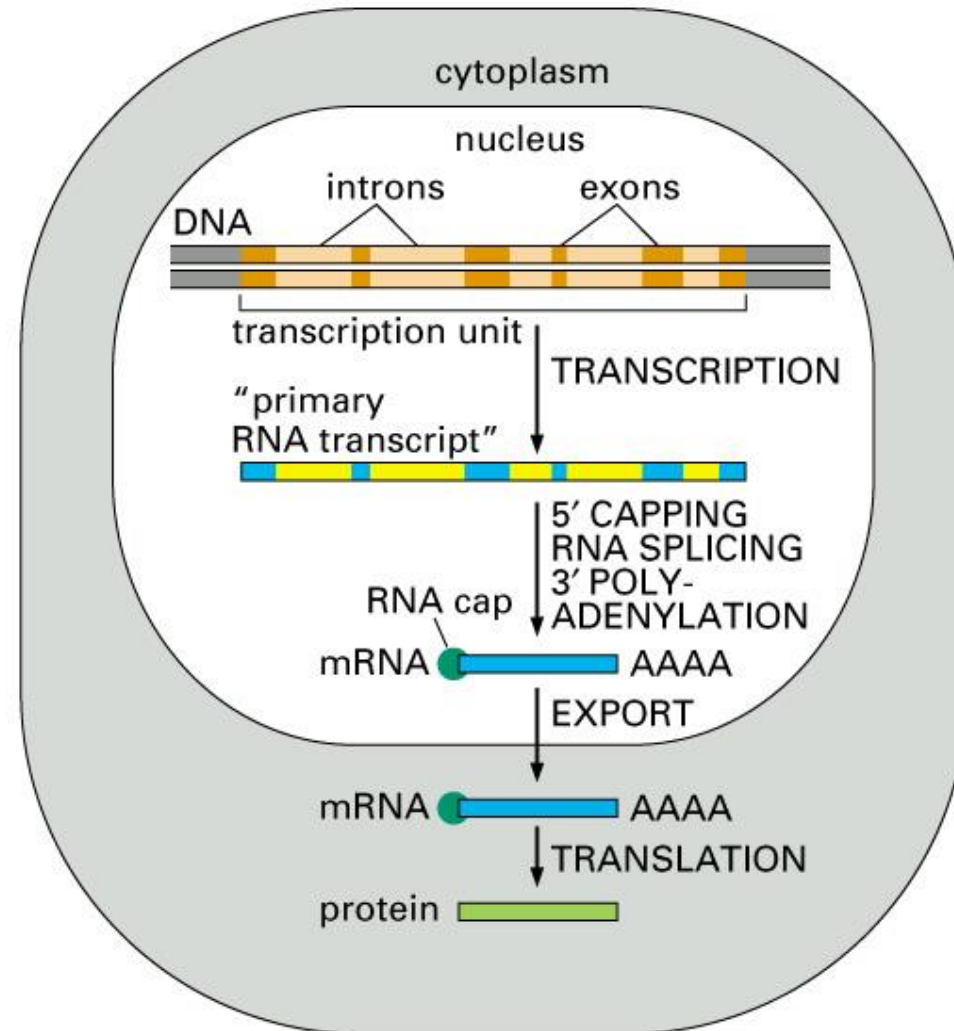
Custo energético

Em eucariotos, o RNA mensageiro é processado

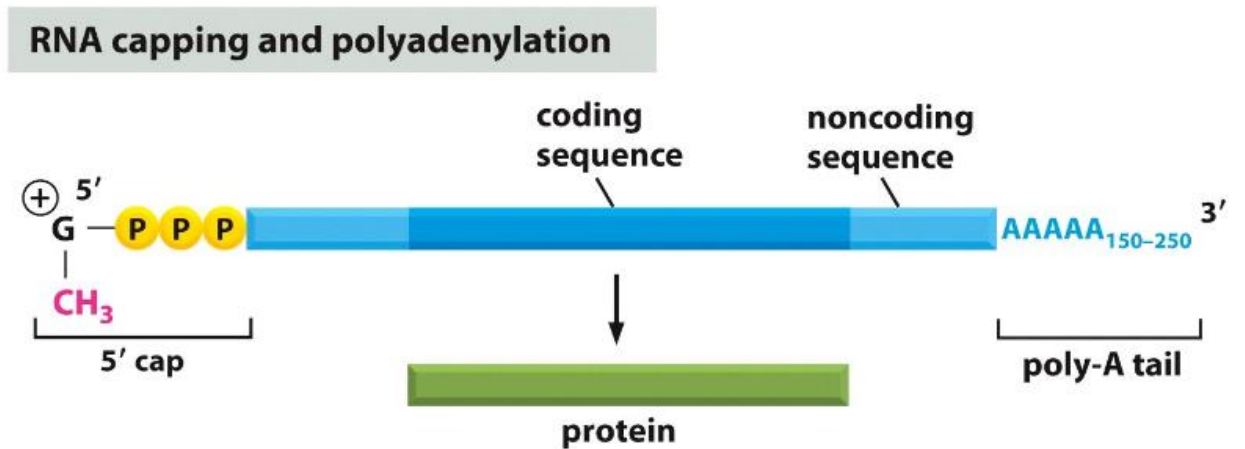
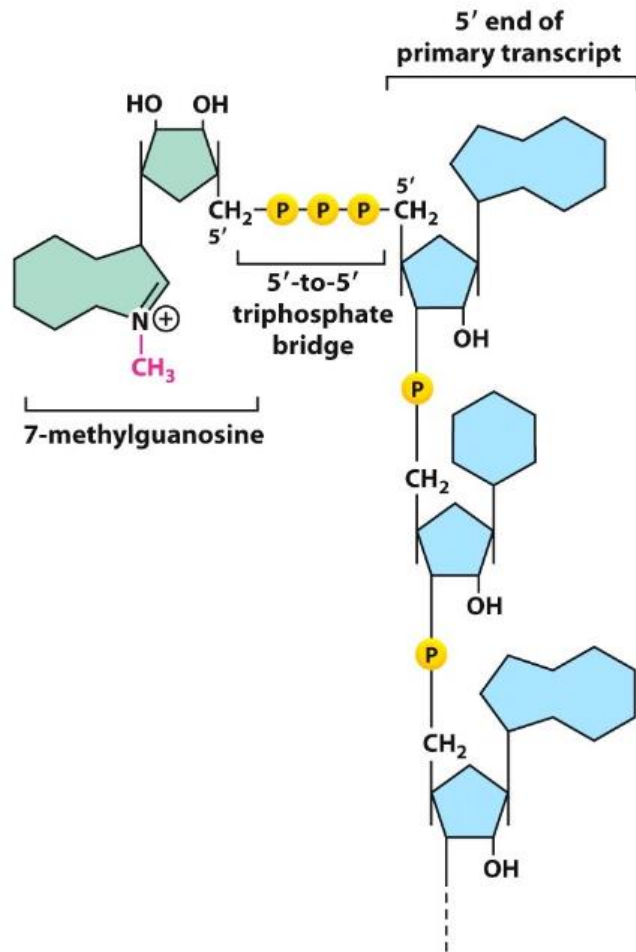
Procaríotos



Eucariotos

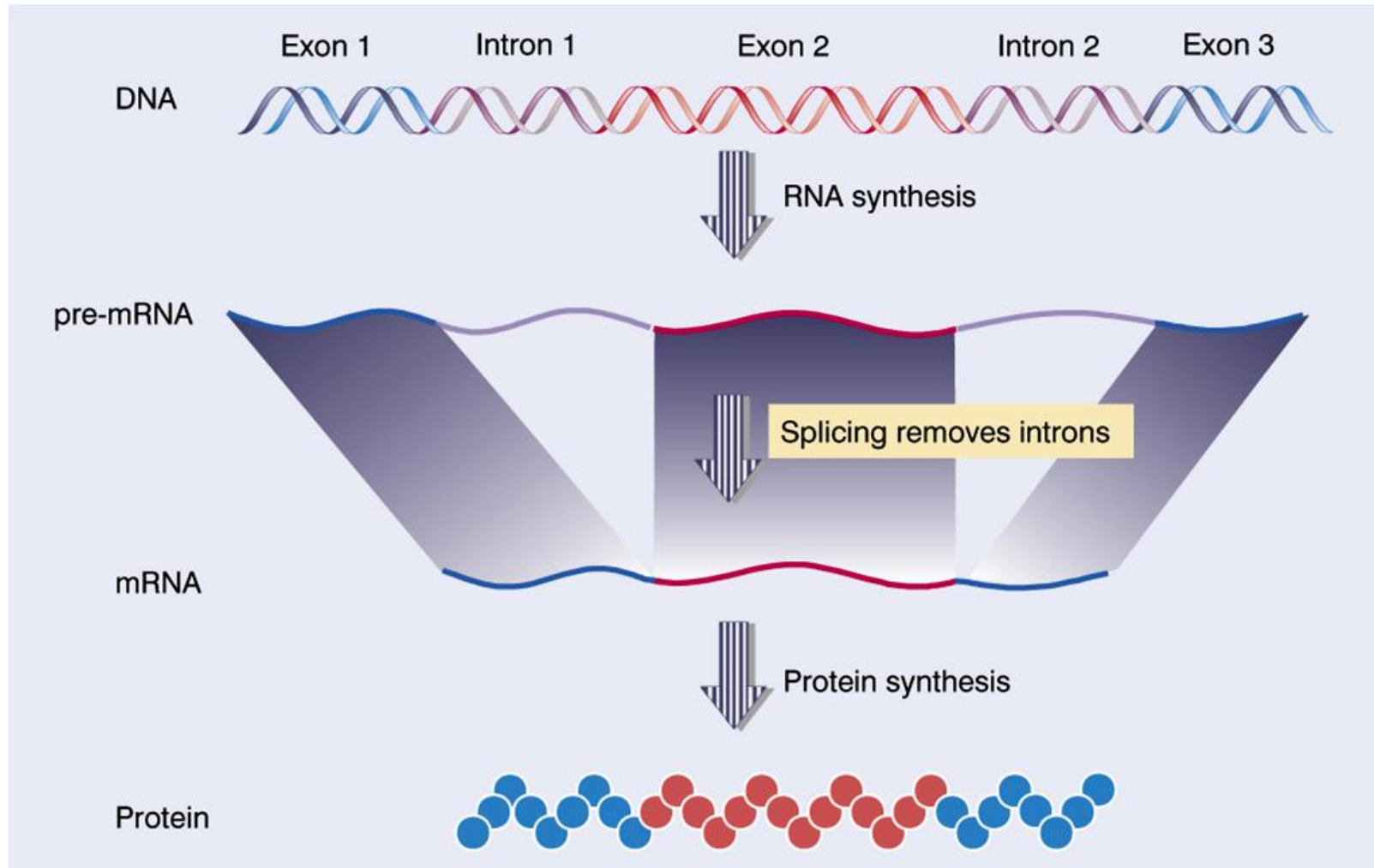


Capping e poli-adenilação de mRNAs

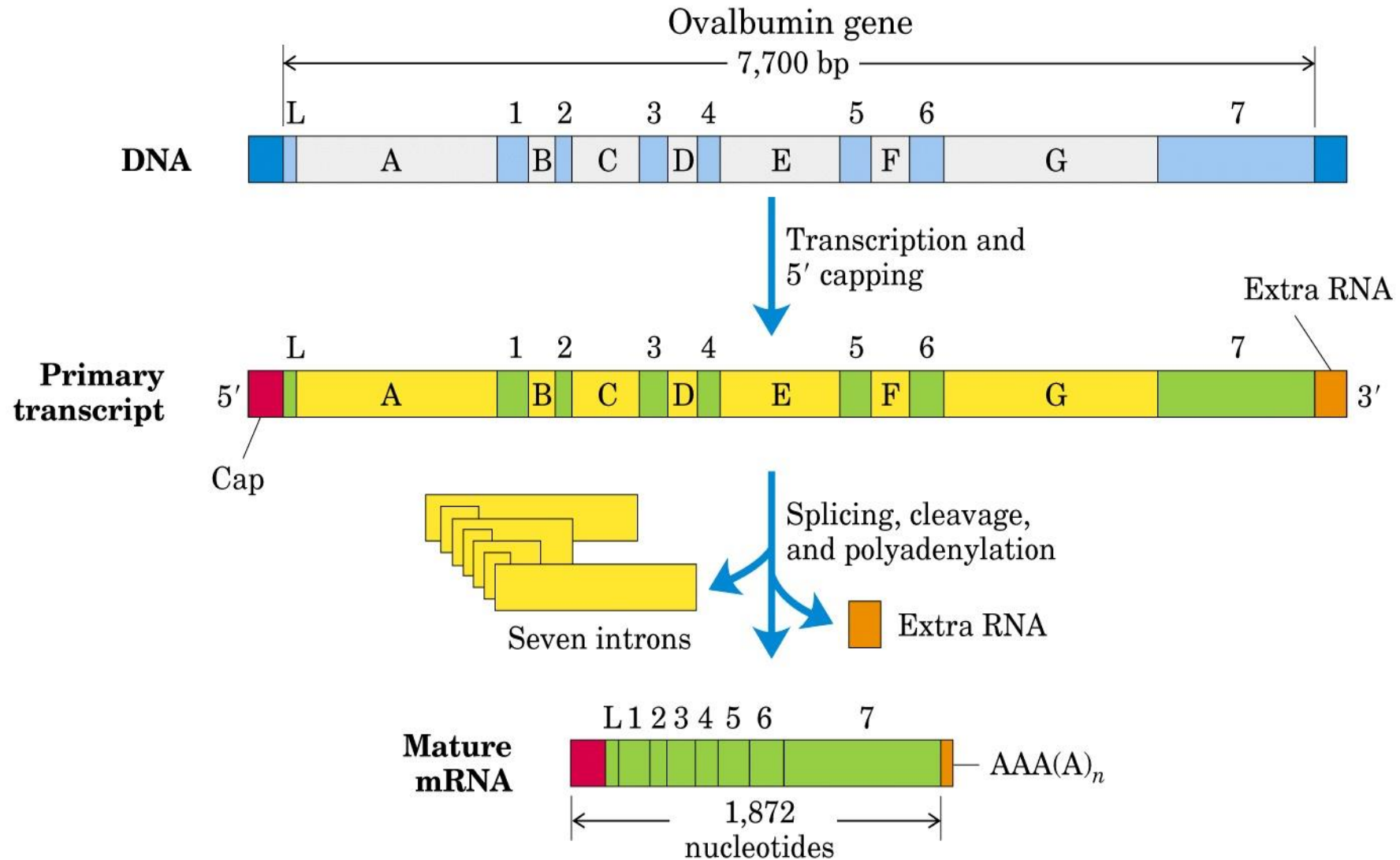


- Protegem RNA de degradação
- Regulam meia-vida do mRNA
- Facilitam identificação de mRNA intacto
- Identificam mRNA para transporte ao citosol
- Identificam mRNA para tradução pelo ribossomo

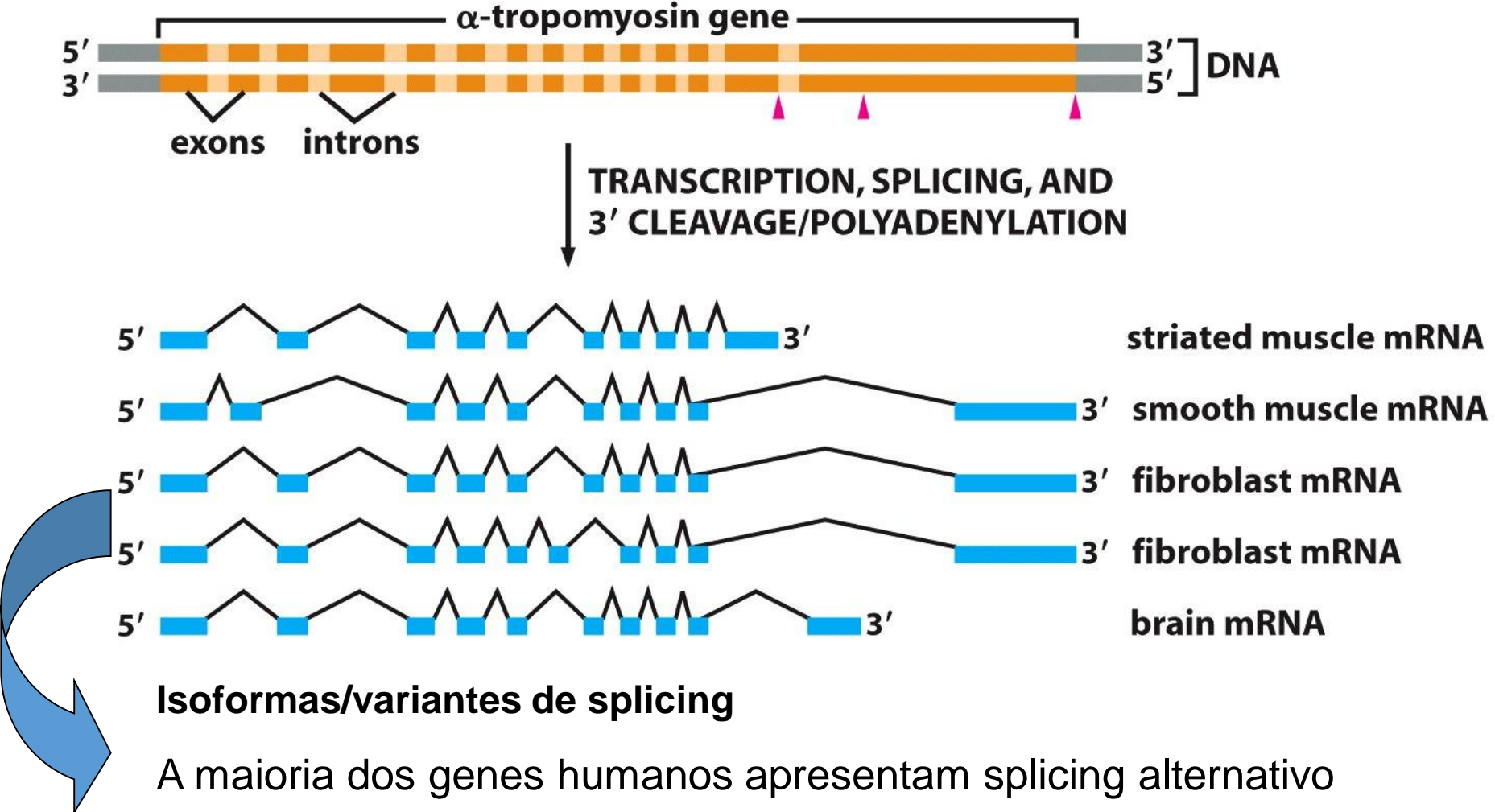
Em eucariotos, o RNA mensageiro é processado



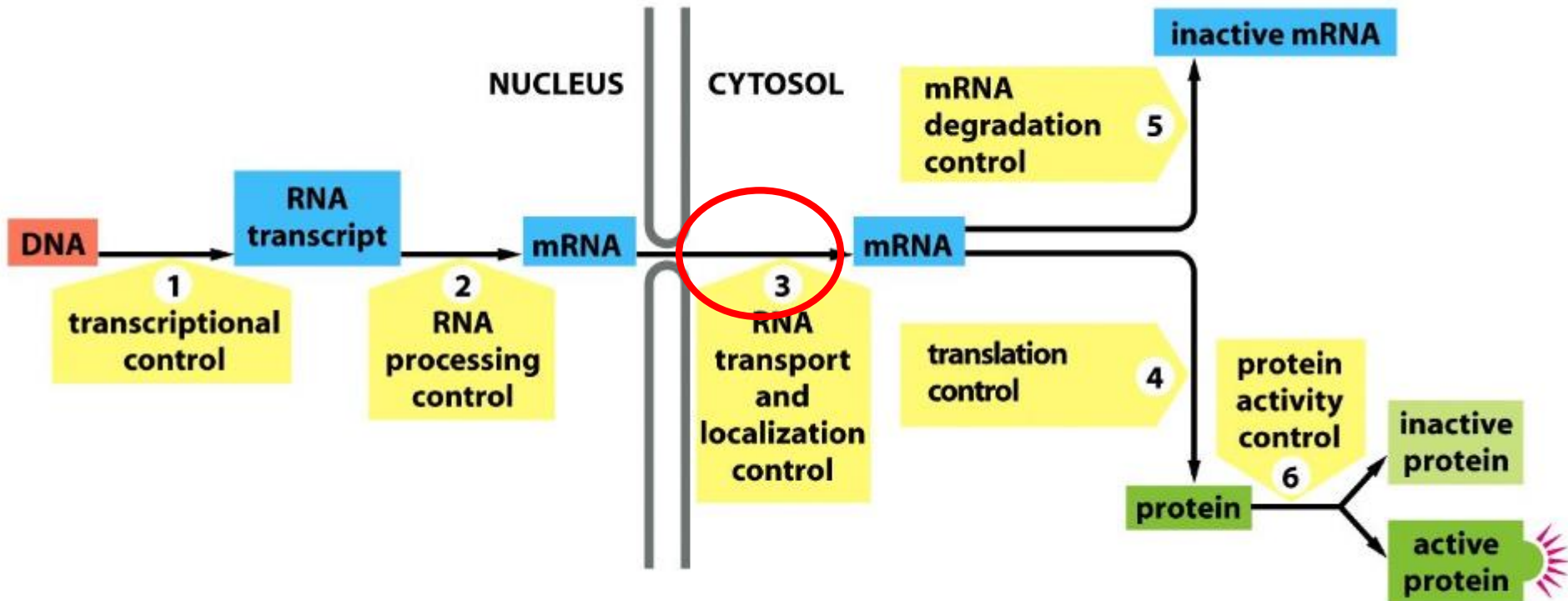
Em eucariotos, o RNA mensageiro é processado



Splicing Alternativo



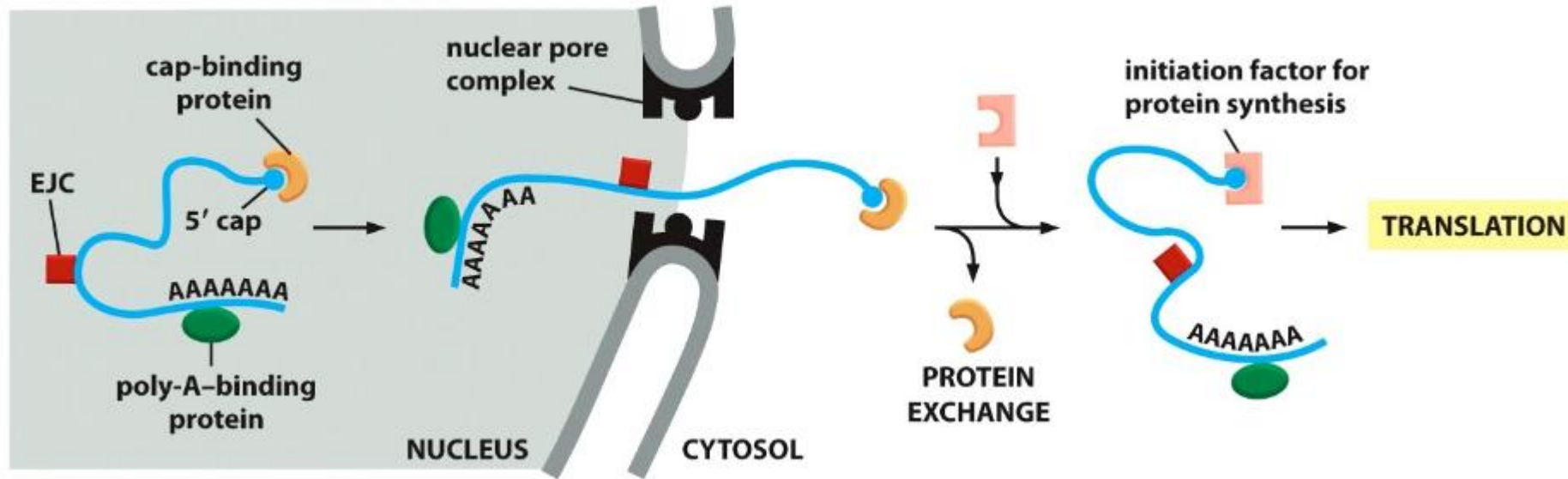
Regulação da Expressão Gênica

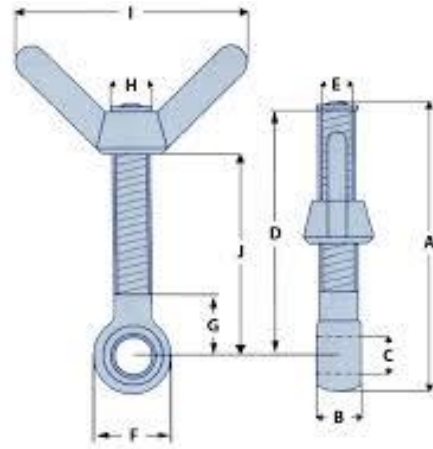
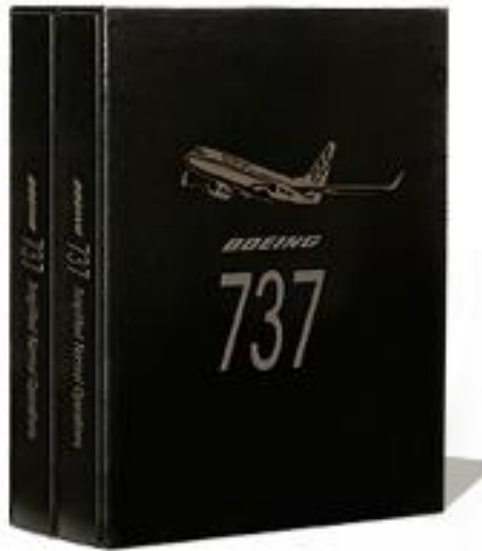
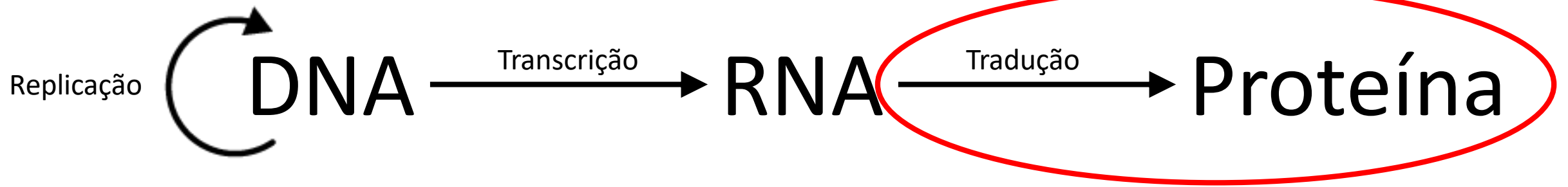


Tempo até resposta biológica

Custo energético

mRNAs maduros são exportados para o citosol





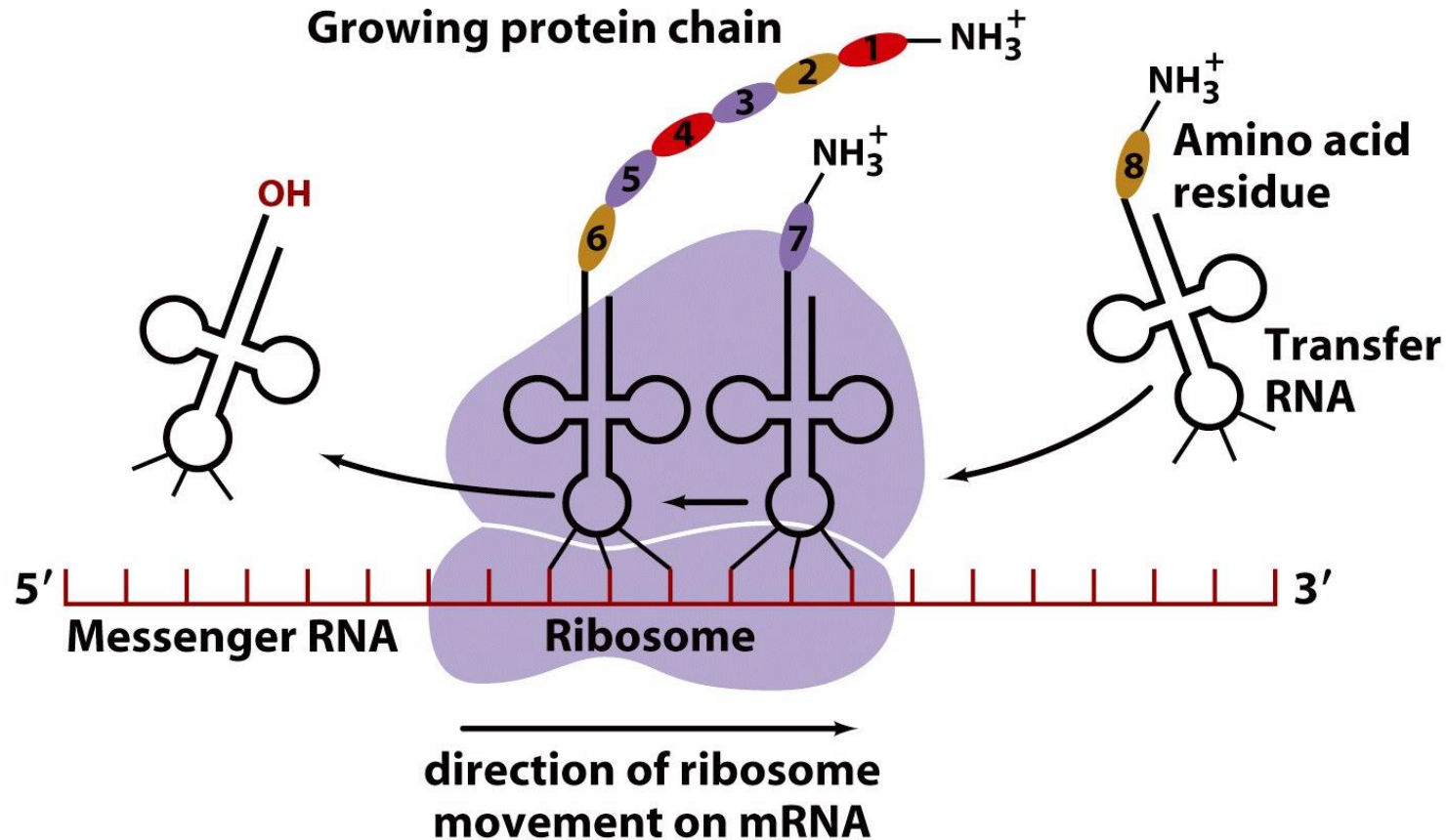
| | | Second base | | | | | | |
|---|------------|-----------------|-----|----------|-----|------|-----|----------|
| | | U | C | A | G | | | |
| U | UUU | Phe | UCU | Ser | UAU | Tyr | UGU | Cys |
| | UUC | F | UCC | S | UAC | Y | UGC | C |
| | UUA | Leu | UCA | STOP | UAA | STOP | UGA | STOP |
| | UUG | L | UCG | STOP | UAG | STOP | UGG | Trp W |
| C | CUU | Leu L | CCU | Pro P | CAU | His | CGU | Arg R |
| | CUC | | CCC | | CAC | H | CGC | |
| | CUA | | CCA | | CAA | Gln | CGA | |
| | CUG | | CCG | | CAG | Q | CGG | |
| A | AUU | Ile Met M | ACU | Thr T | AAU | Asn | AGU | Ser |
| | AUC | | ACC | | AAC | N | AGC | S |
| | AUA | | ACA | | AAA | Lys | AGA | Arg |
| | AUG | | ACG | | AAG | K | AGG | R |
| G | GUU | Val V | GCU | Ala A | GAU | Asp | GGU | Gly G |
| | GUC | | GCC | | GAC | D | GGC | |
| | GUA | | GCA | | GAA | Glu | GGA | |
| | GUG | | GCG | | GAG | E | GGG | |

Código Genético

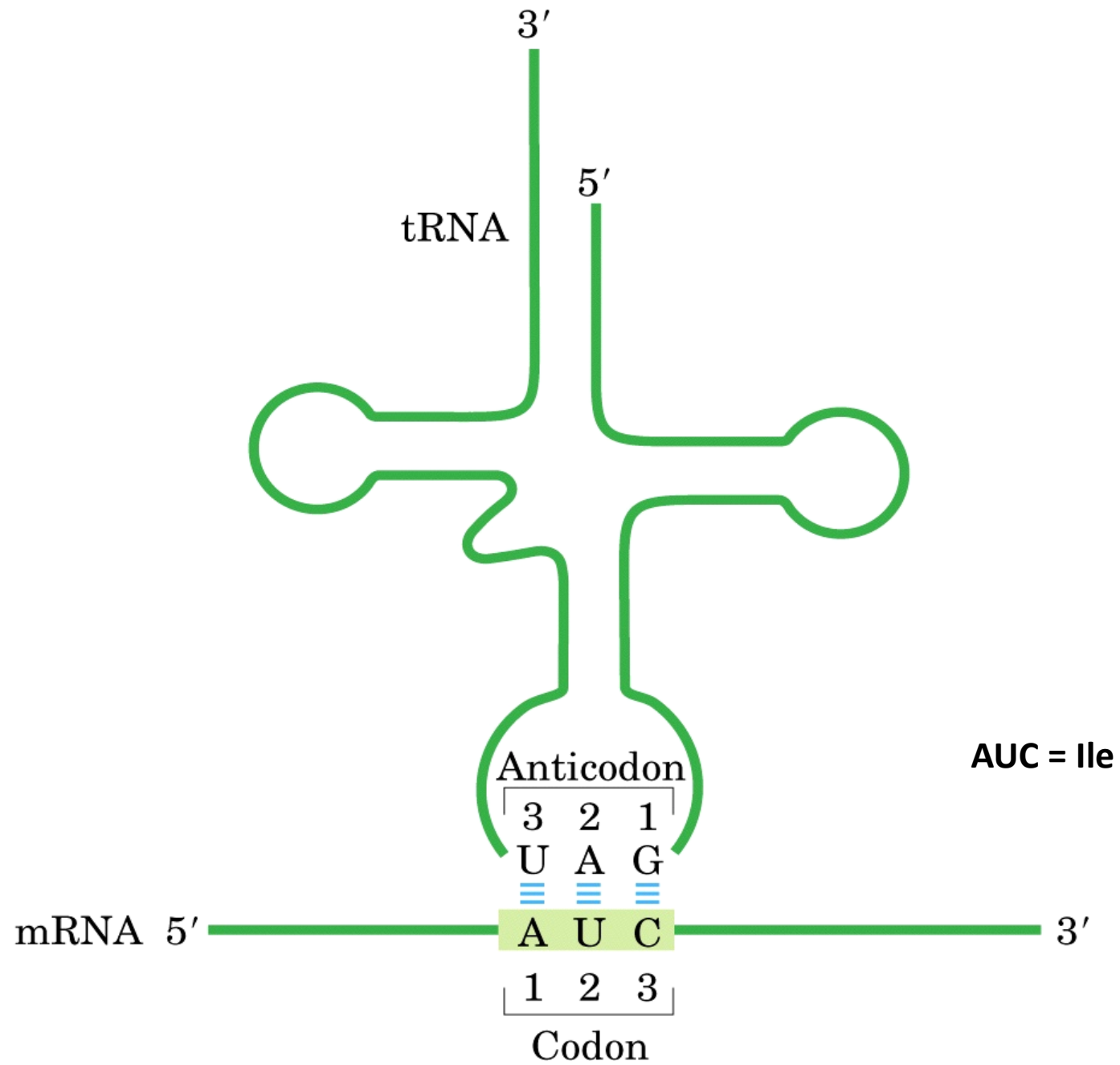
4 bases no DNA x 3
posições = 4^3
= 64 códonos

- 20 aminoácidos
- 3 códonos de terminação
- códon de início é sempre **AUG**

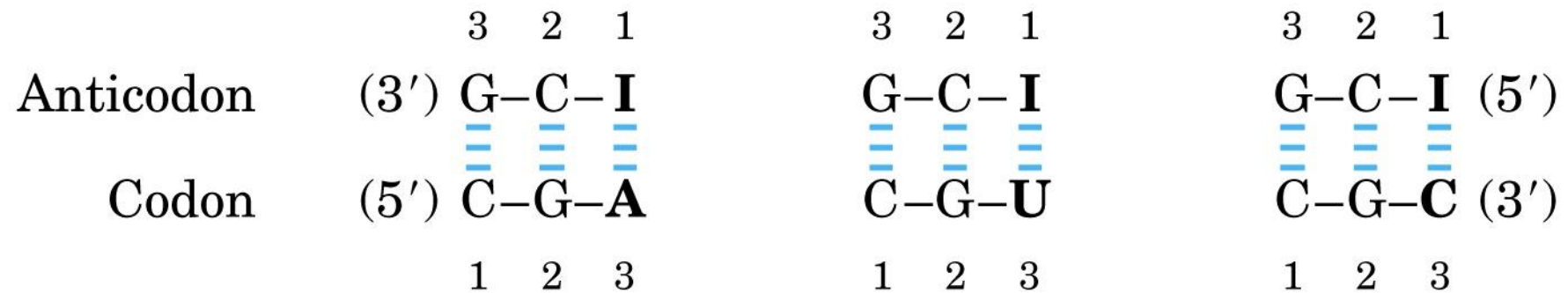
Quem participa da tradução?



- mRNA
- Ribossomos
- Aminoacil-tRNAs
- Fatores de tradução



Pareamento oscilante da terceira base do códon (Wobble base-pairing)

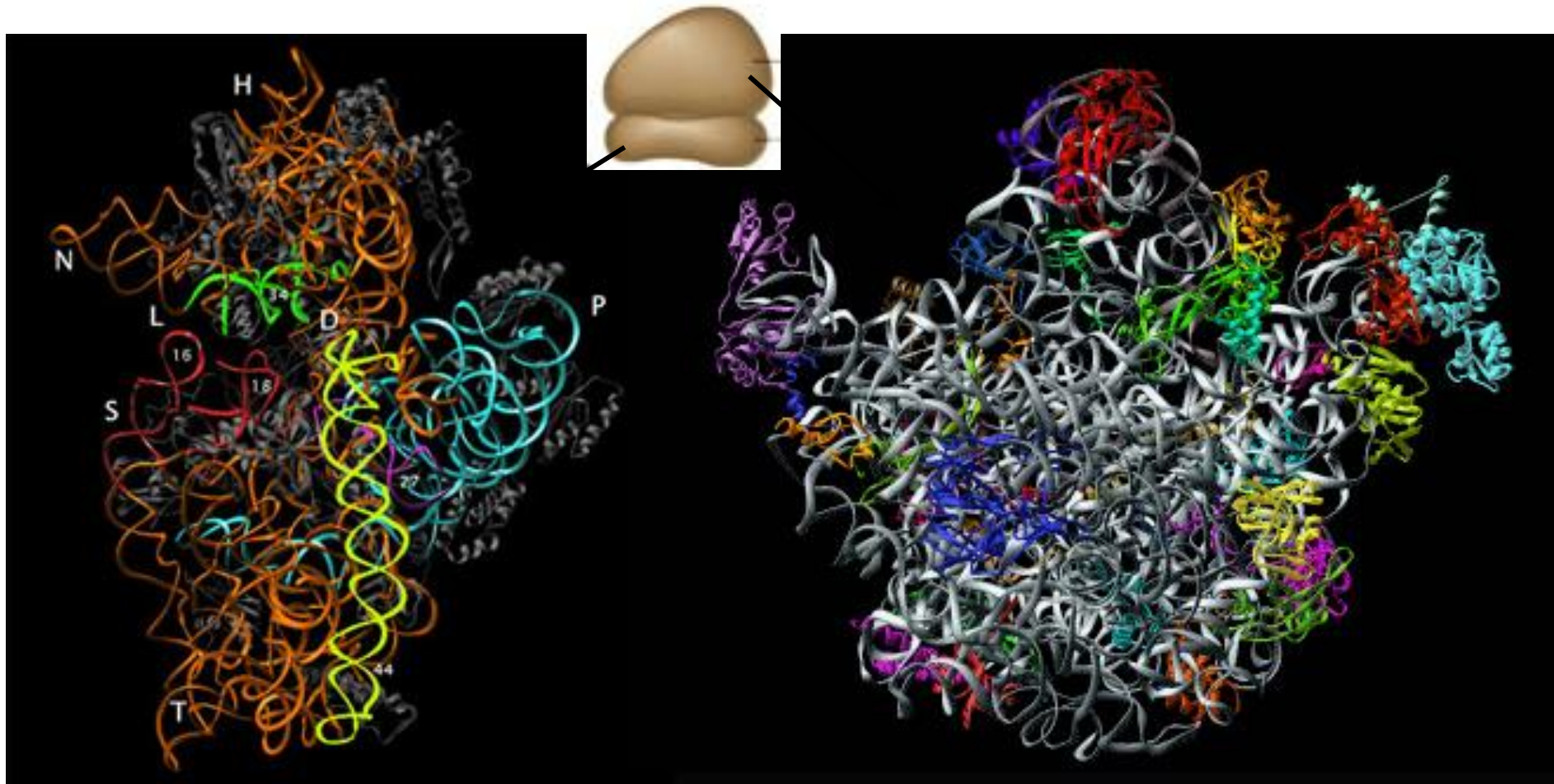


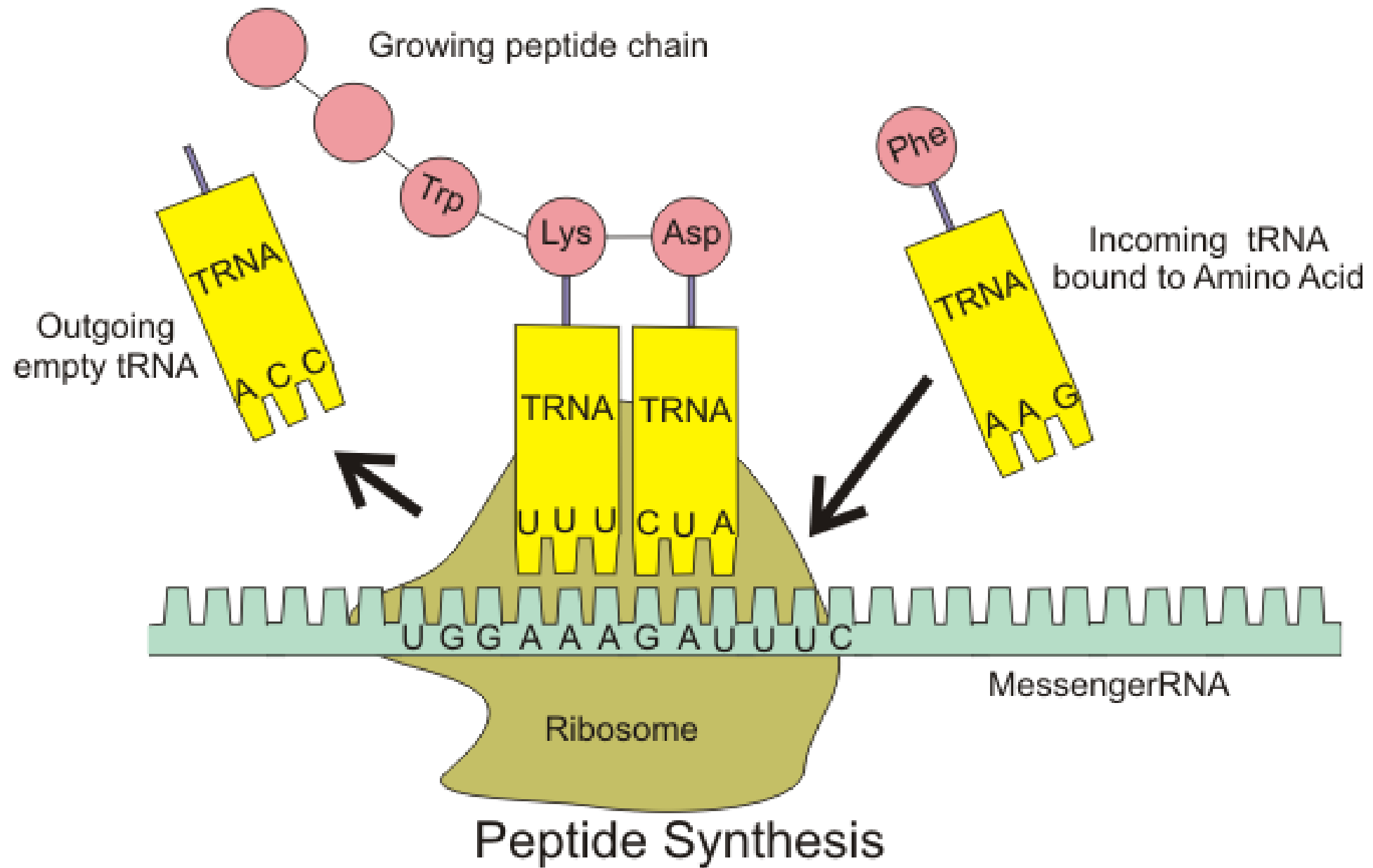
- I = inosinato (nt); hipoxantina (base)
 - ligações de H mais “fracas” com A, U ou C
- Alguns anticódons podem parear com mais de um códon que codifica o mesmo aminoácido
- Permite rápido deslocamento do ribossomo
- Número menor de tRNAs que os 61 códons

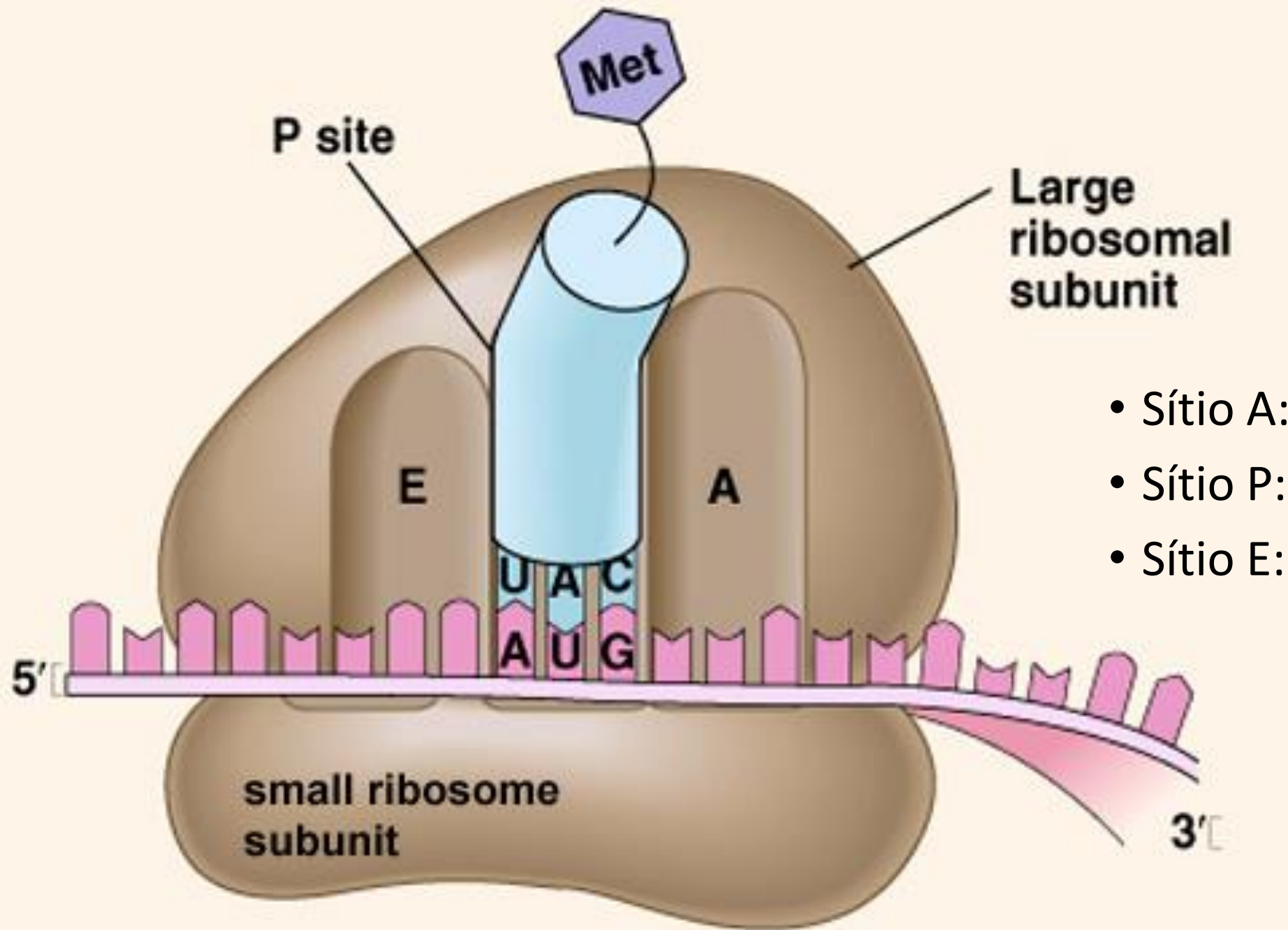
Código Genético

- Universal
 - Praticamente todos os organismos usam a mesma “tabela”
 - Exceção: mitocôndrias, algumas bactérias e ciliados
- Não-sobreposto
 - um códon lido de cada vez
- Degenerado
 - mais de um códon por aminoácido
- Terceira posição do códon menos específica (“wobble”)

Ribossomo é formado por proteínas e rRNA



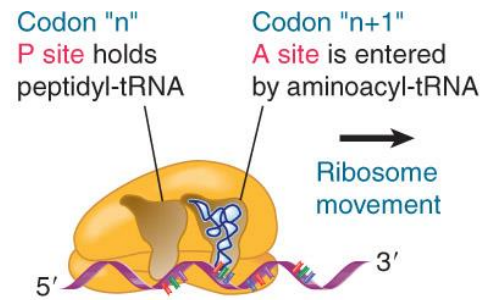




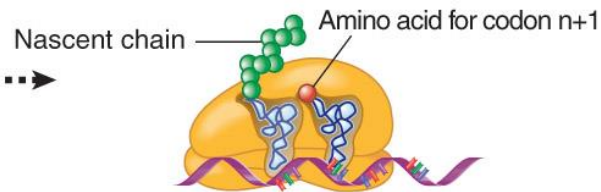
- Sítio A: tRNA-aa
- Sítio P: tRNA-peptídeo
- Sítio E: saída (*exit*)

Etapas da síntese proteica

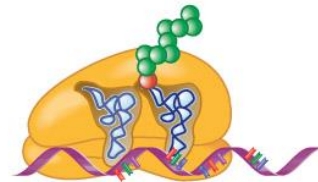
- Ativação do aminoácido
 - Ligação ao tRNA
- Início da tradução
- Elongação
- Terminação
- Dobramento/processamento da proteína pós-tradução



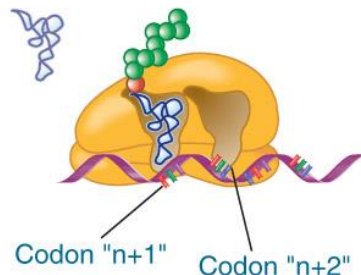
1 Before peptide bond formation peptidyl-tRNA occupies P site; aminoacyl-tRNA occupies A site



2 Peptide bond formation polypeptide is transferred from peptidyl-tRNA in P site to aminoacyl-tRNA in A site



3 Translocation moves ribosome one codon; places peptidyl-tRNA in P site; deacylated tRNA leaves via E site; A site is empty for next aa-tRNA



O ribossomo tem três sítios de ligação ao tRNA

- Sítio A

- entrada do aminoacyl-tRNA

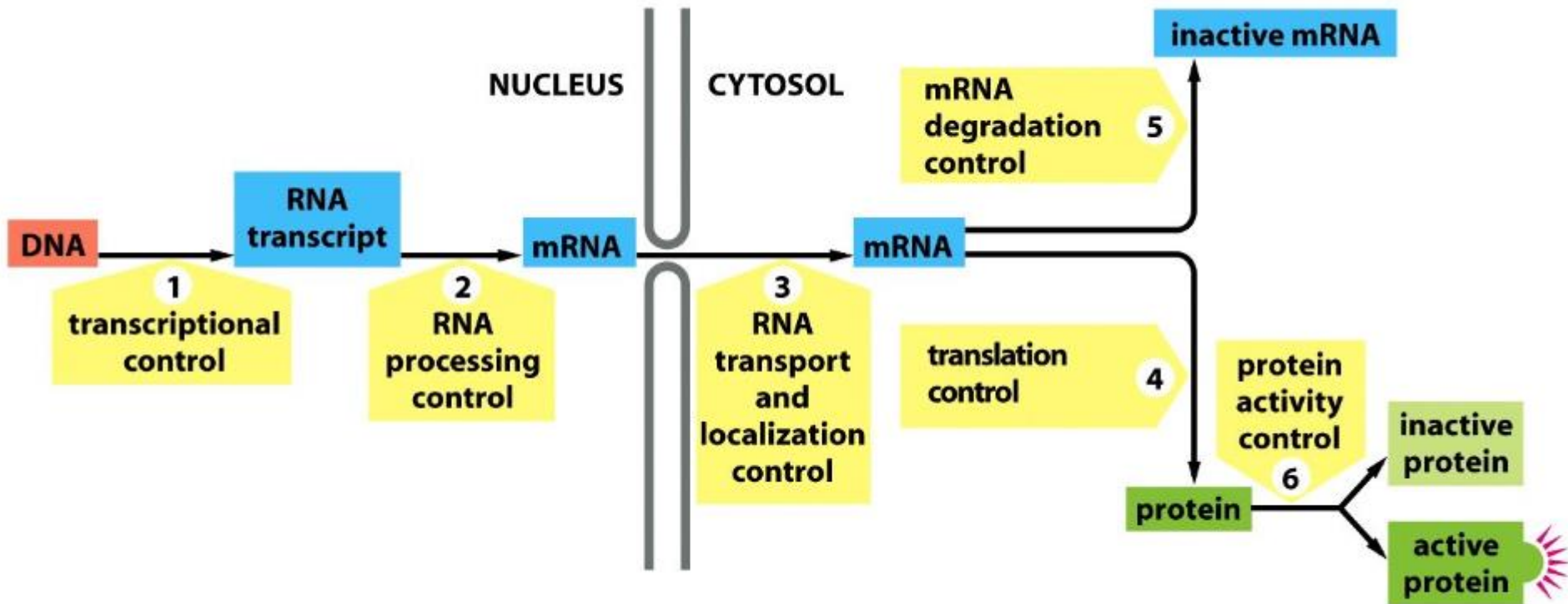
- Sítio P

- onde o peptidil-tRNA se liga (peptídeo nascente ligado ao tRNA)

- Sítio E

- saída do tRNA descarregado (exit)
- Subunidade maior

Regulação da Expressão Gênica

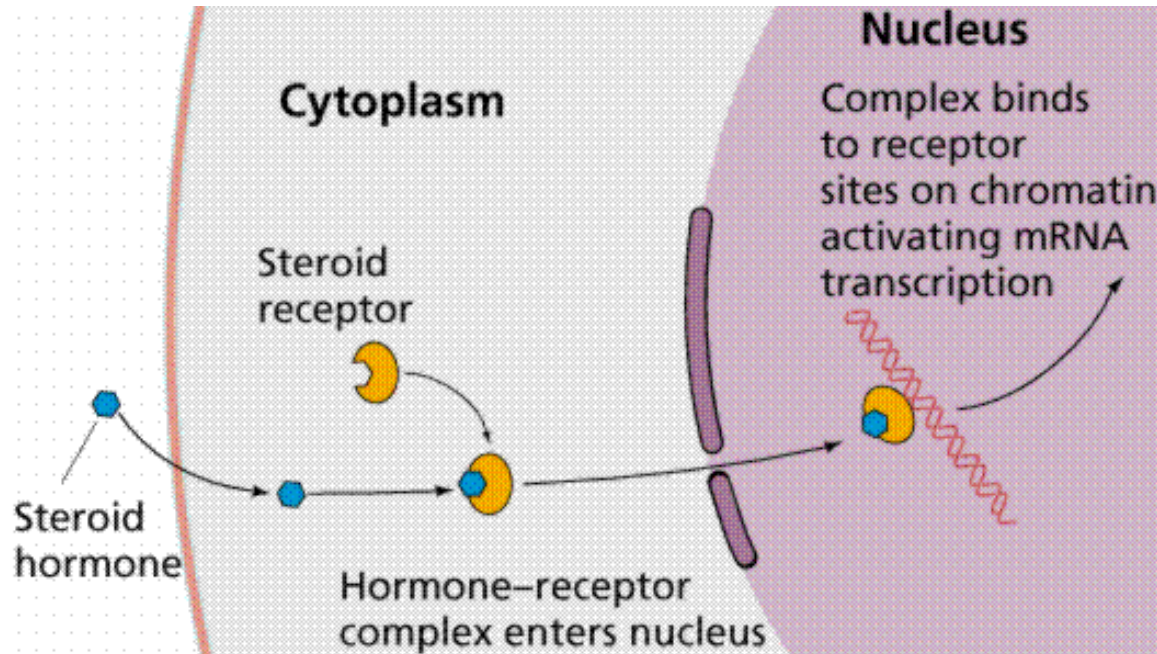


Tempo até resposta biológica

Custo energético

Exemplos de regulação gênica

- Hormônios hidrofóbicos atravessam membrana e ligam receptor
- Receptor translocado para o núcleo, onde liga HRE



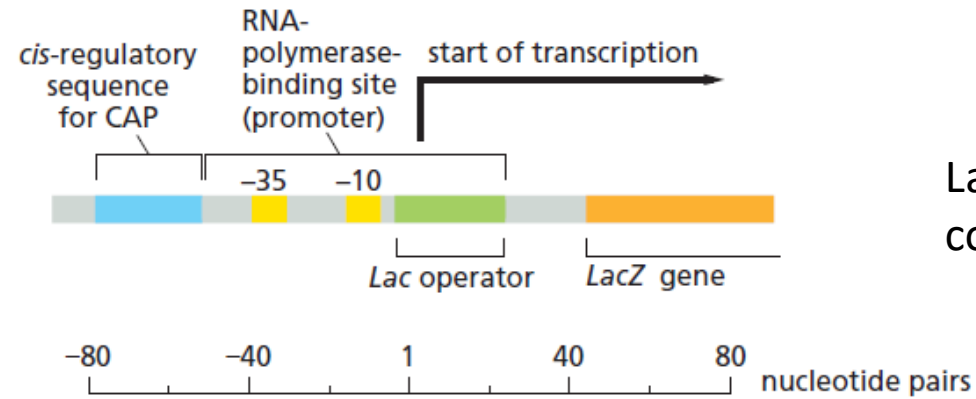
| TABLE 28-4 Hormone Response Elements (HREs) Bound by Steroid-Type Hormone Receptors | |
|--|---------------------------------|
| Receptor | Consensus sequence bound* |
| Androgen | GG(A/T)ACAN ₂ TGTTCT |
| Glucocorticoid | GGTACAN ₃ TGTTCT |
| Retinoic acid (some) | AGGTCAN ₅ AGGTCA |
| Vitamin D | AGGTCAN ₃ AGGTCA |
| Thyroid hormone | AGGTCAN ₃ AGGTCA |
| RX [†] | AGGTCANAGGTCANAG GTCANAGGTCA |

*N represents any nucleotide.

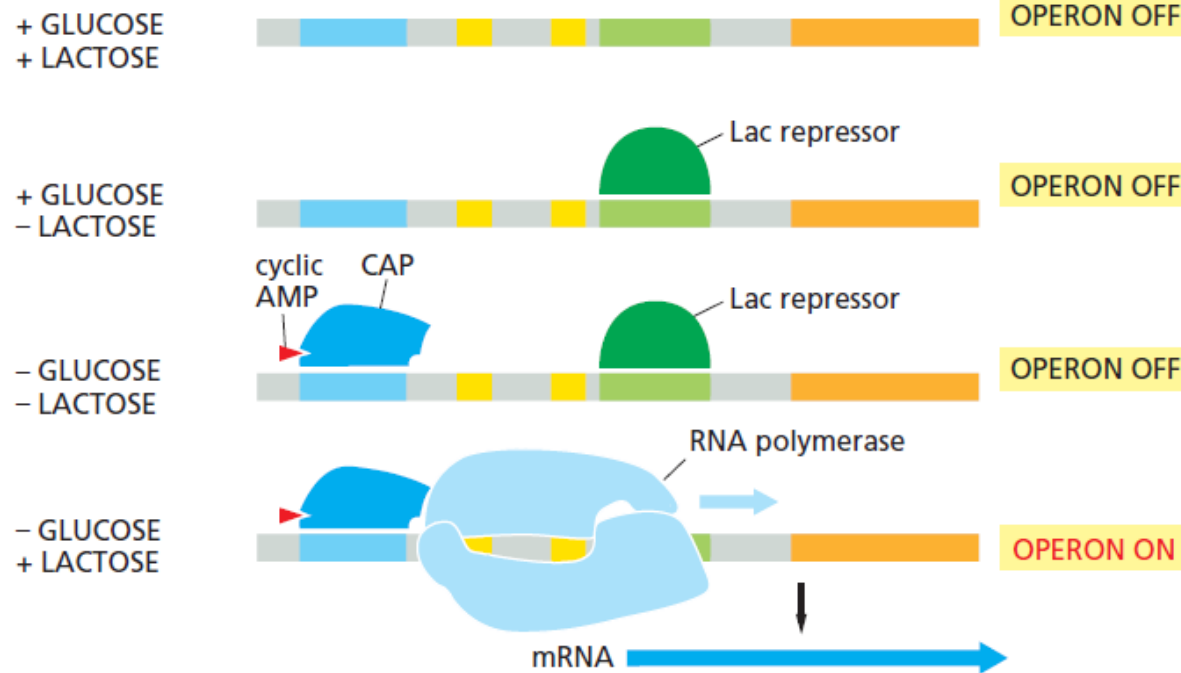
[†]Forms a dimer with the retinoic acid receptor or vitamin D receptor.

Table 28-4
Lehninger Principles of Biochemistry, Fifth Edition
© 2008 W. H. Freeman and Company

Exemplos de regulação gênica



LacZ = enzima para usar lactose como fonte de carbono

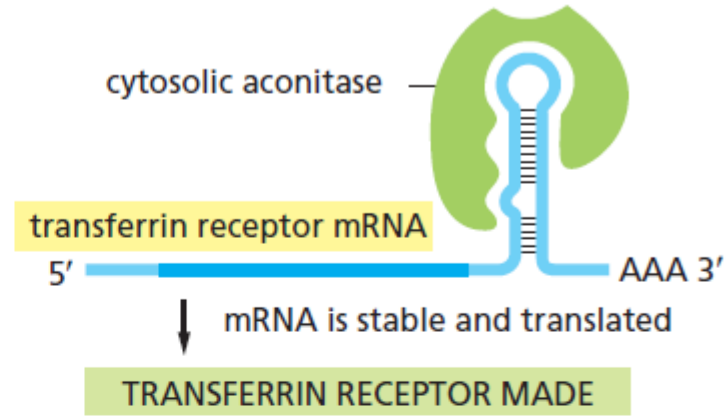
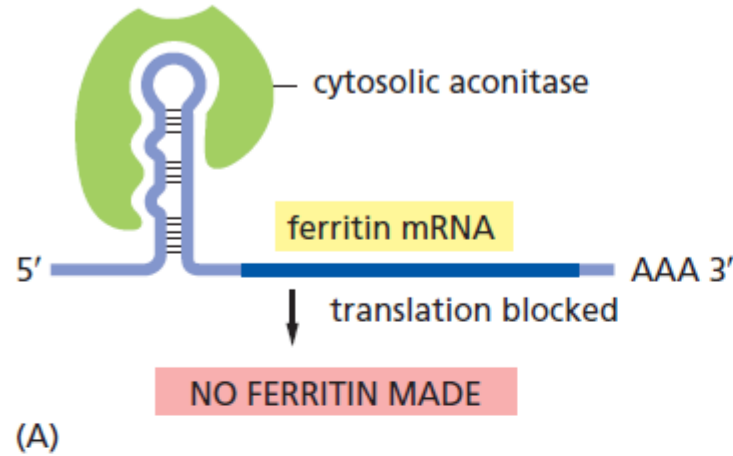


Repressor Lac perde afinidade pelo DNA quando tem lactose

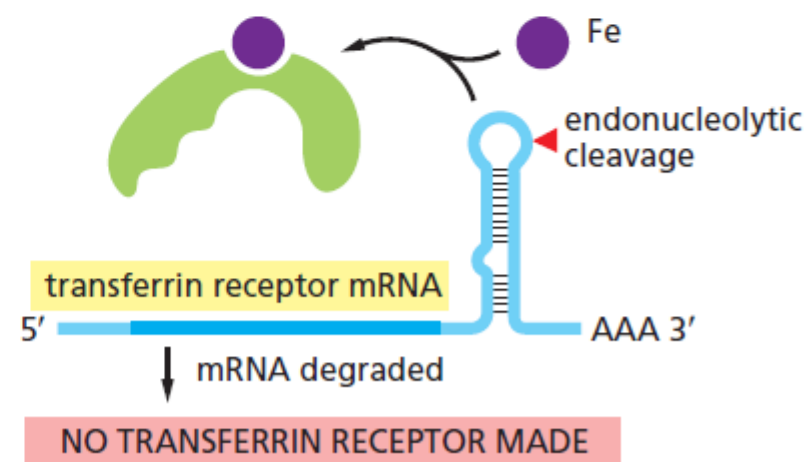
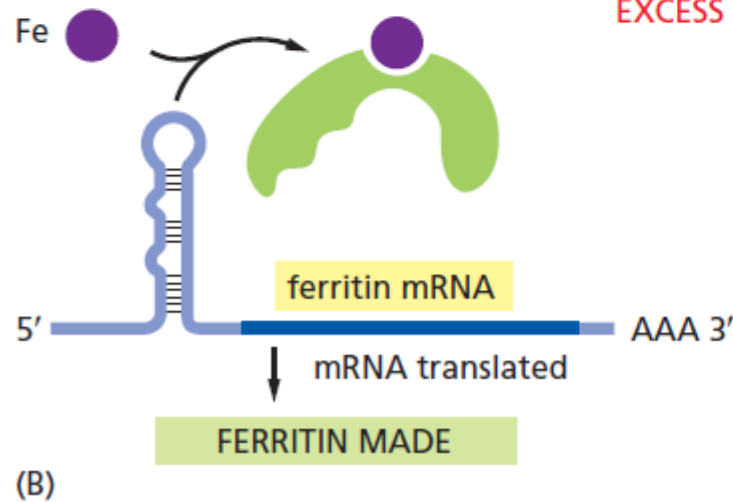
AMP cíclico é produzido quando não tem glicose

Exemplos de regulação gênica

IRON STARVATION



EXCESS IRON



Ferritina: “Depósito” intracelular de Ferro

Transferrina: Transportador de Ferro pela membrana