LGN5809 - Genética Molecular

CONTROLE PÓS-TRANSCRICIONAL DA EXPRESSÃO GÊNICA

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SUMÁRIO

- *Overview* do controle pós-transcricional;
- A relevância da associação RNA – proteína
- Processamento do RNA;
- Splicing alternativo;
- Transporte das mRNPs;
- Degradação do mRNP;
- Próxima aula.

ETAPAS NO CONTROLE DA EXPRESSÃO

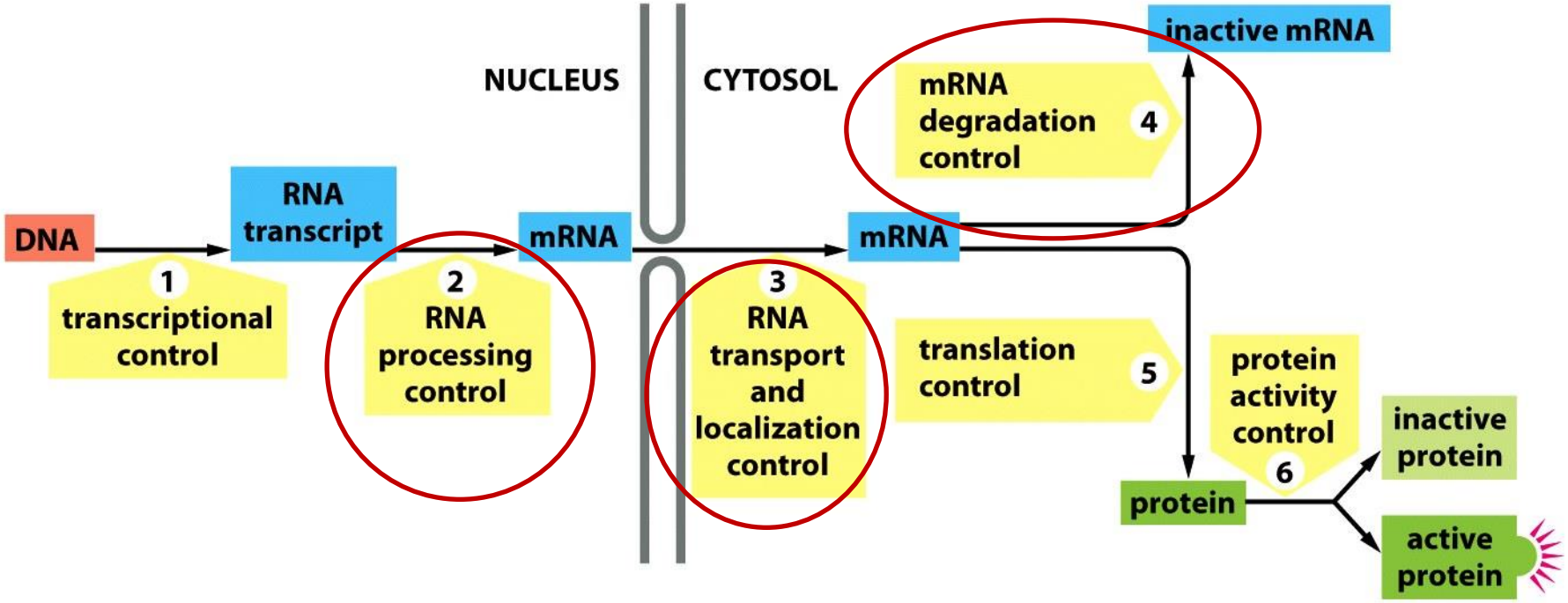
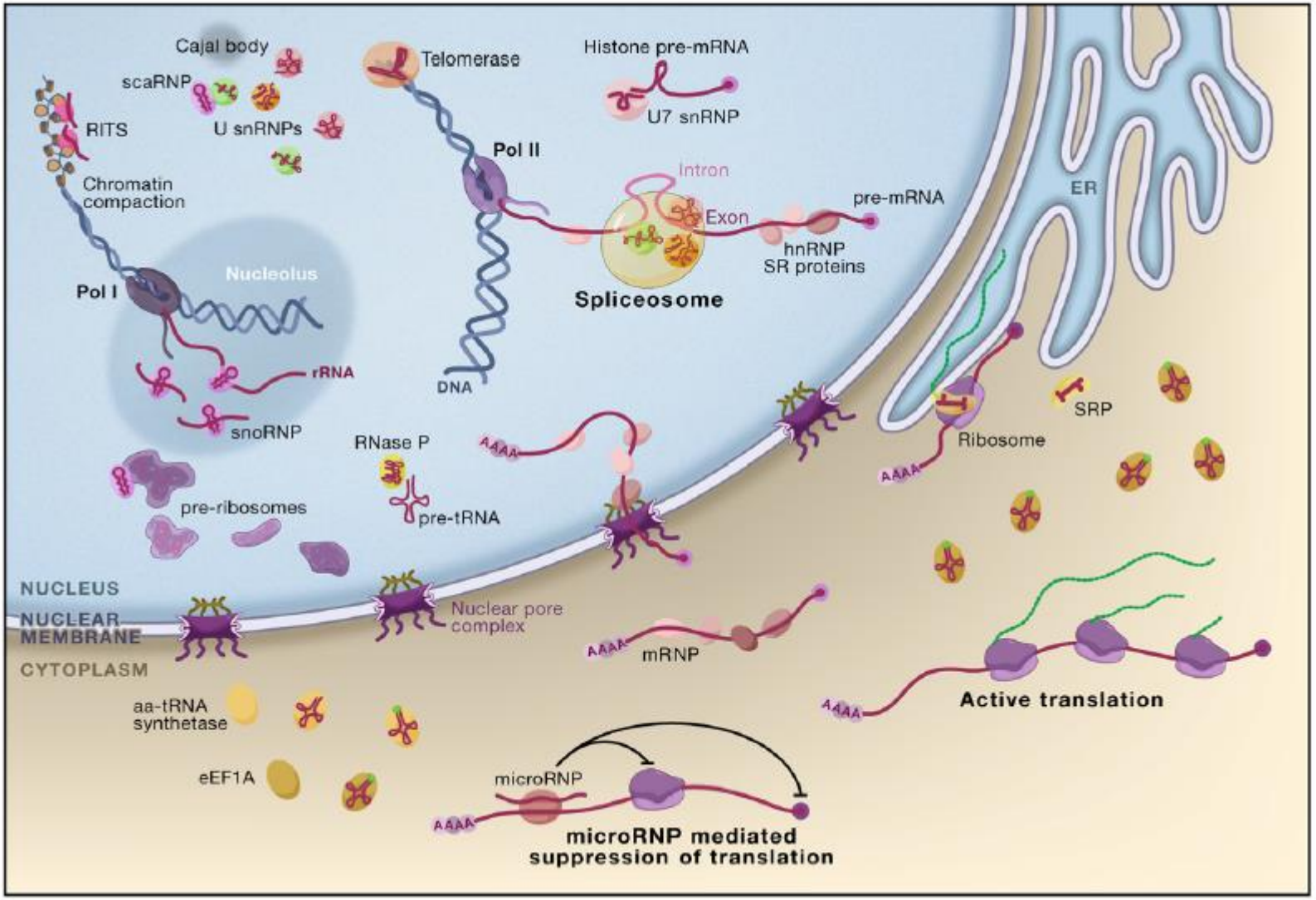


Figure 8-3 Essential Cell Biology 3/e (© Garland Science 2010)

O controle pós-transcricional abrange várias etapas!

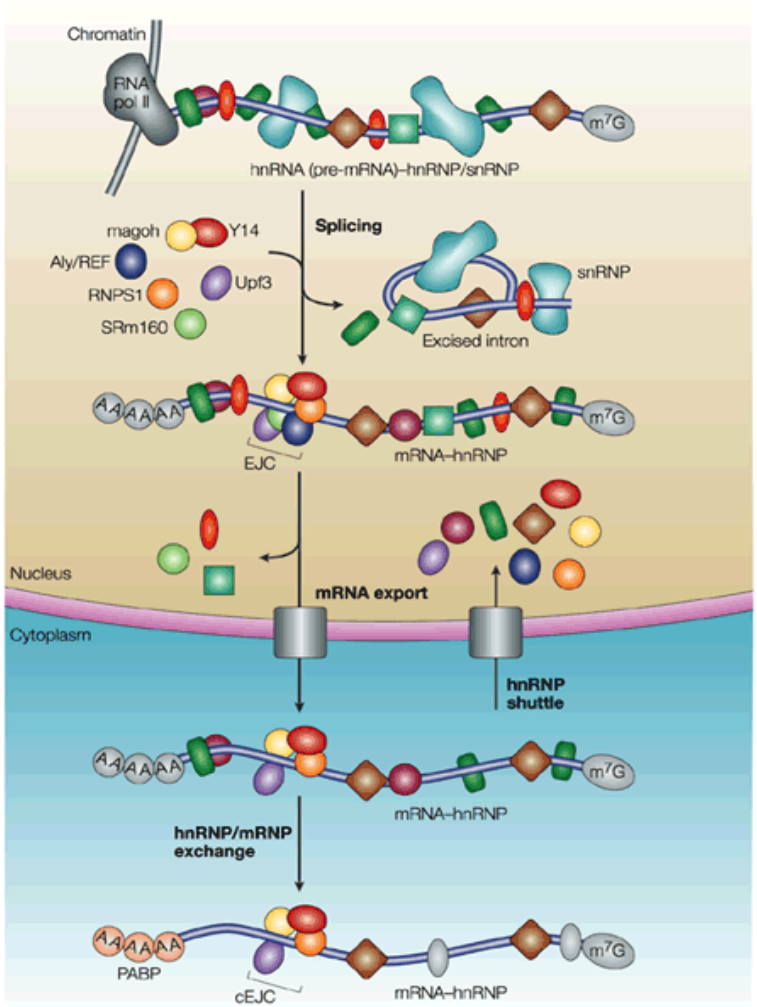
A estabilidade do RNA é altamente dependente de proteínas!



doi: 10.1016/j.cell.2009.02.009

OS RNAs NÃO ESTÃO SOZINHOS!

Interdependência de proteínas e RNA!!!!



Termo correto: hnRNP e mRNP
"heterogeneous nuclear ribonucleoproteins"

"*fibril model*" - Stevenin et al. 1982
"*mRNP code*" – Singh and Valcarcel 2005

Abundantes tanto quanto histonas!!!

doi: 10.1038/nrm760

Available online at www.sciencedirect.com
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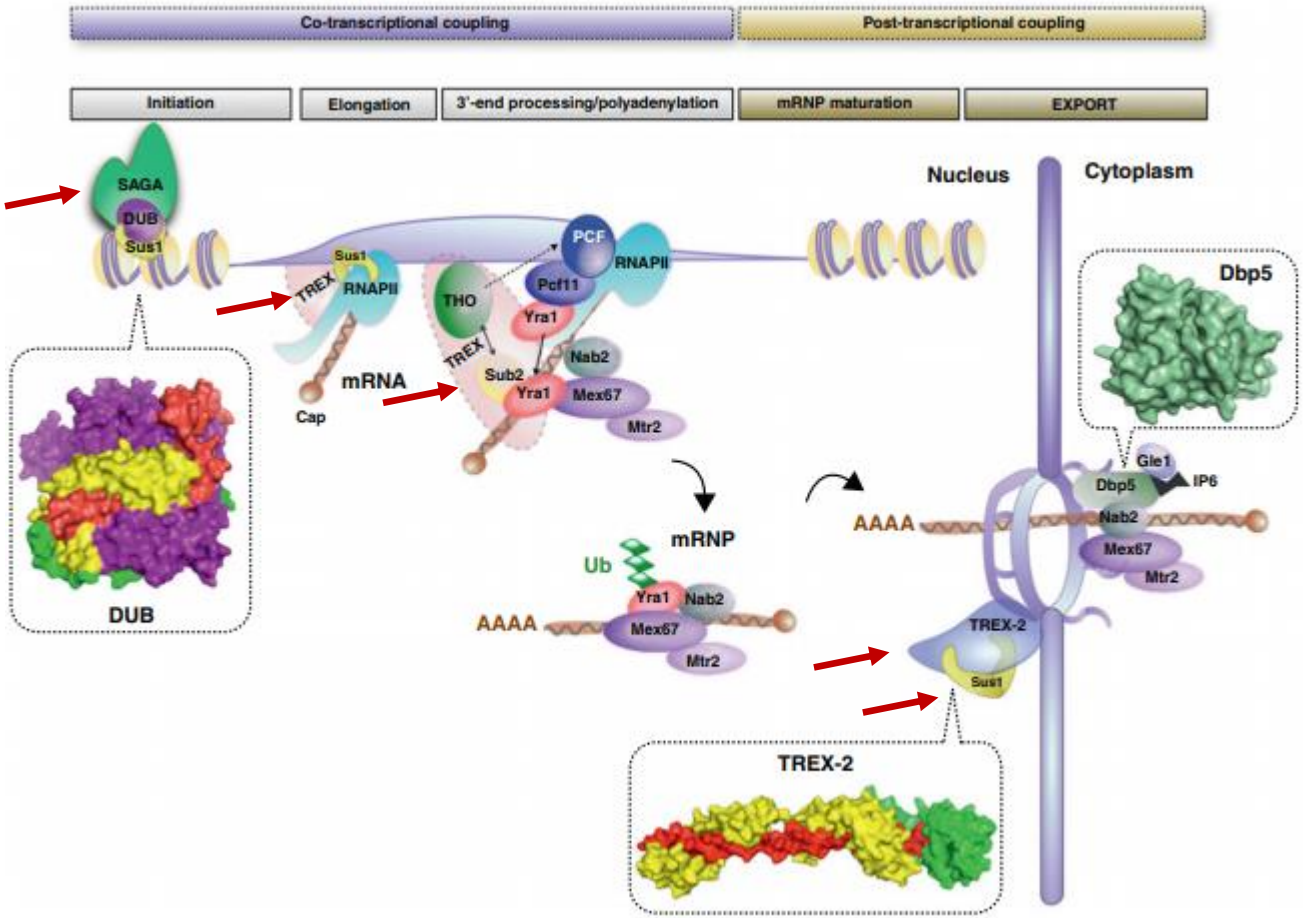
Current Opinion in Cell Biology

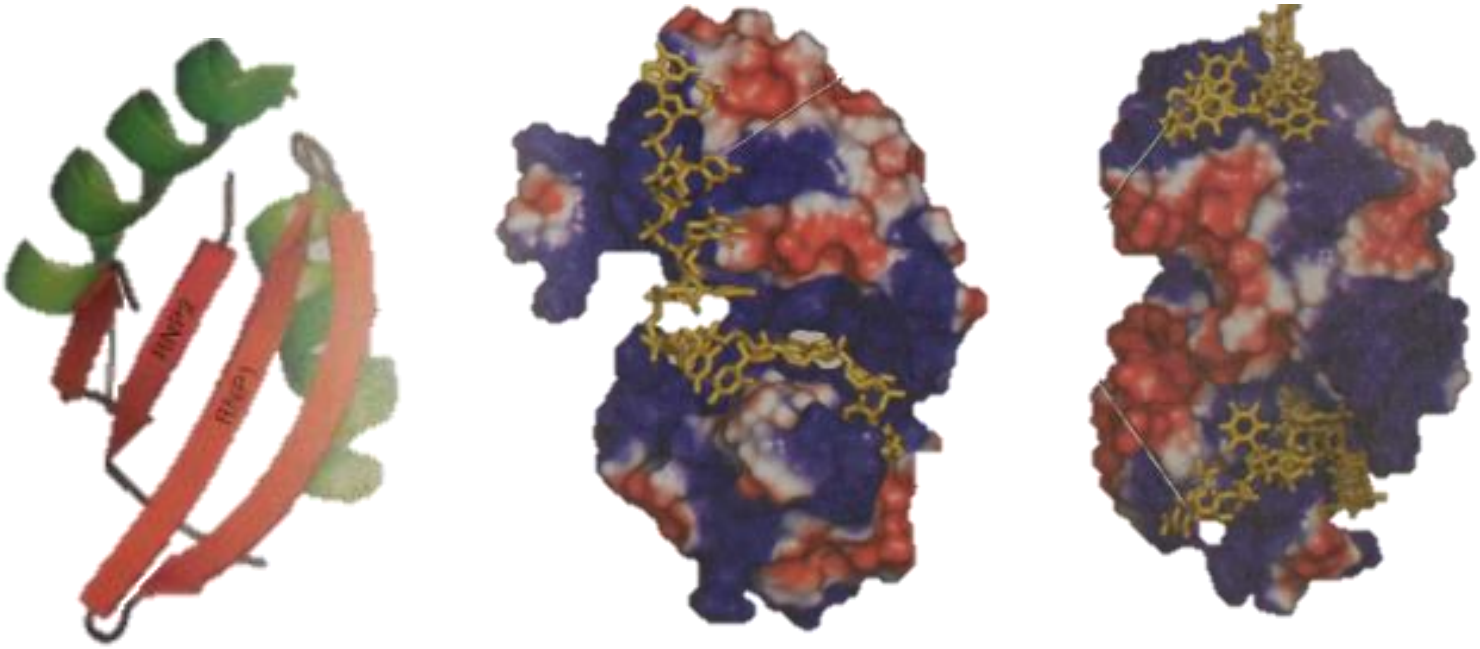
Linking gene regulation to mRNA production and export

Susana Rodríguez-Navarro[1] and Ed Hurt[2]



doi: 10.1016/j.ceb.2010.12.002

MOTIVO RRM ou RBD



Domínio conservado:
1 folha beta de 4 fitas (carga positiva) e 2 alfas

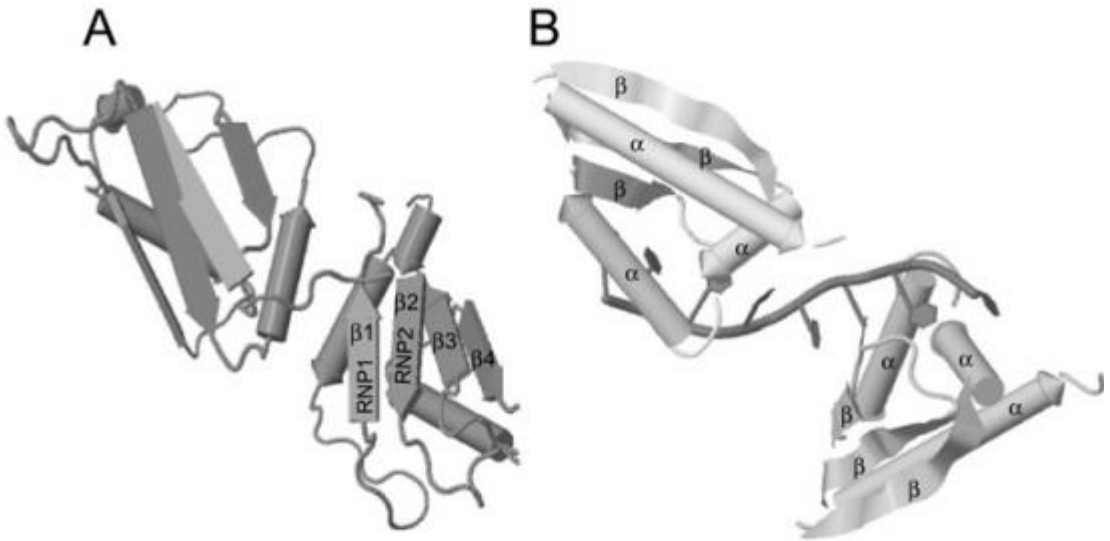
TABLE 1. Major hnRNP proteins

Protein	Molecular weight (kDa)	Domain/functional motif	Preferred binding sequence	Reported function	Shuttling capacity
A1	34	2X RBD, RGG	UAGGG(A/U)	Splicing Export Telomere biogenesis	+
A2/B1	36/38	2X RBD, RGG	$(U/UAGGG)_N$	Splicing Localization	+
C1/C2	41/43	1X RBD	U6	Splicing Stability	–
D (AUF)	44–48	2X RBD, RGG	AU rich	Telomere biogenesis Stability Recombination	+
E1/E2/E3/E4 (αCP1-4 or PCBP1-4)	38, 39	3X KH	C rich	Stability Translation	+
F	53	3X RBD	GGGA	Splicing	Not known
G	43	1X RBD, RGG	CC(A/C)	Splicing	–
H/H′ (DSEF-1)	56	3X RBD	GGGA	Splicing Polyadenylation	Not known
I (PTB)	59	4X RBD	UCUUC	Splicing Localization Polyadenylation	+
K/J	62	3X KH, RGG	C rich	Transcription Stability Translation	+
L	68	4X RBD	CA repeat	Export Stability Riboswitch	Not known
M	68	4X RBD	G or U rich	Splicing	+
P2(FUS/TLS)	72	RBD	GGUG	Avid binding to poly(A) Autoantibody target	+
Q1NSAP	55–70	3X RBD, RGG	GAIT element	Splicing Translation	+
R1/R2	82	RBD	Not known	Retinal development	+
U	120	RGG	Not known	Nuclear retention	–

doi:10.1261/rna.2254110

TABLE 2. Minor hnRNP proteins

Protein	Domain/functional motif	Reported function
9G8	1X RBD	Splicing Export
A0	2X RBD, RGG	Splicing
Aly/REF	1X RBD	EJC Export
ASF/SF2 (SRp30a)	2X RBD	Splicing
CUG-BP (hNab50)	3X RBD	Splicing (intronic enhancer) Myotonic dystrophy Translation
DEK	No significant homology	EJC Splicing
Hrp1/Nab4	2X RBD	Polyadenylation
HuR	3X RBD	Stability Export
magoh	No significant homology	EJC Localization
Npl3/Nop3	2X RBD	Export pre-rRNA processing
PABP1	4X RBD	Translation Stability
RNPS1	1X RBD	Splicing EJC NMD
SC35 (SRp30b)	1X RBD	Splicing
Squid/hrp40	2X RBD	Localization
SRm160	RS domain	EJC Splicing
SRp20	1X RBD	Splicing Export
Upf3	1X RBD	EJC NMD
Y14	1X RBD	EJC NMD Localization
Yra1	1X RBD	Export



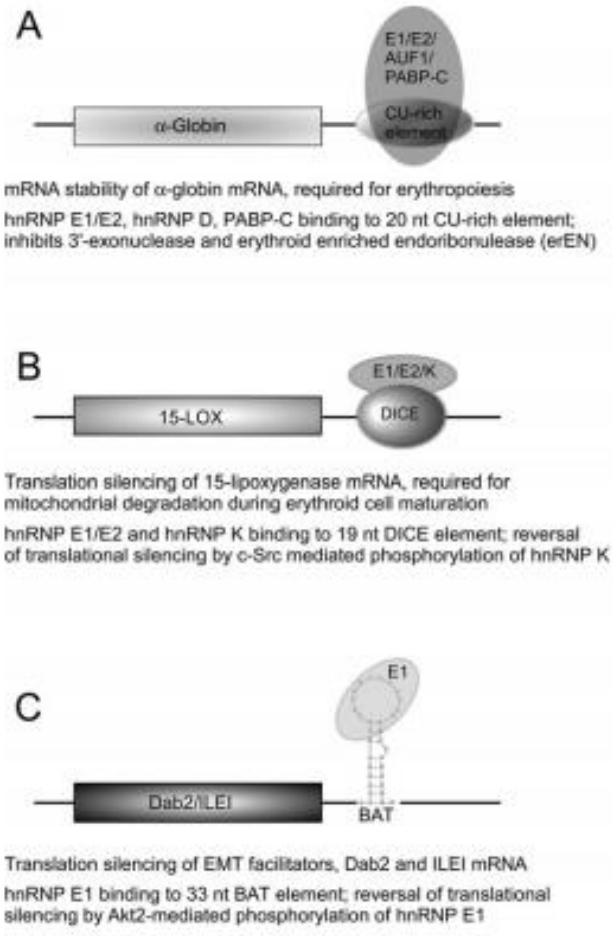
RNP-CS-RBD motif

KH1 domain (polymer:2)

doi:10.1261/rna.2254110

Heterogeneous nuclear ribonucleoproteins (hnRNPs) in cellular processes: Focus on hnRNP E1's multifunctional regulatory roles

E1 é codificada por um gene sem íntron proveniente de um evento de retrotransposição de hnRNP E2. hnRNP E1 é constitutivamente expressada e regula as principais etapas do processo de de expressão processamente de pre-mRNA, estabilidade de mRNA e tradução.

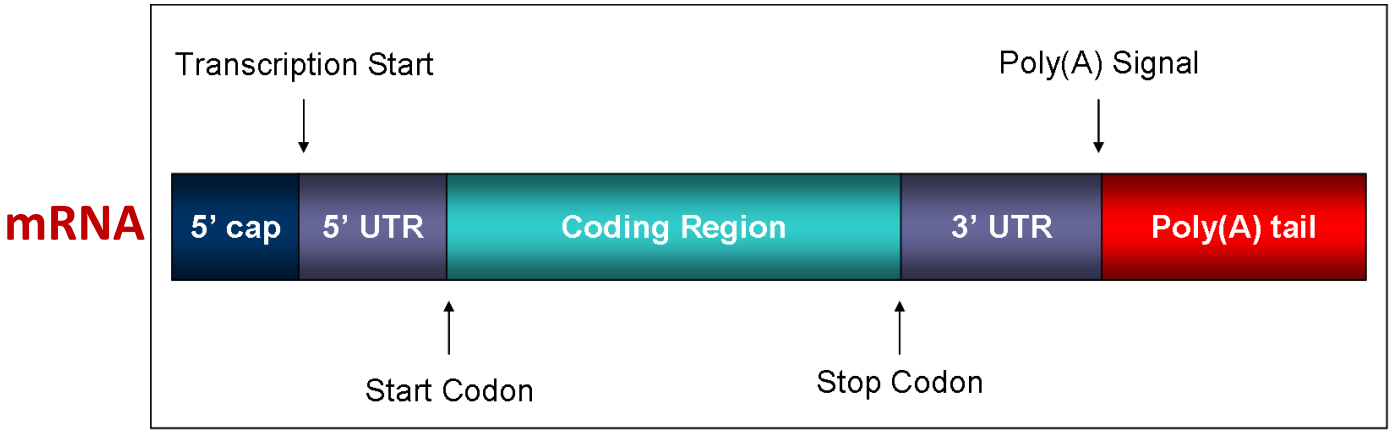


doi:10.1261/rna.2254110

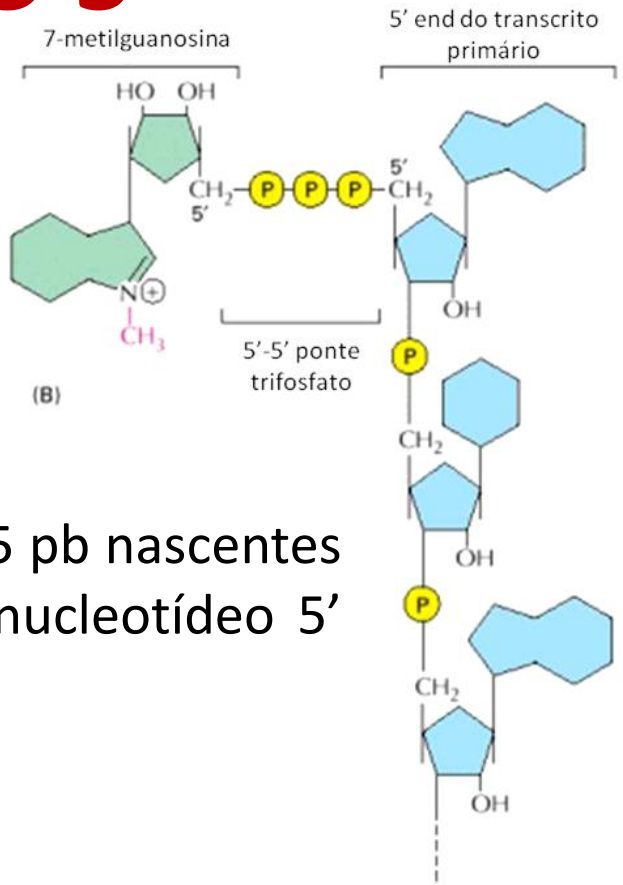
PROCESSAMENTO DO RNA

- **Durante a transcrição** as modificações que podem ocorrer nos transcritos nucleares são basicamente de três tipos:
  - Capeamento ("capping") do terminal 5';
  - Poliadenilação do terminal 3';
  - Montagem de segmentos codificadores ("*splicing*").
- Este conjunto de modificações no transcrito nuclear originará o mRNA, pronto para migrar para o citoplasma.

No RNA transcrito há sequências que sinalização para seu processamento!!



CAPEAMENTO 5’



Logo após a transcrição, APROXIMADAMENTE 25 pb nascentes há a ligação de 7-metilguanosina ao primeiro nucleotídeo 5’ do transcrito de RNA.

FUNÇÕES:

. Proteger o transcrito do ataque de 5’ exonucleases ;

. Facilitar transporte para citoplasma;

. Au

snRNAs e snoRNAs generalmente possuem um capeamento 5’ hiper-metilado

AS RNAPOLs POLIMERIZAM RNA DISTINTOS...ASSIM...

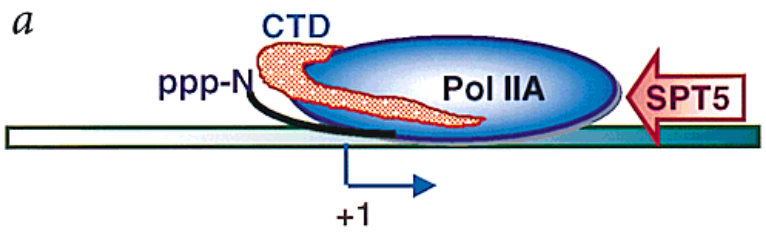
LEMBRA???

Polimerases	RNA transcrito
RNA polimerase I	Pré-rRNA
RNA polimerase II	**mRNA, snRNA, siRNA, miRNA**
RNA polimerase III	tRNA, 5S-rRNAsn RNA u6, outros pequenos RNA estáveis

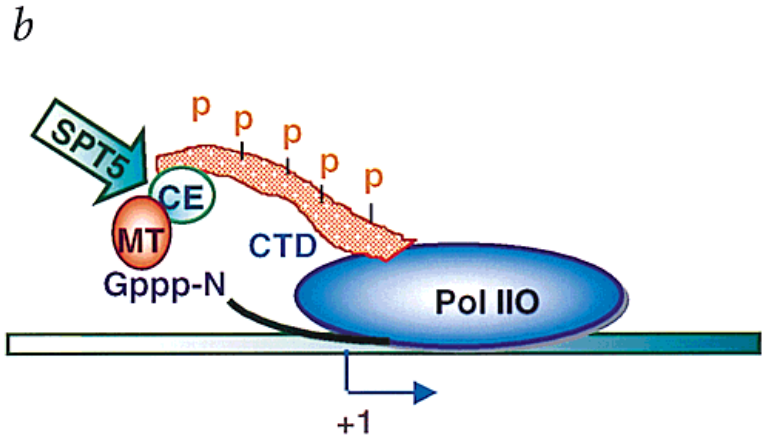
RNAP II - Domínio CTD – Tyr-Ser- Pro-Thr-Ser - Pro -Ser

TFIIH - fosforila a Ser5

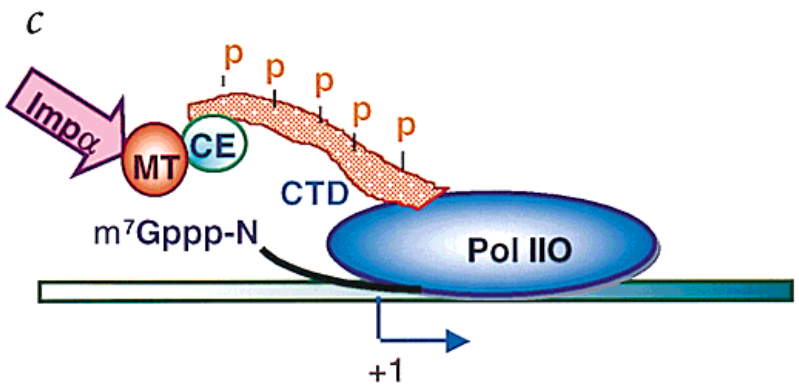
Ligação ao complexo dimérico de capeamento



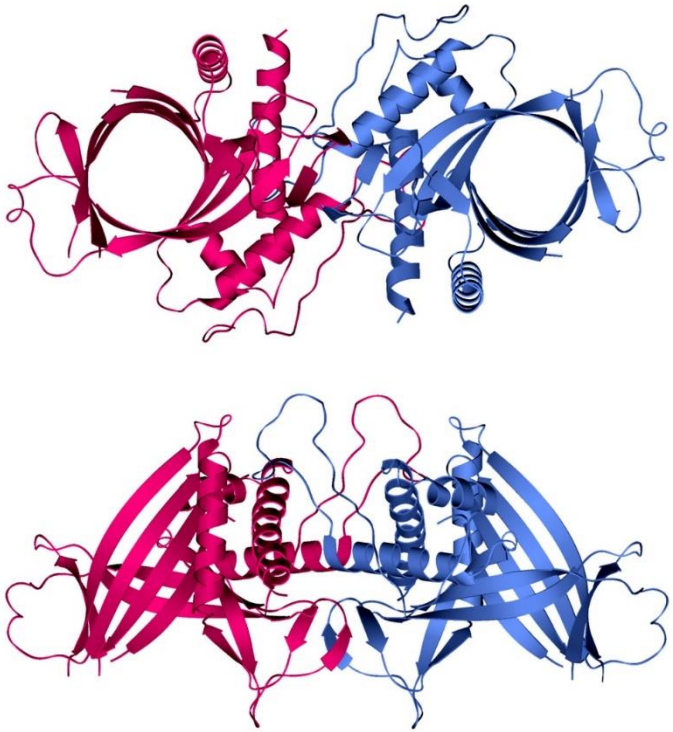
a) RNAPolII com CTD fosforilado Ser2 iniciando a transcrição produz de 20 a 25 pb e pausa com a ajuda da enzima SPT5 que se liga ao complexo



b) CTD é fosforilada e a enzima de capeamento (CE - capping enzyme) se liga modificando o inicio da fita nascente.
MT se liga a CE (mamiferos) ou ao P-CTD (levedura)



c) Impα estimula a MT a ligar cap G que é metilado, e a RNAPolII se altera para continuar a elongação.



As proteínas trifosfatase usado no capeamento não são conservada!

- Divalent cation-dependent RNA triphosphatases of DNA – vírus e fungo,
- Divalent cation-independent RNA triphosphatases – nematóides, mamíferos e outros animais.

Molécula alvo para desenvolvimento de antifúngios e antivirais!!

- Yu L, Martins A, Deng L, Shuman S. (1997) Structure-function analysis of the triphosphatase component of vaccinia virus mRNA capping enzyme. J Virol. 1997;71:9837-9843.
- Ho CK, Pei Y, Shuman S. (1998) Yeast and viral RNA 5’ triphosphatases comprise a new nucleoside triphosphatase family. J Biol Chem. 1998;273:34151-34156.

POLIADENILAÇÃO

Após o término da transcrição – clivagem terminal do RNA;
Adição de cerca de 200 resíduos de adenilato (AMP)

FUNÇÕES:

. Facilitar transporte para o citoplasma;
. Estabilizar o mRNA;
. Facilitar a tradução.

Também influenciada pela CTD da RNApolII

Mas não dependente de fosforilação!

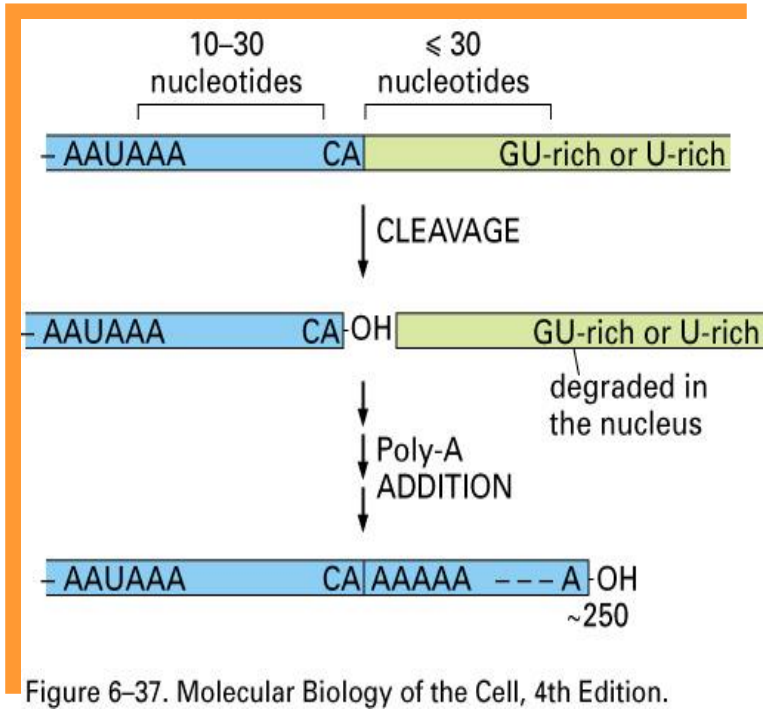


Figure 6–37. Molecular Biology of the Cell, 4th Edition.

REGIÕES DE CLIVAGEM NÃO SÃO CANÔNICAS!!

Gene	Distance from poly-A signal site
D-globin	600bp
A-globin	1500bp
Globin-mice	100-300
Globin-human	100-300
DHFR-mice	1000-2000
a-Amylase	2000-4000
Ovalbumin	800-1000
Gastirin	192

Em plantas sinal de poli-A - AAUAAA
Leveduras – sequências ricas em AU

ALGUMAS PROTEÍNAS ENVOLVIDAS NO POLIADENILAÇÃO

- **CPSF:** Clivagem e poliadenilação fator específico – composto por 4 subunidades: 160kd, 100kd, 70kd e 30 KD cada. Se liga a AAUAAA e interage com a Pol A polymerase. Cliva o fator de estimulação (CStF).

- **CStF:** Identifica os locais ricos em GU n U. Interage com os fatores de clivagem CF-1 e CF 2.

- **CF1 and CF-2:** Enzimas que clivam a extremidade 3'.

- **PAP:** Poli A polimerase é uma proteína monomérica, necessita da ligação com poli-A binding protein-II (PAB-II). Contém os domínios RBD, sítios ricos em serina e treonina para fosforilação.

- **PAB-II:** Estímulo da PAPs e controle do tamanho de causa poliA.

ENTENDENDO O PROCESSO

• CPSF e CStF se ligam aos seus sítio especificos – sinal poliA e sequências ricas em GU/U respectivamente.

• Juntas em seus respectivos locais de ligação, formam um *looping* colocando junto também o sítio de clivagem.

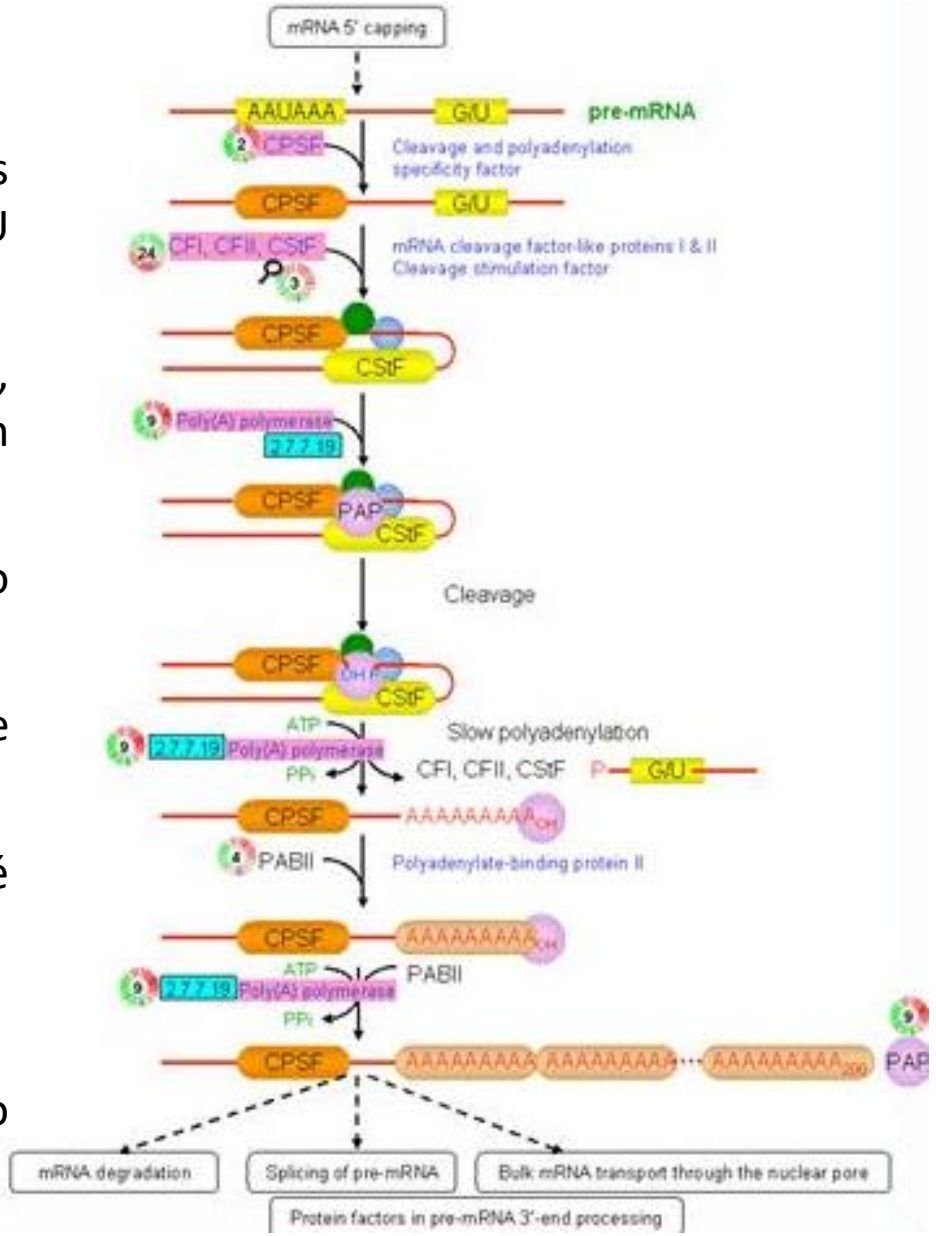
•CF1 e CF2 se unem a CPSF e CStF e clivam o RNA.

•PAP se liga ao complexo de fatores de clivagem.

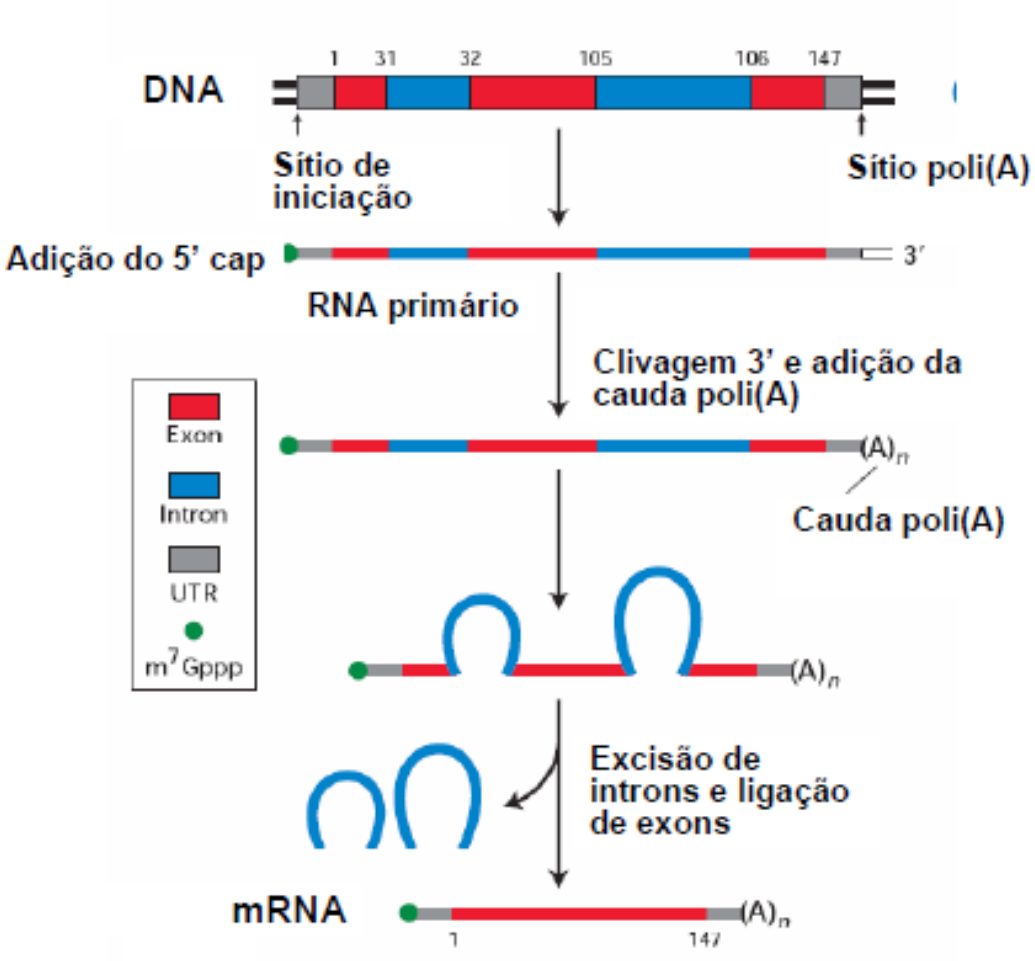
•O RNA é clivado e CStF com o resto do 3' é liberado.

•A clivagem e adição do poli A é simultâneo

•Para PAP ser ativada, é requerido o estímulo da PAB-II.



SPLICING



NEM SEMPRE OCORRE NESSA ORDEM!

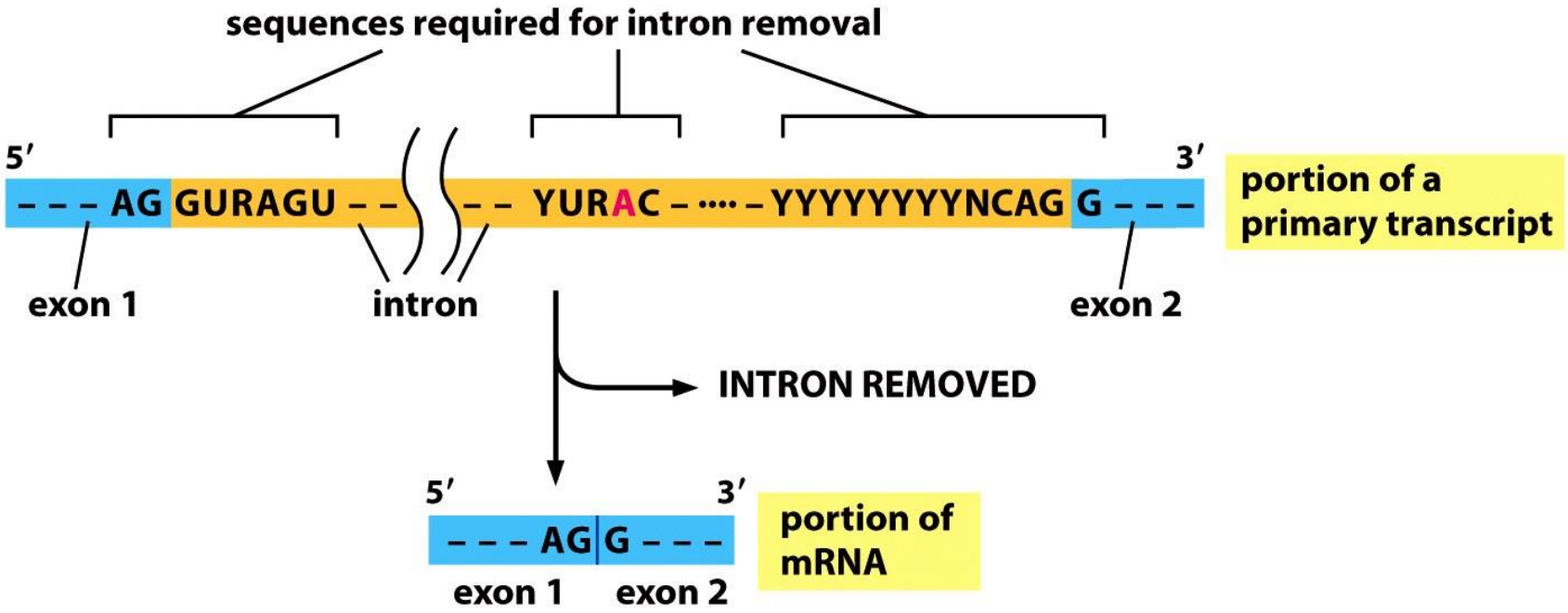
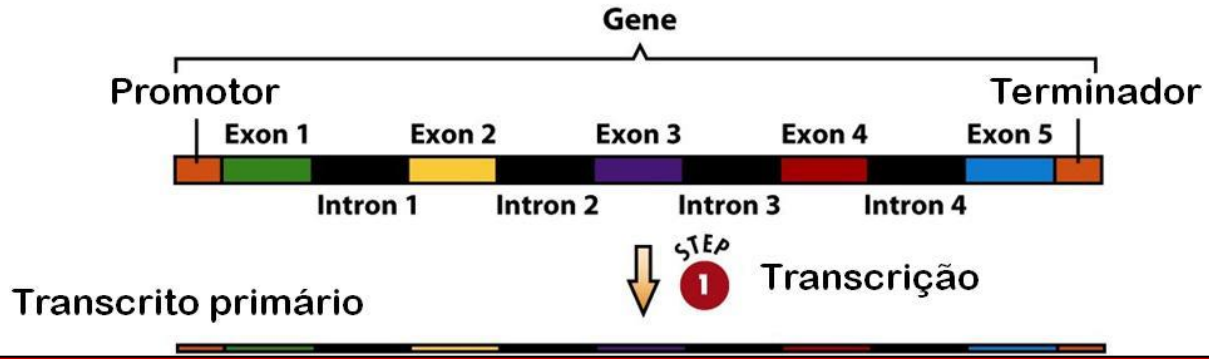


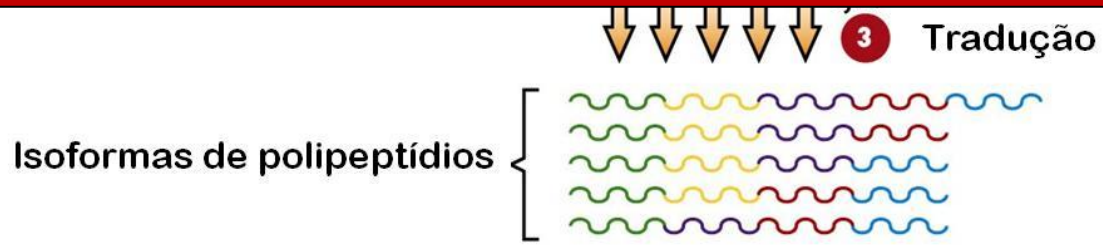
Figure 7-19 Essential Cell Biology 3/e (© Garland Science 2010)

Sequências especificas e snRNA (*small nuclear* RNA – pequenos RNA nucleares) auxiliam junto a proteínas (RPN) na retirada do introns

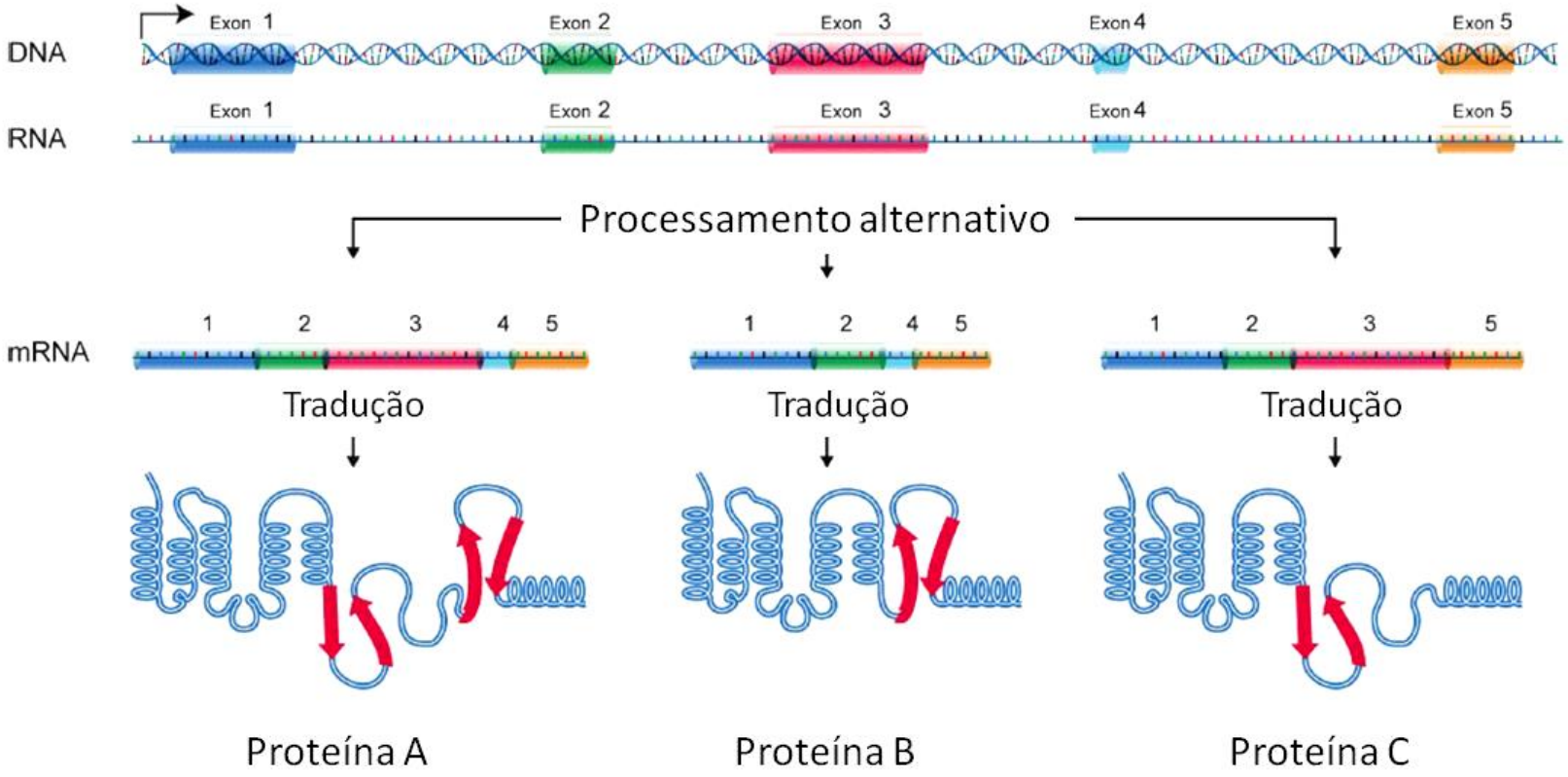
ISOFORMAS DE PROTEÍNAS



"Evolution can seek new solutions without destroying the old … the genetic material does not have to duplicate to provide a second copy of an essential gene in order to mutate to a new function." Walter Gilbert

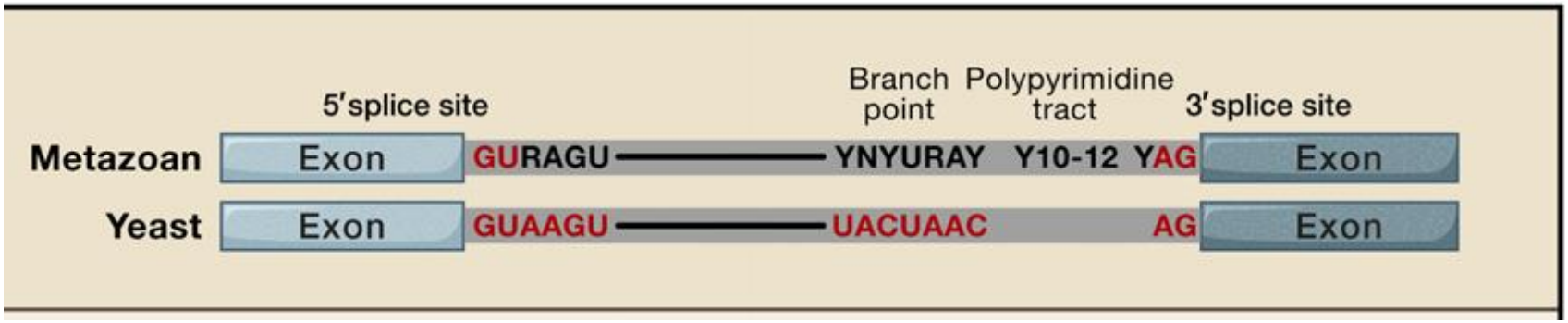


SPLICING ALTERNATIVO GERANDO DIVERSAS PROTEÍNAS



Para proporcionar uma enorme diversidade de proteínas em eucariotos superiores os *spliceossomos* devem não somente catalizar o *splicing* com grande precisão mas também exibir um alto grau de flexibilidade permitindo uma rápida resposta ao sinais regulatórios.

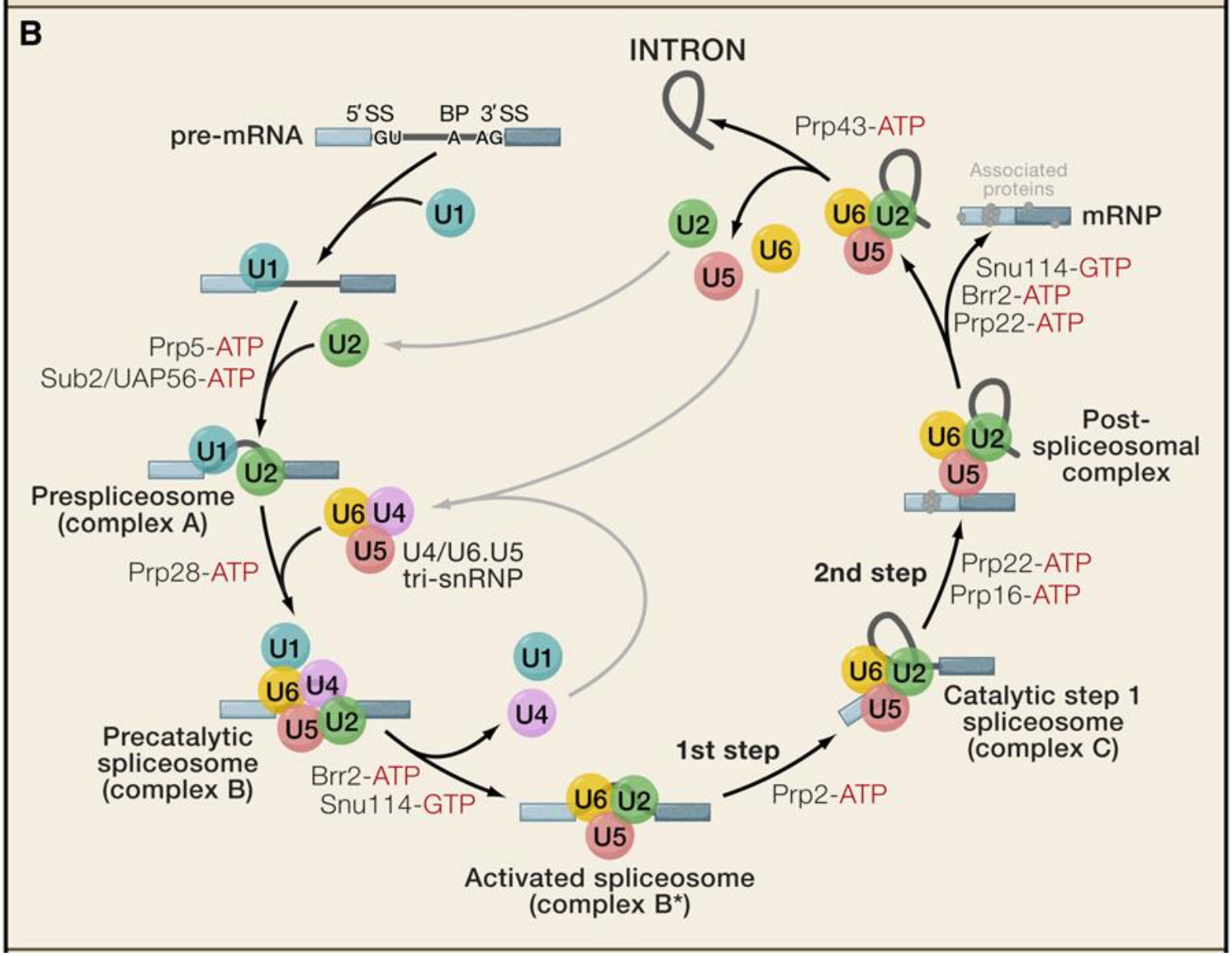
Sequências chaves para o controle do *splicing* …



- 5’SS;
- Branch point – sitio de ligação de vários snRNP;
- Sequencias ricas em pirimidinas;
- 3’SS;

Mas como ocorre?

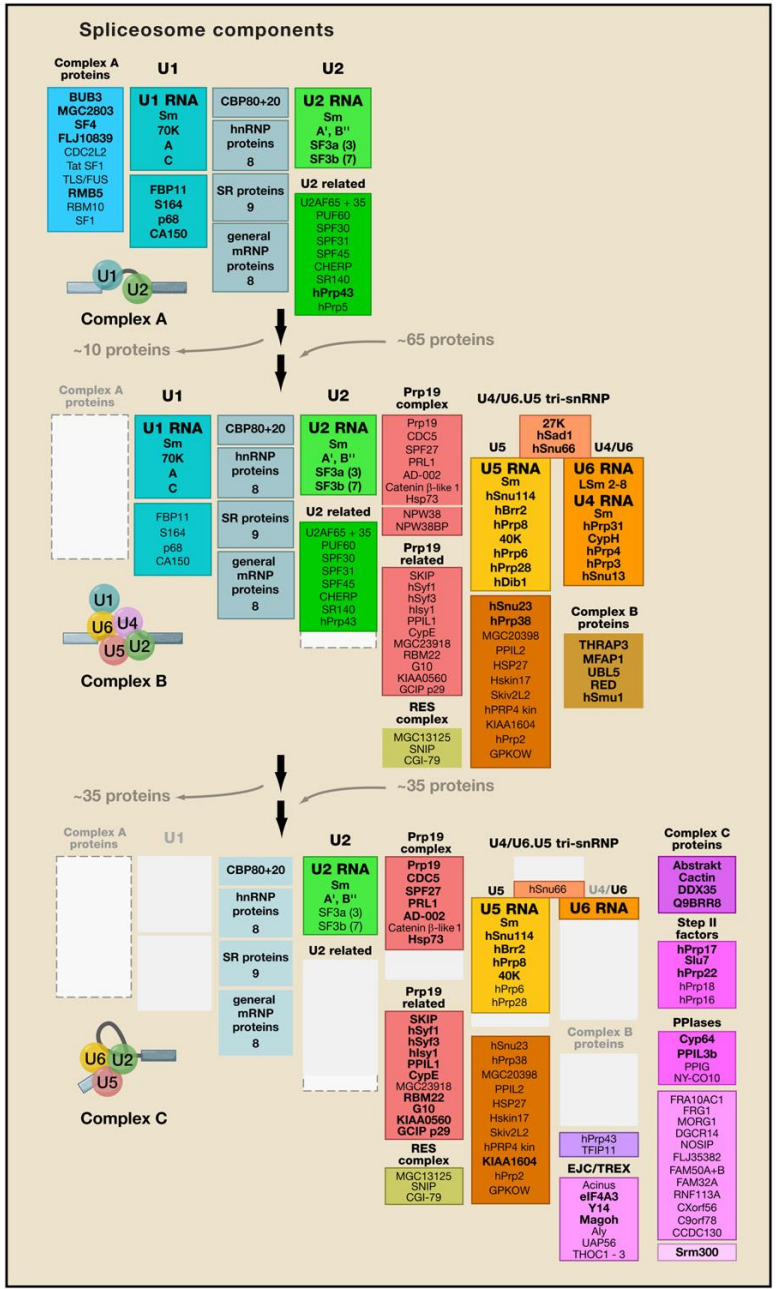
Doi: 10.1016/j.cell.2009.02.009



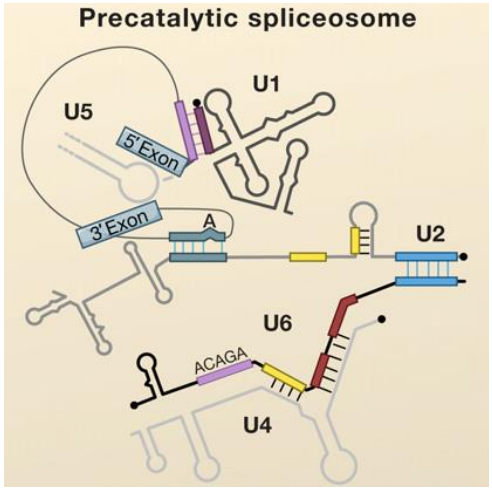
snRNP e DExD/H-type RNA-dependent ATPases/helicases ALTAMENTE CONSERVADAS, assim como as GTPases

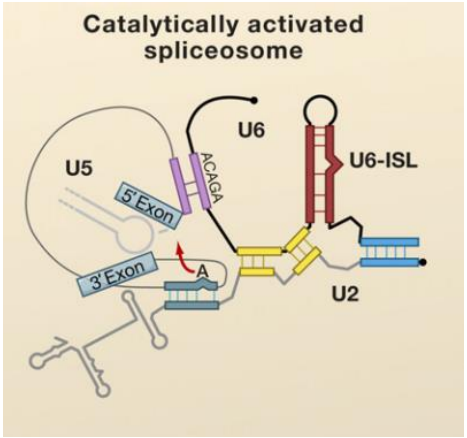
doi: 10.1016/j.cell.2009.02.009

É muita proteína envolvida!!!



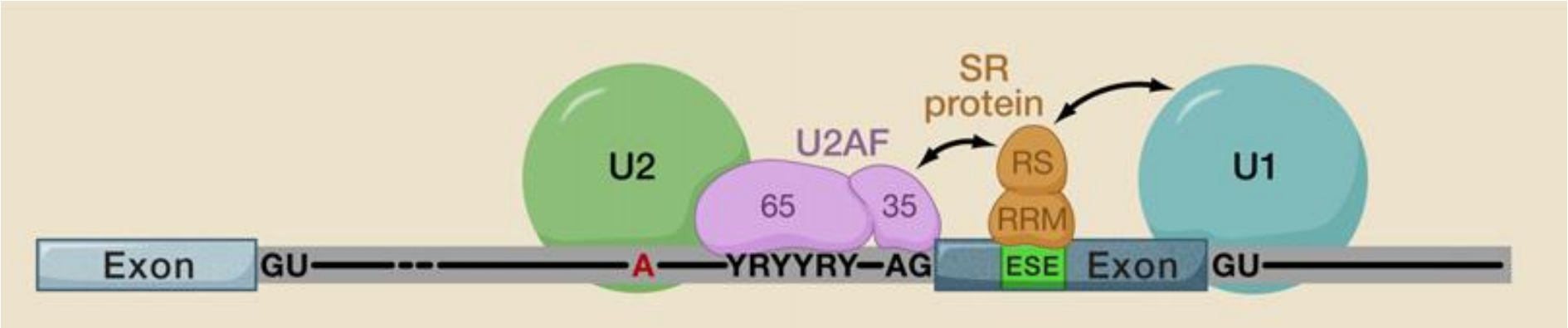
RNA-RNA, RNA-proteína e proteína-proteína regem o processo alternativo do RNA!!





Doi: 10.1016/j.cell.2009.02.009

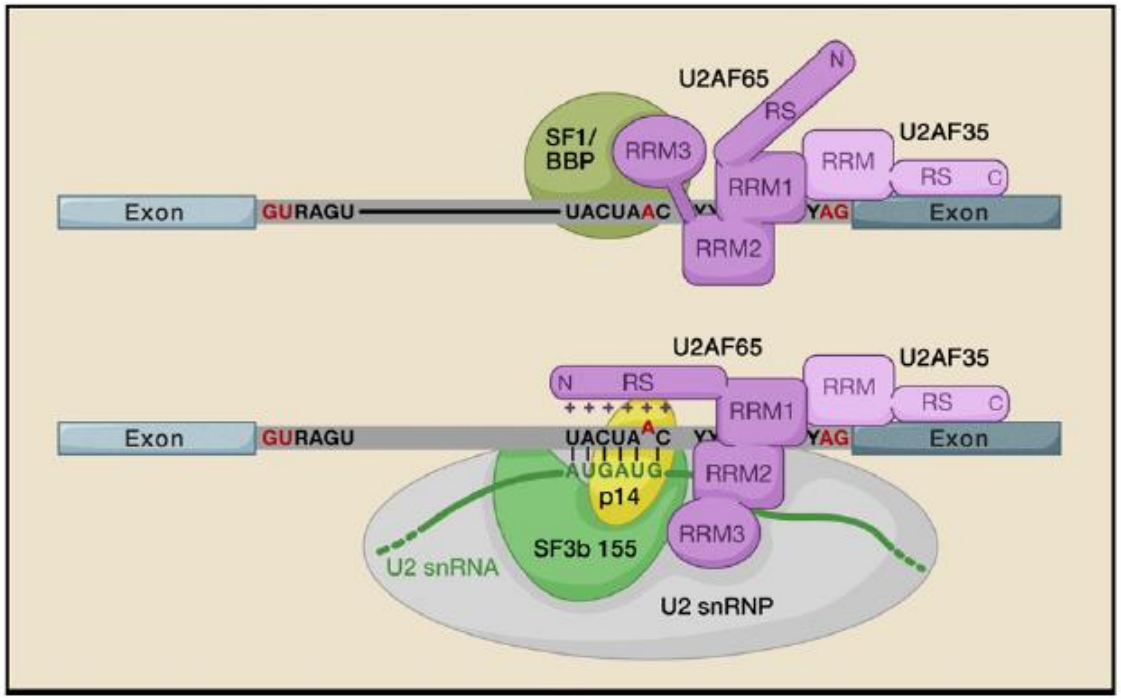
E o *splicing* alternative …



- RRM – RNA recognition motifs;
- RS – arginine serine rich domain;
- SR – serine rich domain;
- U2FA – U2 auxiliary factors;
- ESE – Exonic splicing enhancer.

Doi: 10.1016/j.cell.2009.02.009

Interações moleculares entre o *branch site* e 3'SS colaboram com a modelagem do RNA e o *splicing* alternativo!



Tamanho da sequência intrônica e exônica influencia também!!!

doi: 10.1016/j.cell.2009.02.009

Leading Edge
Review

Cell

The Spliceosome: Design Principles of a Dynamic RNP Machine

Markus C. Wahl,[2,3,*] Cindy L. Will,[1,*] and Reinhard Lührmann[1,*]
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[2]Makromolekulare Röntgenkristallographie
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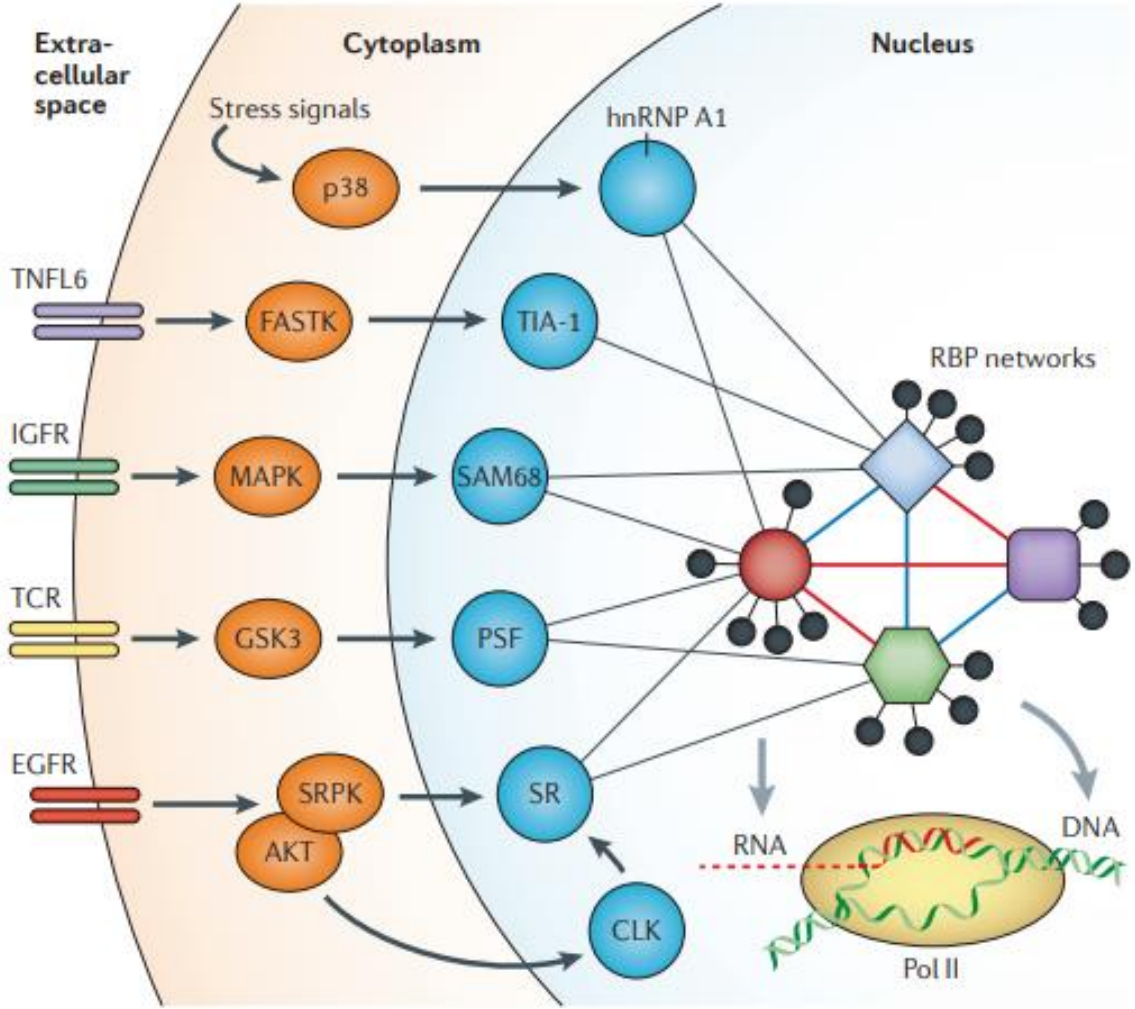
DOI 10.1016/j.cell.2009.02.009

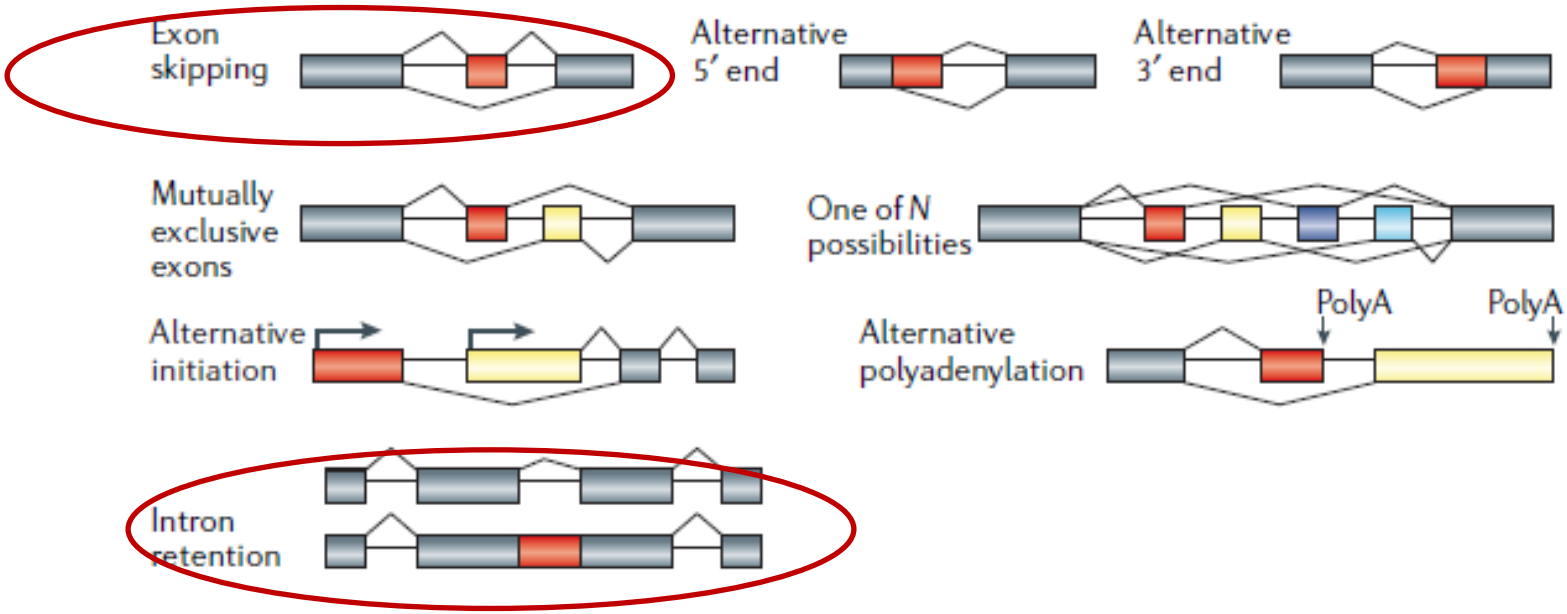
Tudo começa com sinalização celular!!



doi:10.1038/nrg3778

Alternative splicing and RNA selection pressure — evolutionary consequences for eukaryotic genomes

Yi Xing[*‡§] *and Christopher Lee*[*]



doi:10.1038/nrg1896

Apesar de ser uma loucura...

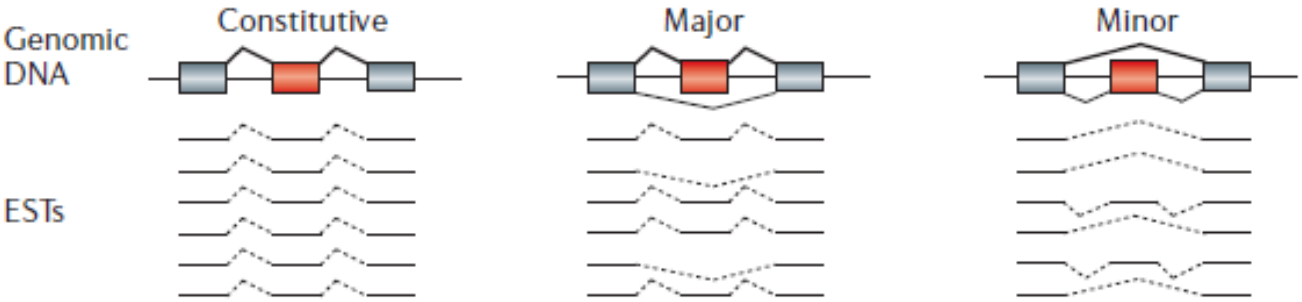
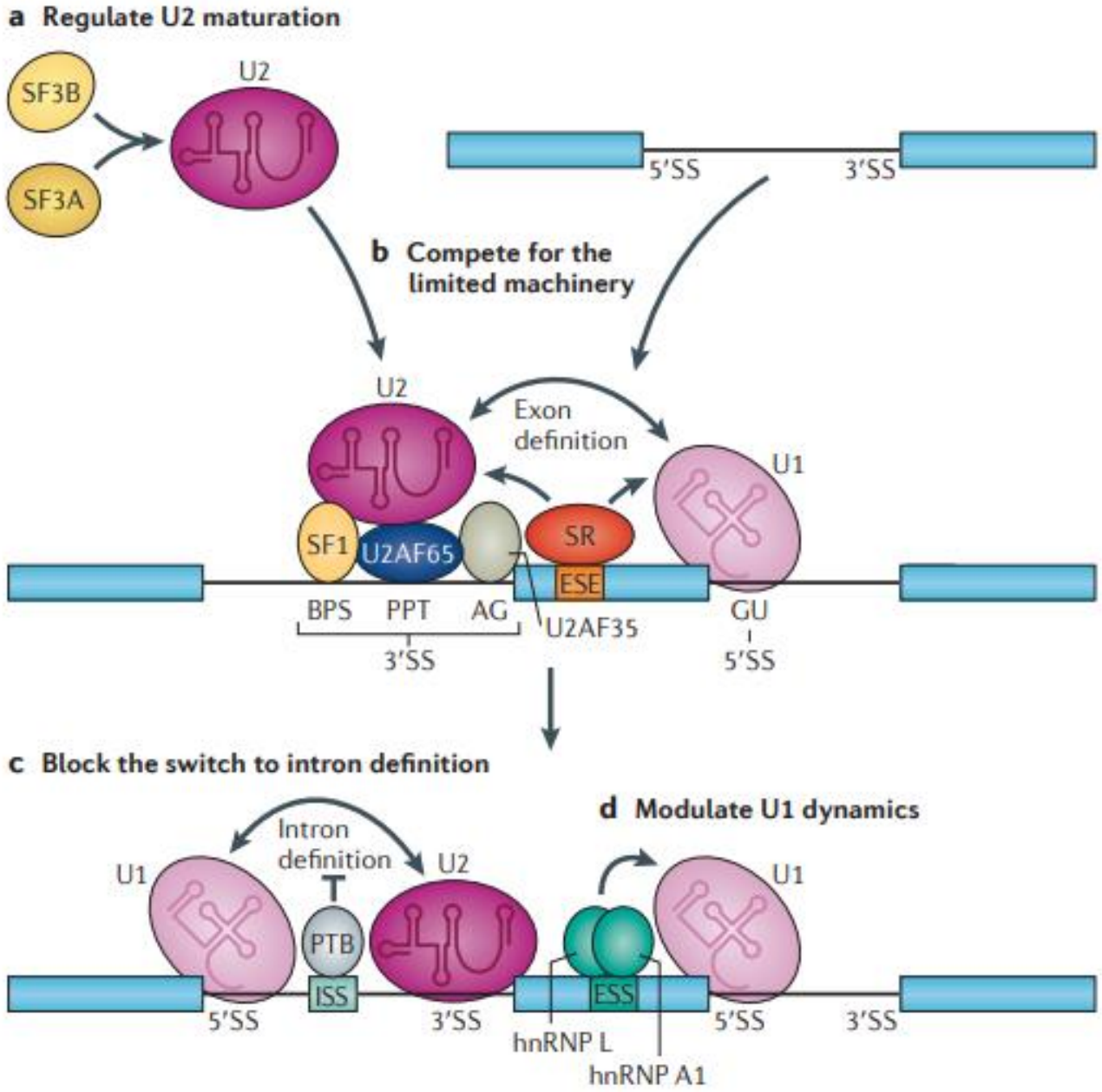


Table 1 | **Characteristics of ancestral alternatively spliced exons**

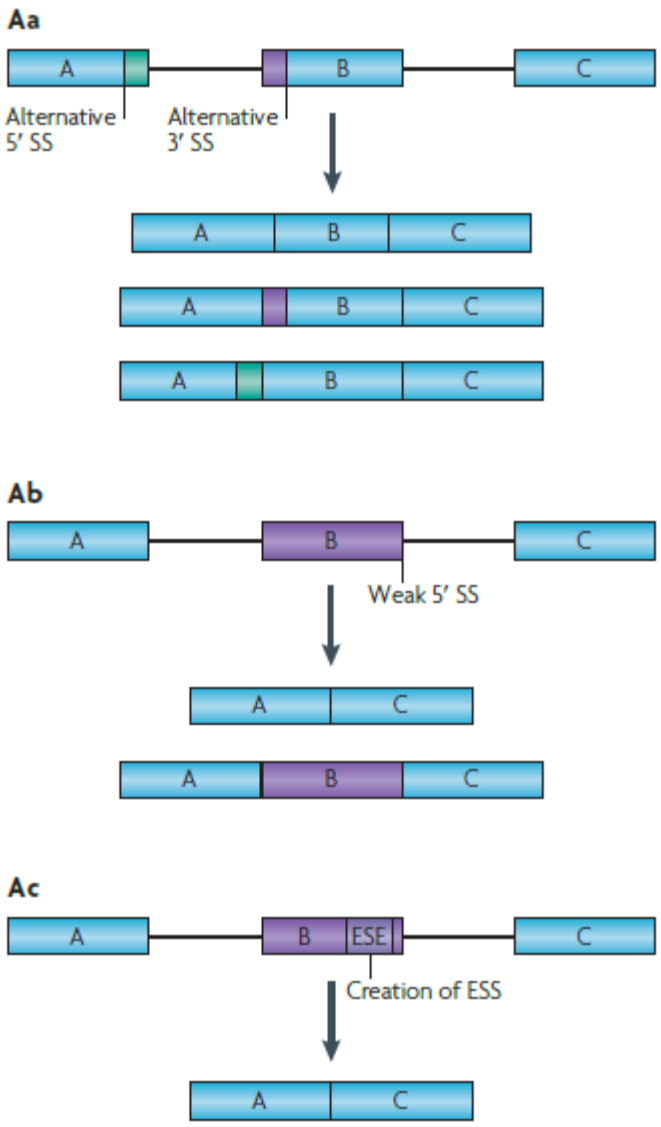
Characteristic	Ancestral alternatively spliced exons compared with constitutive exons	References
Exon size	Shorter	67,79,81
Frame preservation	More likely to be a multiple of 3 nucleotides	81–83,94,99
Intronic conservation	Highly conserved	15,65,77,81,82

doi:10.1038/nrg1896



doi:10.1038/nrg3778

Surgimento do *splicing* alternativo



Alternative splicing and evolution: diversification, exon definition and function

Hadas Keren, Galit Lev-Maor and Gil Ast

Mutações reduzindo o reconhecimento de um éxon:

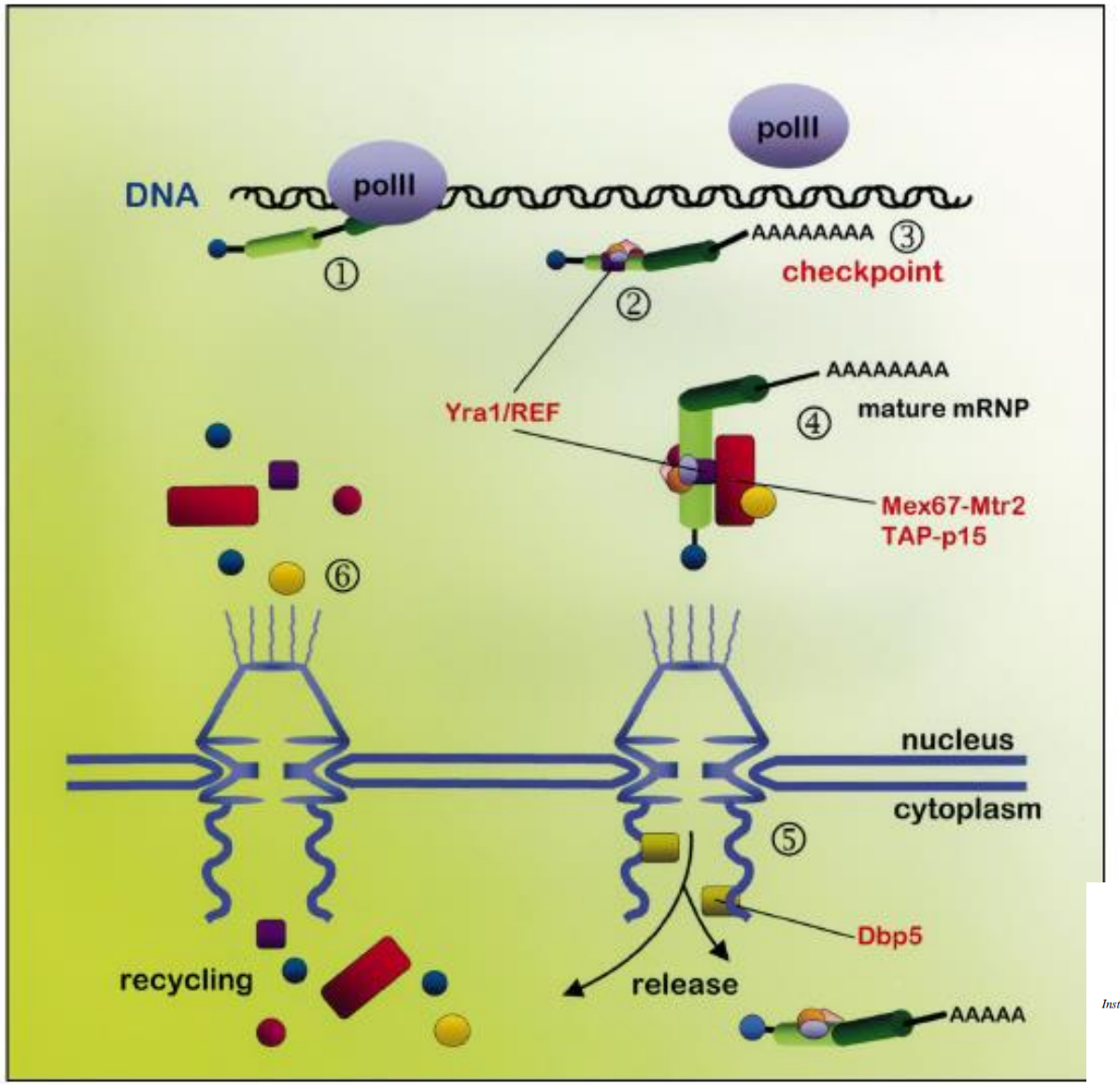
Aa – novos alternativos 5′ SS ou 3′ SS

Ab – Redução do reconhecimento 5′ SS.

Ac – Mutações em éxons ou íntrons

doi:10.1038/nrg2776

EXPORTAÇÃO DO mRPN



Sinalização 20 a 25 pb *upstream* de exons para a ligação aos receptores de exportação – *splicing* e exportação acoplados!!!

Minireview

Nuclear export of mRNA

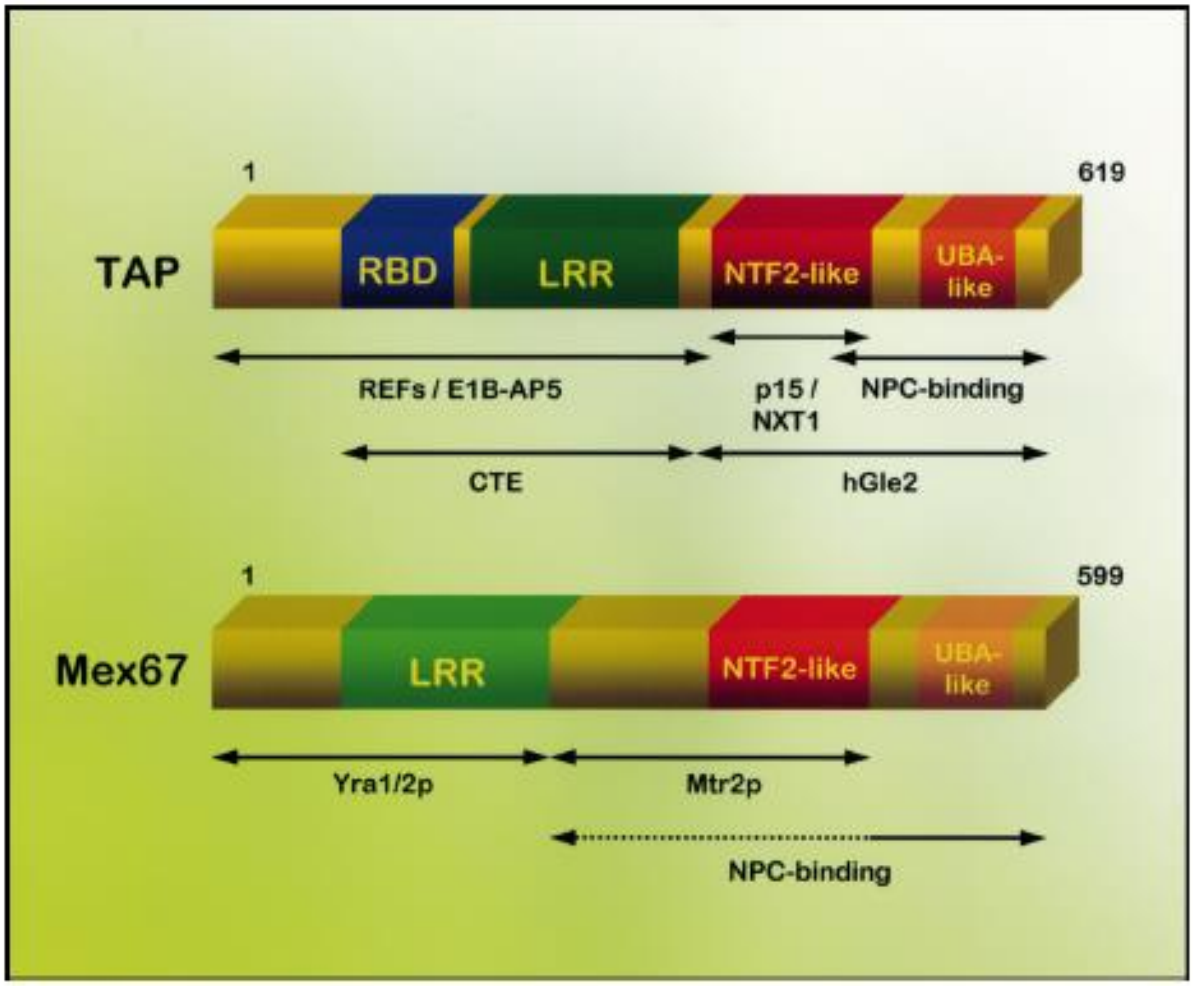
Daniel Zenklusen, Françoise Stutz*

Institute of Microbiology, Centre Hospitalier Universitaire Vaudois, 44, rue du Bugnon, 1011 Lausanne, Switzerland

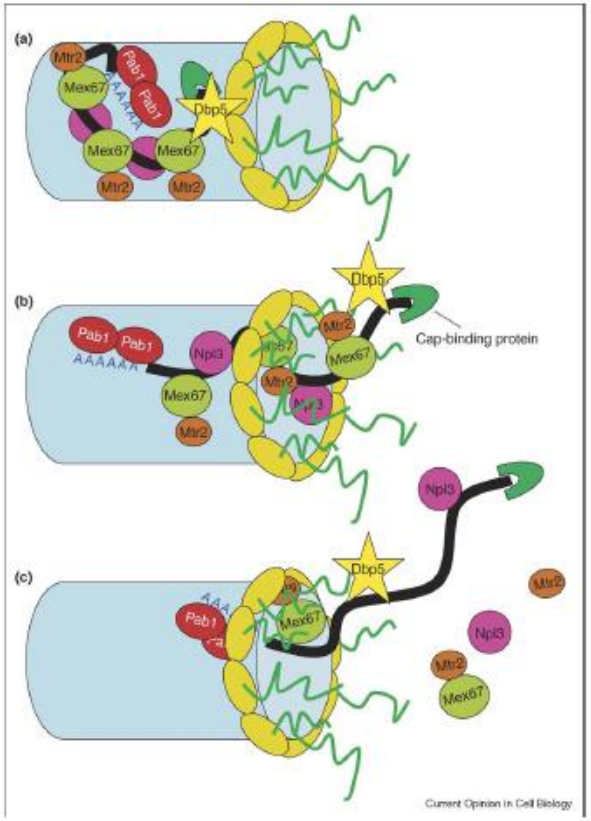
Received 4 May 2001; accepted 4 May 2001

First published online 17 May 2001

Edited by Gunnar von Heijne



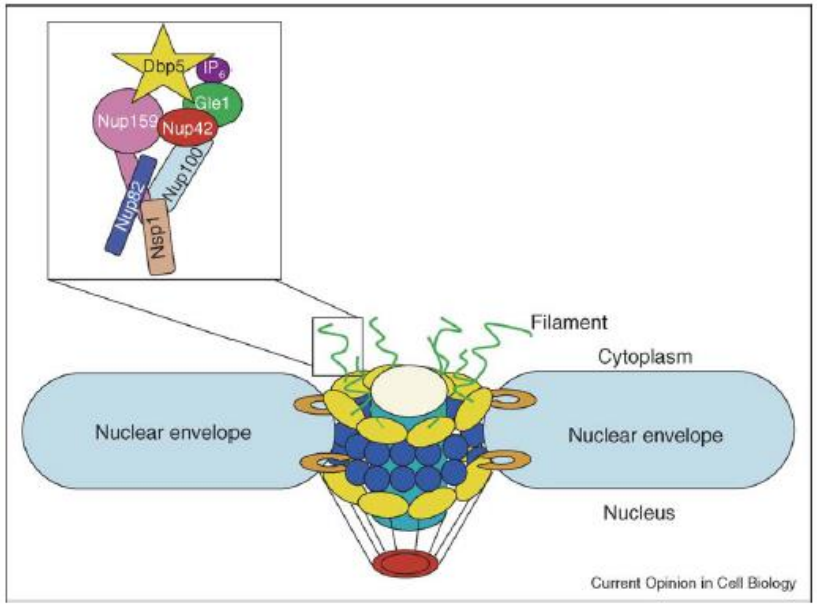
São heterodímeros com domínios não canônicos de ligação ao RNA (RDB)!



ELSEVIER

Transport of messenger RNA from the nucleus to the cytoplasm

Charles N Cole[1,2] and John J Scarcelli[1]



Dbp5 – proteína chave na alteração da mRNP citoplasmática!

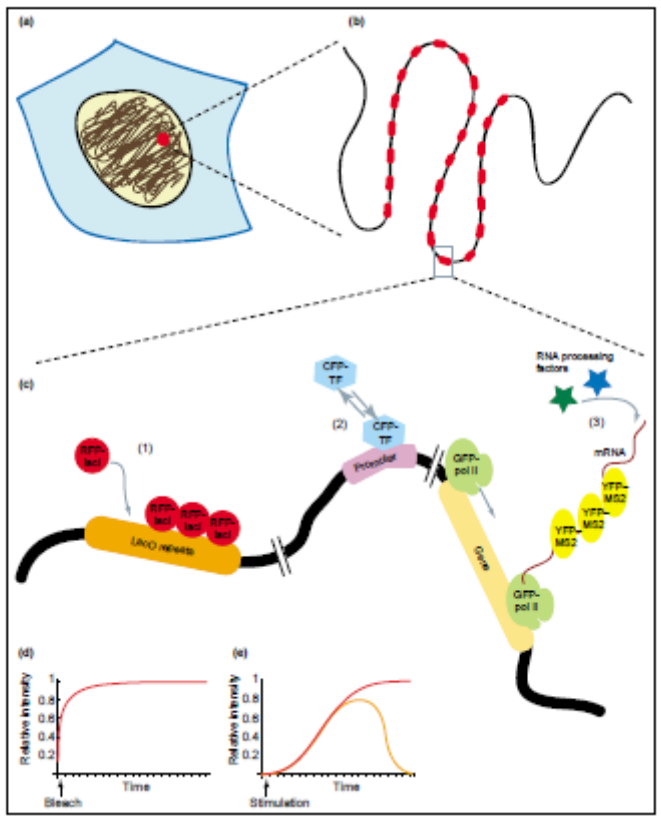
Mas como isso funciona???
Ninguém sabe…☺

doi 10.1016/j.ceb.2006.04.006

ELSEVIER

Dynamics of transcription and mRNA export

Xavier Darzacq[1,2], Robert H Singer[1] and Yaron Shav-Tal[1]



Importante o uso de *single cells* e marcadores fluorescentes

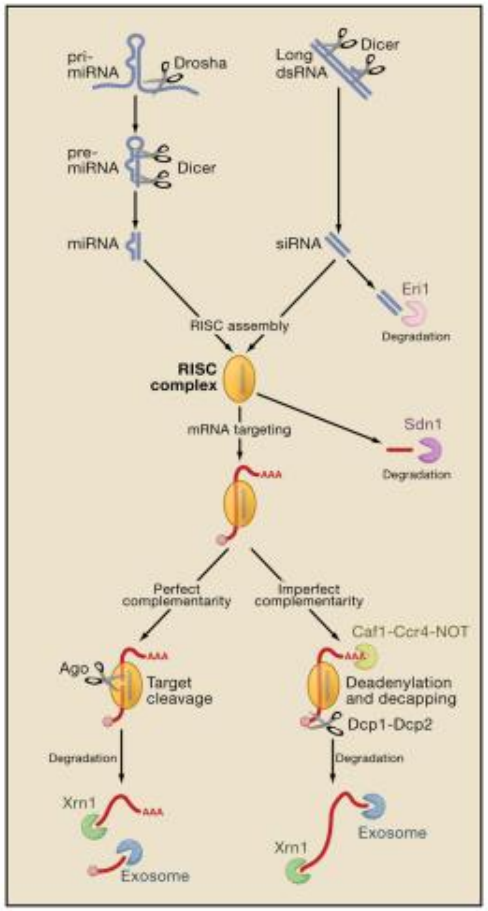
(1) lacO ligado a uma proteina de fluorescencia
(2) Analise de TF por promotores
(3) Produção de mRNAs contendo MS2 *repeats*

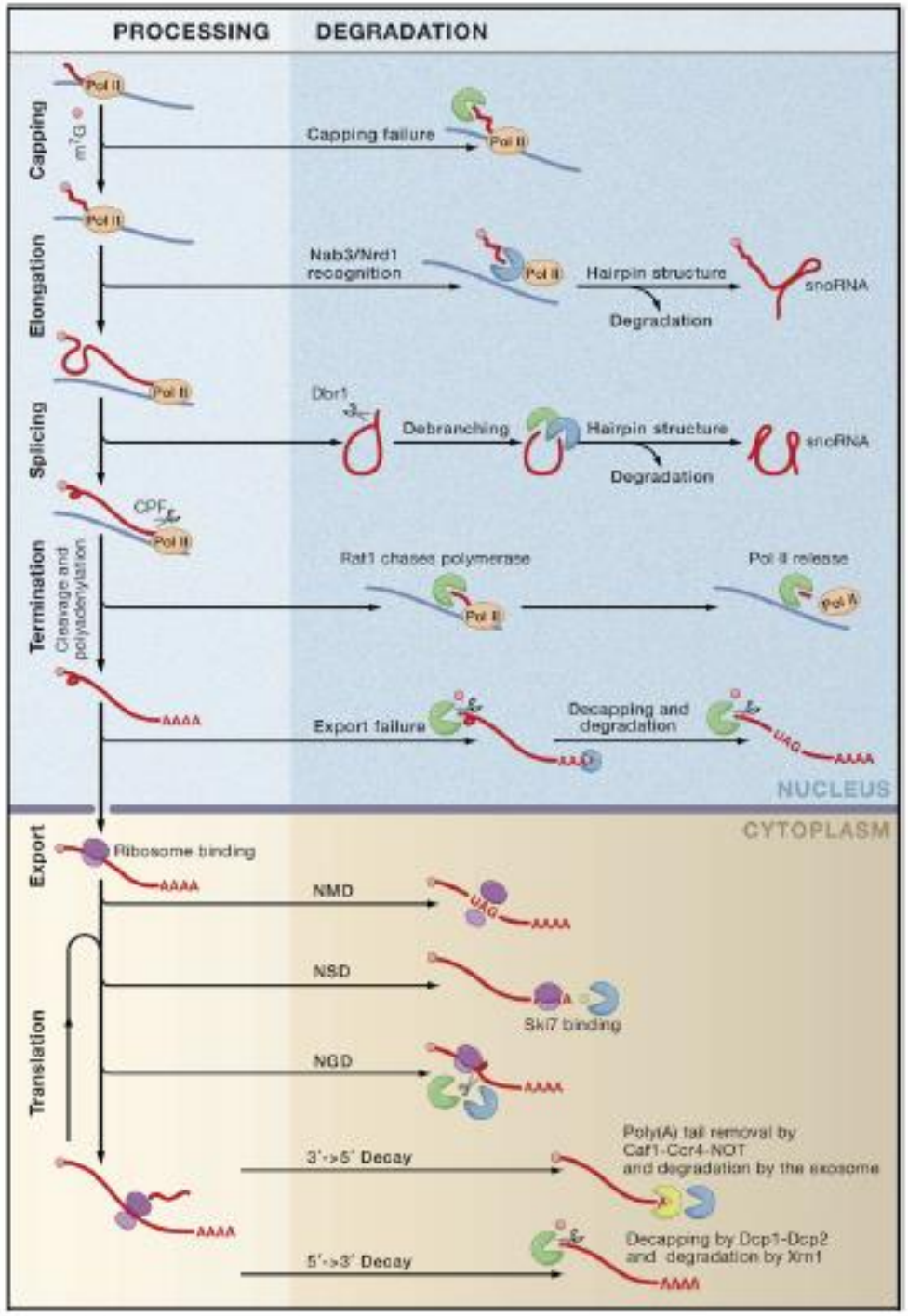
doi: 10.1016/j.ceb.2005.04.004

Leading Edge
Review

The Many Pathways of RNA Degradation

Jonathan Houseley[1] and David Tollervey[1,*]
[1]Wellcome Trust Centre for Cell Biology, University of Edinburgh, EH9 3JR, UK
*Correspondence: d.tollervey@ed.ac.uk
DOI 10.1016/j.cell.2009.01.019





doi: 10.1016/j.cell.2009.01.019

Table 1. Human Homologs of Yeast RNA Degradation Factors

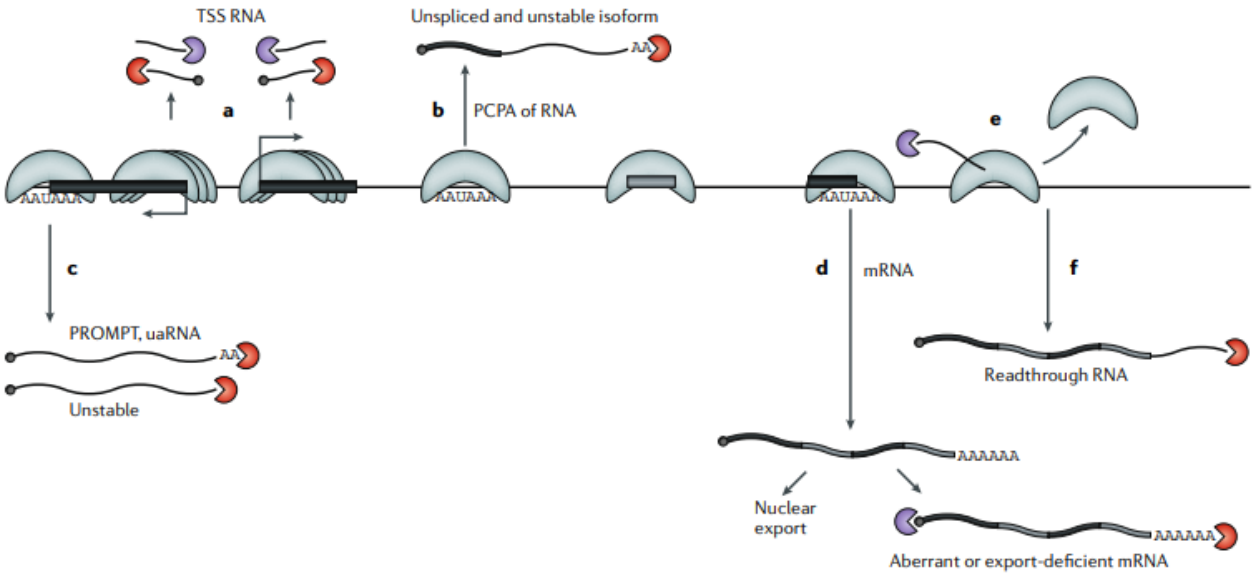
Complex	Protein Names in Yeast	Human Homologs (% Identity)	Information
5′-End Processing Enzymes			
	Dcp1	DCP1B (34%), DCP1A (33%)	Member of decapping complex with Dcp2
	Dcp2	DCP2 (37%)	Catalytic pyrophosphatase subunit of decapping complex
	Rat1	XRN2 (41%)	Nuclear 5′ exonuclease
	Xrn1 (Kem1)	XRN2 (36%), XRN1 (35%)	Cytoplasmic 5′ exonuclease
Core Exosome			Highly conserved RNA decay complex
	Rrp44 (Dis3)	Rrp44 (44%)	Only catalytic component of the core exosome; 3′ hydrolytic exonuclease and endonuclease activity
	Csl4	EXOSC1 (48%)	Member of core exosome complex
	Rrp4	EXOSC2 (41.6%)	Member of core exosome complex
	Rrp40	EXOSC3 (35.1%)	Member of core exosome complex
	Rrp41	EXOSC4 (35.4%)	Member of core exosome complex
	Rrp42	EXOSC7 (35.1%)	Member of core exosome complex
	Rrp43	EXOSC8 (29%)	Member of core exosome complex
	Rrp45	EXOSC9 (34.8%)	Member of core exosome complex
	Rrp46	EXOSC5 (29.1%)	Member of core exosome complex
	Mtr3	EXOSC6 (27%)	Member of core exosome complex
Exosome-Associated Factors			
	Rrp6	EXOSC10 (32%)	Nuclear-specific exosome component; 3′ hydrolytic exonuclease
	Rrp47 (Lrp1)	C1D (32%)	Nuclear exosome cofactor
	Mpp6	MPP6 (distantly related)	Nuclear exosome cofactor
	Ski7	unclear	Cytoplasmic exosome cofactor, connects exosome to Ski complex
TRAMP Complexes			Nuclear RNA degradation factors involved in RNA quality control and cryptic RNA degradation
	Trf4 (Pap2)	POLS (37%)	Nuclear poly(A) polymerase, TRAMP4 complex component
	Trf5	PAPD5 (36%)	Nuclear poly(A) polymerase, TRAMP5 complex component
	Air1, Air2	ZCCHC3 (37%, 39%)	TRAMP complex components
	Mtr4	SKIV2L2 (52%)	Helicase, TRAMP complex component, has TRAMP-independent functions
Ski Complex			Cytoplasmic mRNA degradation complex, also involved in viral suppression
	Ski2	SUPV3L1 (60%), SKIV2L (38%)	Cytoplasmic helicase, member of exosome cofactor Ski complex
	Ski3	unclear	Member of exosome cofactor Ski complex
	Ski8	unclear	Member of exosome cofactor Ski complex
Sen1-Nrd1-Nab3 Complex			Involved in Pol II termination and processing/degradation of snoRNA, snRNA, and cryptic ncRNAs
	Sen1	LOC91 431 (36%)	Helicase
	Nrd1	unclear	RNA-binding protein
	Nab3	unclear	RNA-binding protein
Lsm Complexes			7 member ring-shaped RNA chaperone complexes
	Lsm1	LSM1 (46%)	Member of cytoplasmic Lsm1-7 complex
	Lsm2	LSM2 (63%)	Member of both Lsm complexes
	Lsm3	LSM3 (41%)	Member of both Lsm complexes
	Lsm4	LSM4 (31%)	Member of both Lsm complexes
	Lsm5	LSM5 (51%)	Member of both Lsm complexes

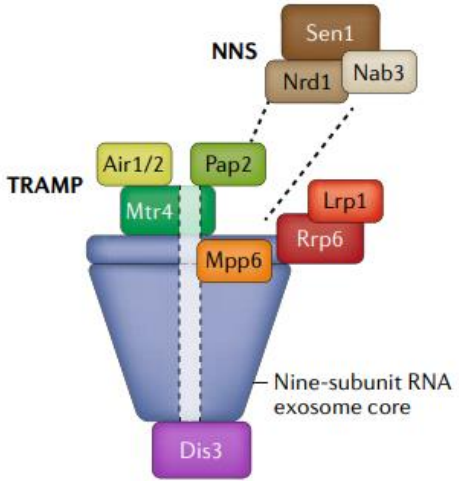
doi: 10.1016/j.cell.2009.01.019

REVIEWS

Controlling nuclear RNA levels

Manfred Schmid and Torben Heick Jensen**

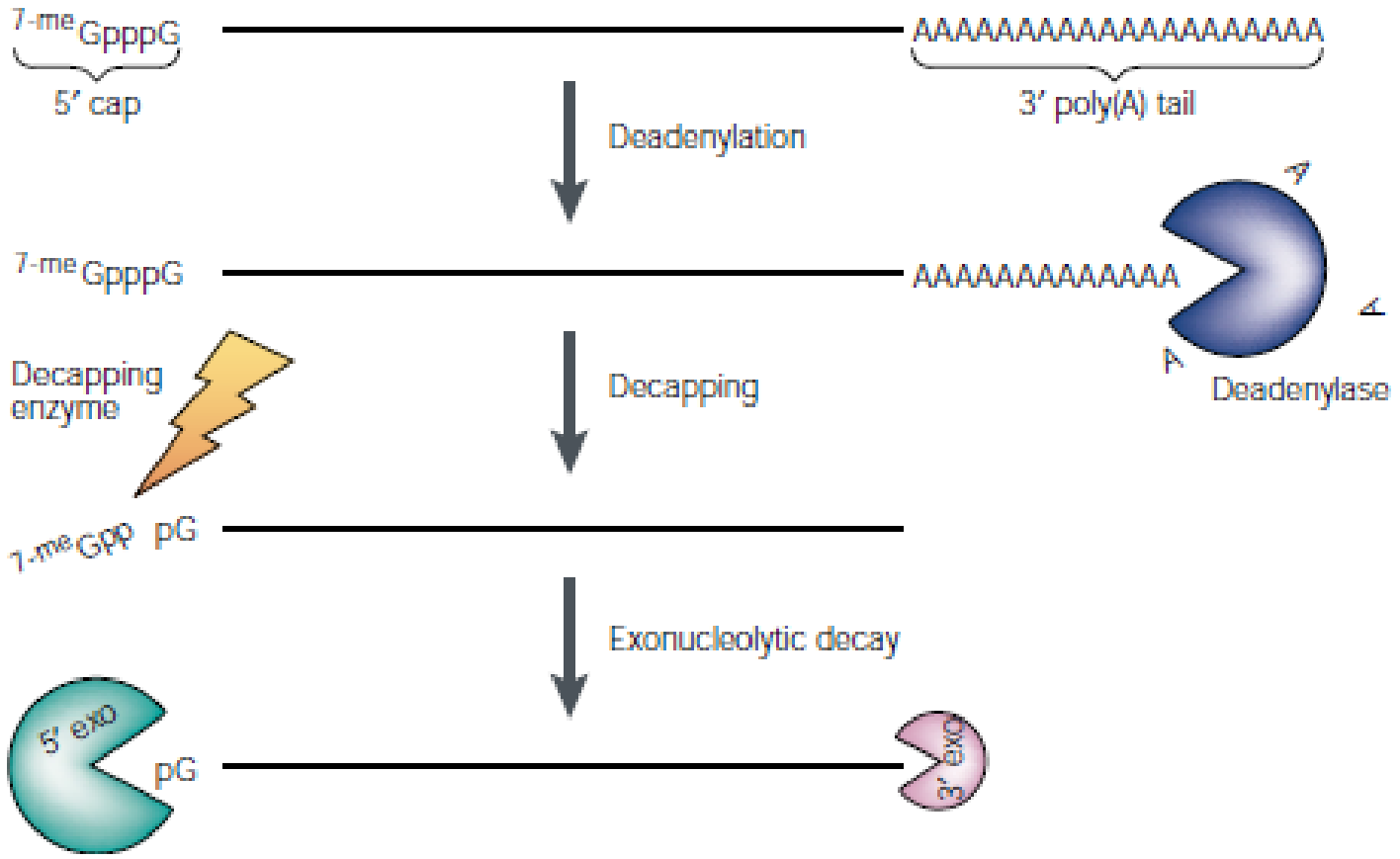


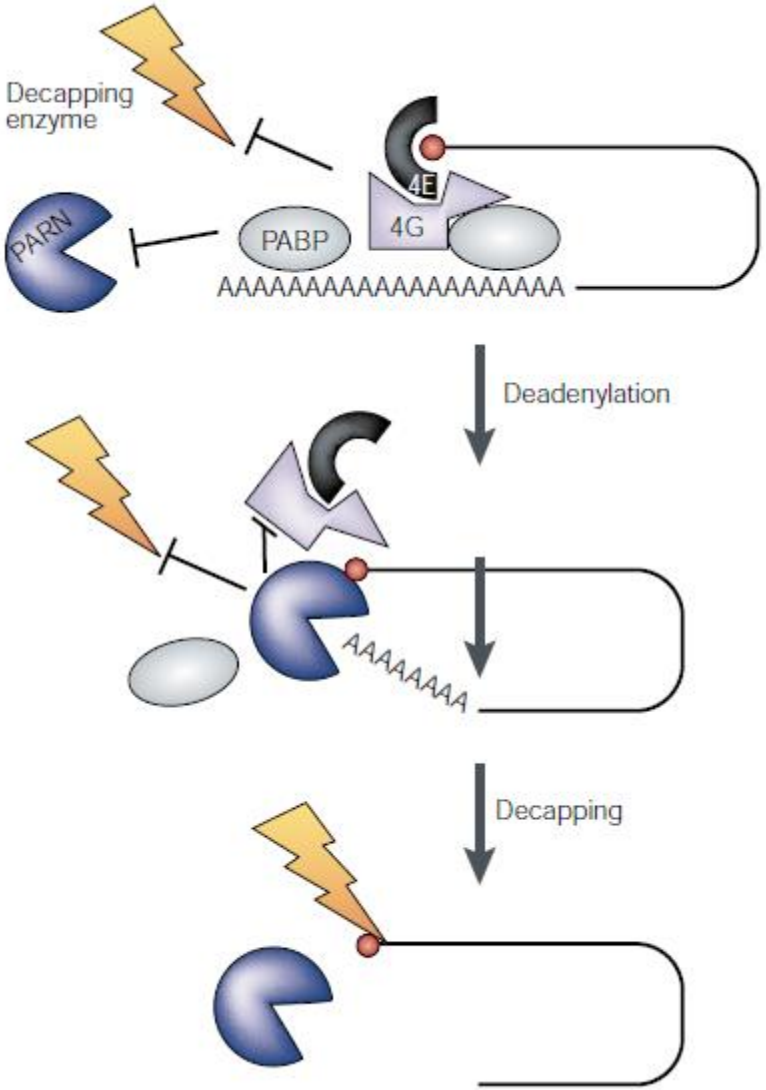


doi:10.1038/s41576-018-0013-2

THE CAP-TO-TAIL GUIDE TO mRNA TURNOVER

Carol J. Wilusz, Michael Wormington*‡ and Stuart W. Peltz*‡*



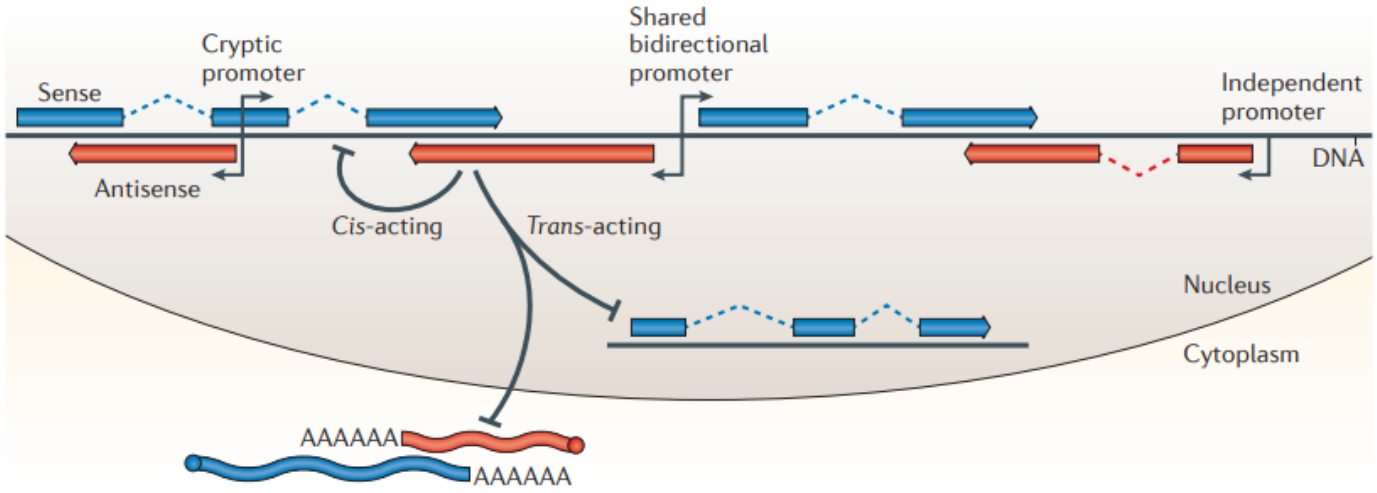


Fatores de tradução também protegem o mRNP durante a producão de proteínas.

NON-CODING RNA

Gene regulation by antisense transcription

Vicent Pelechano[1] and Lars M. Steinmetz[1–3]



doi:10.1038/nrg3594

Table 1 | **Examples of functional antisense transcription across all kingdoms of life**

Mechanism of action	Antisense locus	Effects	Species	Refs
DNA methylation	*LUC7L*	Methylates *HBA1* promoter CpG island, which represses its expression	Humans	76
	Air	Regulates *Igf2r* imprinting by DNA methylation	Mice	77,78
Chromatin modifications	*XIST* and *TSIX*	Inactivates X chromosome gene expression	Mammals	2
	ANRIL	Represses the tumour suppressor locus *CDKN2B–CDKN2A* by both histone H3 lysine 27 (H3K27) methylation and DNA methylation	Humans	57, 80
	BDNF-AS	Represses *BDNF* by histone modification	Mammals	81
	HOTAIR	Silences the *HOXD* locus in *trans* by the recruitment of Polycomb proteins	Humans	3
	COOLAIR	Represses *FLC* sense gene by H3K4 demethylation and recruits Polycomb proteins, which increase H3K27me3 levels	Plants	85, 86
	COLDAIR	Antisense to *COOLAIR*; represses *FLC* sense gene by the recruitment of Polycomb proteins	Plants	88
	AS to *PHO84*	Represses *PHO84* by histone deacetylation both in *cis* and in *trans*	*S. cerevisiae*	4,69
	RTL	Silences transcription of the *Ty1* retrotransposon in *trans* through chromatin modification and post-transcriptionally controls its retrotransposition	*S. cerevisiae*	70, 73
Transcriptional interference	*RME2*	Represses *IME4* by transcriptional interference in *cis* and functions after transcription initiation of *IME4*	*S. cerevisiae*	5,99
Isoform variation	*ZEB2-AS*	Induces exon skipping in *ZEB2*, which produces an alternative isoform that has increased translation efficiency	Humans	7
Translation efficiency	AS to *Uchl1*	Increases translation efficiency of *Uchl1* using a SINEB2 domain	Mice	33
	SymR	Decreases translation efficiency of *SymE* by competing with binding of the 30S ribosome	Enterobacteria	6
RNA stability	*BACE1-AS*	Increases stability of *BACE1* by masking an microRNA-binding site	Humans	105, 106
	WDR83 and *DHPS*	Increase their mutual stability by forming a duplex within their 3′ untranslated regions	Humans	53

doi:10.1038/nrg3594

PRÓXIMA AULA – 31 de Maio

Aula “Controle Traducional e Pós Traducional Da Expressão Gênica e Processamento e direcionamento de proteínas “– início 9 horas

Apresentação:
Fosfoproteoma e proteoma subcelular.