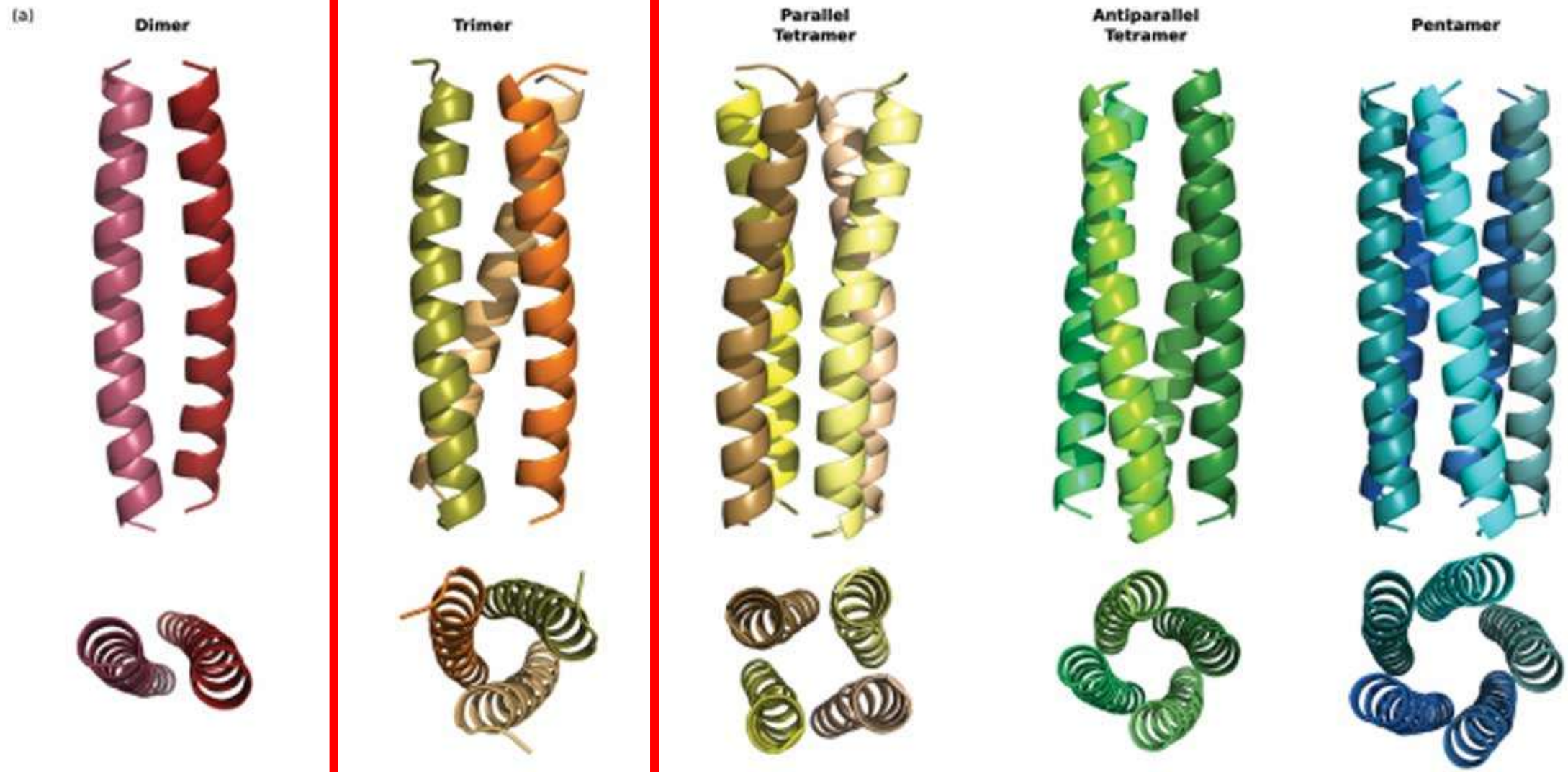
Risposte quesiti 1° Parte corso

Elementi Mobili

Elementi mobili LINE1:

- ORF1, ci sono due domini, un Recognition Motif ed un Coiled Coil.

Quali sono le funzioni del Coiled Coil Domain ?

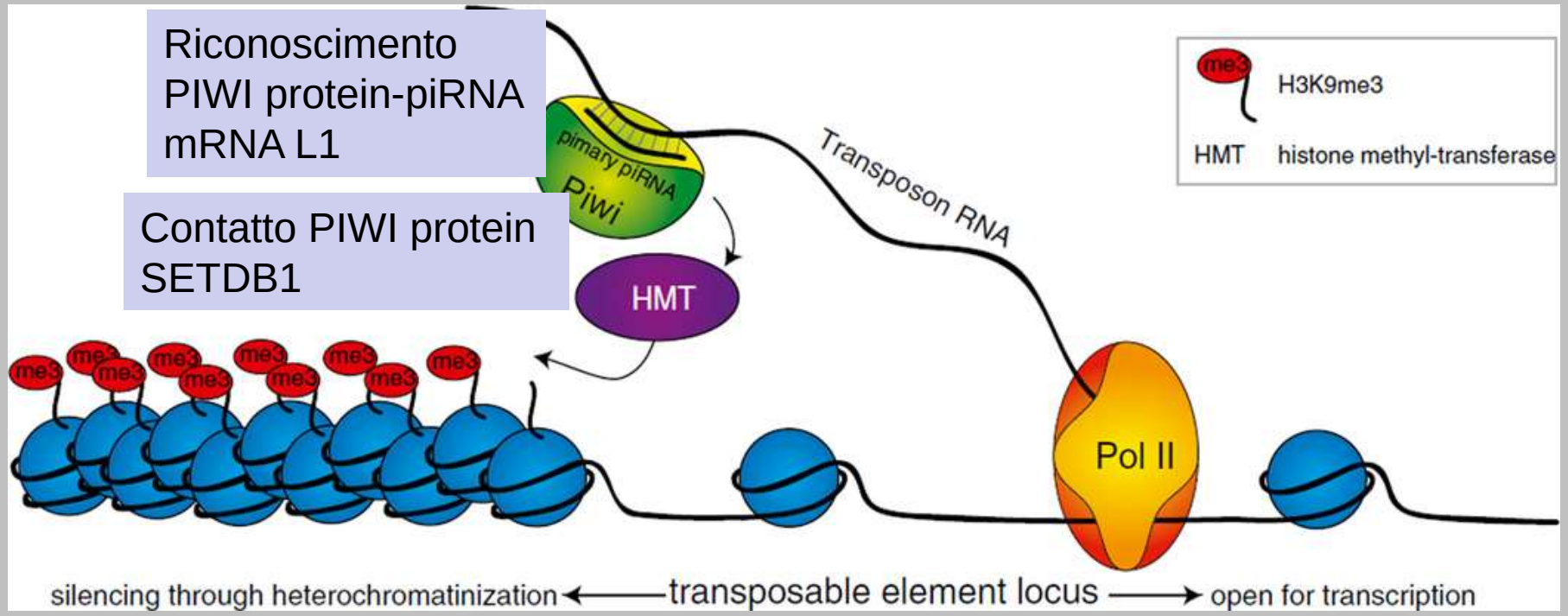

(a)
Dimer
Trimer
Parallel
Tetramer
Antiparallel
Tetramer
Pentamer

Silenziamento Elementi Mobili

- Silenziamento dei trasposoni: - La metil-transferasi SETDB1 compie la trimetilazione della lisina 9 H3, o richiama la proteina HP1?

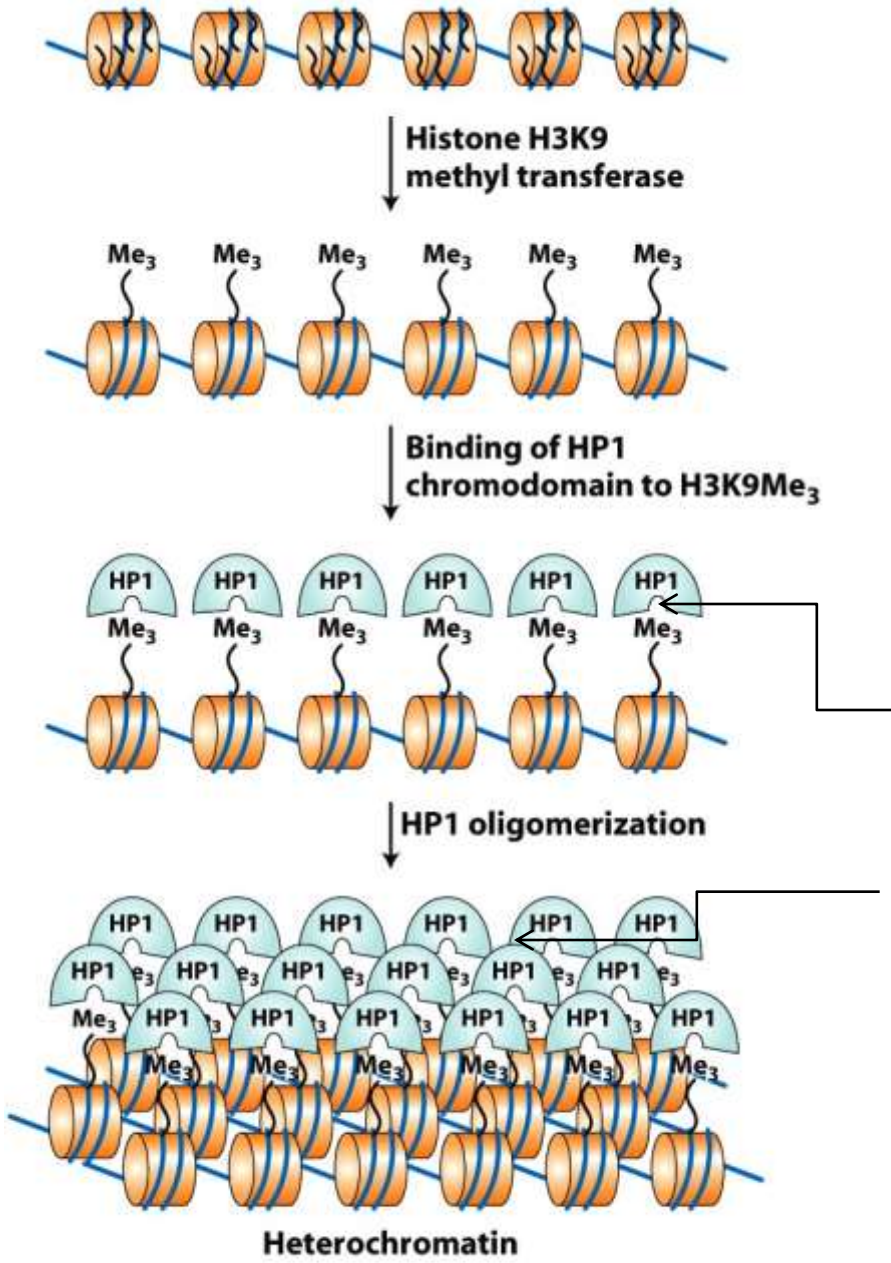
- -

nuclear function of the piRNA pathway



SETDB1 = HMT Histone Methyl Transerase

Riconoscimento
H3K9 – HP1 (Cromodominio)



Cromodominio

Figure 6-34a
Molecular Cell Biology, Sixth Edition

Inibizione Elementi Mobili - PIWI proteins

- Le PIWI sono geni?

Sono proteine codificati da mRNA trascritti dalla RNA pol II da geni corrispondenti, e che vanno ad interagire con i pi-RNA

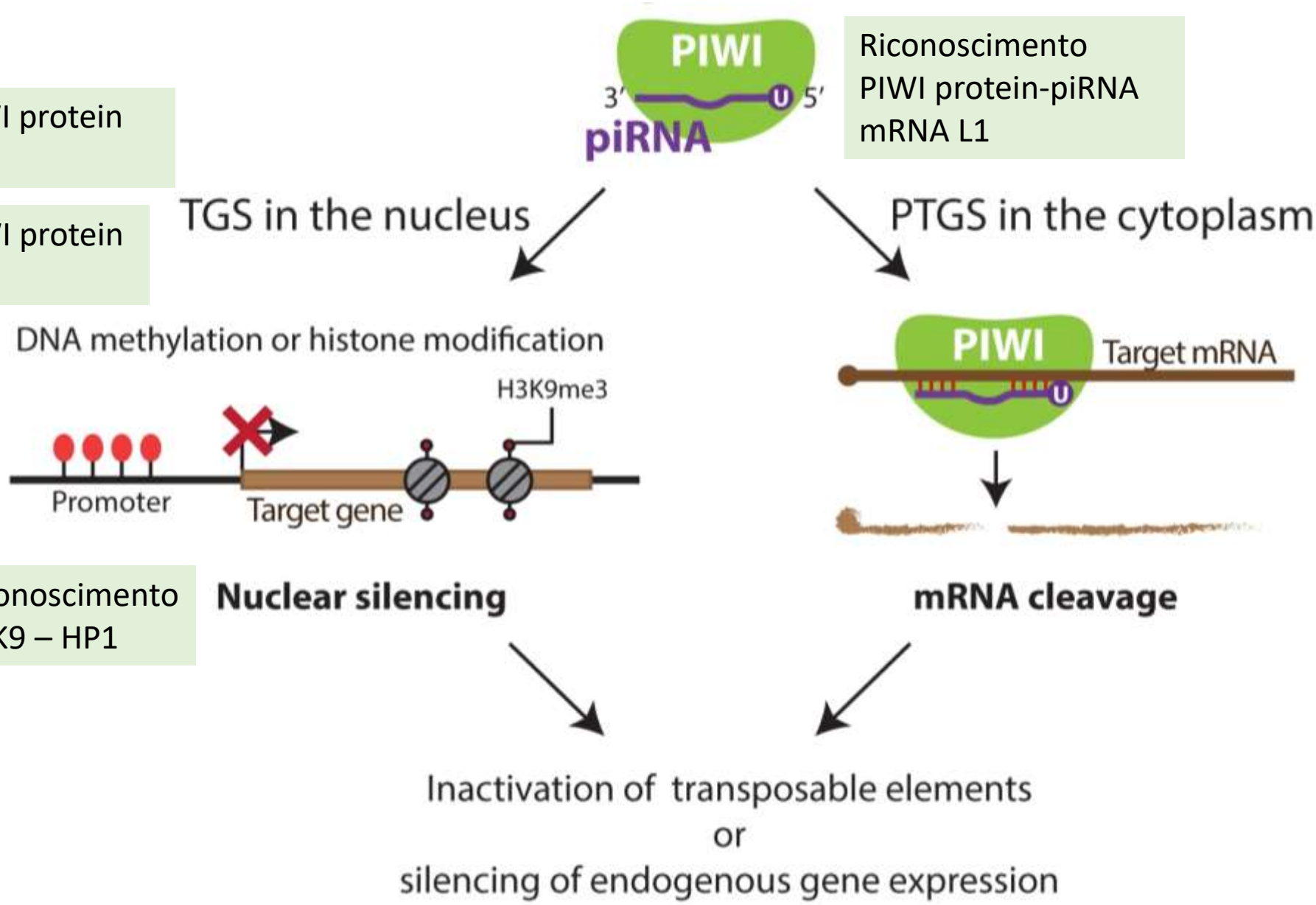
- Non ho compreso la differenza fra i piRNAs, PIWI, MIWI

transcriptional gene regulation (TGS) or post-transcriptional gene regulation (PTGS) by PIWI –piRNA complexes

Contatto PIWI protein
SETDB1

Contatto PIWI protein
DNMT

Riconoscimento
H3K9 – HP1



Riconoscimento
PIWI protein-piRNA
mRNA L1

A PIWI protein

B

Human	Mouse
PIWIL1 (HIWI)	PIWIL1 (MIWI)
PIWIL2 (HILI)	PIWIL2 (MILI)
PIWIL3 (HIWI3)	–
PIWIL4 (HIWI2)	PIWIL4 (MIWI2)

Inibizione Elementi Mobili - piRNA

- Maturazione Pi RNA?

E' una via quella di maturazione molto complessa e con grandi differenze tra organismi

“piRNA is the most diverse class of regulatory RNAs in general. According to piRBase, the number of unique piRNA sequences in the mouse is **over 68 million”**

Inibizione Elementi Mobili - Tudor Domains

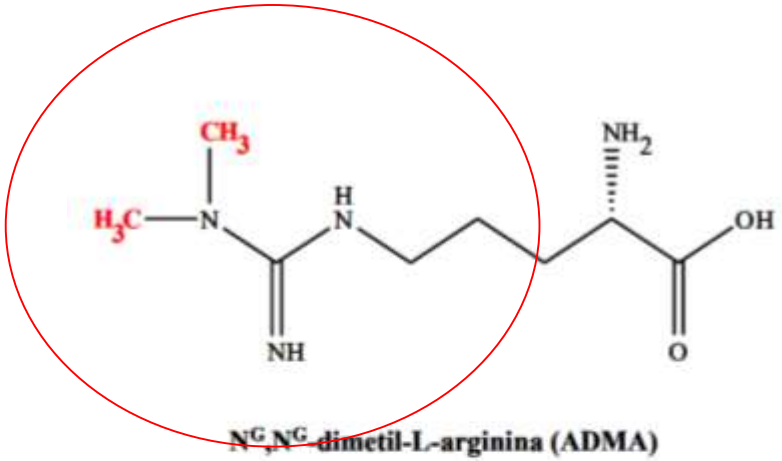
- Non ho compreso la differenza fra i piRNAs, PIWI, MIWI e la connessione con le proteine TUDOR.
- Linee germinali e contributo delle proteine appartenenti alla famiglia di Tdrd.
- In che modo i domini Tudor partecipano alla biogenesi dei pi-RNA in relazione alla dimetilazione asimetrica delle Arginine?

Inibizione Elementi Mobili - Tudor Domains

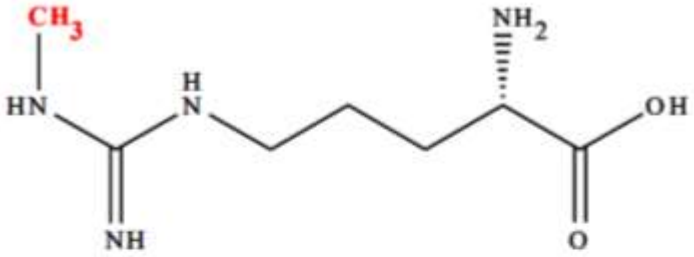
- I domini tudor permettono un riconoscimento proteina proteina tra gli enzimi che maturano i piRNA. In particolare le proteine con le Arg di-metilate in modo simmetrico.
- l'espressione sia dei messaggeri per PIWI proteins che dei piRNA è particolarmente efficiente nella linea germinale
- Quando si interrompe il riconoscimento (es trd12 KO) si interrrompe il processo e ci sono disastri come l'atrofia testicolare (esempio modello murino)

Di-metil arginina asimmetrica (ADMA) e simmetrica (SDMA)

Asimmetrica

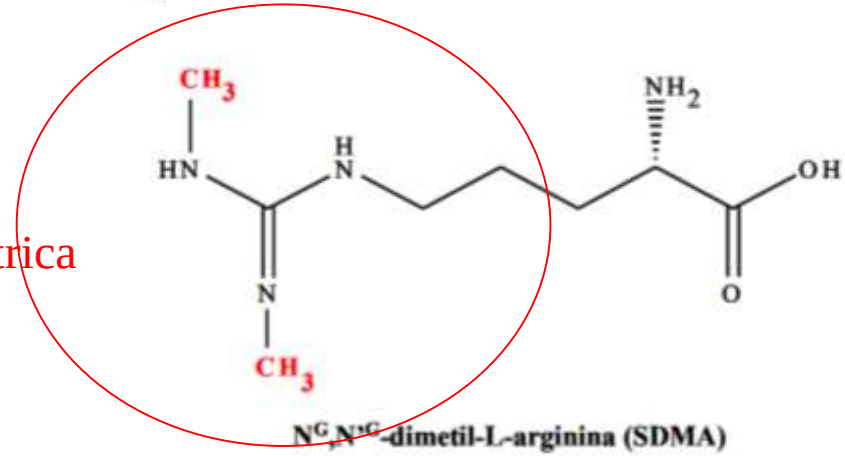


N^{G},N^{G}-dimetil-L-arginina (ADMA)



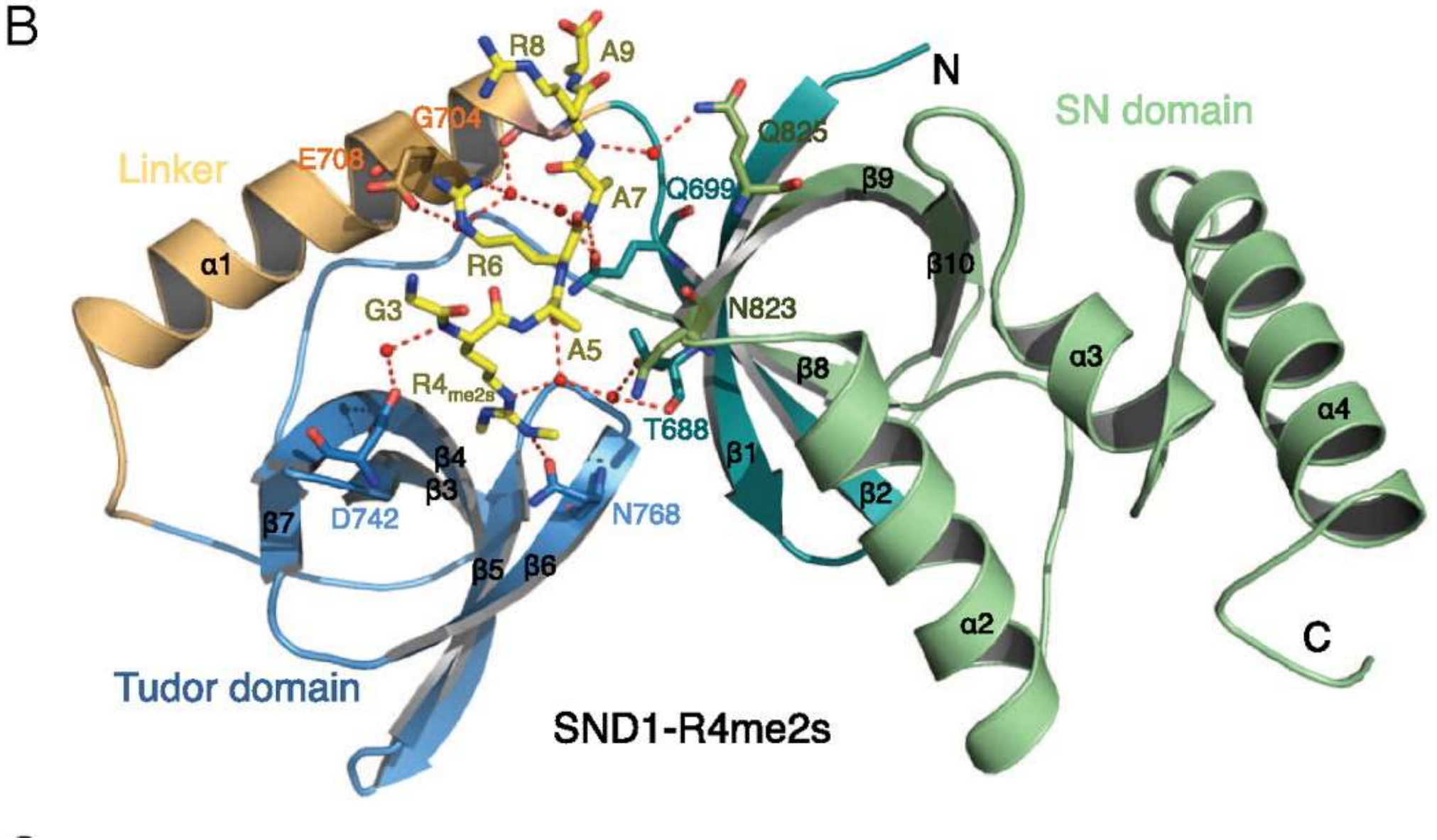
N^{G}-monometil-L-arginina (NMMA)

Simmetrica

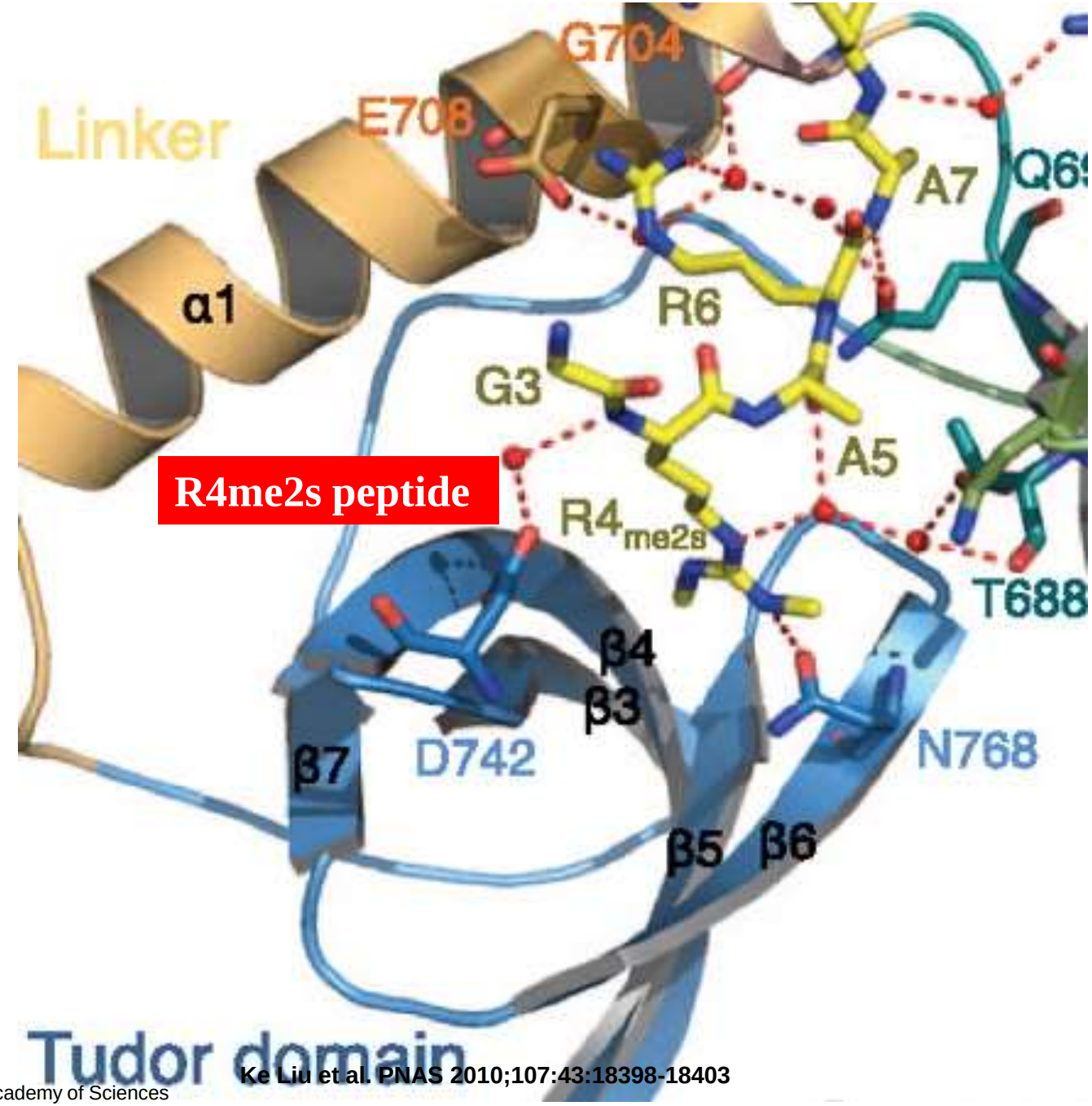


N^{G},N'^{G}-dimetil-L-arginina (SDMA)

Crystal structures of human extended Tudor domain protein in complex with PIWIL1 peptides.

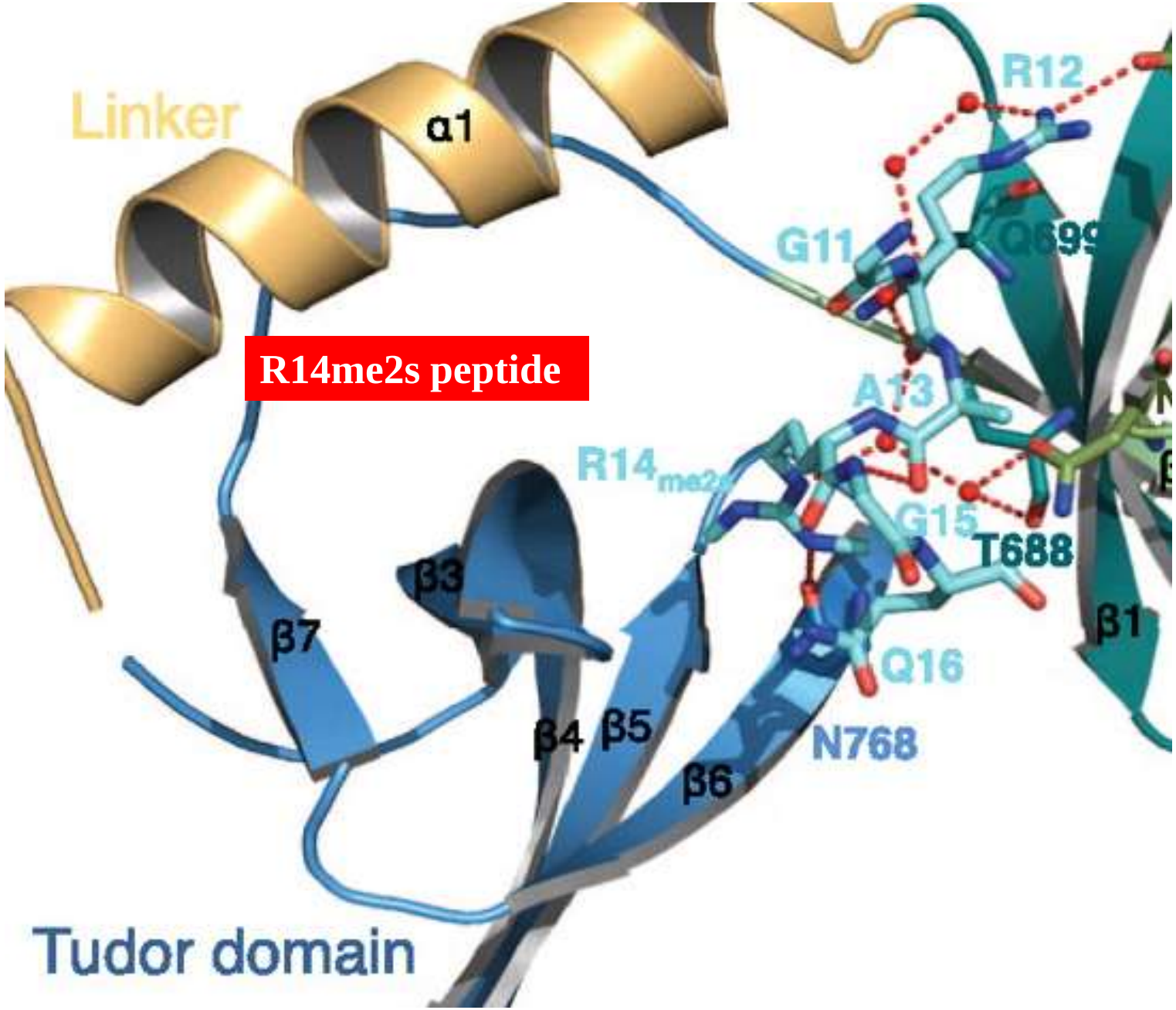


Ke Liu et al. PNAS 2010;107:43:18398-18403



Ke Liu et al. PNAS 2010;107:43:18398-18403

Crystal structures of human extended Tudor domain in complex with PIWIL1 peptides.



Extended Tudor domain preferentially binds Piwi peptides with symmetrical methylation of Arg R me2s

Peptide name	Sequence	Kd (μM) (pH 7.5, 50mM NaCl)	Kd (μM) (pH 7.5, 150mM NaCl)
R4me2s	TGRARARARGRARGQE	10±1	47±6
R4me2a	TGRARARARGRARGQE	42±2	188±38
R4me1	TGRARARARGRARGQE	19±1	97±20
Histone H3K4me1/2/3	ARTKQTARKST	No detectable binding	No detectable binding
Histone H3K9me1/2/3	ARTKQTARKSTGGKA	No detectable binding	No detectable binding

Nessun legame agli istoni metilati!

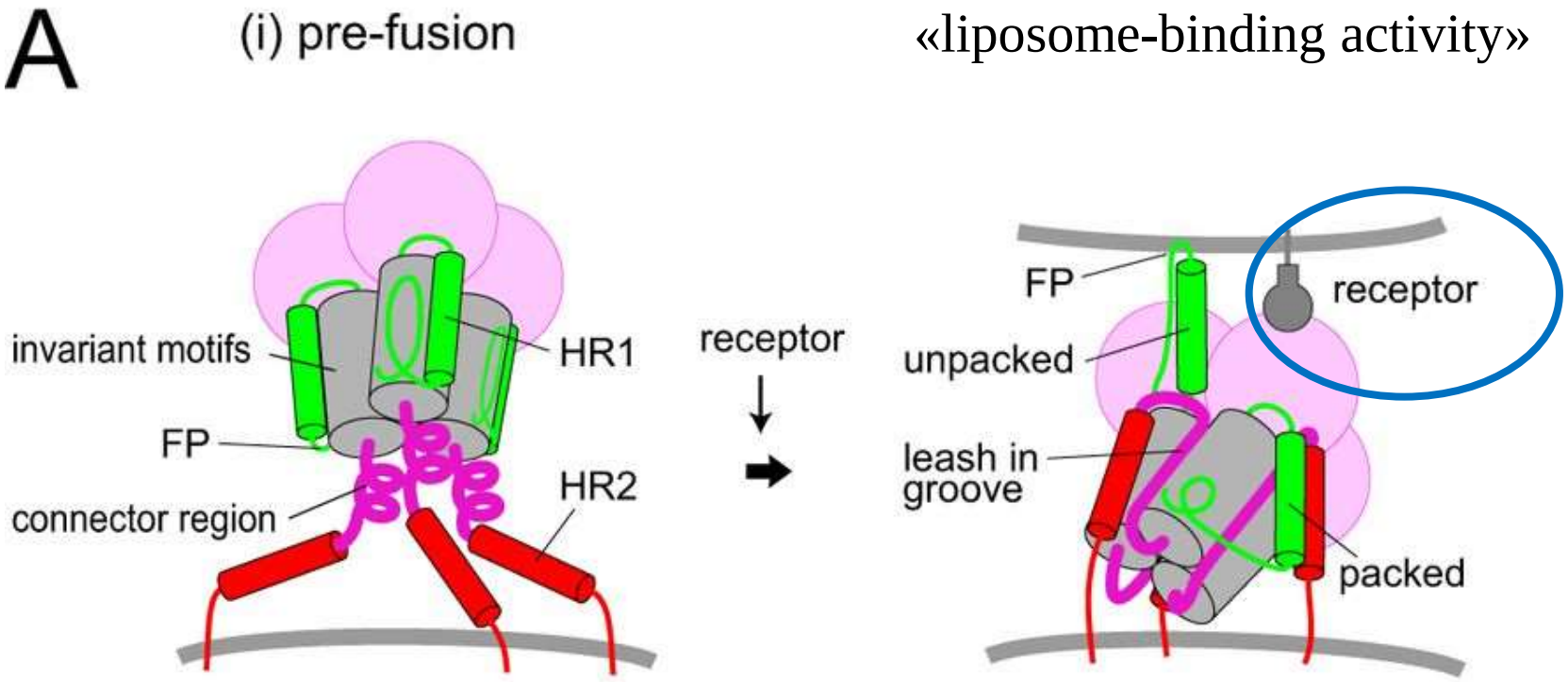
R: modified arginine; K: modified lysine; *: all Kd are measured by fluorescence polarization except H4R3me2s, which was measured by ITC.

Ke Liu et al. PNAS 2010;107:43:18398-18403

Coronavirus - Proteasi

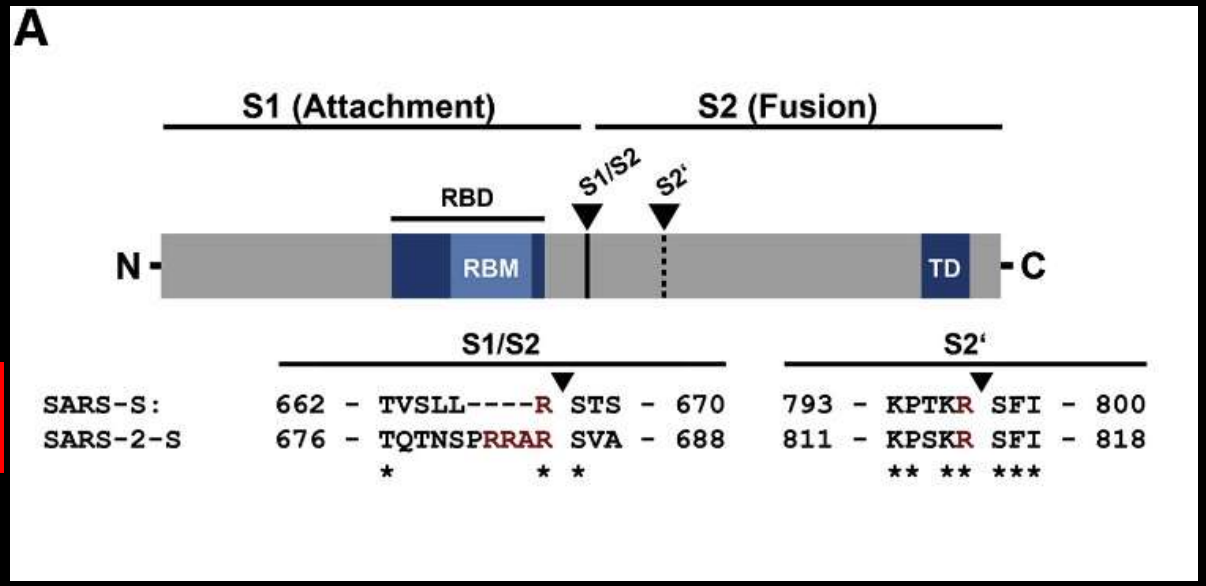
- Non mi è chiaro il concetto di protein priming

Schematic diagram of S protein activation.



Miyuki Kawase et al. J. Virol. 2019; doi:10.1128/JVI.00785-19

Figure 1

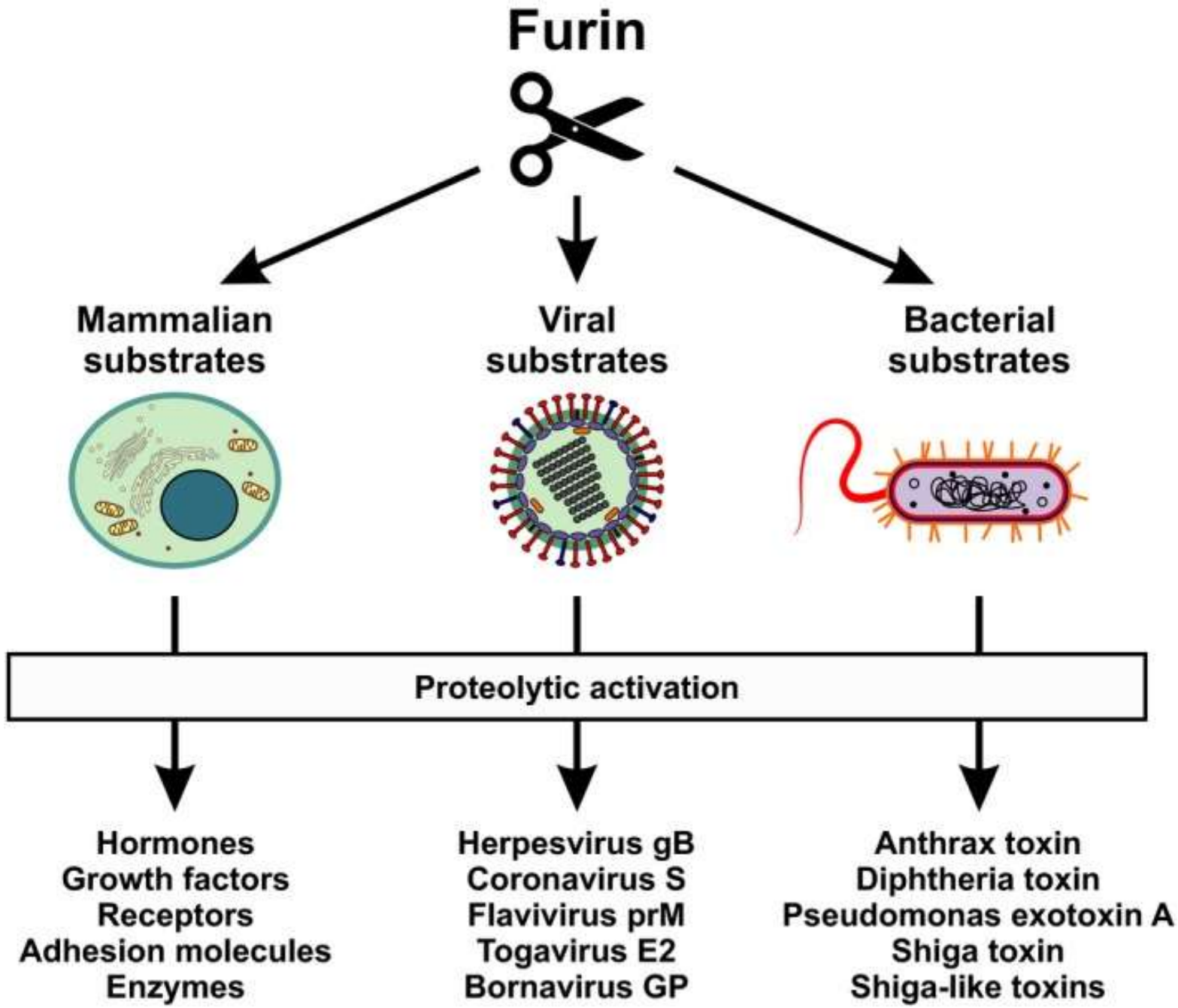


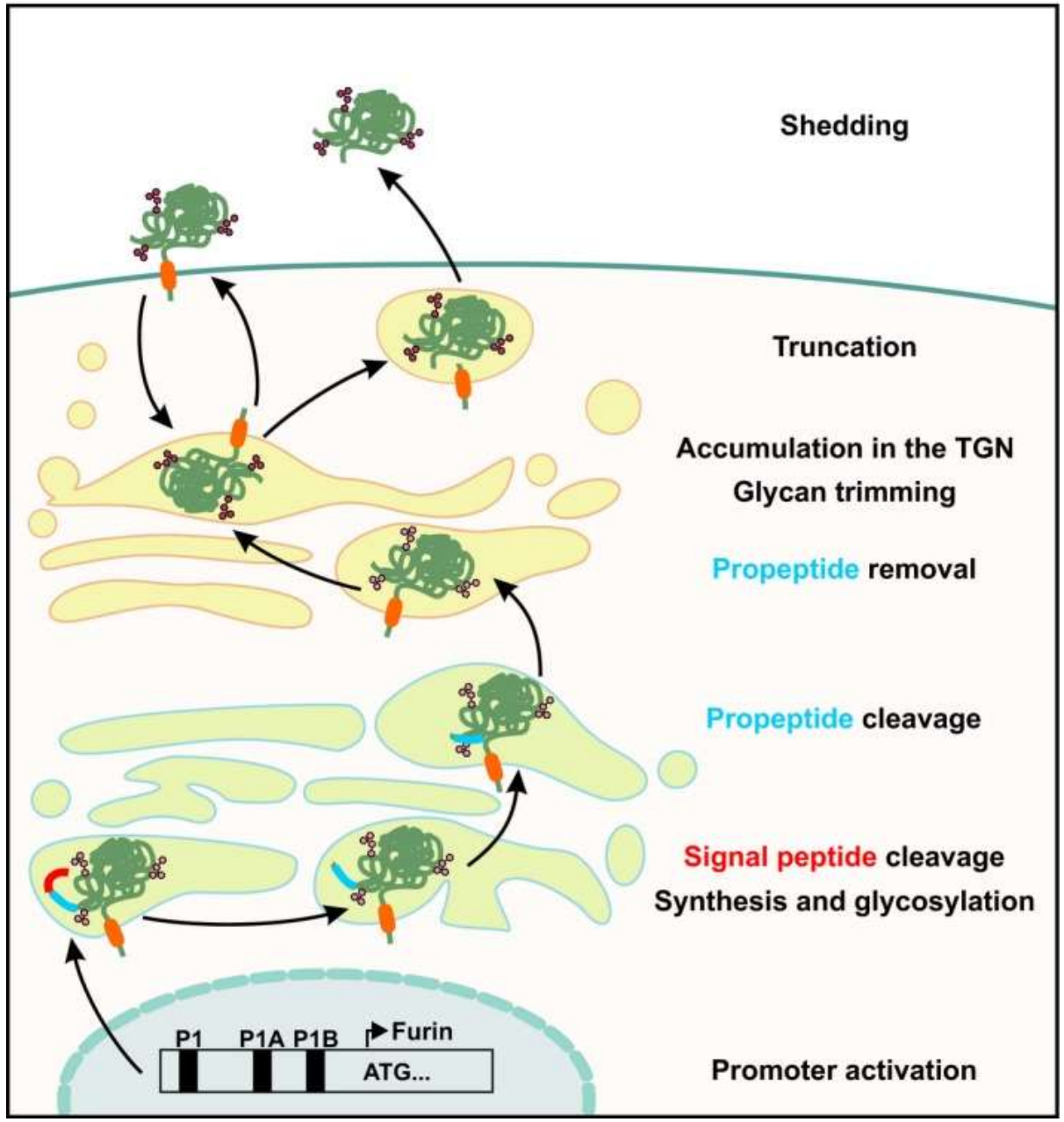
Virus entry requires **S protein priming** by cellular proteases, which cleave S protein at the S1/S2 and the S2' site and allows fusion of viral and cellular membranes, a process driven by the S2 subunit

Cell DOI: (10.1016/j.cell.2020.02.052)

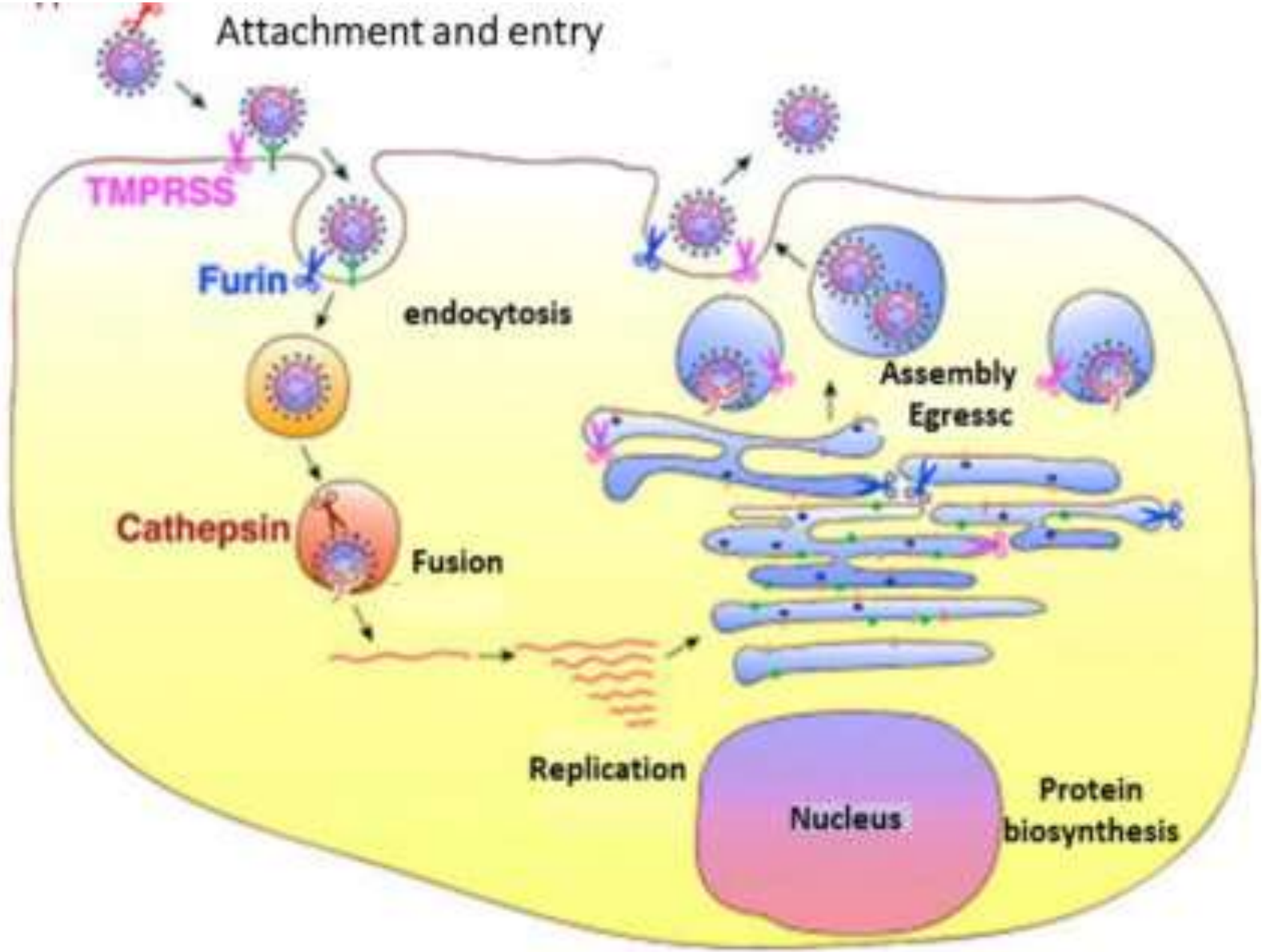
Coronavirus - Proteasi

- Proteasi dell'ospite FURINA e TMPRSS2 hanno la medesima funzione nella maturazione dello Spike in tessuti diversi o sono molecole diverse? non ho compreso bene la differenza fra le due.


Furin
Mammalian substrates
Viral substrates
Bacterial substrates
Proteolytic activation
Hormones
Growth factors
Receptors
Adhesion molecules
Enzymes
Herpesvirus gB
Coronavirus S
Flavivirus prM
Togavirus E2
Bornavirus GP
Anthrax toxin
Diphtheria toxin
Pseudomonas exotoxin A
Shiga toxin
Shiga-like toxins


Shedding
Truncation
Accumulation in the TGN
Glycan trimming
Propeptide removal
Propeptide cleavage
Signal peptide cleavage
Synthesis and glycosylation
P1
P1A
P1B
Furin
ATG...
Promoter activation

FURINA


Attachment and entry
TMPRSS
Furin
endocytosis
Cathepsin
Fusion
Replication
Assembly
Egressc
Nucleus
Protein
biosynthesis

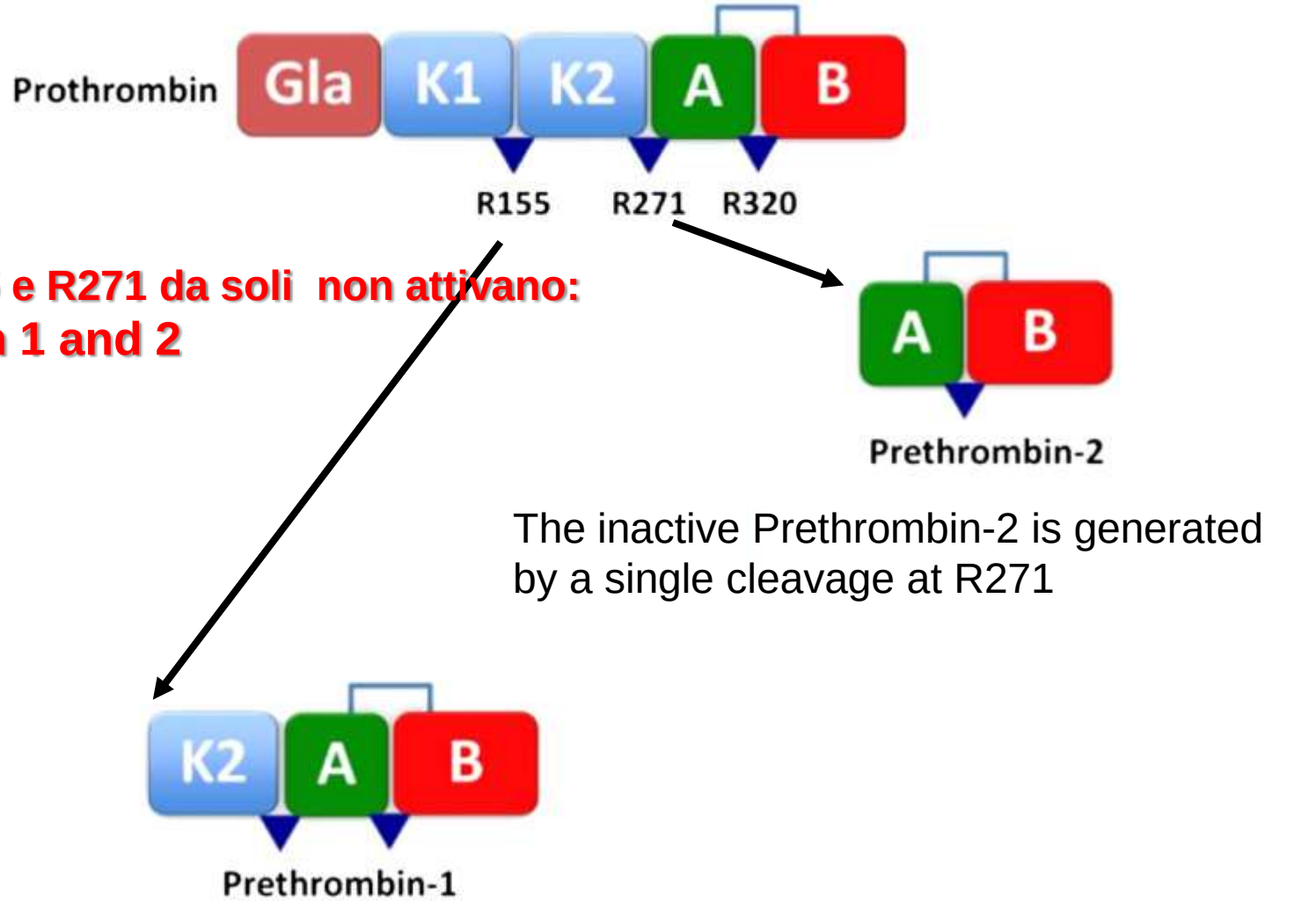
Coronavirus - Proteasi

- Stavo rivedendo la lezione 6 nella parte riguardo l’inibizione della proteina Tmprss2 per ridurre lo sviluppo virale; non riesco a capire come questa proteina possa interagire con lo spike, si trova vicino al sito di interazione tra ace2 e spike?

Trombina Attività ed attivazione

- Lei ha detto che questo enzima possiede due specifici siti di taglio riconosciuti dal complesso protrombinasi e attraverso una ricerca personale ho scoperto che questi siti sono rispettivamente
- - tra arginina e triptofano in pozione N terminale
- - tra arginina e isoleucina in posizione C terminale
- Volevo chiederle se fossero informazioni corrette poiché nella sua lezione ha parlato di un’esposizione (a seguito del taglio) della porzione ammino-terminale che corrisponde ad una isoleucina-valina nel nuovo peptide libero...

Schematic representation of prothrombin activation.

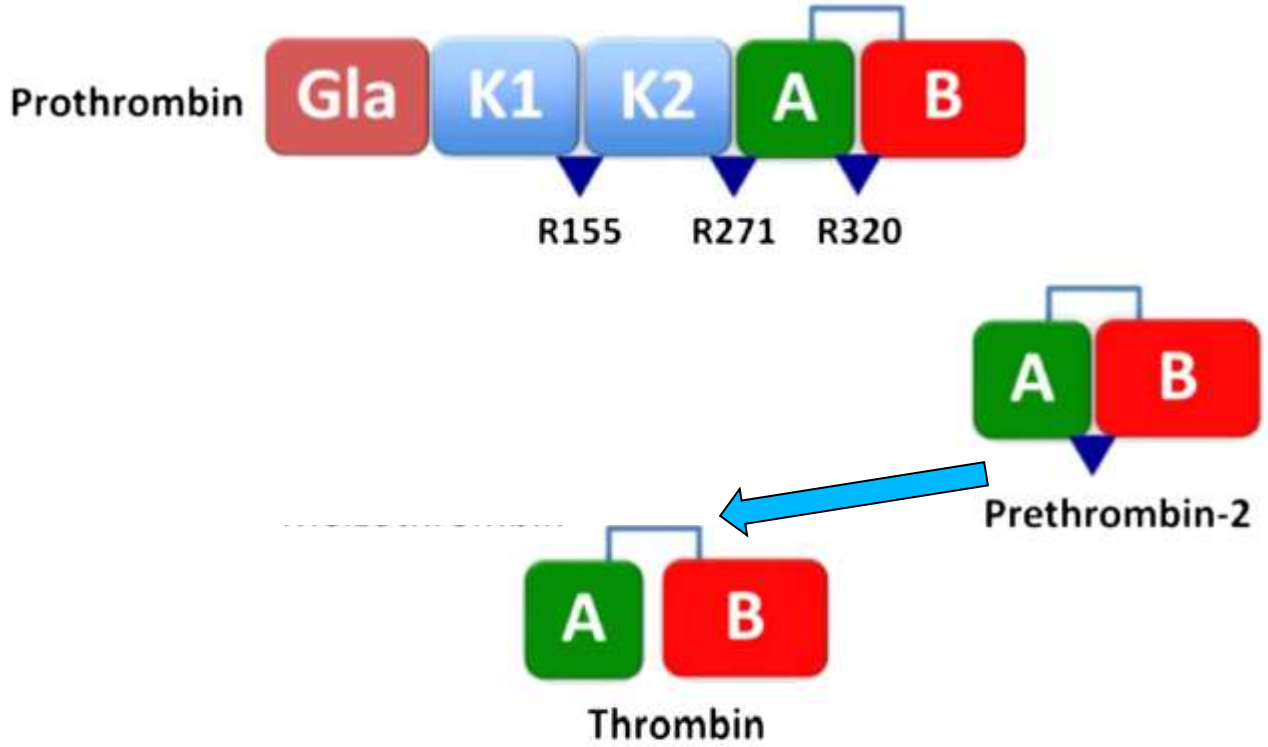


I tagli in R155 e R271 da soli non attivano:
Prethrombin 1 and 2

The inactive Prethrombin-2 is generated by a single cleavage at R271

Zhiwei Chen et al. PNAS 2010;107:45:19278-19283

Cleavage at R271 and R320 produce Thrombin



Cleavage at R320 separates the A and B chains and generates an active protease.

The inactive Prethrombin-2 is generated by a single cleavage at R271

Zhiwei Chen et al. PNAS 2010;107:45:19278-19283

Prothrombin is activated to thrombin by two proteolytic cleavages

Table 1 Sites of cleavage in the human vitamin K-dependent zymogens*

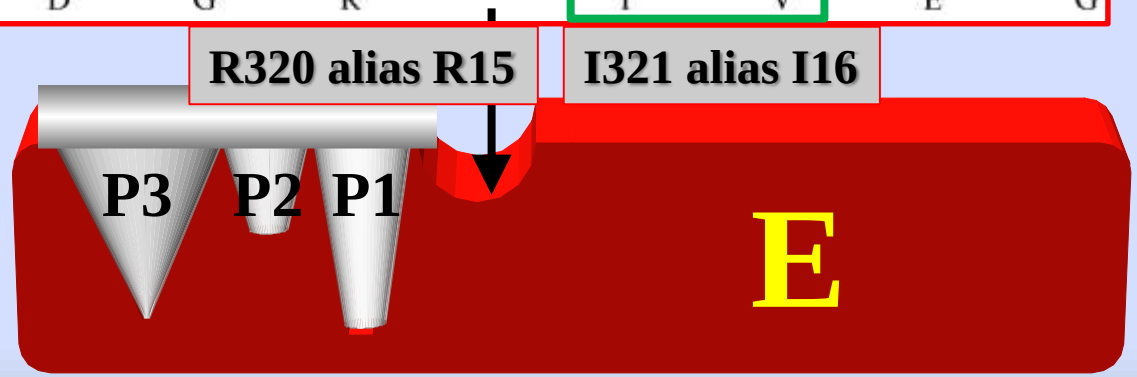
Enzyme	Substrate†	P_4	P_3	P_2	P_1	↓	$P_{1'}$	$P_{2'}$	$P_{3'}$	$P_{4'}$
Xa/Va	II	I	E	G	R		T	A	T	S
	$II_{(15–16)}$	I	D	G	R		I	V	E	G

R271

Enzyme	Substrate†	P_4	P_3	P_2	P_1	↓	$P_{1'}$	$P_{2'}$	$P_{3'}$	$P_{4'}$
Xa/Va	II	I	E	G	R		T	A	T	S
	$II_{(15–16)}$	I	D	G	R		I	V	E	G

R320 alias R15

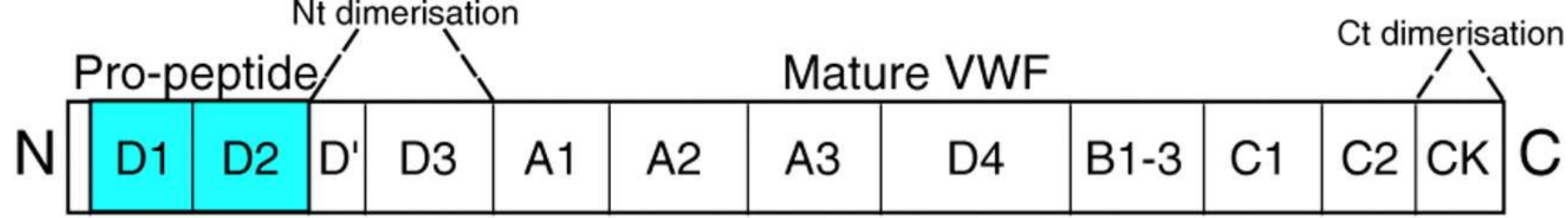
I321 alias I16



Fattore von Willebrand

- VWF: - Può rispiegare, passaggio per passaggio, la biosintesi del Fattore di Von Willebrant come al minuto 49 della lezione del 27 aprile, dal pre-poli-peptide al multimero maturo?

A

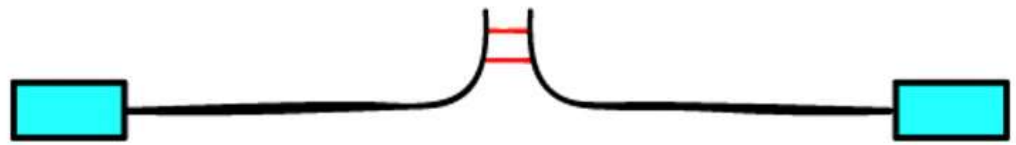


B

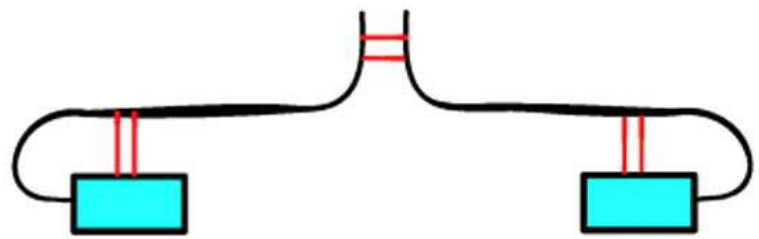
Location and process

VWF

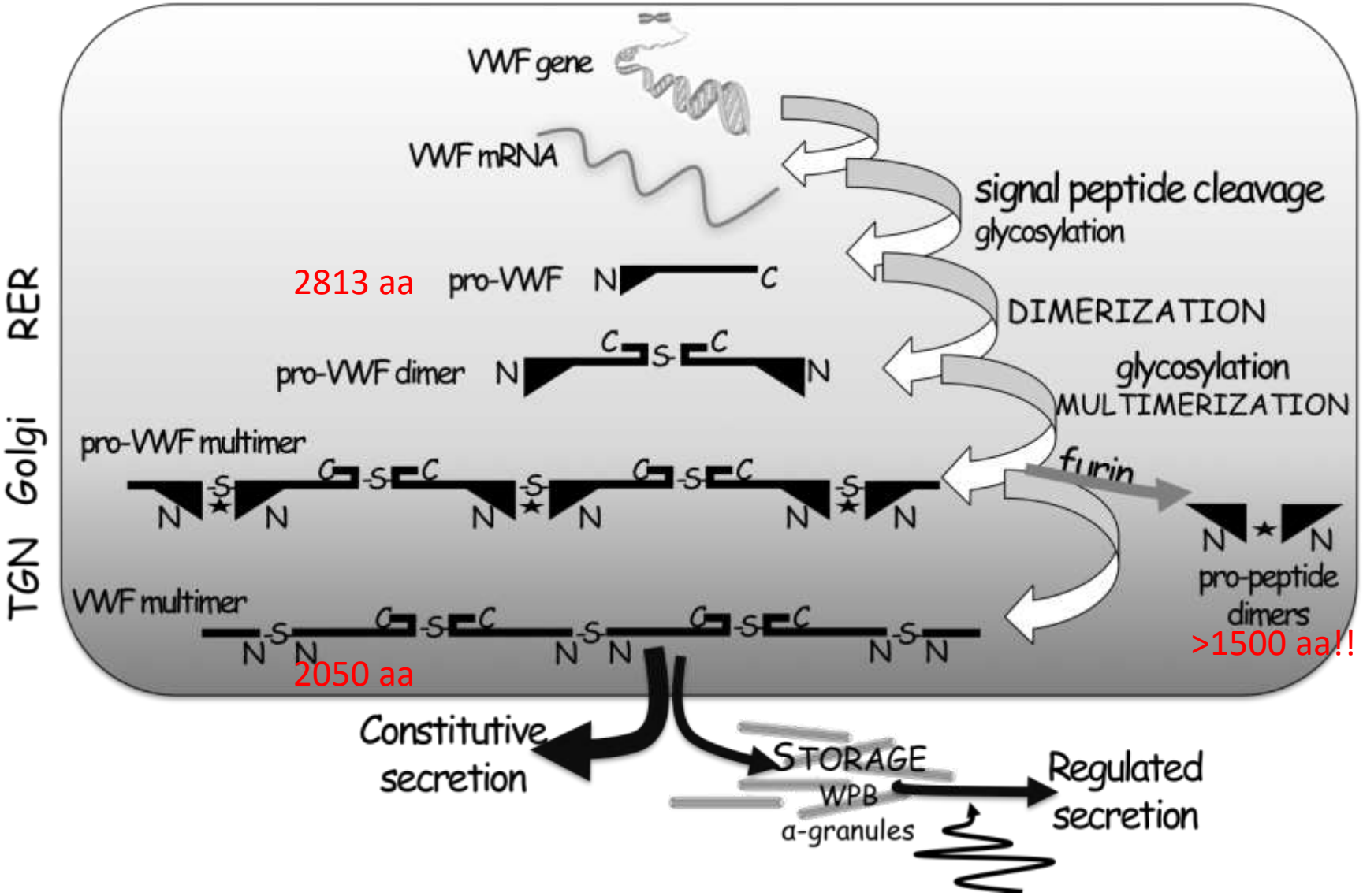
ER
C-terminal dimerisation



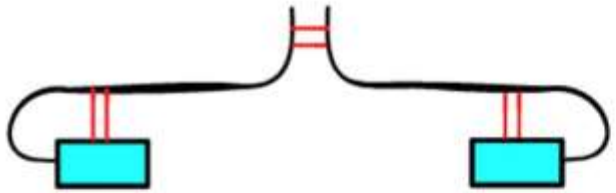
ER and Golgi
N-terminal intrachain
disulphide bonds form



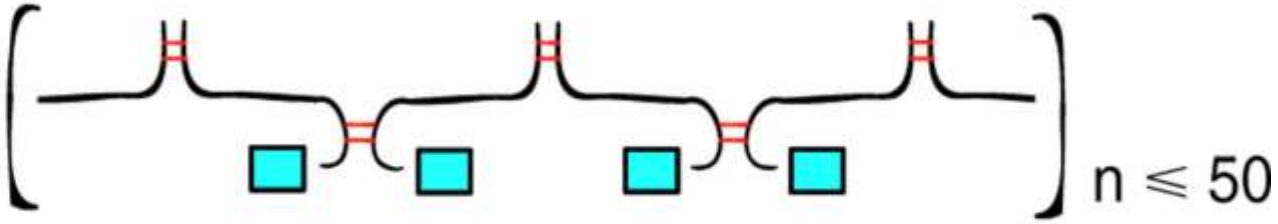
Von Willebrand factor (VWF) –biosynthesis-



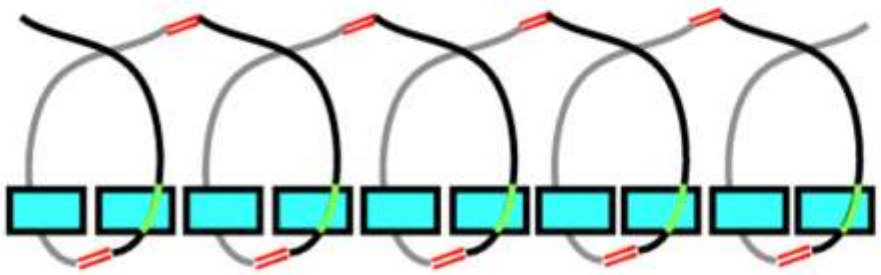
ER and Golgi
N-terminal intrachain disulphide bonds form



Golgi and TGN
N-terminal interchain disulphide bonds replace intrachain disulphide bonds and cleavage of pro-peptide



TGN and WPB
Ionic interaction between mature VWF and pro-peptide occurs, resulting in tubulation



Key:

Propeptide — mature VWF — Disulphide bond — Ionic interaction

Biosynthesis of VWF