

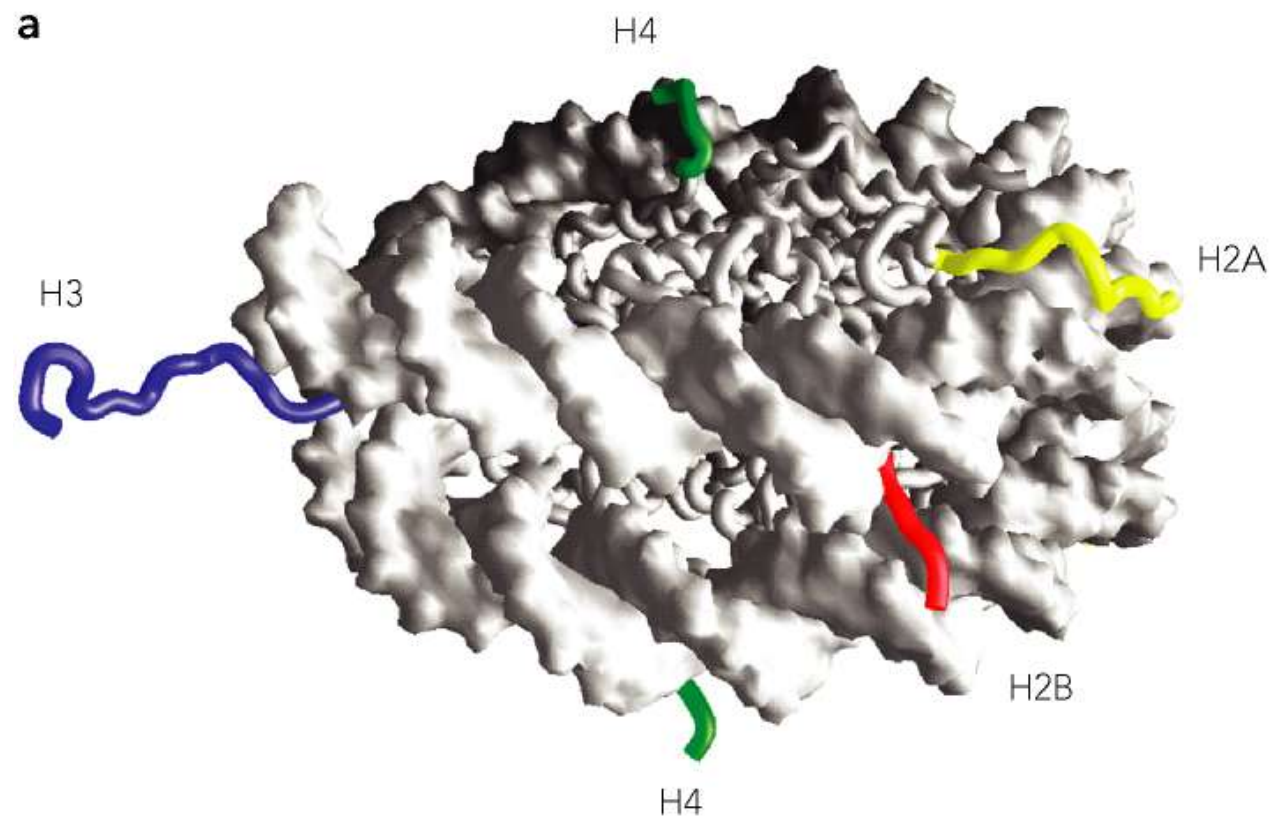
La cromatina telomerica e la sua modificazione

Telomeres also bind to nucleosomes, which are rich in modified histones.

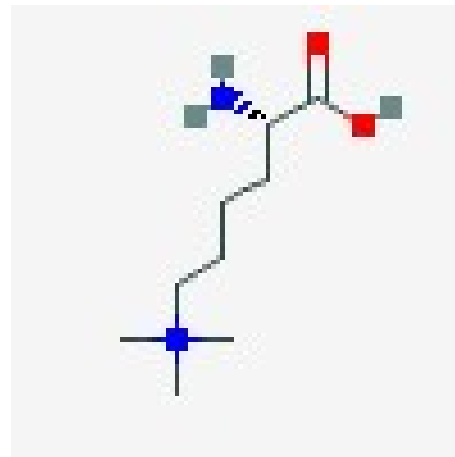
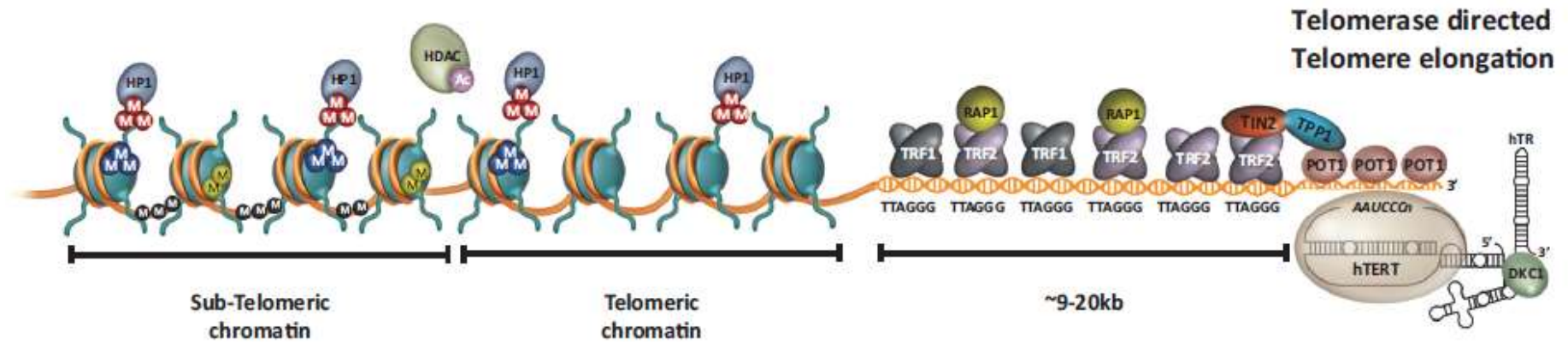
Major histone modifications *found in telomeres are*

-H3K9 and H4K20 trimethylation

-low abundance of acetylated H3 and H4



Telomeres in germ and stem cells



Kme3 = trimetillisina



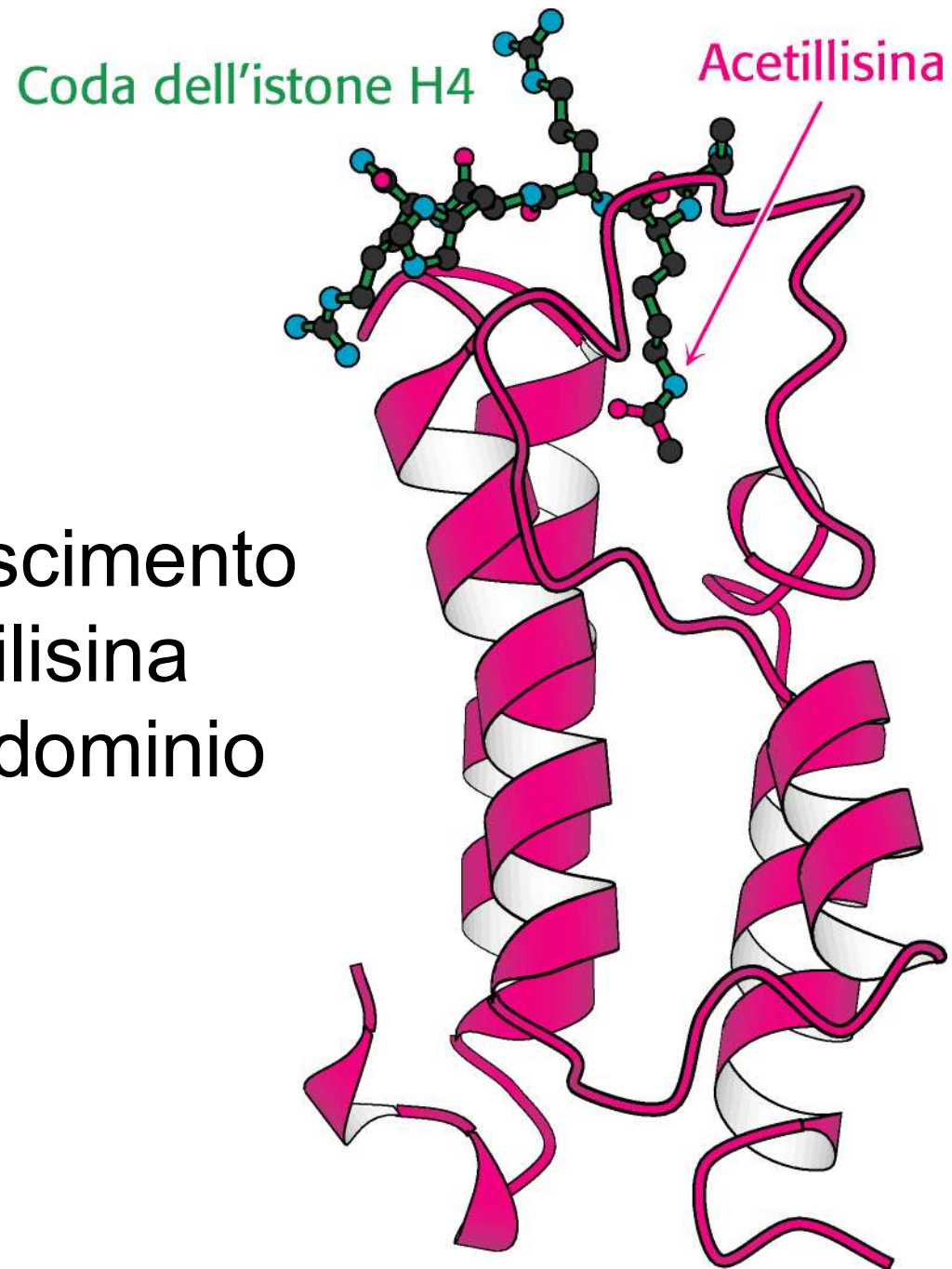
Telomeres also bind to nucleosomes, which are rich in modified histones.

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Riconoscimento
acetilisina
bromodominio



Un enzima deacetilante specifico: SIRT6

SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin

The Sir2 deacetylase regulates chromatin silencing and lifespan in *Saccharomyces cerevisiae*.

In mice, deficiency for the Sir2 family member SIRT6 leads to a shortened lifespan and a premature ageing-like phenotype.

SIRT6 is a chromatin-associated NAD⁺-dependent, histone H3 lysine 9 (H3K9) deacetylase that modulates telomeric chromatin.

SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin

SIRT6 contributes to the propagation of a specialized chromatin state at mammalian telomeres,

Deacetylation is required for proper telomere metabolism and function.

chromatin regulation by SIRT6 is linked to telomere maintenance and a human premature ageing syndrome

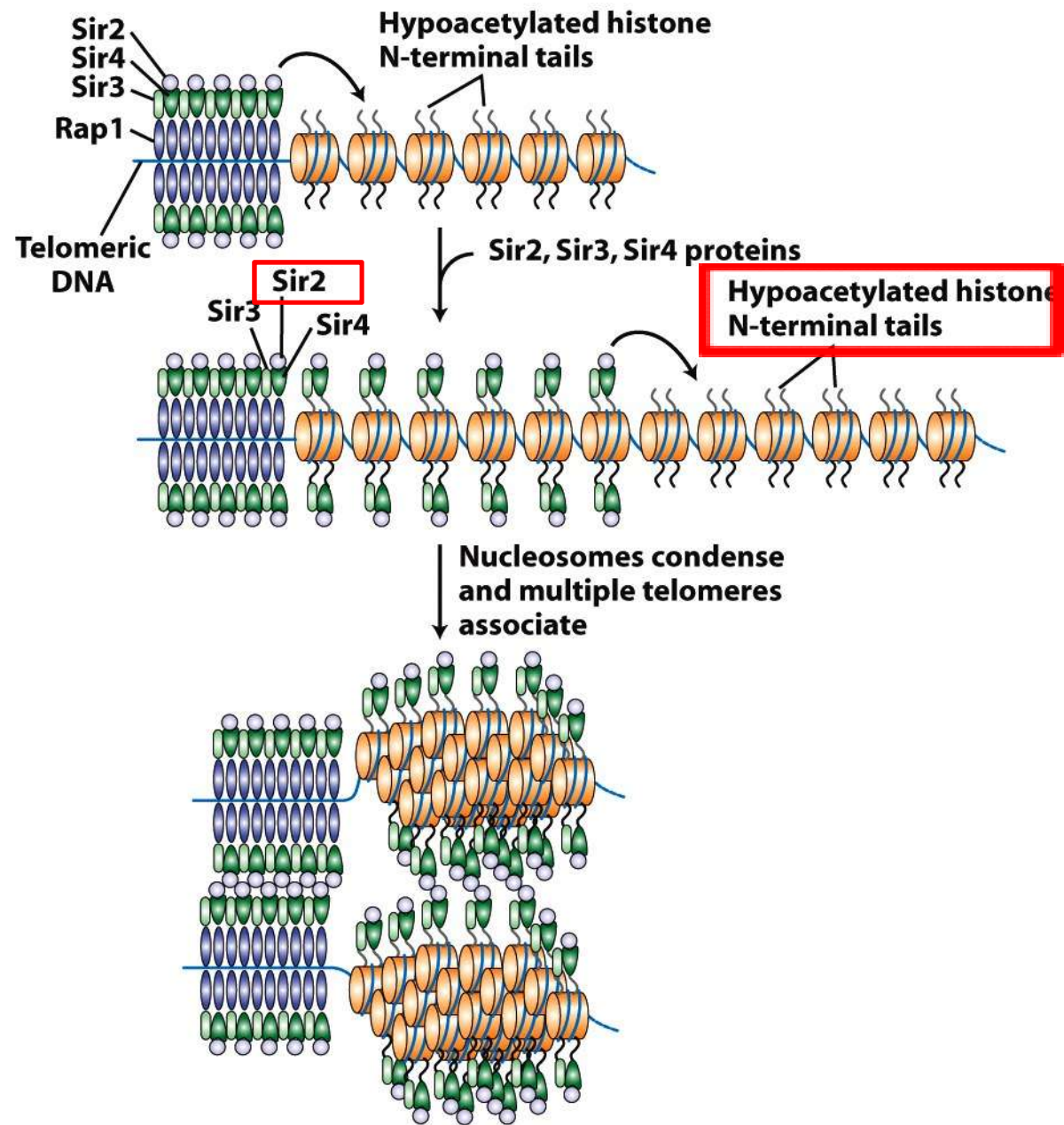


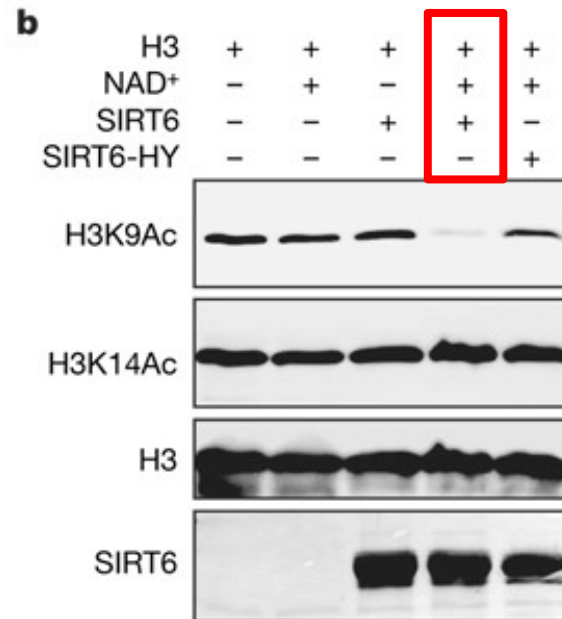
Figure 7-35
Molecular Cell Biology, Sixth Edition
 © 2008 W. H. Freeman and Company

SIRT6 (sir 2) deacetylates lysine 9 of histone H3 at telomeric chromatin

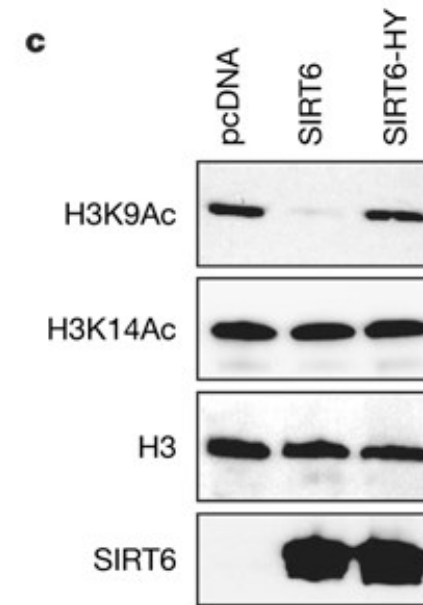
a

Peptide	Activity
H2AK5Ac	-
H2AK13Ac	-
H2BK5Ac	-
H2BK12Ac	-
H2BK15Ac	-
H2BK20Ac	-
H3K9Ac	+
H3K14Ac	-
H3K27	-
H4K5Ac	-
H4K8Ac	-
H4K12Ac	-
H4K16Ac	-

histone tail peptides



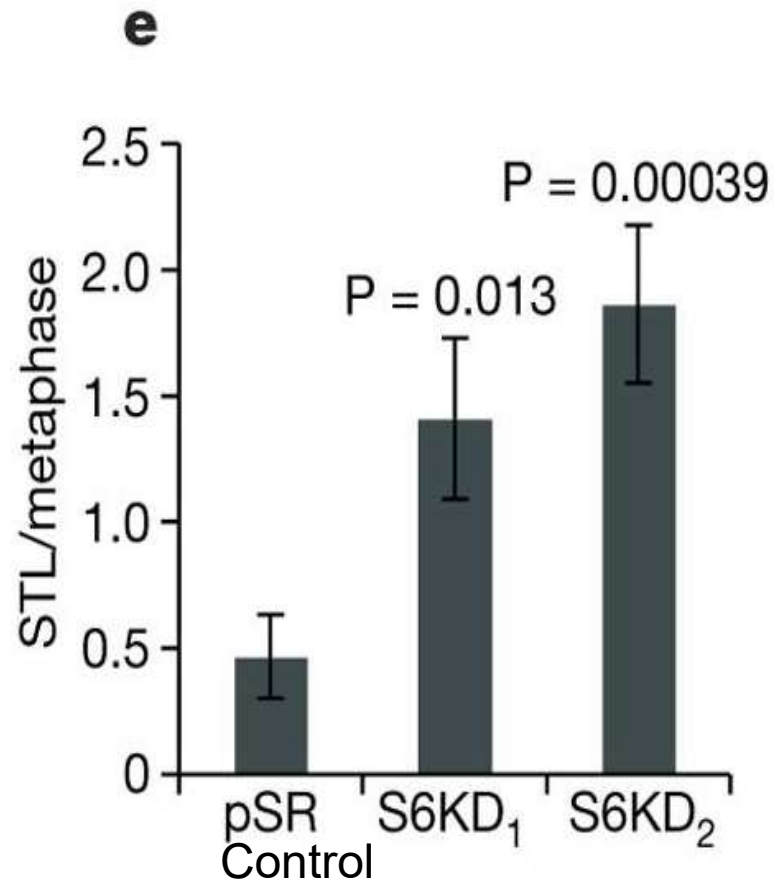
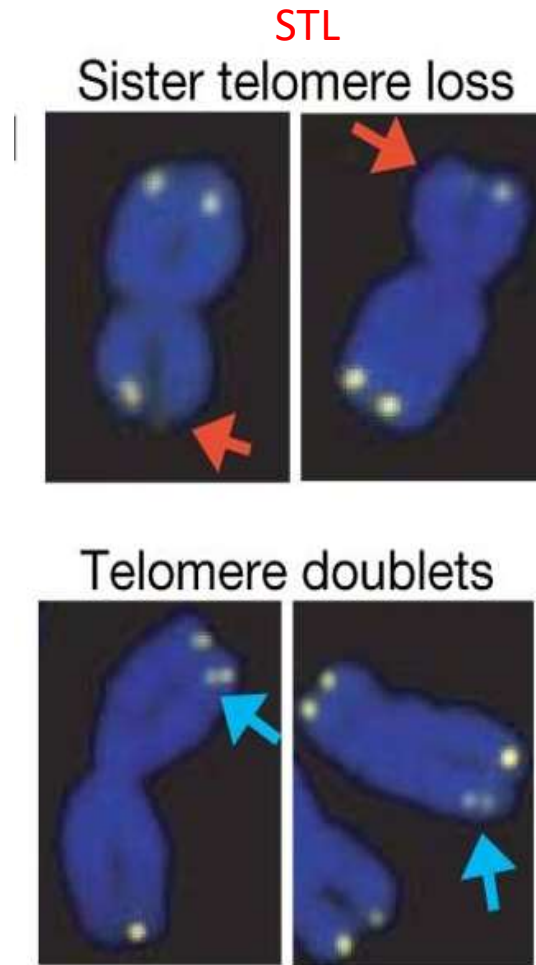
full-length histone H3



293T cells overexpressing SIRT6

SIRT6-HY: *catalytic H133Y SIRT6 mutant protein*

SIRT6 knockdown (S6KD) cells



d, Representative S6KD metaphases showing aberrant telomere signals. Red arrows, sister telomere loss; blue arrows, telomere doublets. e, Quantification of sister telomere loss

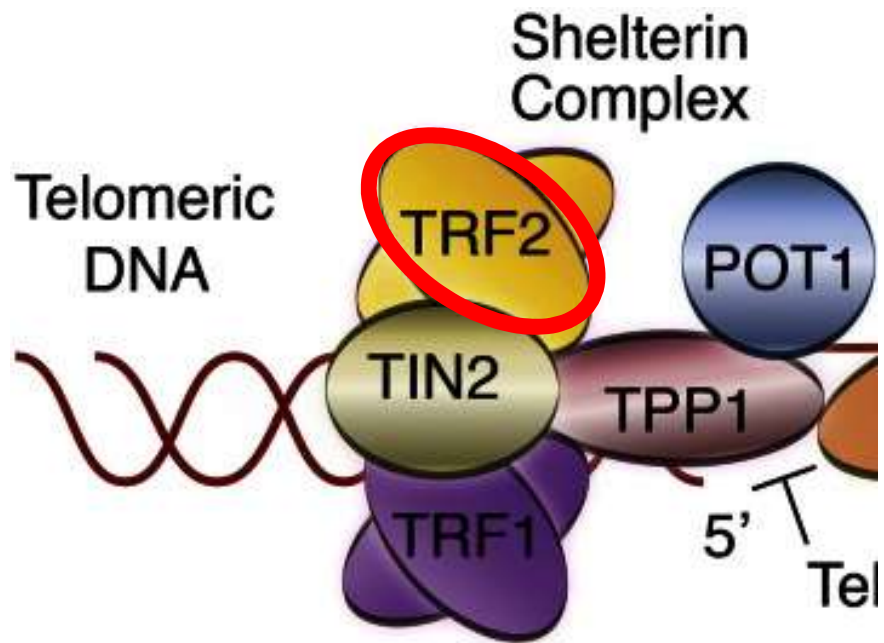
SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin

SIRT6 associates specifically with telomeres

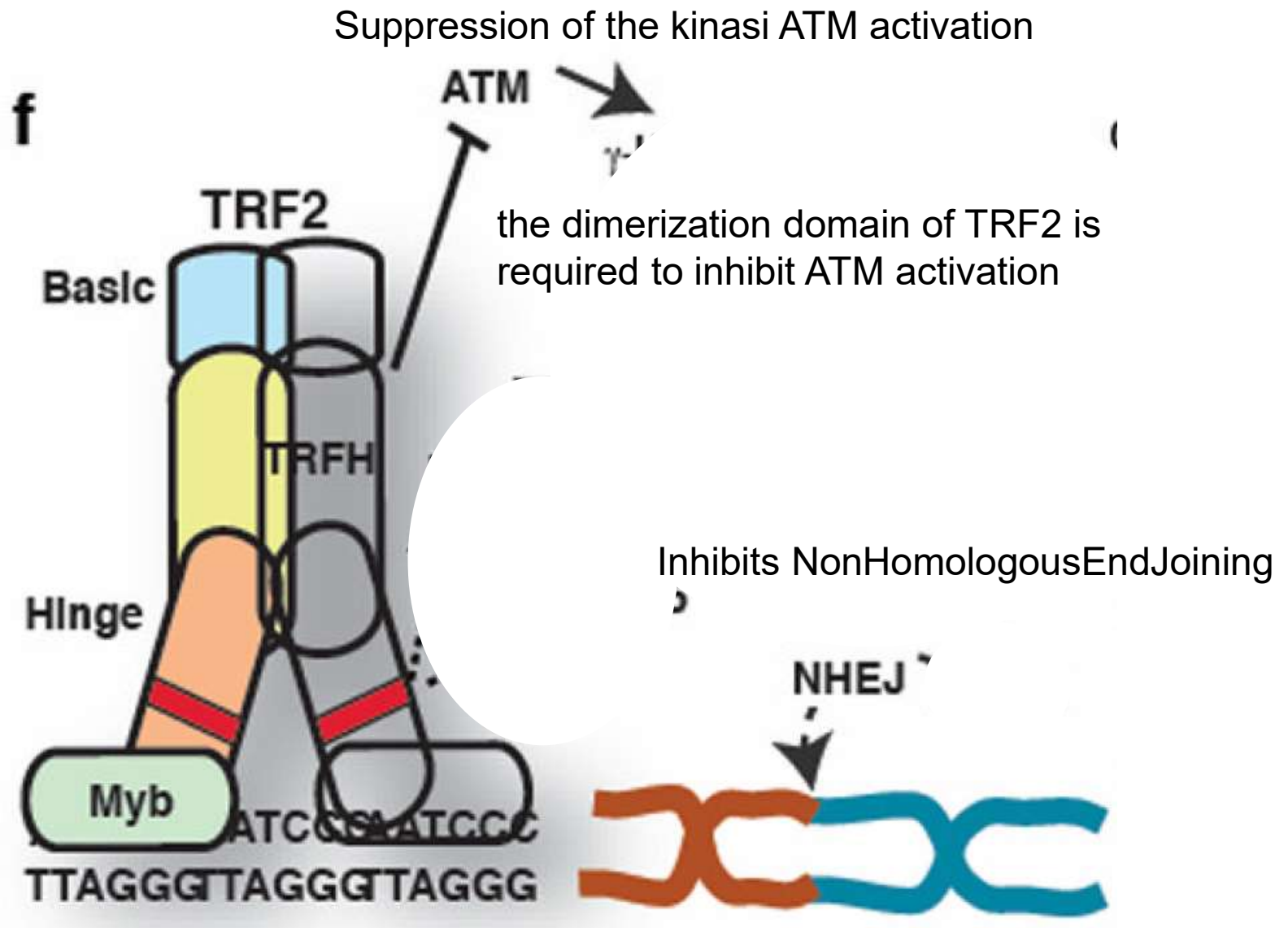
SIRT6 depletion leads to telomere dysfunction with end-to-end chromosomal fusions and premature cellular senescence.

SIRT6-depleted cells exhibit abnormal telomere structures

PROTEZIONE DEL TELOMERO



mechanism for TRF2-mediated chromosome end protection

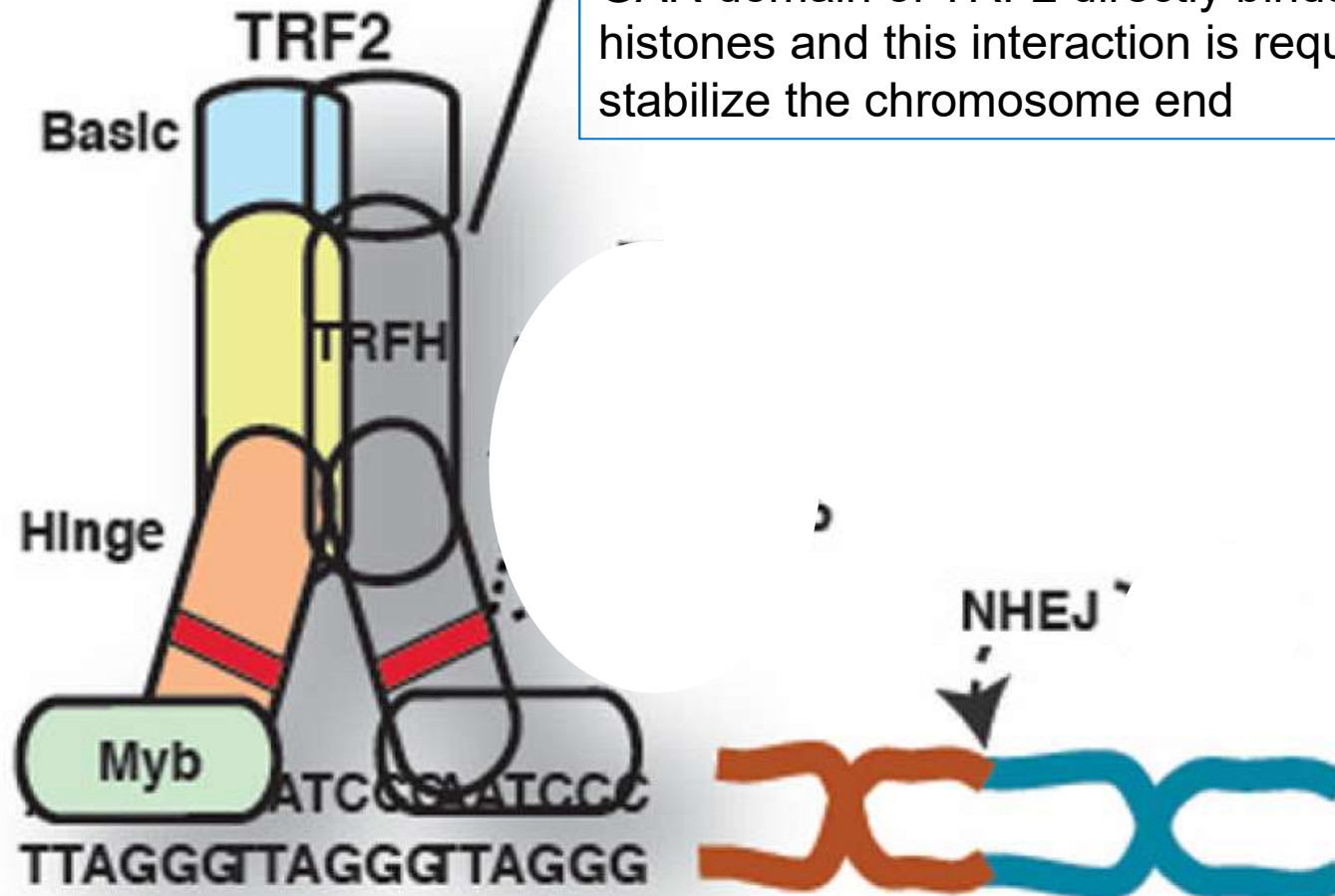


mechanism for TRF2-mediated chromosome end protection

The GAR/Basic domain, which is located at the N terminus of TRF2, is rich in Gly/Arg residues and highly basic

ATM

GAR domain of TRF2 directly binds to core histones and this interaction is required to stabilize the chromosome end



Telomeric nucleosomes

Telomeric nucleosomes are hypersensitive to micrococcal nuclease.

Reconstituted nucleosomes on TTAGGG repeats show higher mobility than on other sequences.

Telomeric chromatin is enriched for heterochromatin modification, such as trimethylation of H3K9 and H4K20, and loss of these marks affects telomere length regulation.

Direct binding of the GAR domain of TRF2 and core histones.

In vitro binding assay for the GAR domain of TRF2 and core histones.

Core histones:

mono-nucleosomes were purified using from HeLa cell nuclei digested with micrococcal nuclease-

mono-nucleosome peaks were collected and partially digested with trypsin to generate the **tailless** histones

Recombinant GST-fused TRF2

GAR domain (GST-Basic) and GST protein (GST) were captured by glutathione-conjugated beads and incubated with core histones purified from HeLa cells.

Beads were washed extensively and then subjected to SDS-PAGE.

Direct binding of the GAR domain of TRF2 and core histones.

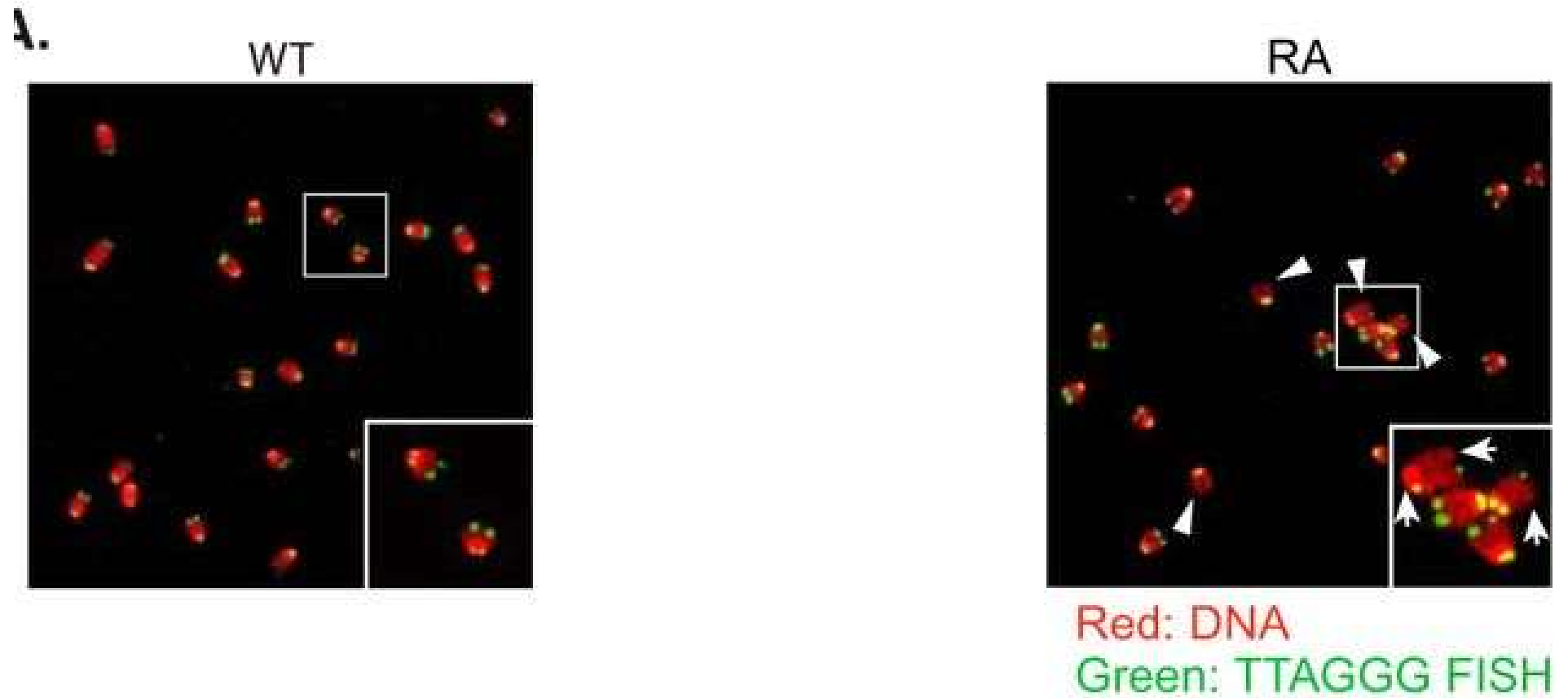
basic domain

E.

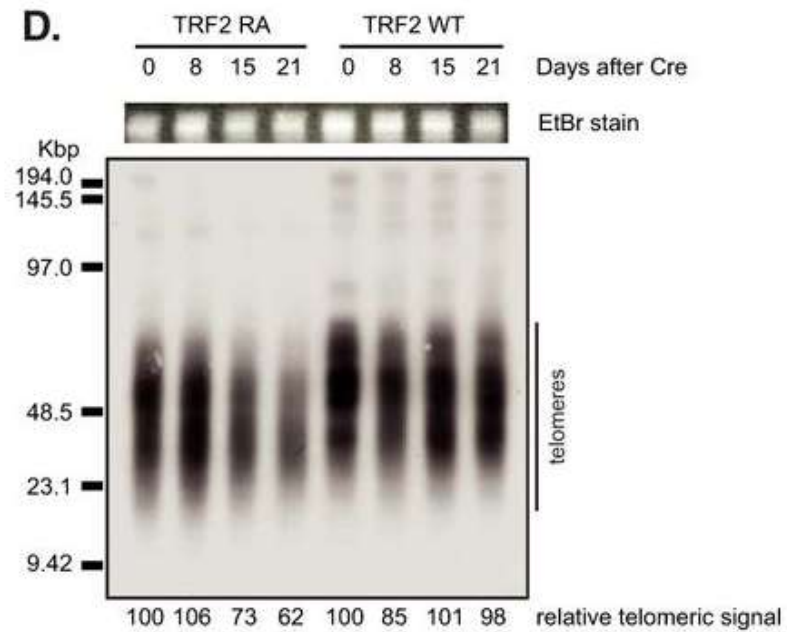
		* * *	
		MAPP <u>GMRLRSGR</u> ---STGAPLTRGSC	
LANA			
hTRF2	MAGGGGSSDGS	GRAAGRRA	SRSSGRARRGRHEPGLGGPAERGAGEARLEEAVNRW
mTRF2	MAGGGGSSDSS	GRAASRRASRS	GGRARRGRHEPGLGGAAERGAGEARLEEAVNRW
Basic2-45	AGGGGSSDGS	GRAAGRRA	SRSSGRARRGRHEPGLGGPAERGAGE (+)
Basic2-24	AGGGGSSDGS	GRAAGRRA	SRSSG (-)
Basic2-30	AGGGGSSDGS	GRAAGRRA	SRSSGRARRGR (+)
Basic10-37		GSGRAAGRRA	SRSSGRARRGRHEPGLGG (+)
			core histone binding
Basic RA	AGGGGSSDGS	GRAAGRRA	SRSSGAAAAGAHEPGLGGPAERGAGE

latency-associated nuclear antigen (LANA)
viral element essential for the DNA replication and genome maintenance
during latency

Rapid telomere DNA loss by loss of histone binding of TRF2.



Rapid telomere DNA loss and t-circle generation by loss of histone binding of TRF2.



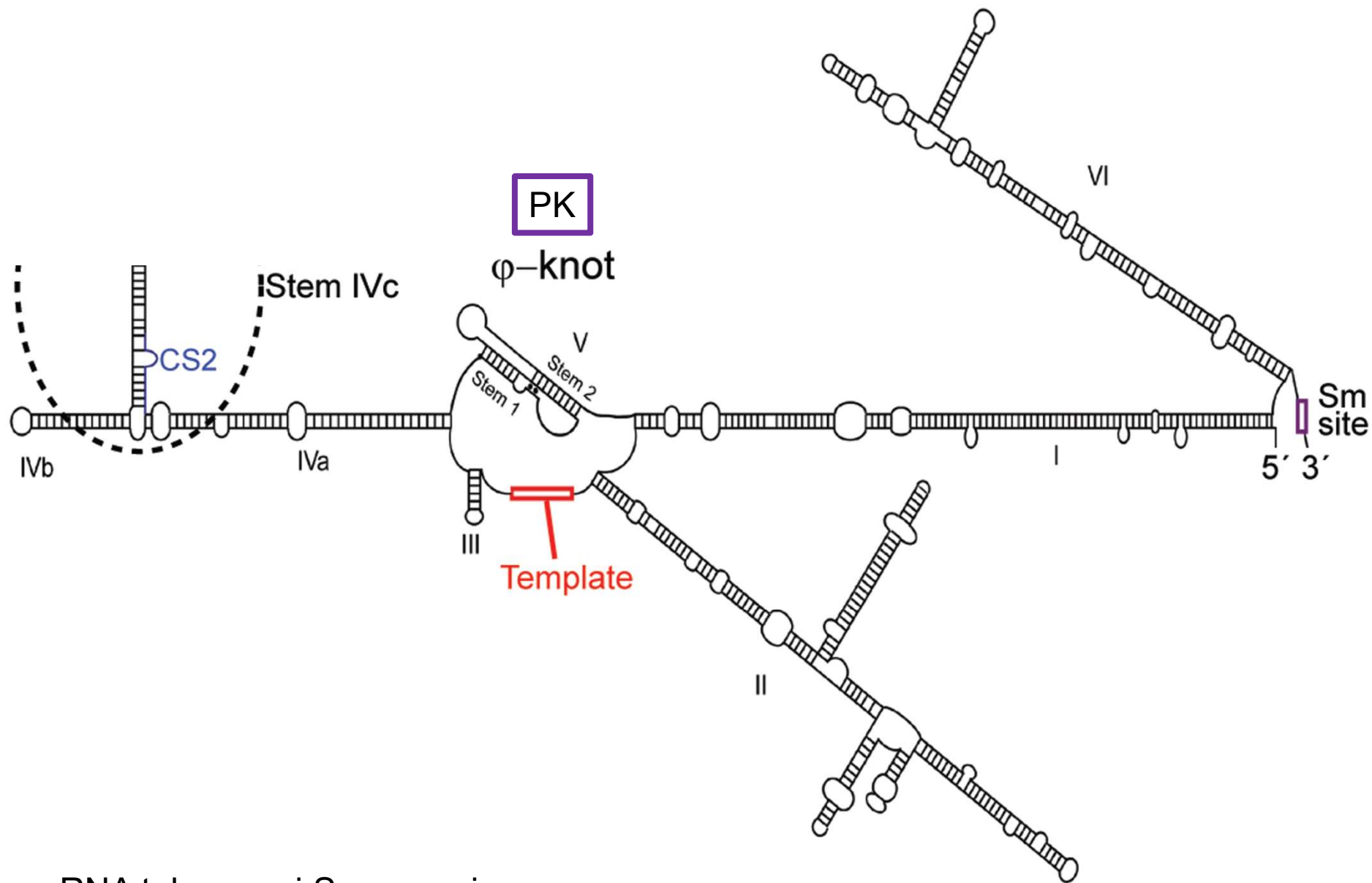
Akimitsu Konishi et al. *J. Biol. Chem.* 2016;291:20798-20810

RNA TEMPLATO DELLA TELOMERASI

hTR is a 451-nucleotide RNA

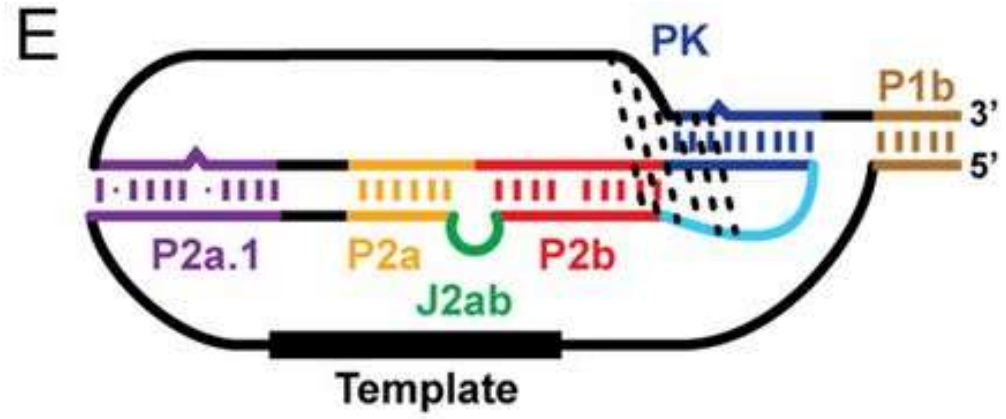
contains a box H/ACA motif at its 3' end essential for hTR stability and for its assembly with hTERT mediated by the presence of the box H/ACA-binding dyskerin complex, which is composed of four proteins:

dyskerin, NOP10, NHP2 and GAR1.

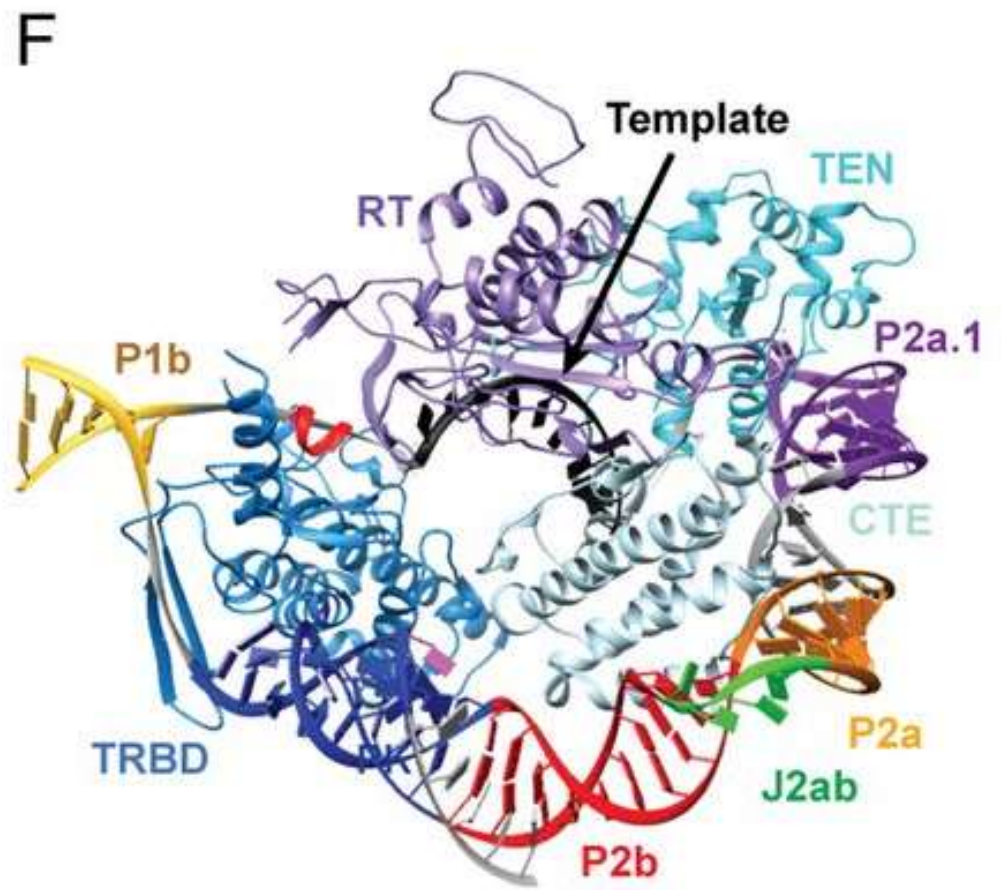
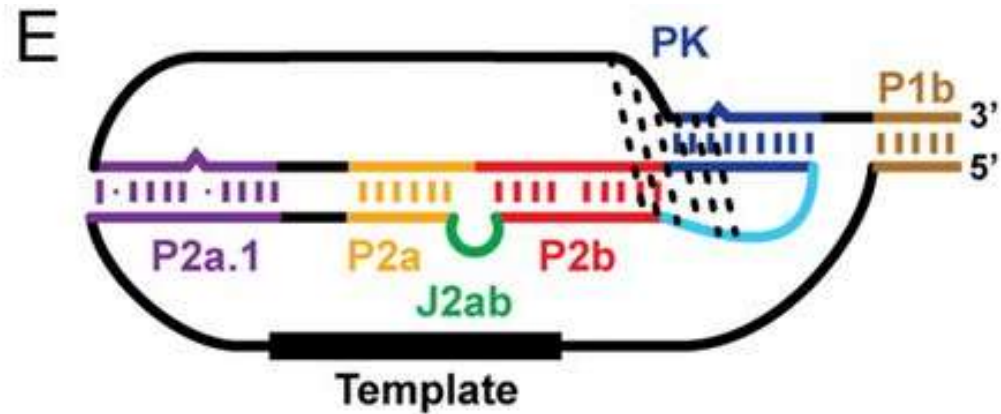


RNA telomerasi Saccaromices

human TERT-t/PK.

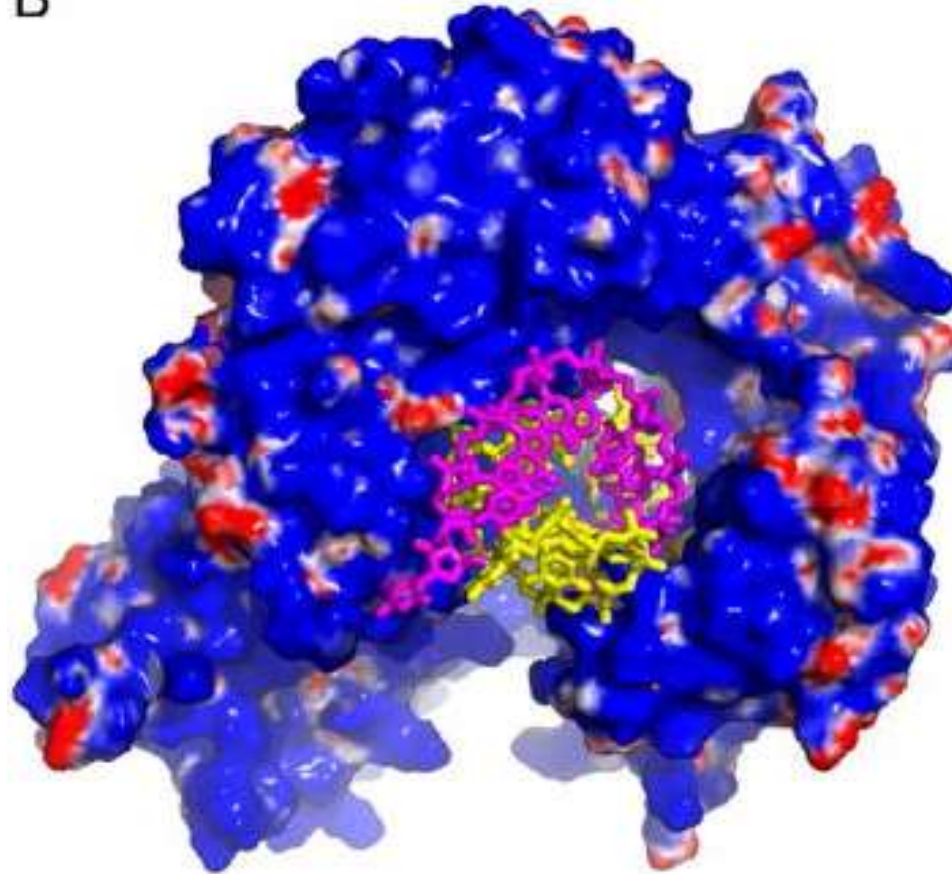


human TERT-t/PK.



RNA (magenta stick)–DNA (yellow stick) hairpin cocrystallized with tcTERT

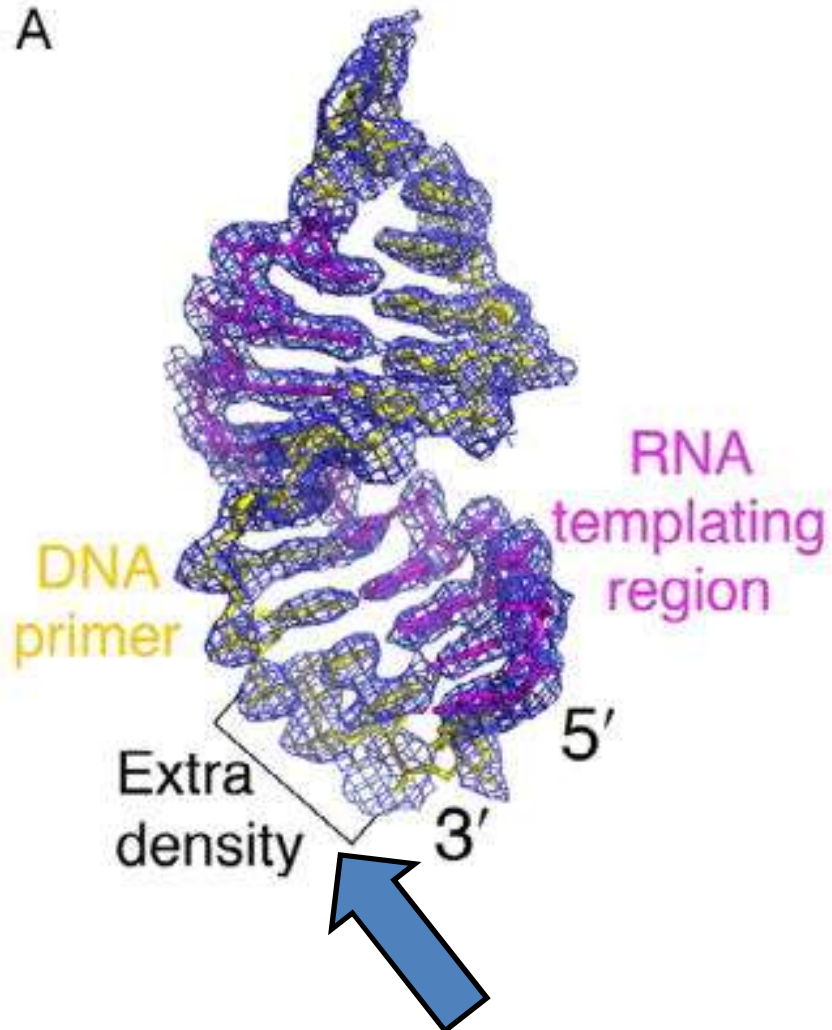
B



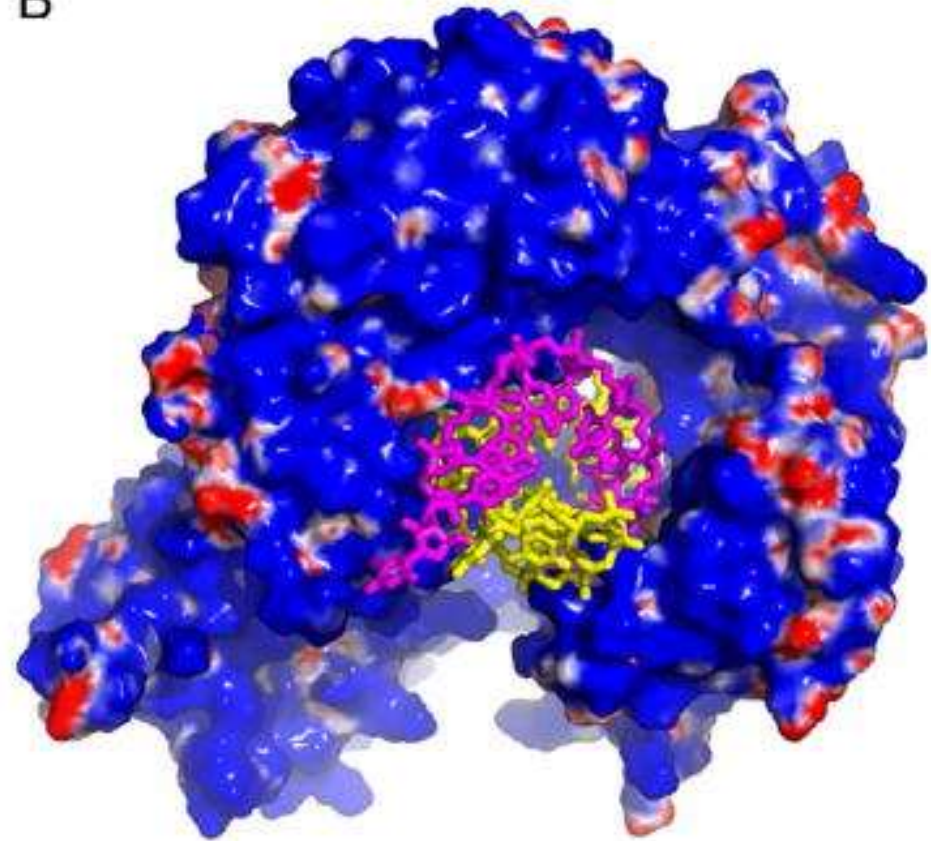
tcTERT surface charge representation,
the RNA–DNA hybrid (stick) docked in the
interior cavity of the TERT ring

RNA (magenta stick)–DNA (yellow stick) hairpin cocrystallized with tcTERT

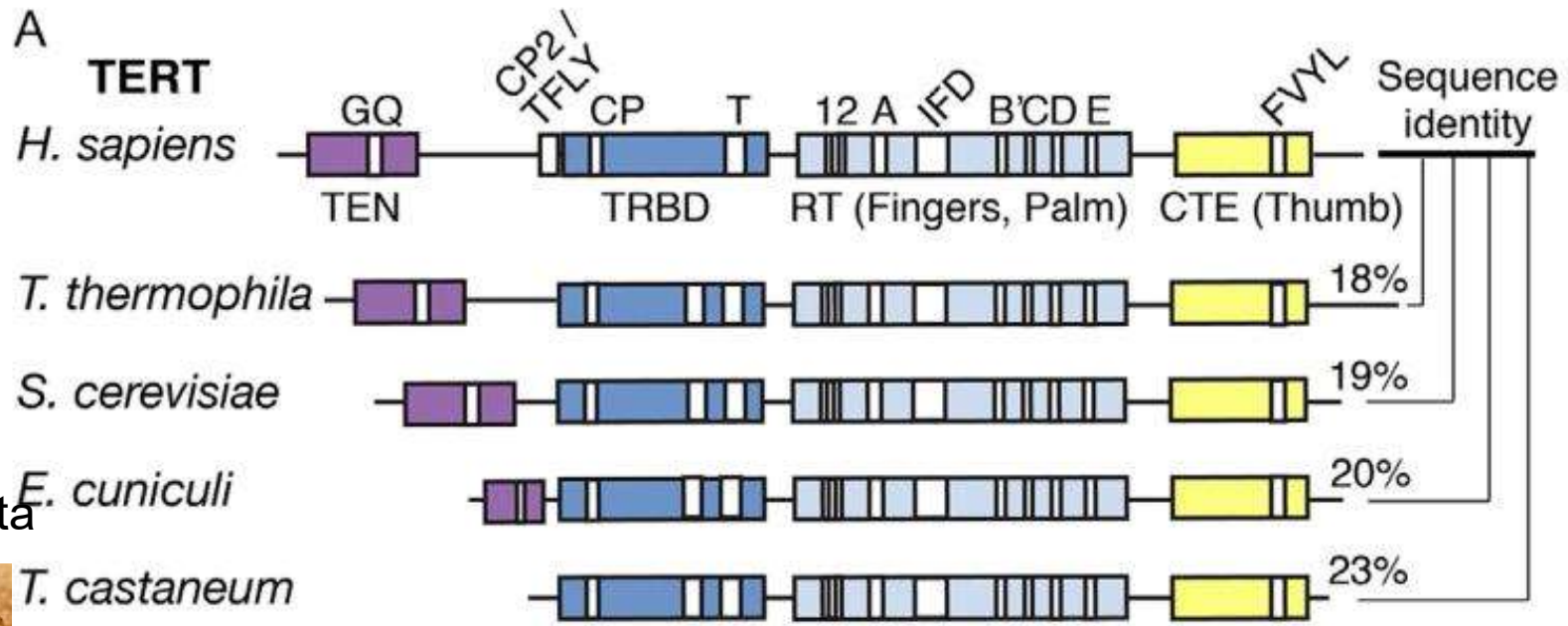
A



B



three additional nucleotides at the 3'-end of the telomeric DNA !!



Fungo
parassita



B

