

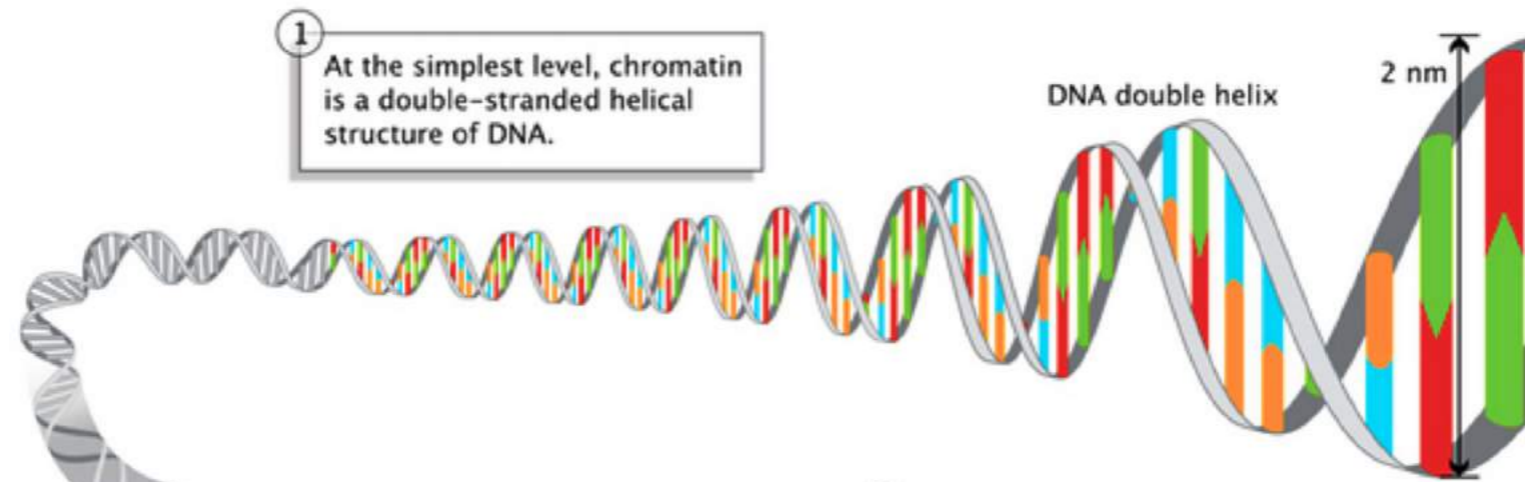


Gli enzimi nucleari come macchine molecolari coordinate per permettere l'espressione del genoma

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Il DNA genomico umano:

$3,3 \times 10^9$ bp (aploide)

2 nm - diametro

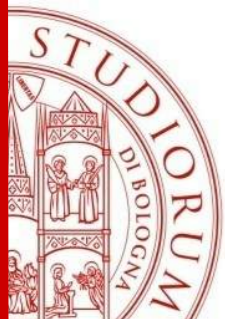
~ 2 m - lungo

~ 4,3 cm/cromosoma in media

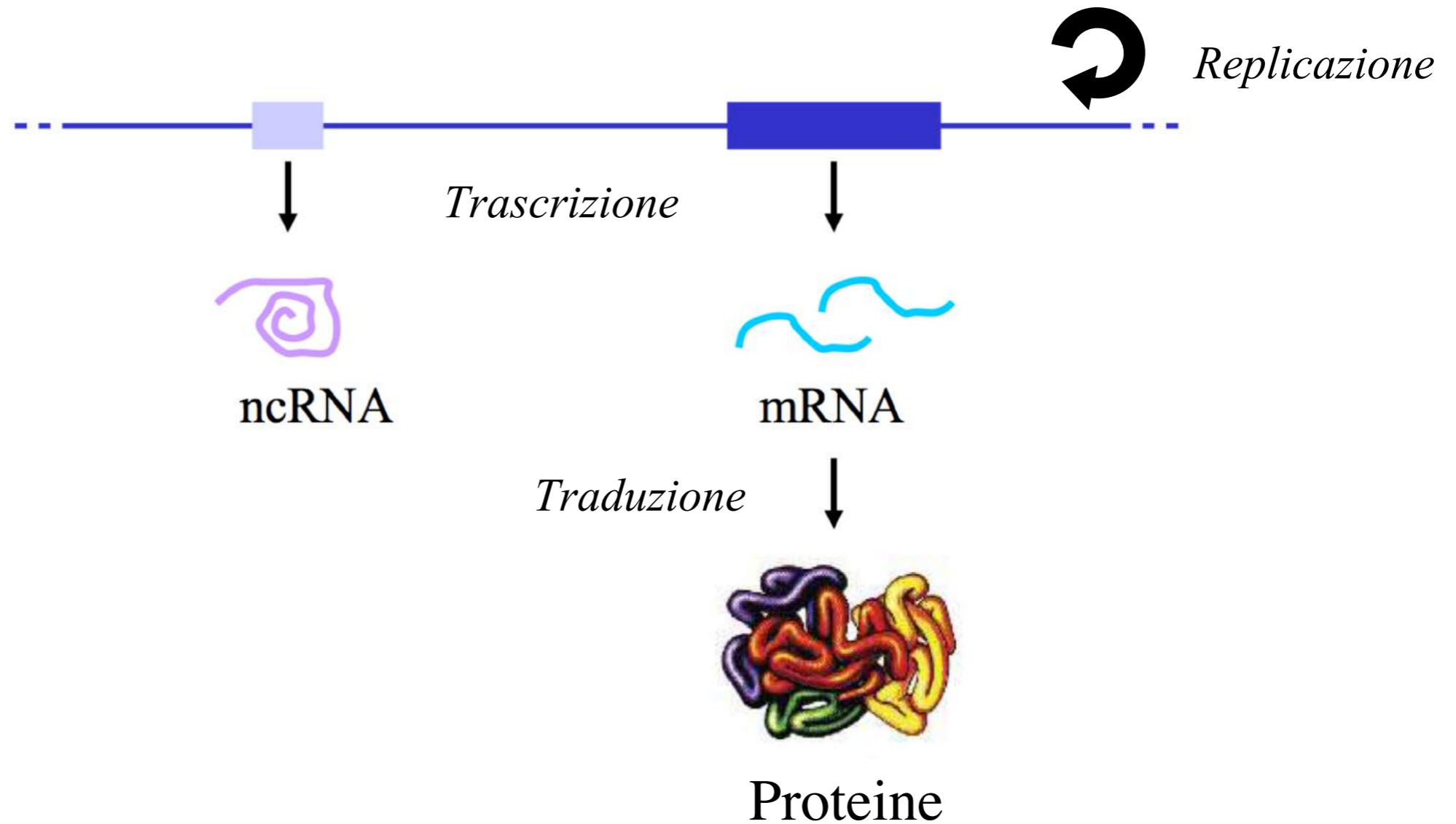
Figure 1: Chromatin has highly complex structure with several levels of organization.

The simplest level is the double-helical structure of DNA.

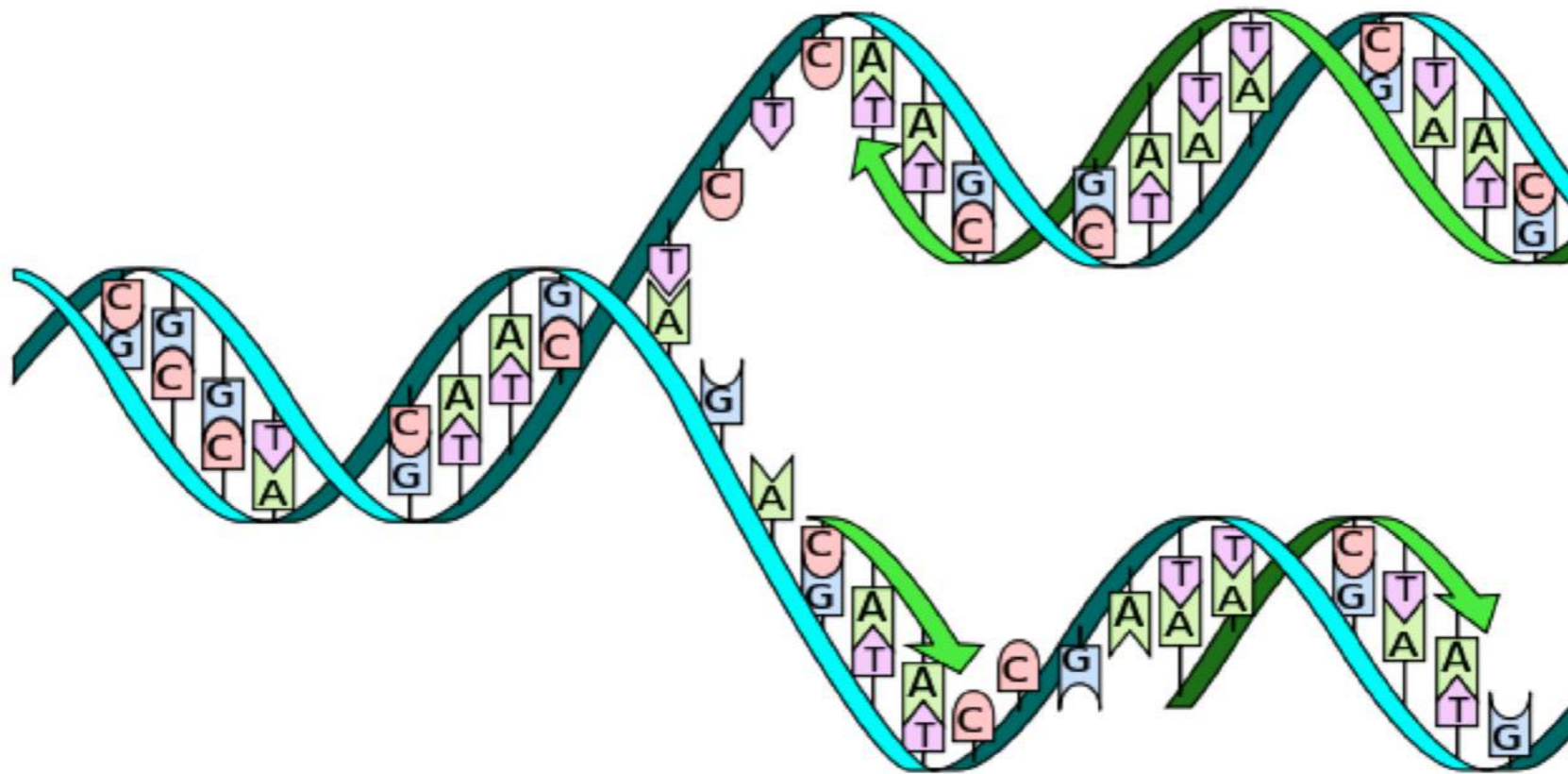
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Il dogma centrale di F. Crick (1958)



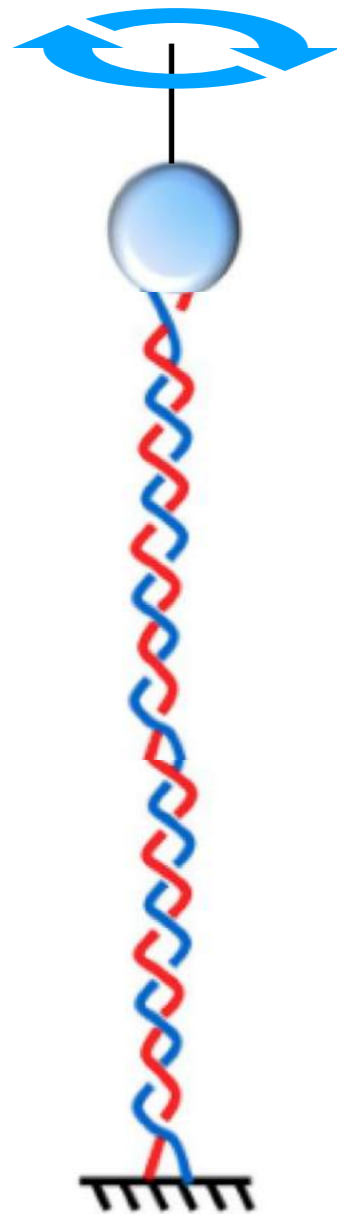
Necessità della separazione dei filamenti della doppia elica per la replicazione



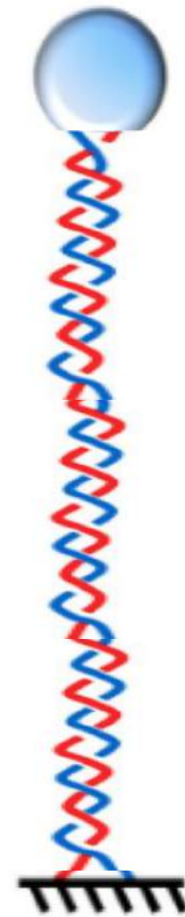
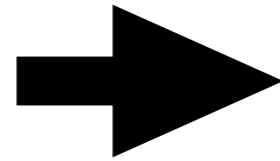
Avvolgimenti e superavvolgimenti del DNA

Twist

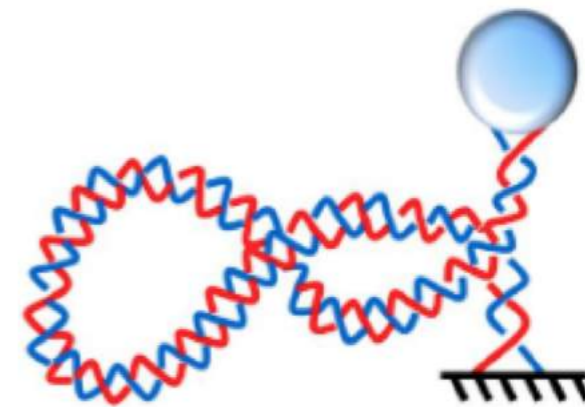
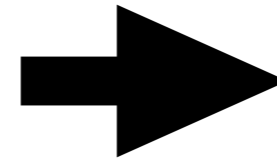
Writhe / Supercoil



ca. 8 avvolgimenti

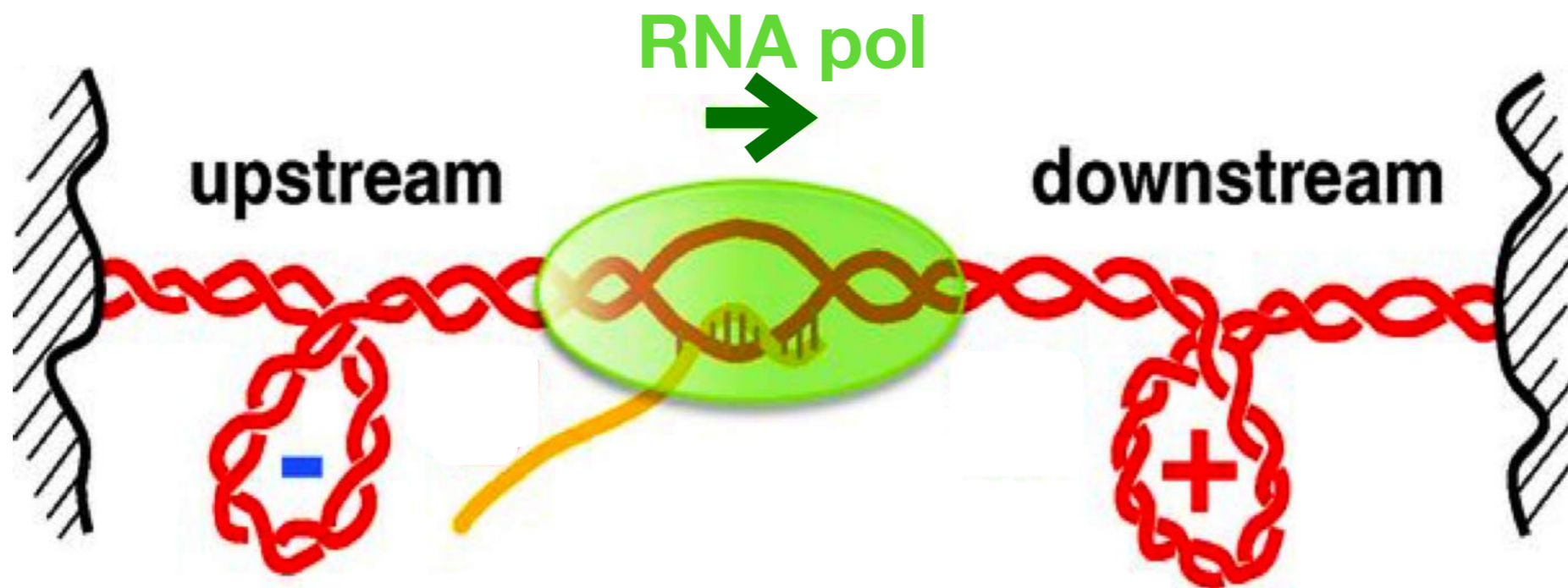


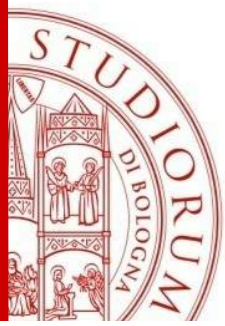
ca. 10 avvolgimenti



ca. 8 avvolgimenti
+
2 superavvolgimenti

Supercoiling twin-domain model

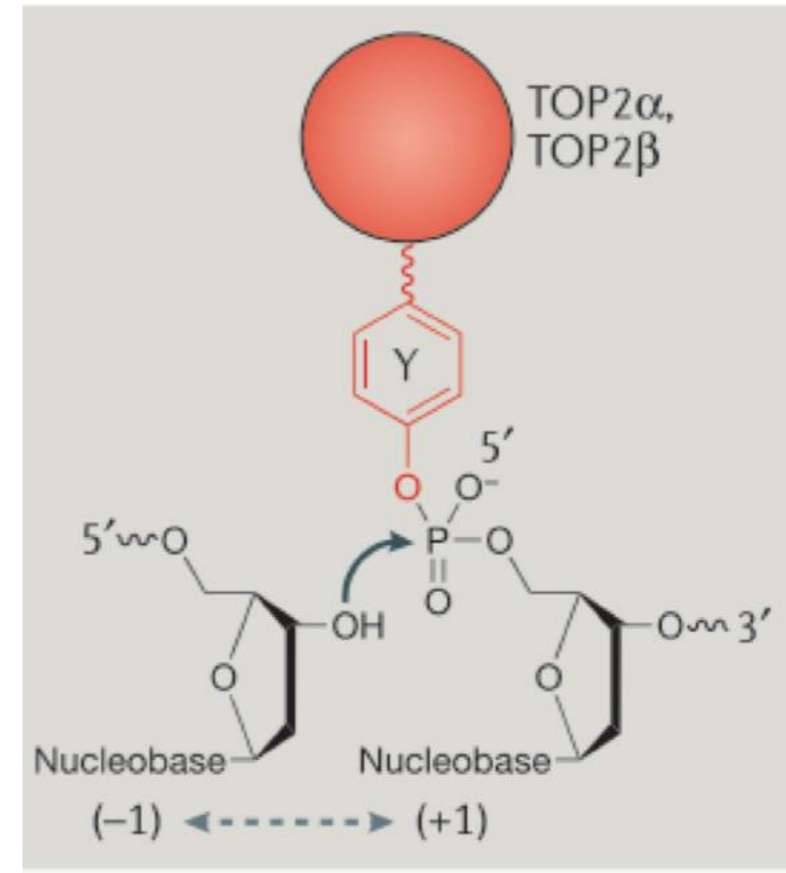
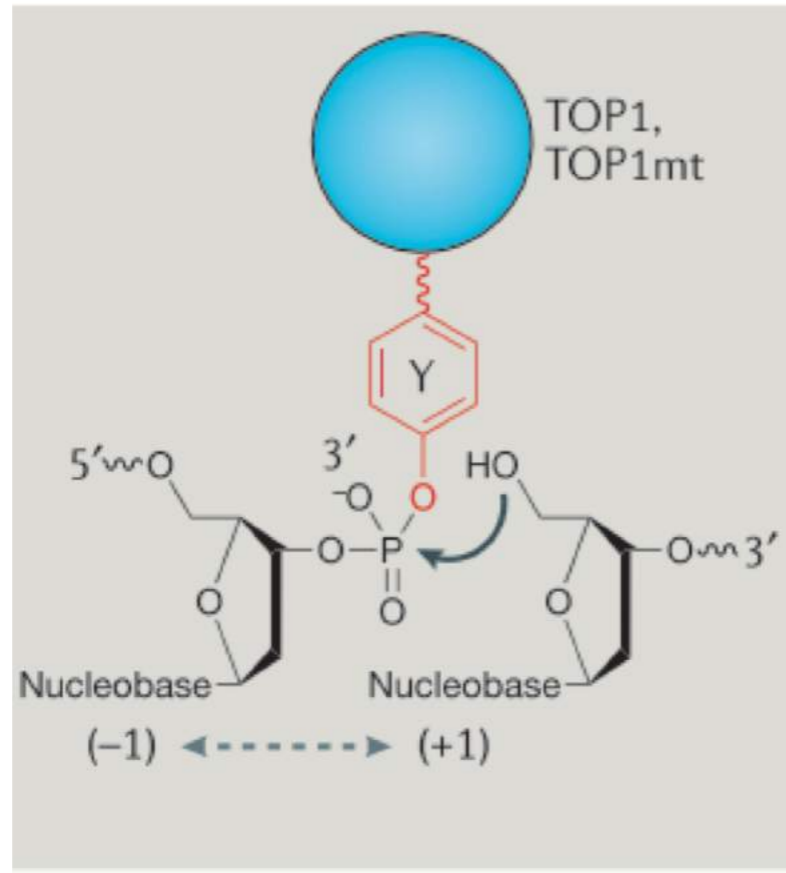




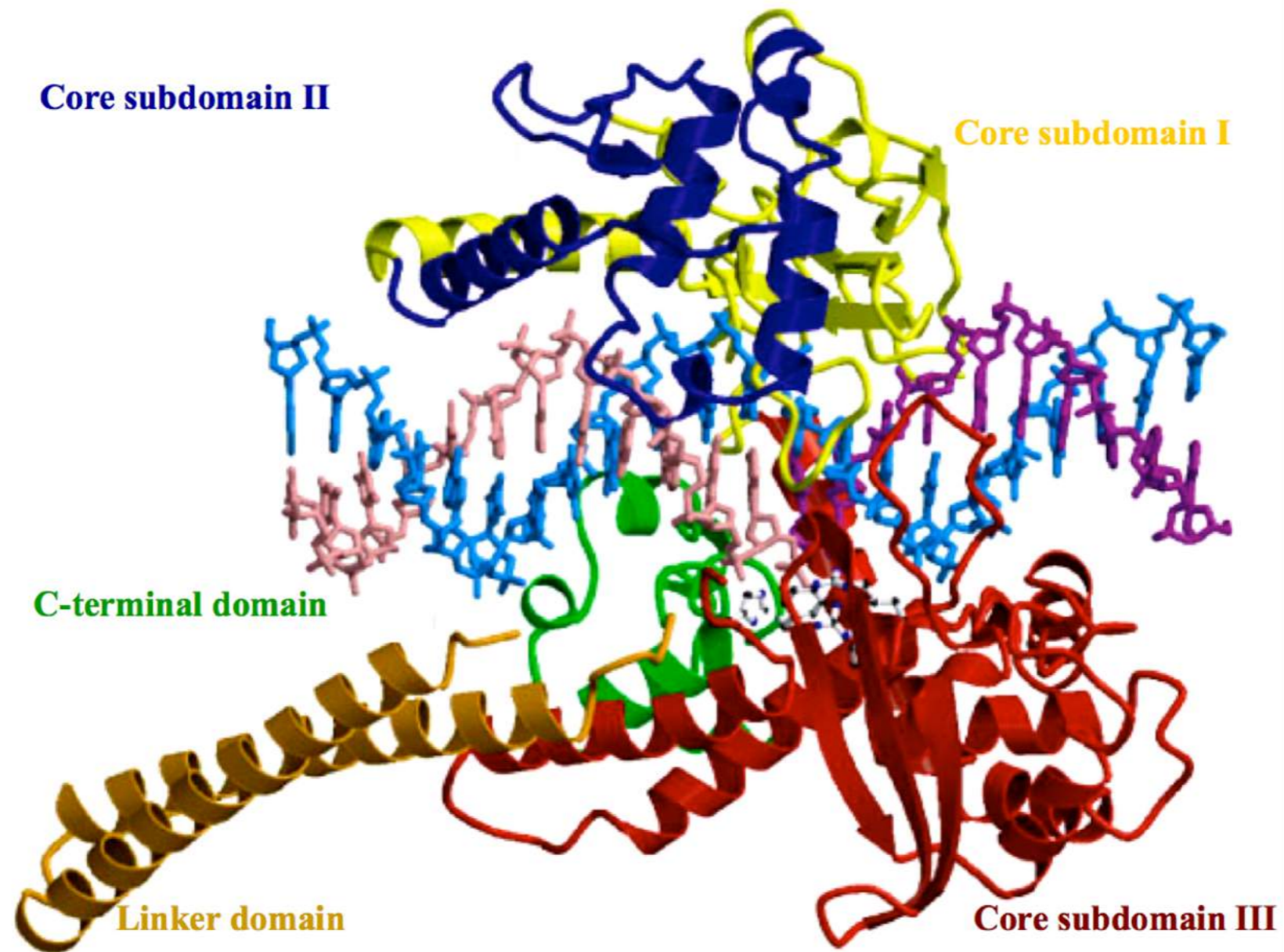
DNA Topoisomerase Family

SPECIES	Type I-A	Type I-B	Type II-A	Type II-B
<i>E. coli</i>	ω protein (Topo I) Topo III	(Topo I-B *)	DNA gyrase Topo IV	(Topo VIII**)
<i>S. cerevisiae</i>	Topo III	Topo I	Topo II	Spo11/Topo VI
Thermophilic archaea	Reverse gyrase	Topo V		Topo VI (Topo VIII**)
<i>D. melanogaster</i>	Topo III	Topo I	Topo II	Spo11/Topo VI
<i>H. sapiens</i>	Topo III α Topo III β	Topo I mtTopo I	Topo II α Topo II β	Spo11/Topo VI

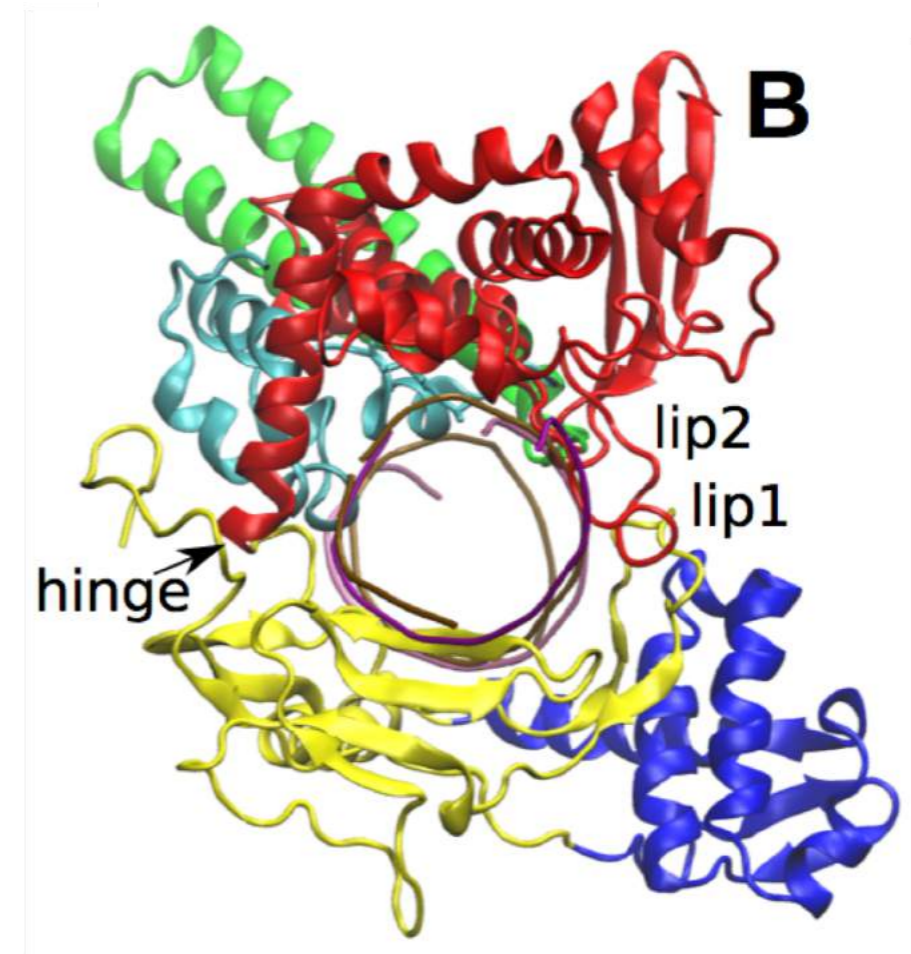
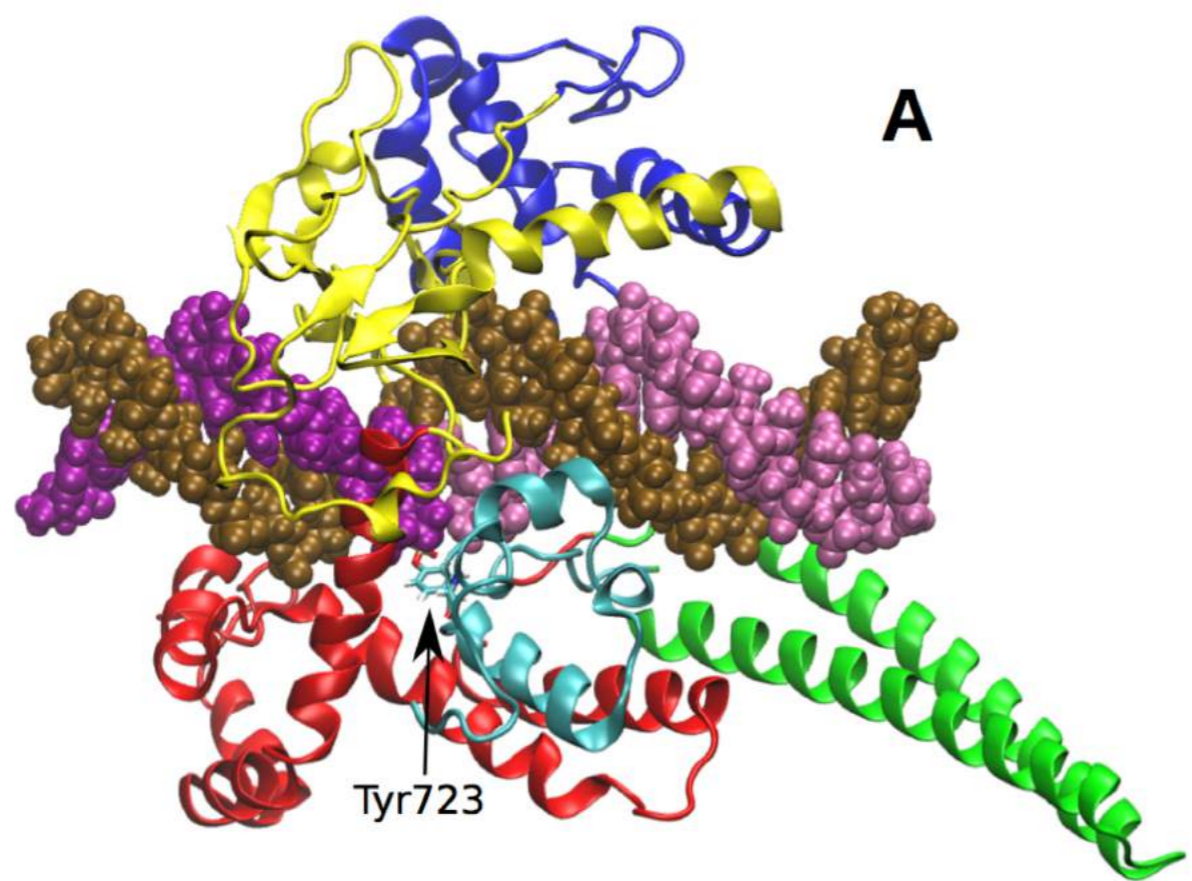
Reazione di taglio/ricucitura delle Topoisomerasi



DNA topoisomerase IB

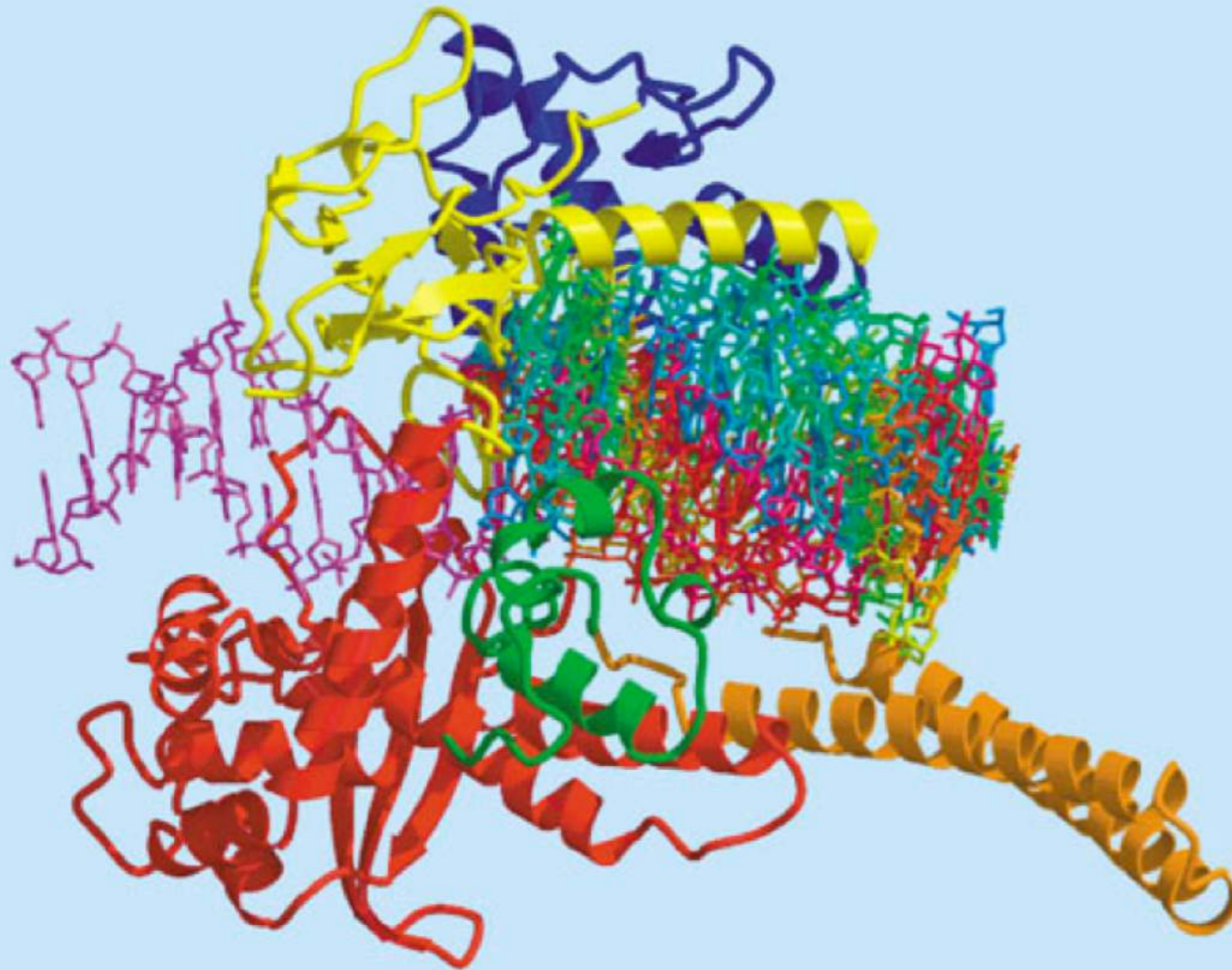


DNA topoisomerase IB

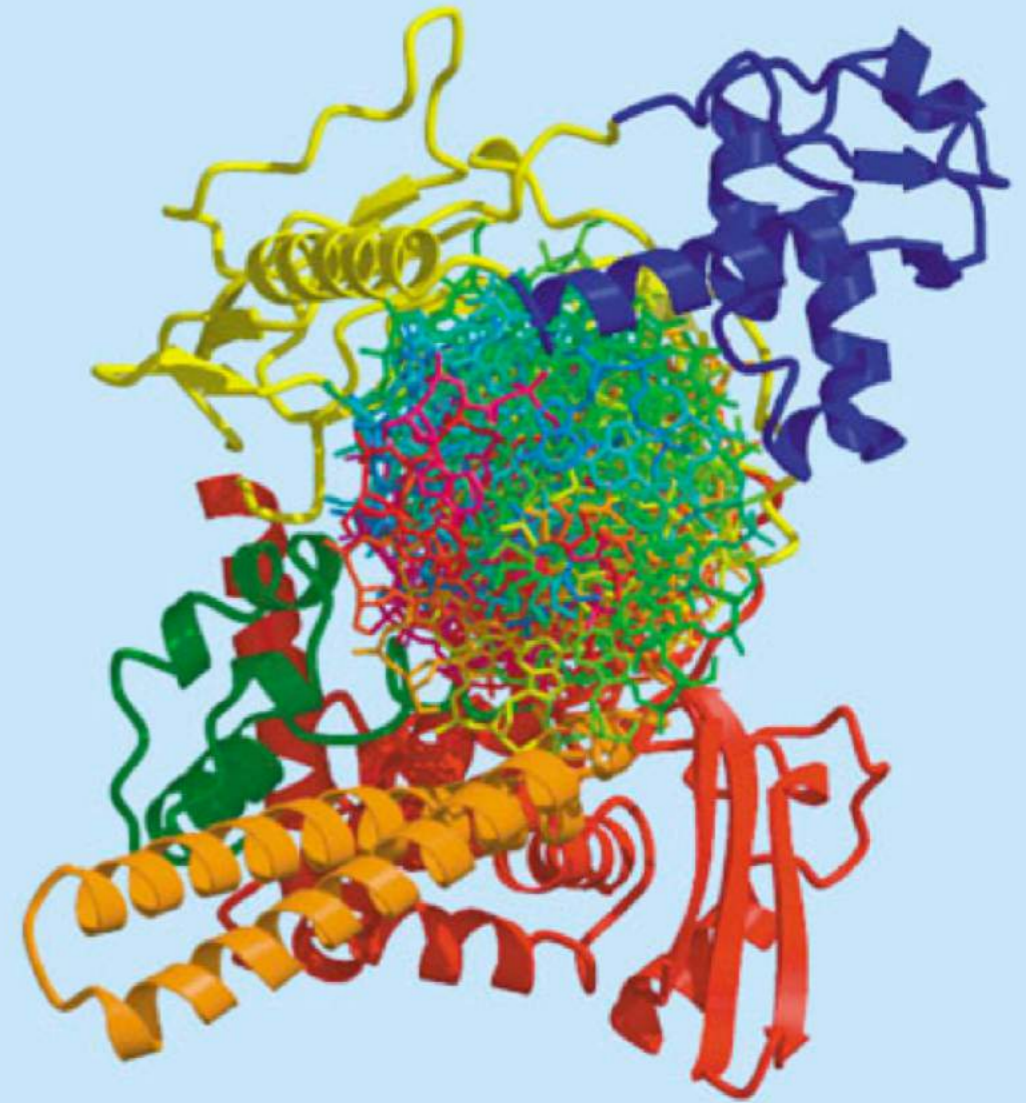


DNA topoisomerase IB

(a)

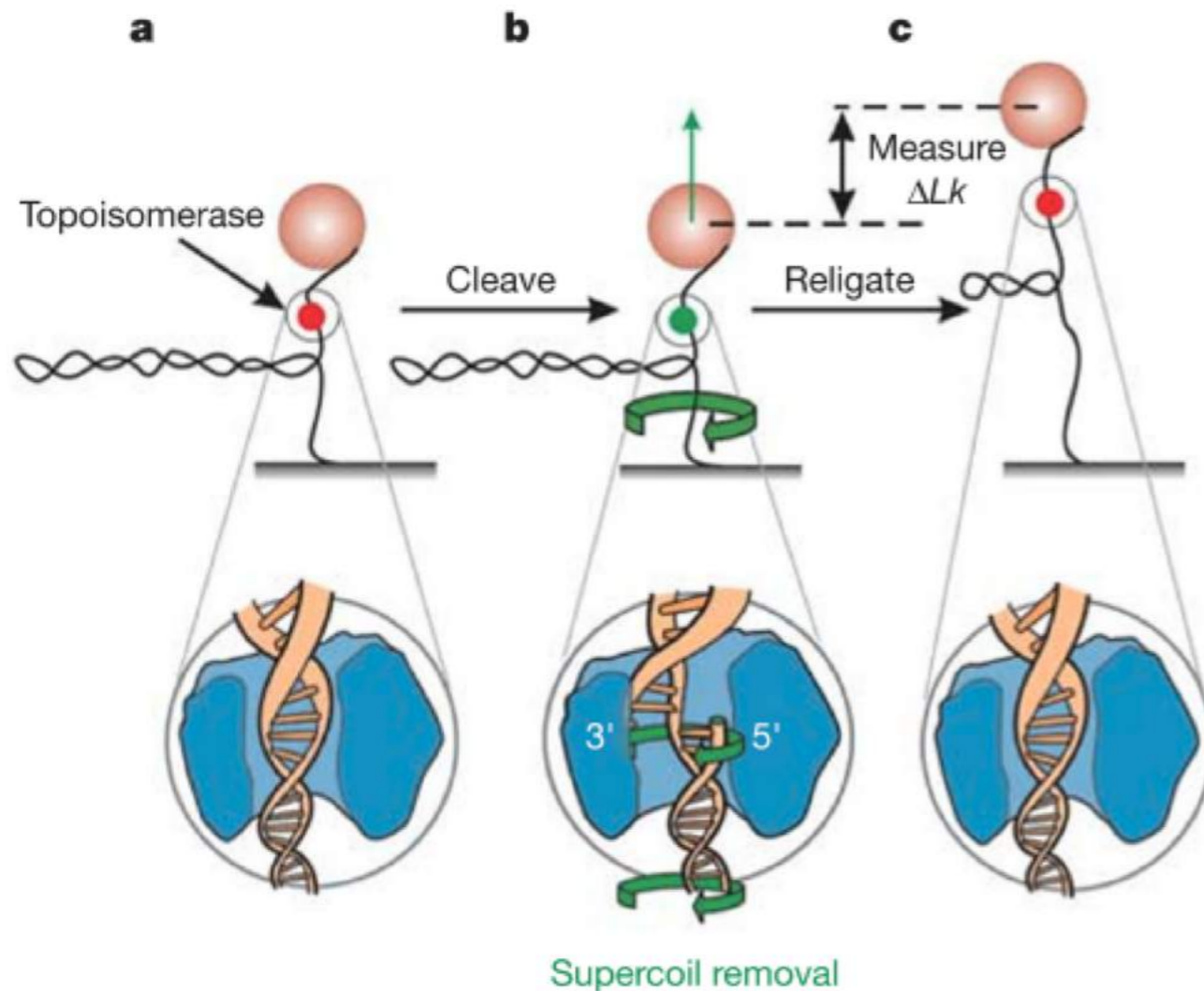


(b)



Current Opinion in Structural Biology

Risultati da studi su singola molecola (e biochimici)

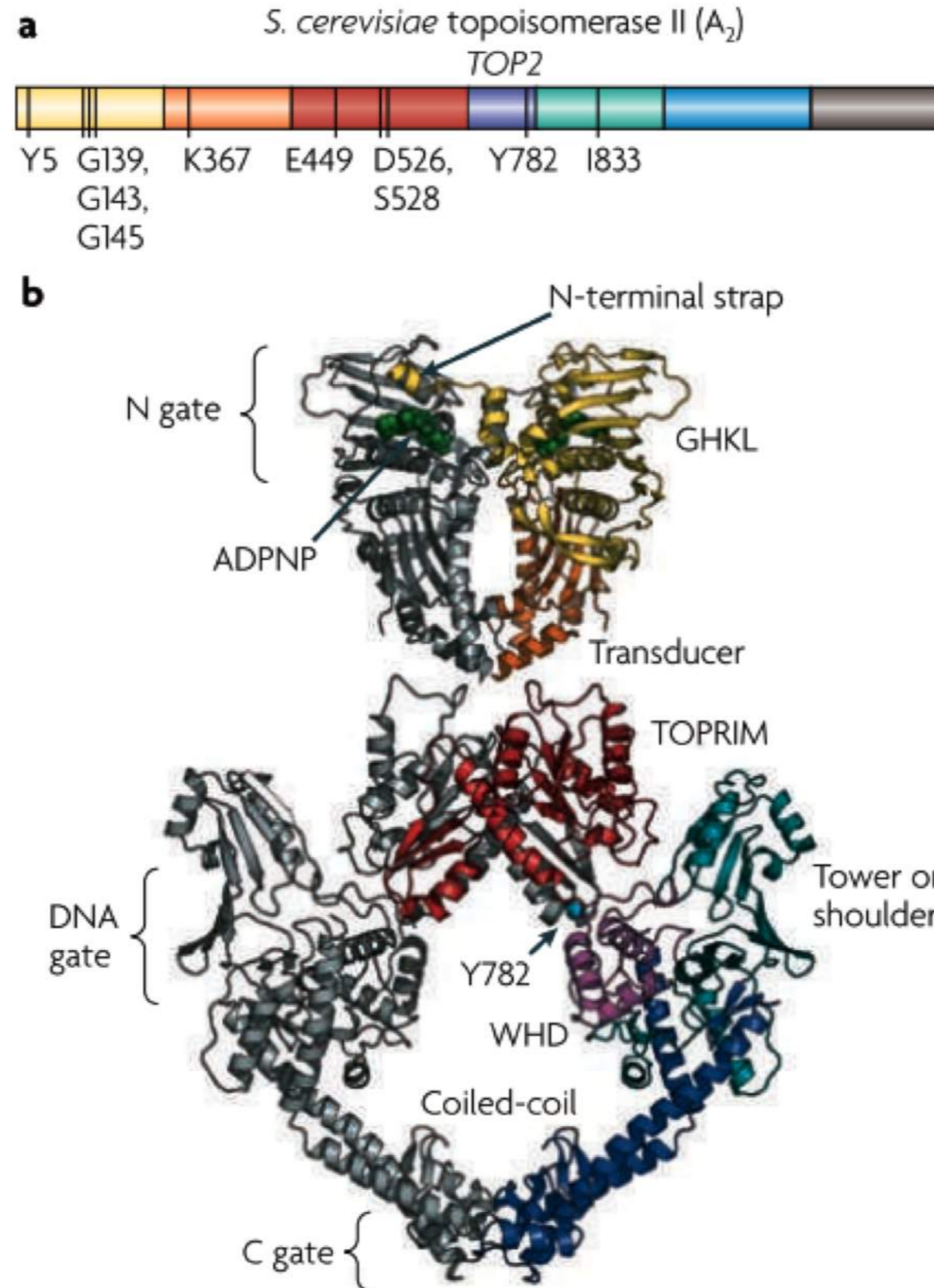


Unlike a nicking enzyme, TopoII does not release all the supercoils at once, but it typically does so in multiple steps.

The number of supercoils removed per step follows an exponential distribution.

The enzyme is found to be torque-sensitive, as the mean number of supercoils per step increases with the torque stored in the DNA.

DNA topoisomerasi II



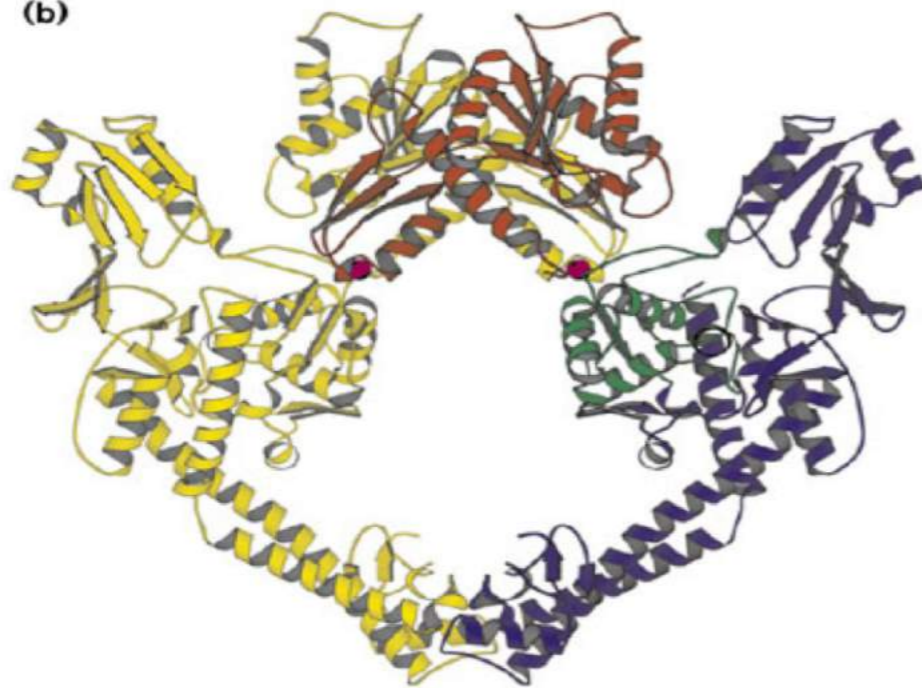
DNA topoisomerasi II varia la conformazione tridimensionale

S. cerevisiae dimeric Top2

(a)



(b)

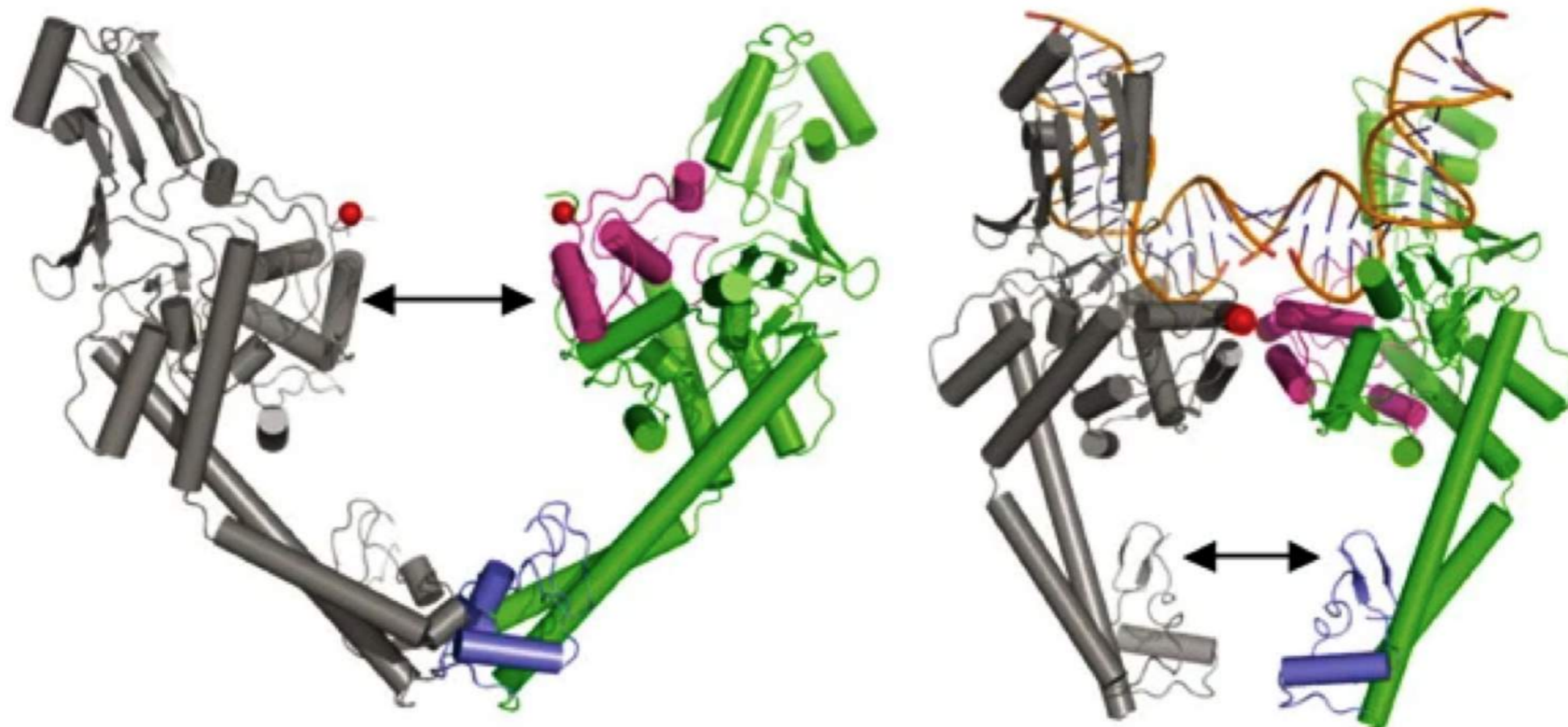


(c)

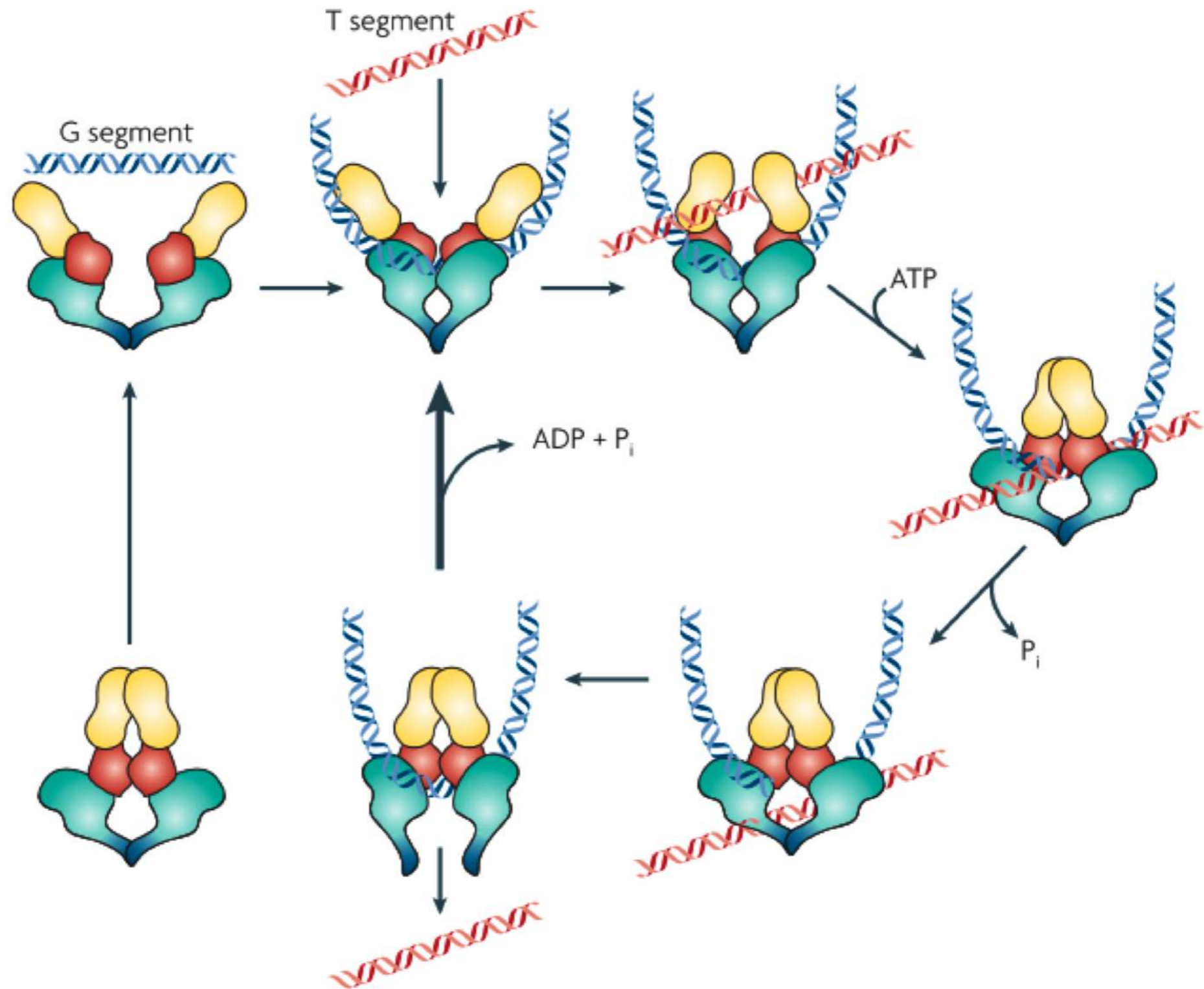


Current Opinion in Structural Biology

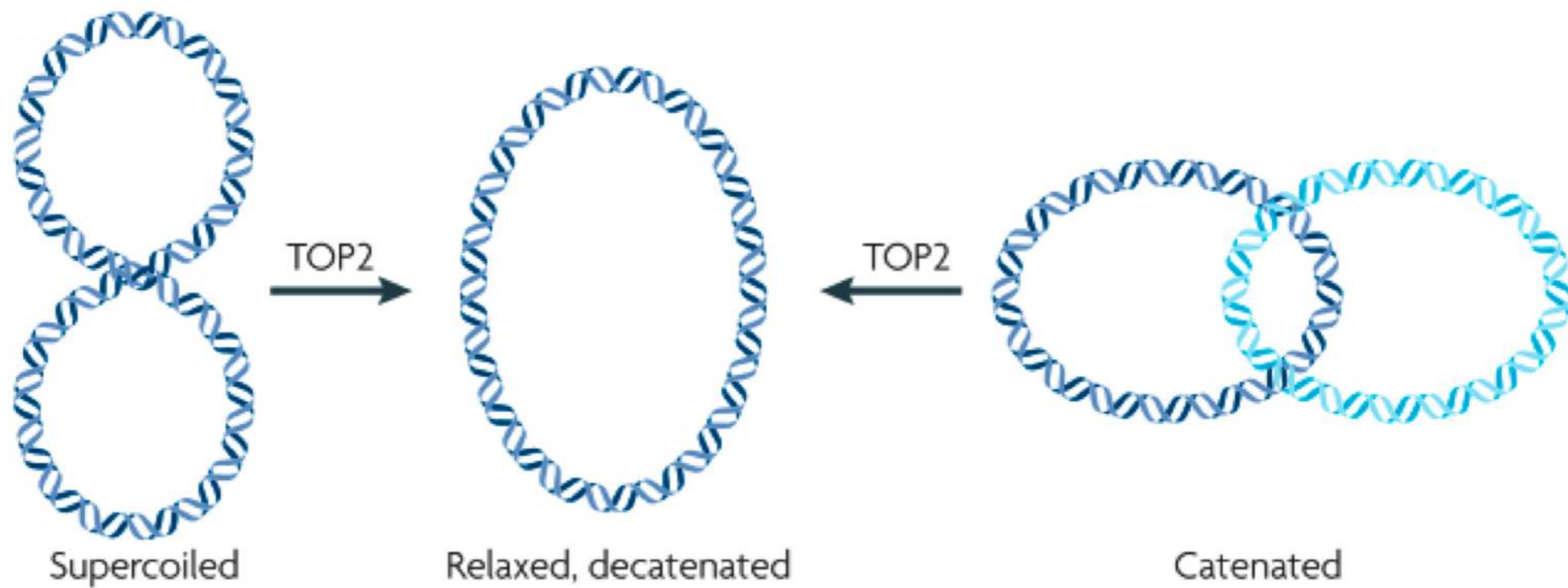
Il *gate* DNA e il *gate* sono alternativamente aperti

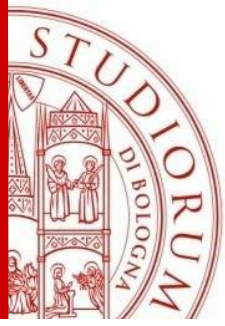


Il lavoro della topoisomerasi II dipende dalle modifiche conformazionali dell'enzima

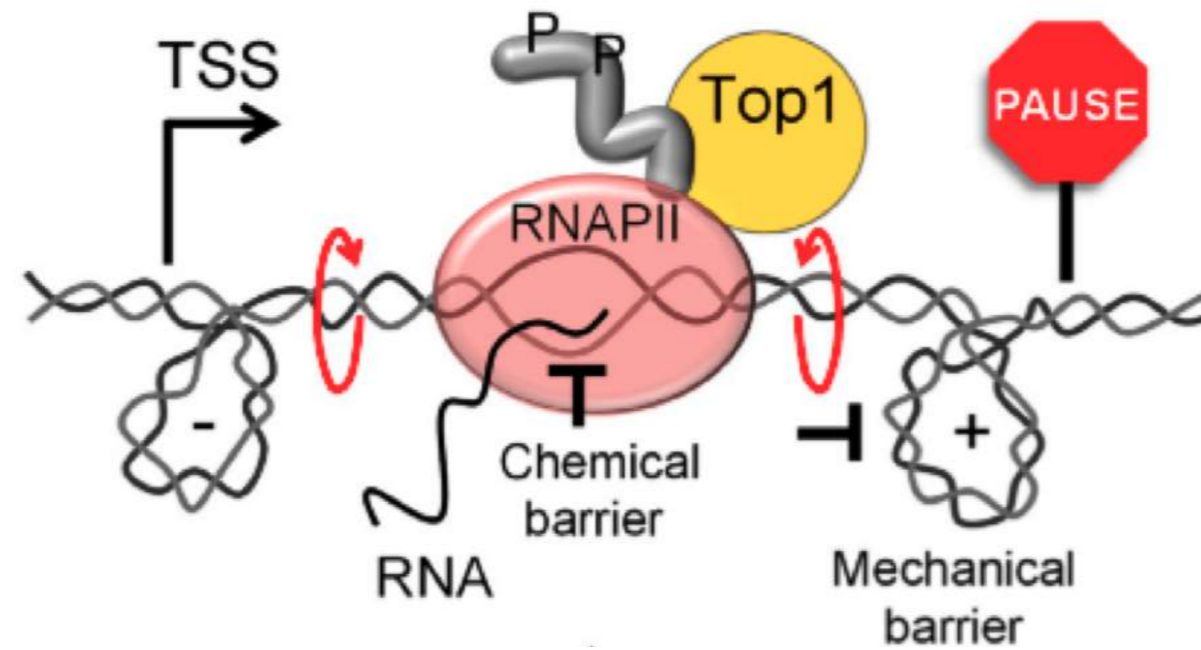


DNA topoisomerasi II cambia il *writhe* (superavvolgimenti) del DNA

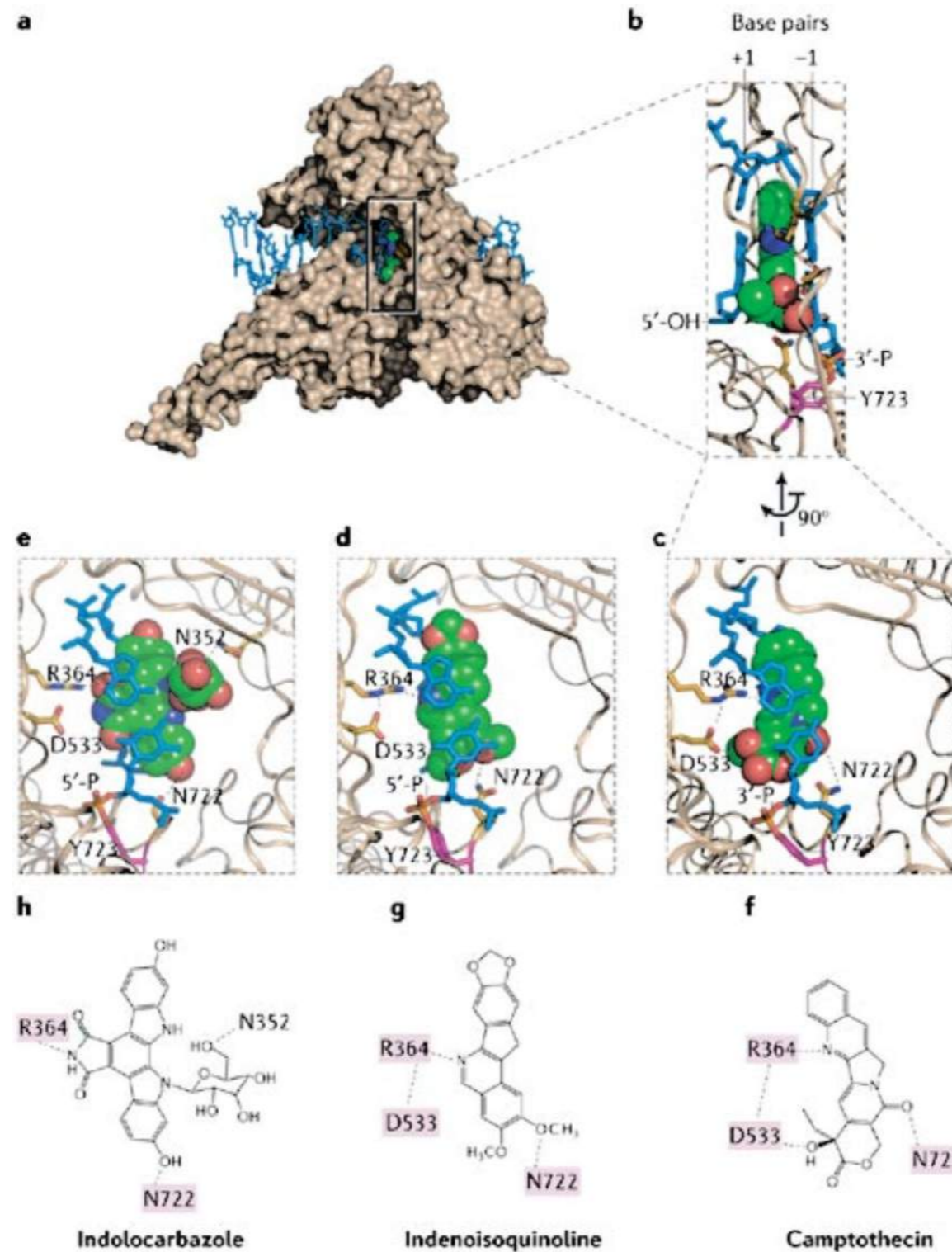
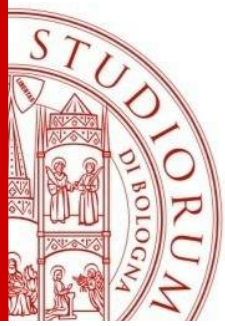




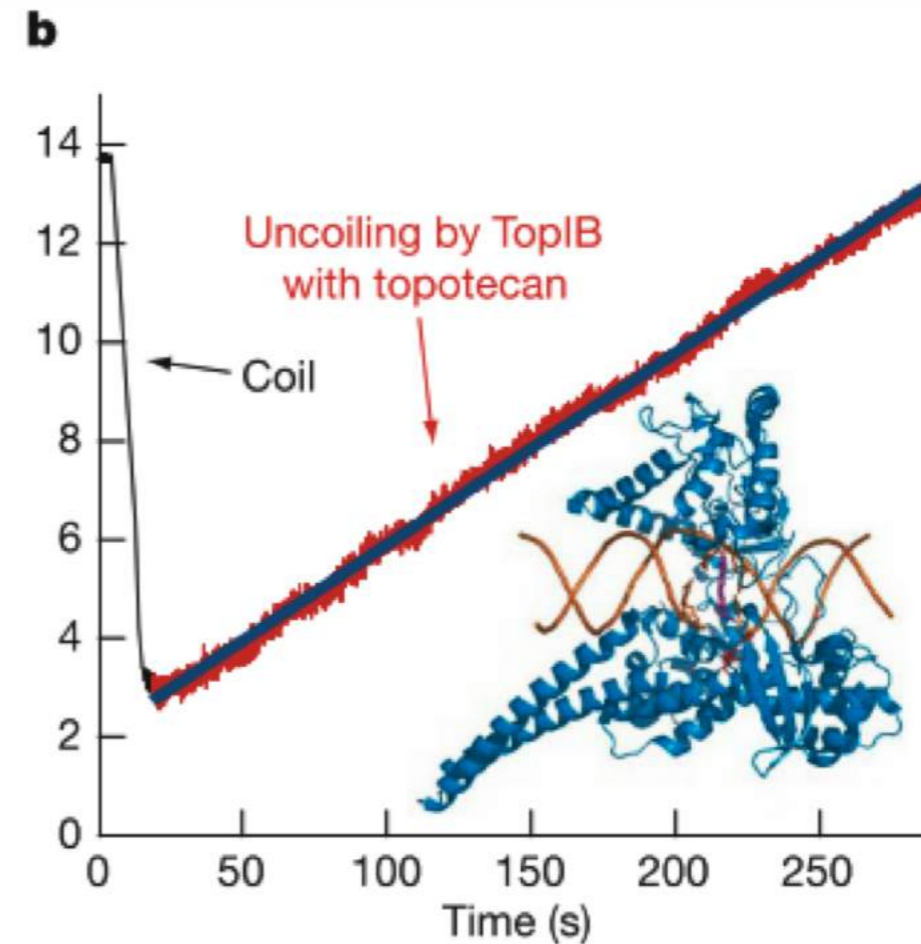
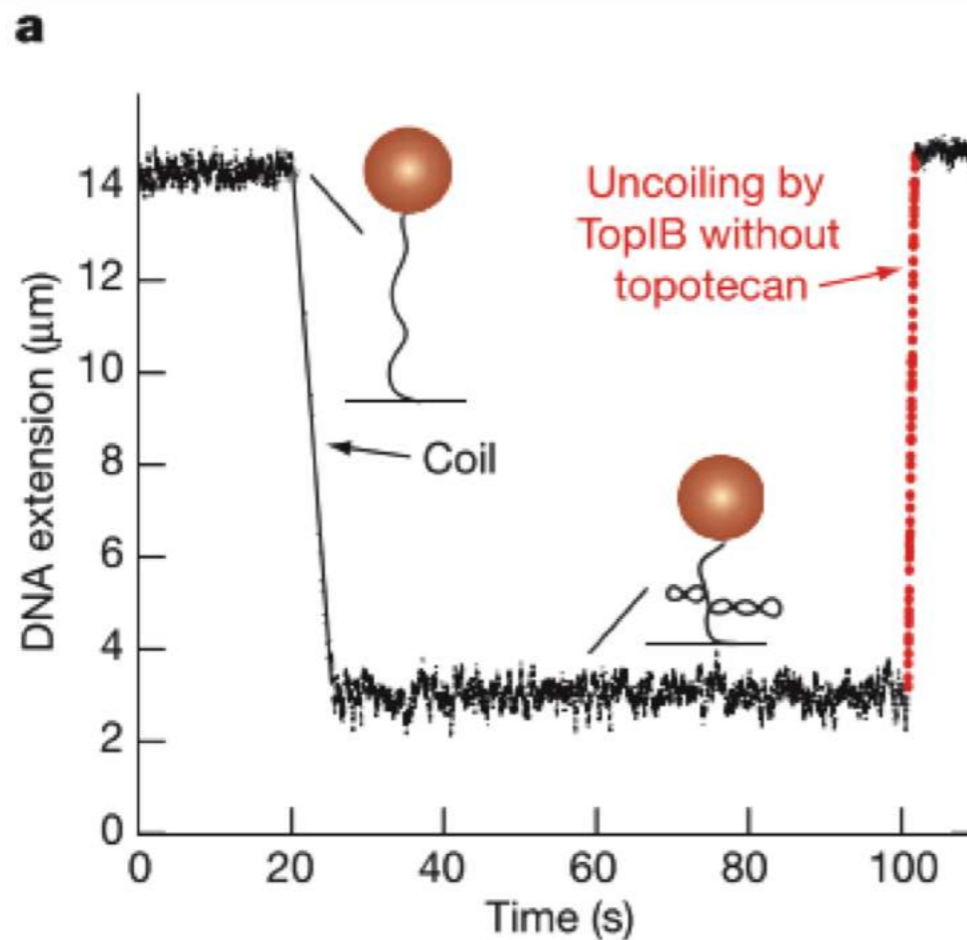
Durante la trascrizione, il lavoro della Topoisomerasi I è coordinato con quello della RNA polimerasi.

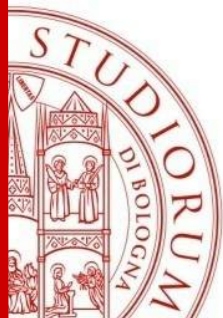


Interferire con il lavoro della Top1 può essere letale per la cellula, perchè risulta in rotture del genoma.



Interferire con il lavoro della Top1 può essere letale per la cellula, perchè risulta in rotture del genoma.





Le DNA topoisomerasi sono i bersagli specifici di diversi ed efficaci farmaci

Farmaci antitumorali

Antracicline, epipodofillotossine

Topoisomerasi II

Camptotecine

Topoisomerase IB

Antibiotici antibatterici



Chinolonici

DNA girasi (Topoisomerasi di tipo II)