

# RNA polymerase

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Structural Biology  
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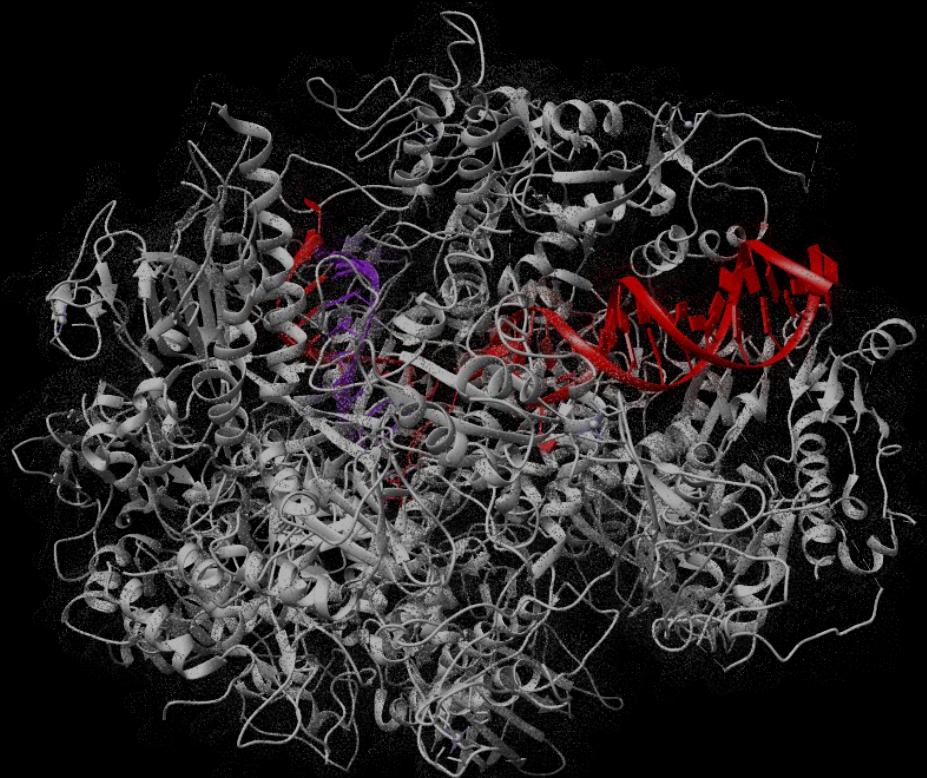
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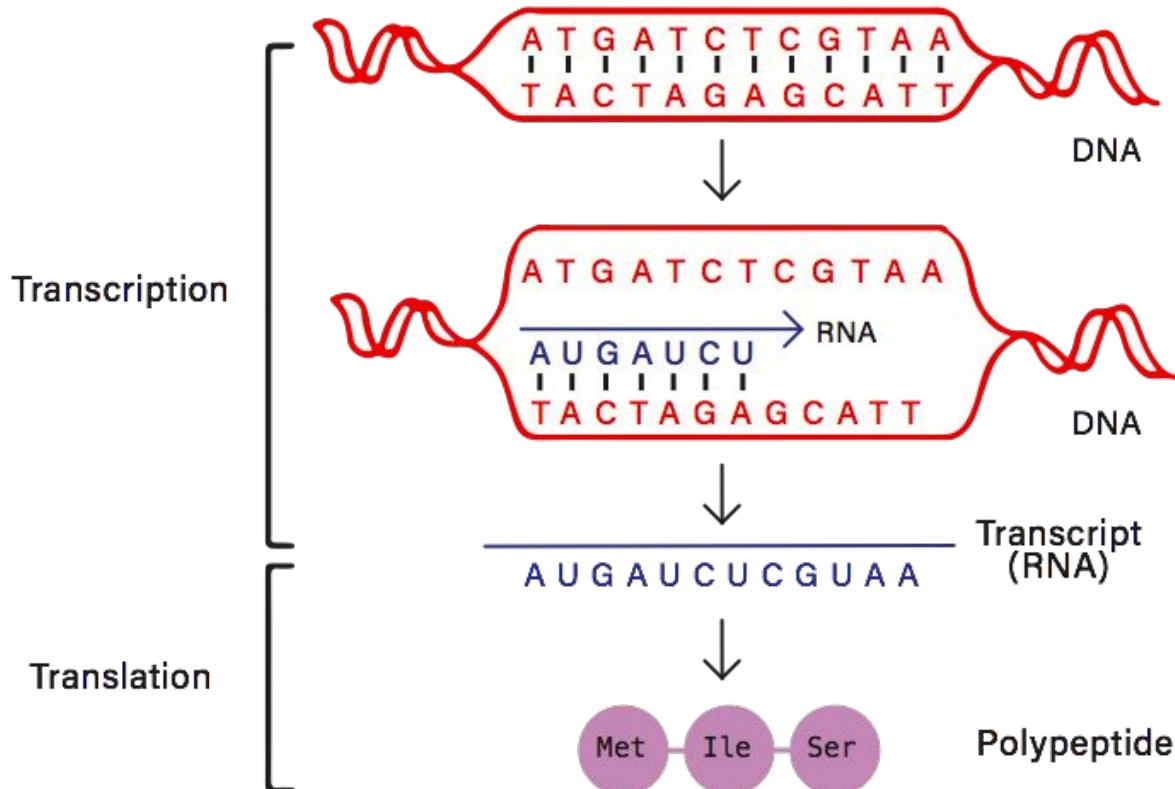
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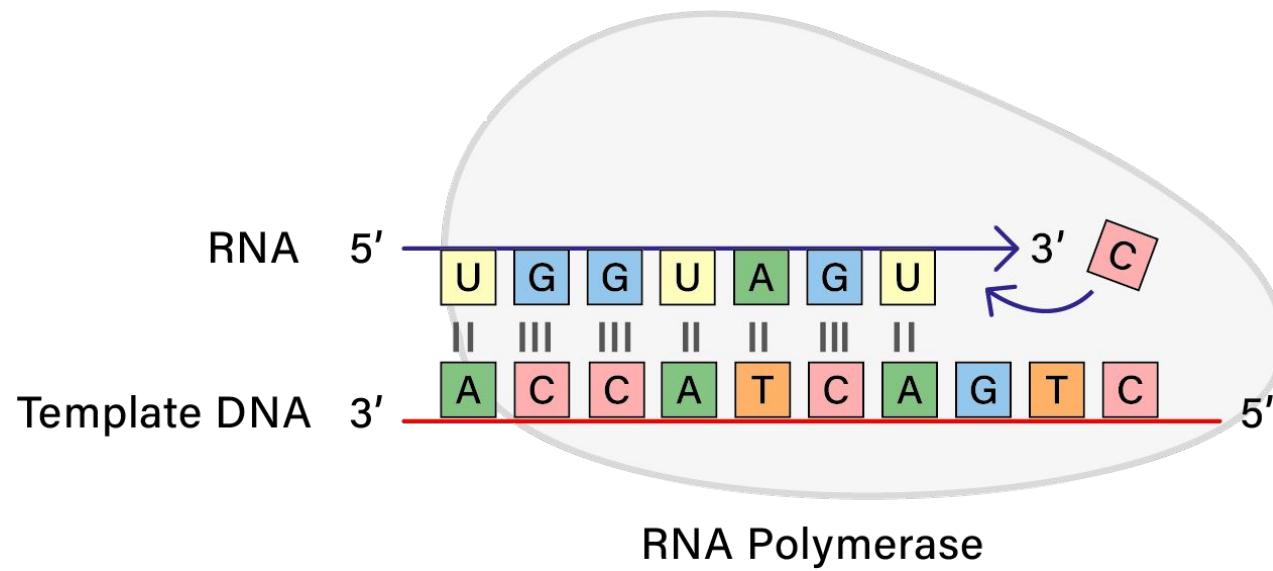


## INTRODUCTION

# TRANSCRIPTION



# TRANSCRIPTION

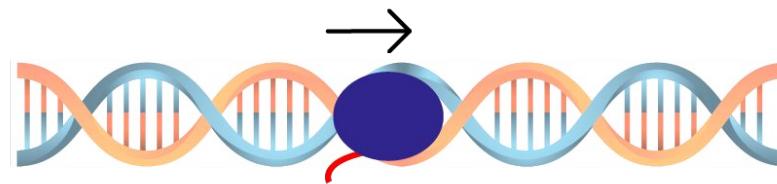


# TRANSCRIPTION: Stages

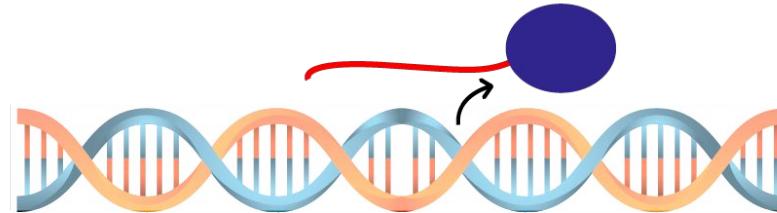
Initiation



Elongation



Termination



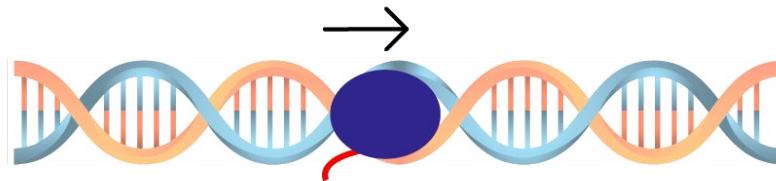
# RNA POLYMERASES I, II and III

- DNA-dependent RNA polymerases
- *Bacteria and Archaea* → Only one RNA polymerase
- Present in all eukaryotes' **nucleus**

RNA polymerase I	RNA polymerase II	RNA polymerase III
rRNA	mRNA miRNA snRNA snoRNA	5S rRNA tRNA

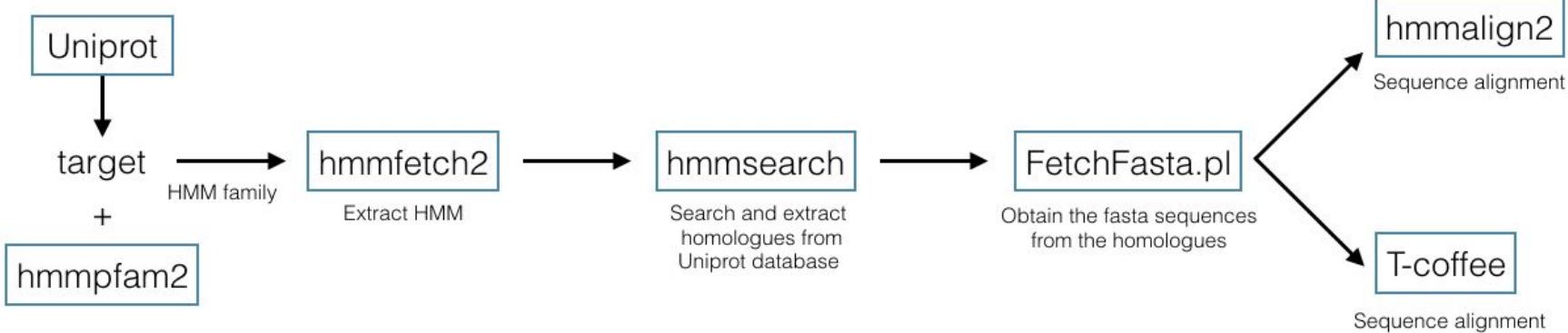
# AIM OF THE PROJECT

- RNA polymerase II and Elongation cycle
- The aim of this project is:
  - To identify the main structures involved in the elongation cycle.
  - To find the residues whose interactions are essential for the enzyme function.
  - To analyze their conservation among different species' genome.

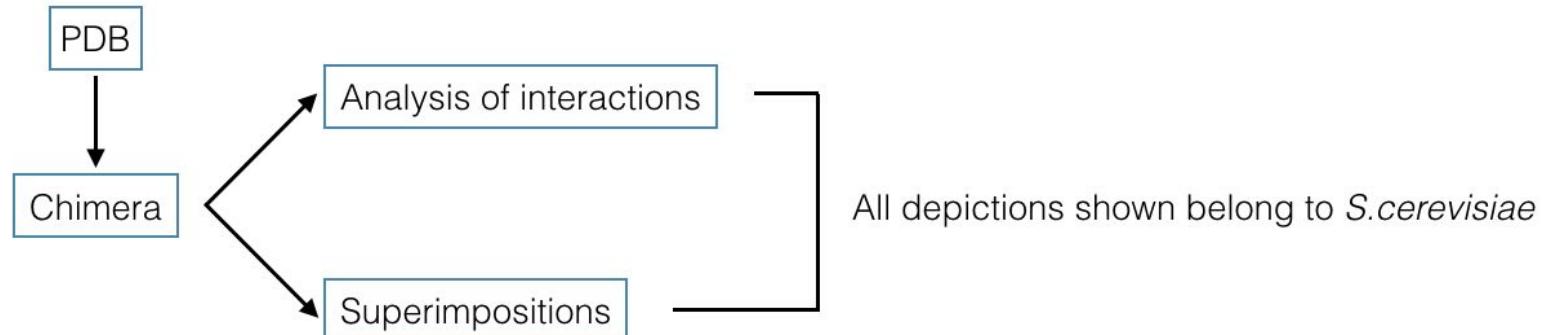


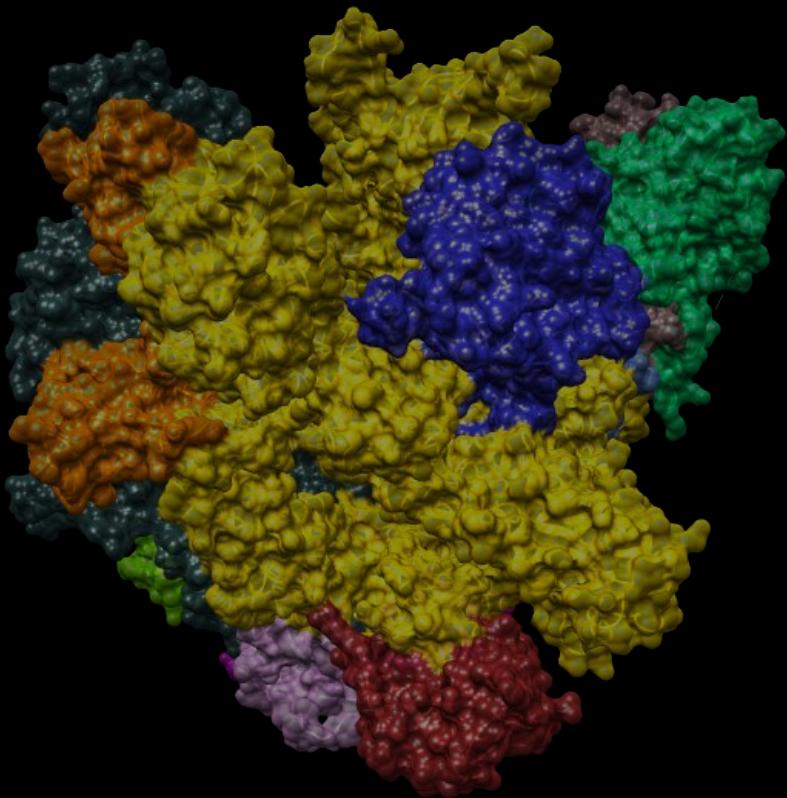
# METHODS

## Sequences



## Structures

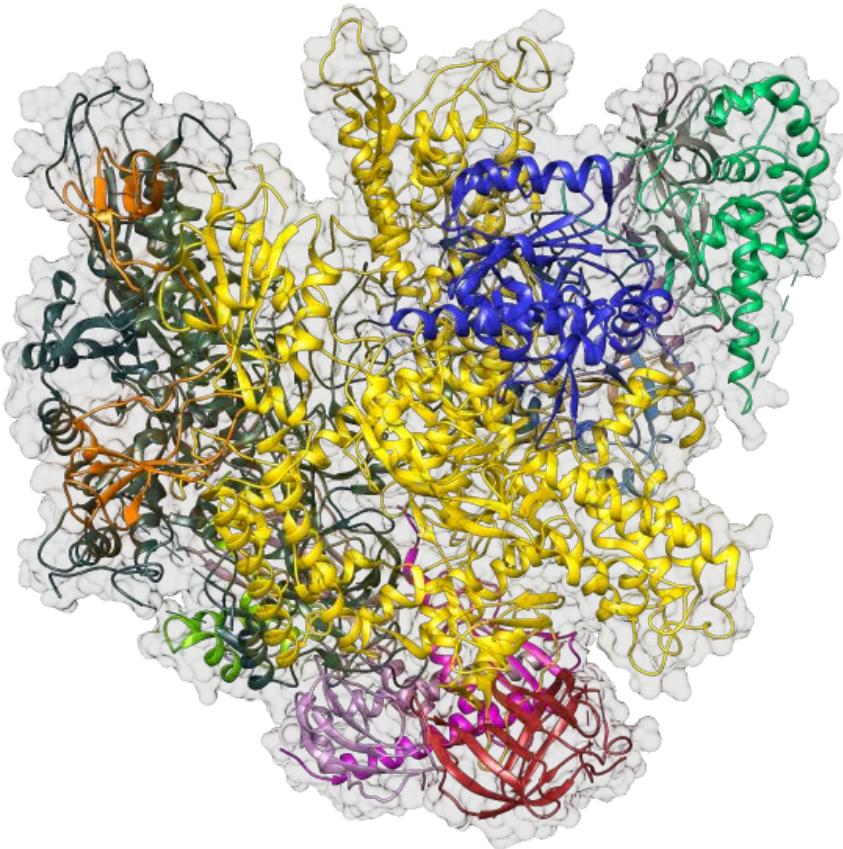
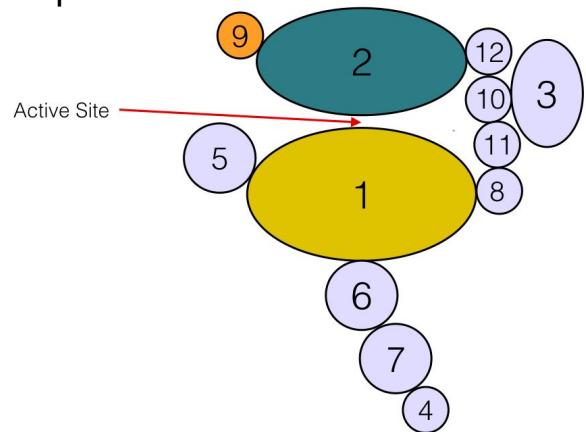




## STRUCTURAL FEATURES OF RNA POLYMERASE II

# RNA polymerase II

- 550 kDa
- 12 subunits (Rpb1-Rpb12)
  - Rpb1
  - Rpb2
  - Rpb9

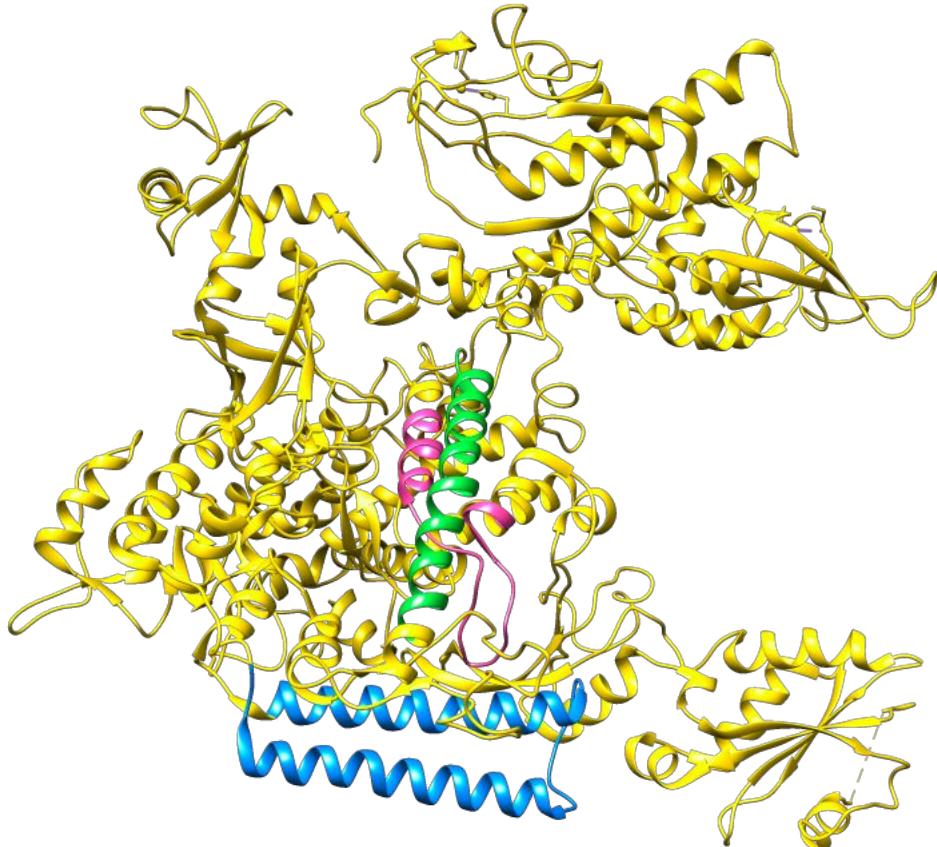


## RNA polymerase II: Rpb1

- Largest subunit (220 kDa)

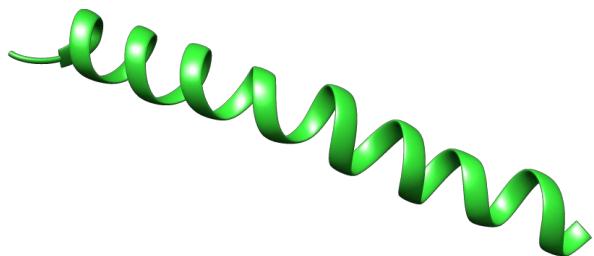
### SCOP information:

- **Class:** Multi-domains protein
- **Fold:** Beta and beta-prime subunits of DNA-dependent RNA polymerase
- **Superfamily:** Beta and beta-prime subunits of DNA-dependent RNA polymerase
- **Family:** RNA polymerase beta-prime

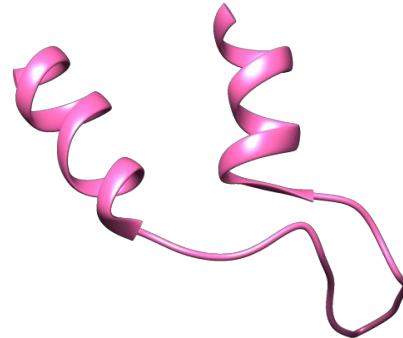


# RNA polymerase II: Rpb1

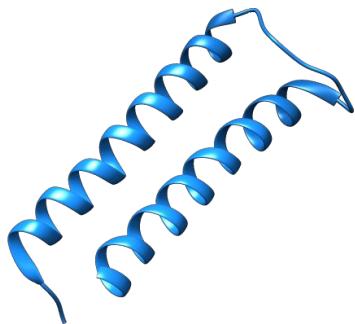
$\alpha$ -helix  
(Bridge helix)



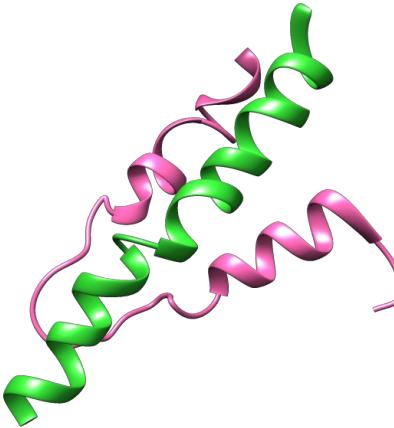
$\alpha$ -hairpin  
(Trigger loop)



$\alpha$ -hairpin  
( $\alpha$ 20- $\alpha$ 21)



three helix  
bundle (BH+TL)

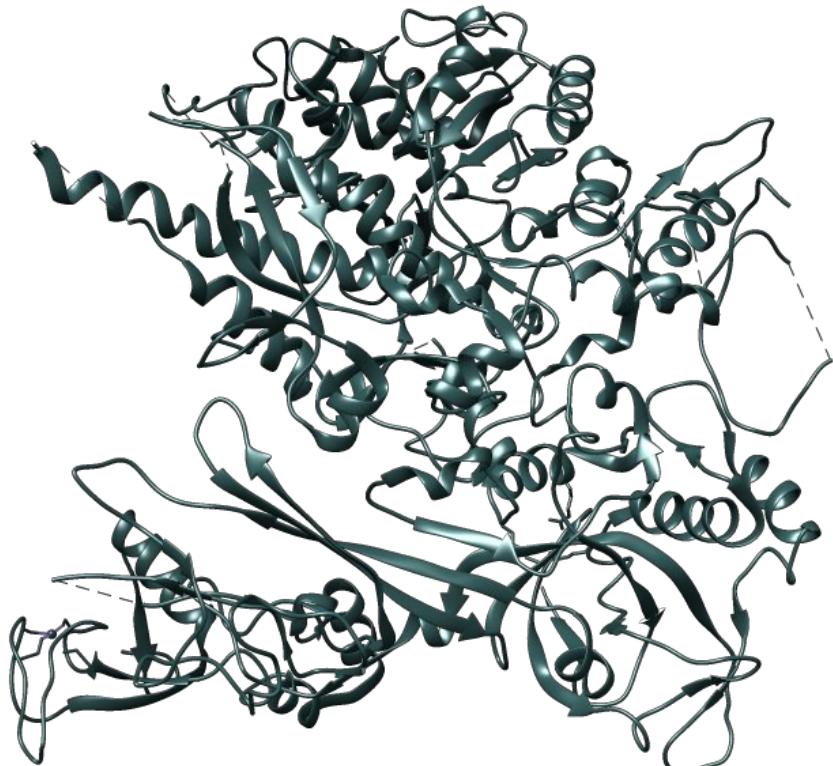


# RNA polymerase II: Rpb2

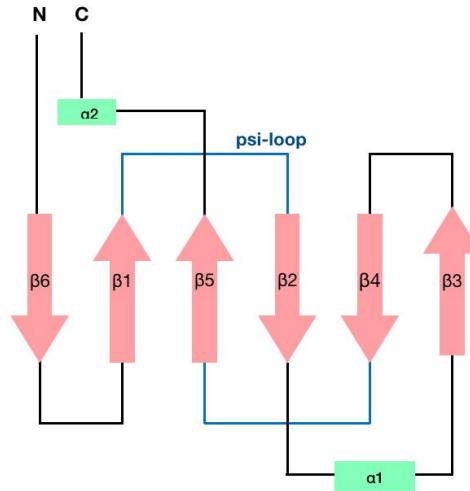
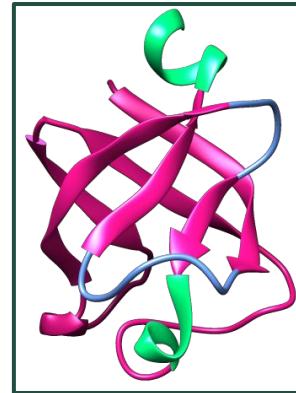
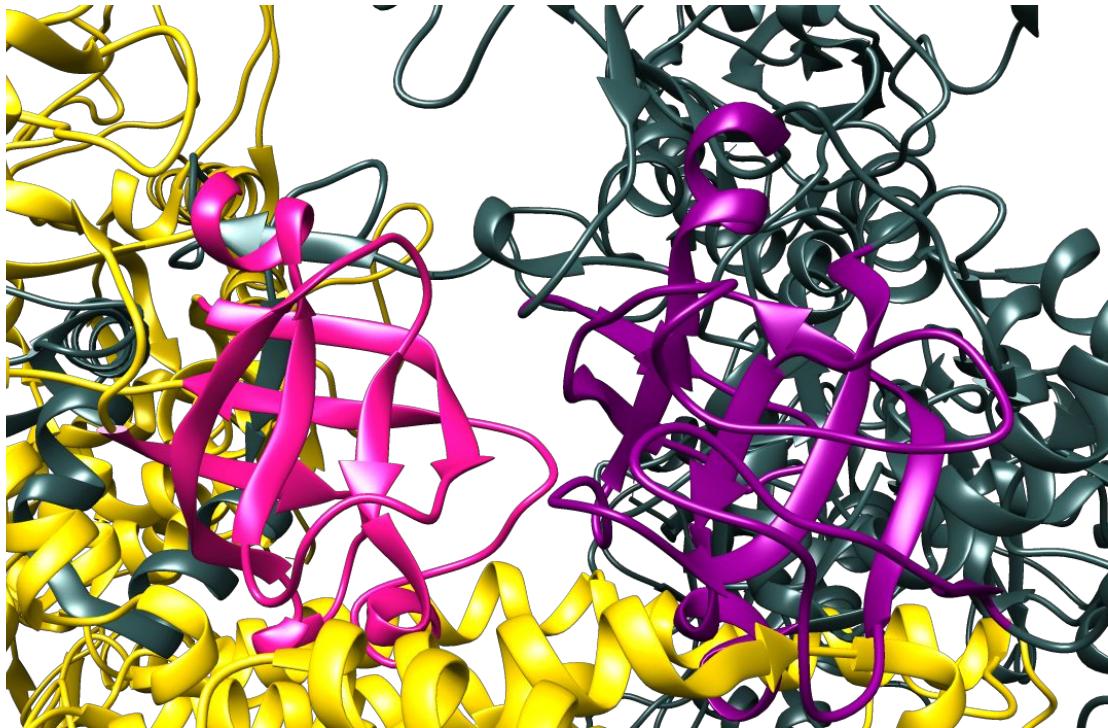
- 2nd largest subunit (140 kDa)

## SCOP information:

- **Class:** Multi-domains protein
- **Fold:** Beta and beta-prime subunits of DNA-dependent RNA polymerase
- **Superfamily:** beta and beta-prime subunits of DNA-dependent RNA polymerase
- **Family:** RNA polymerase beta



## RNA polymerase II: Rpb1 and Rpb2 double-psi $\beta$ -barrels

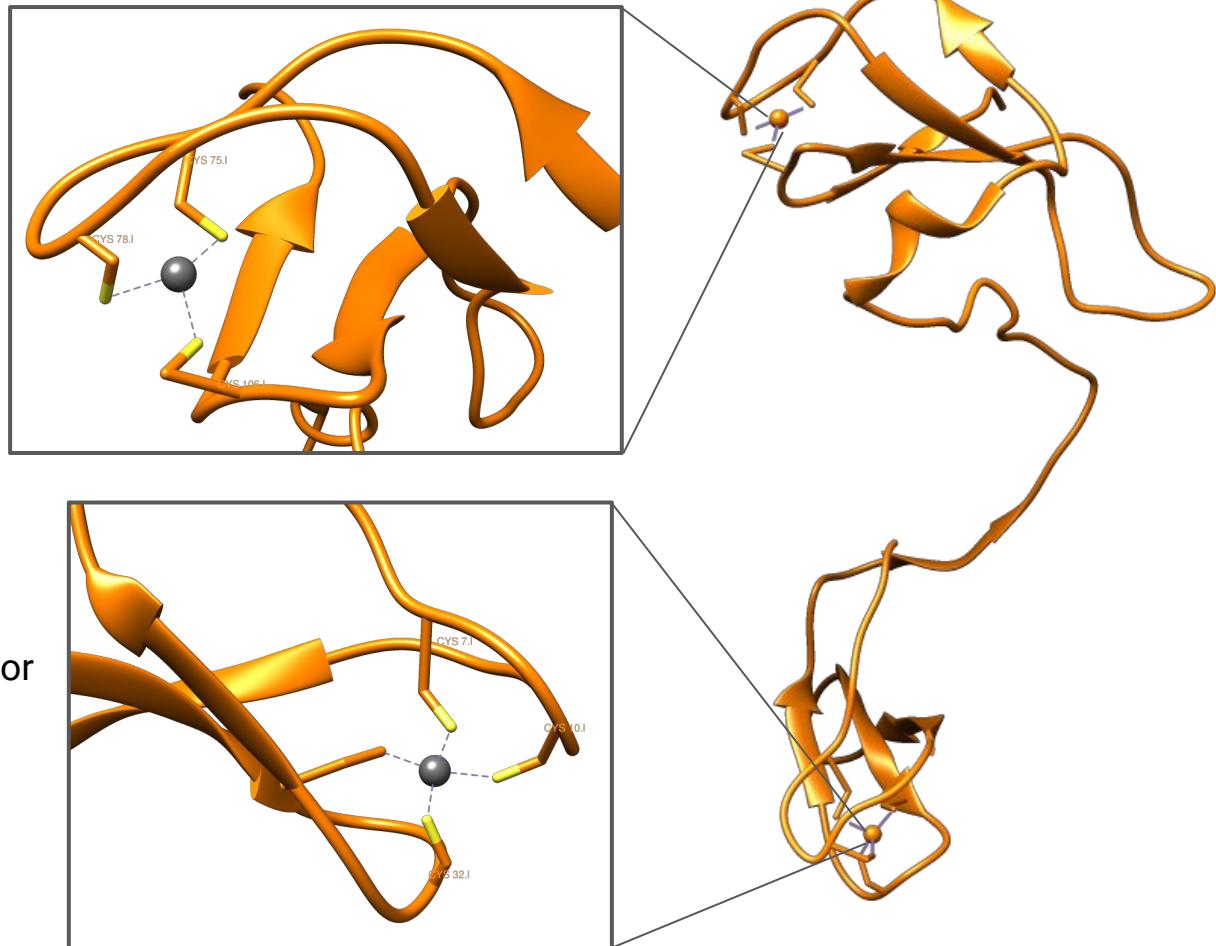


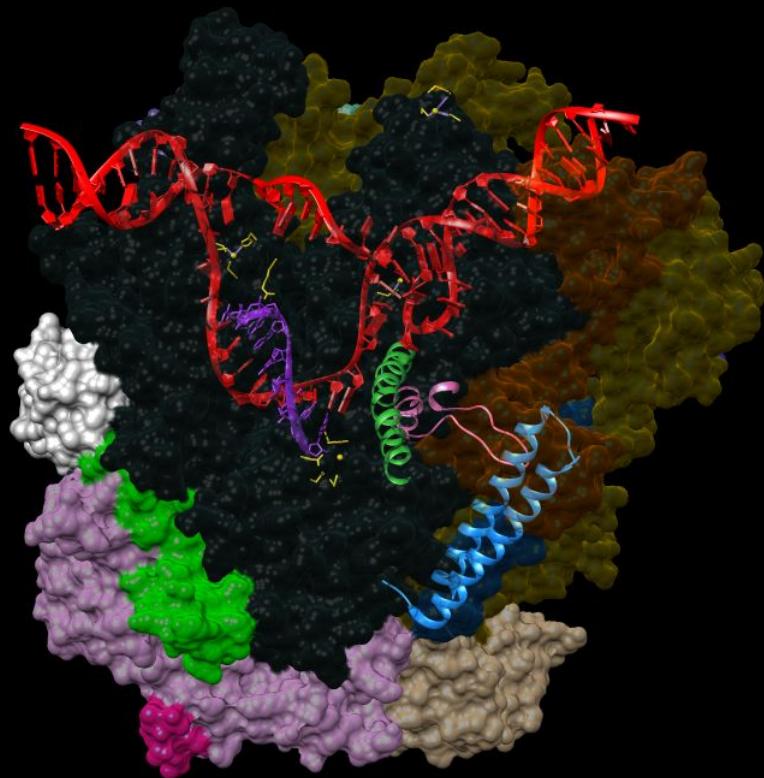
# RNA polymerase II: Rpb9

- 14.5 kDa
- 2 Zn binding motifs

## SCOP information:

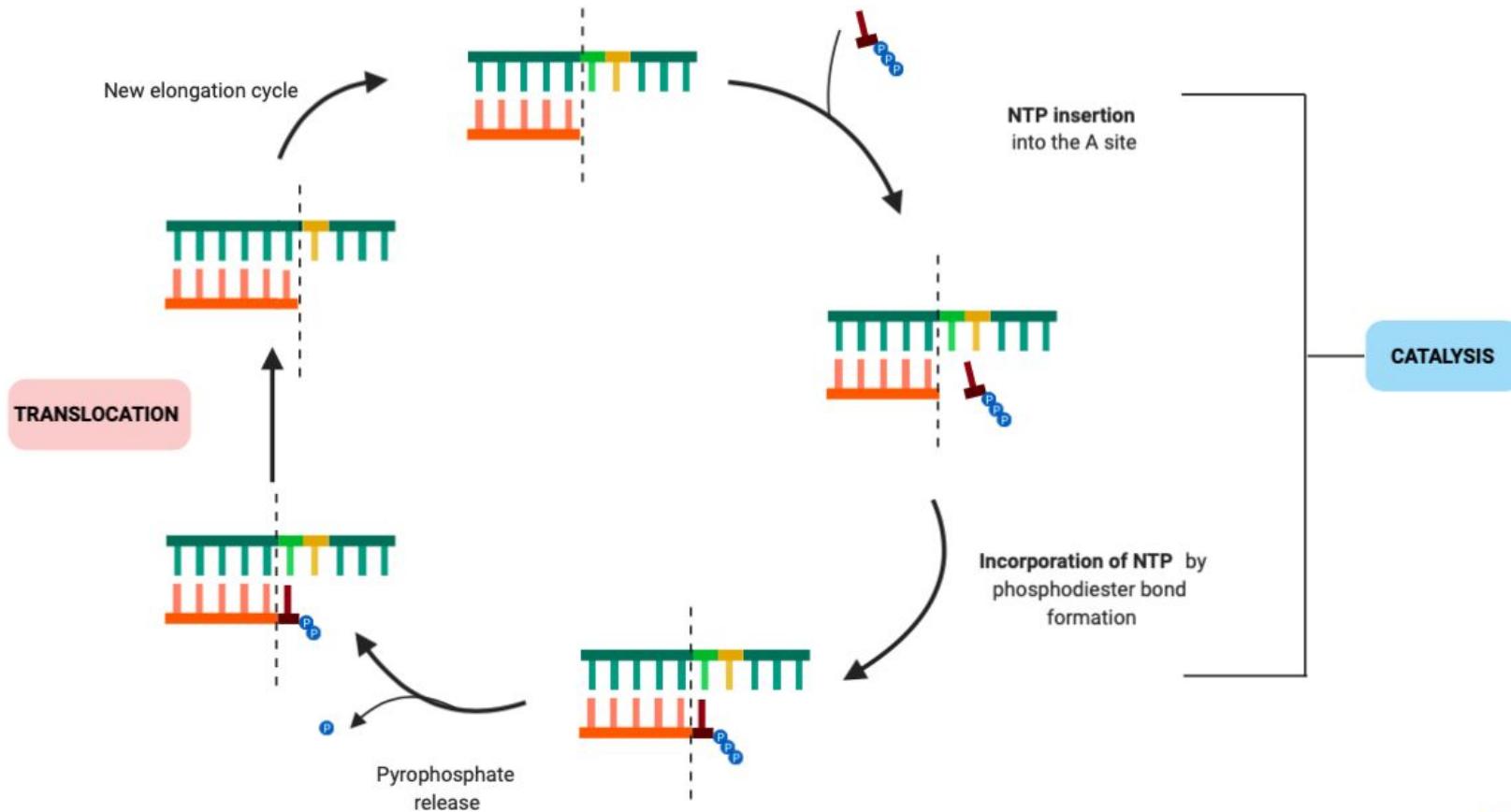
- **Class:** Small proteins
- **Fold:** Rubredoxin-like
- **Superfamily:** Zn beta-ribbon
- **Family:** Transcriptional factor domain



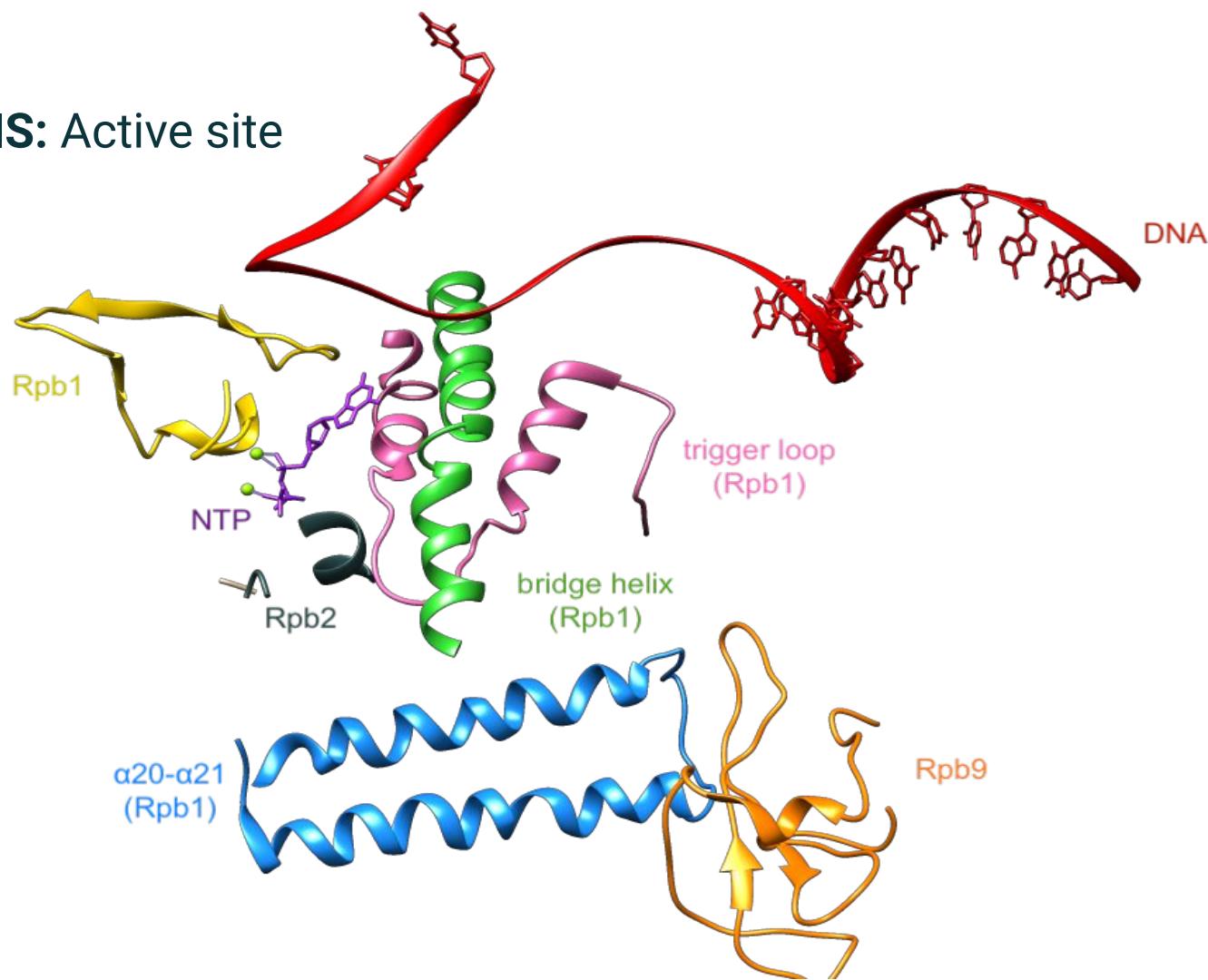


## THE ELONGATION CYCLE

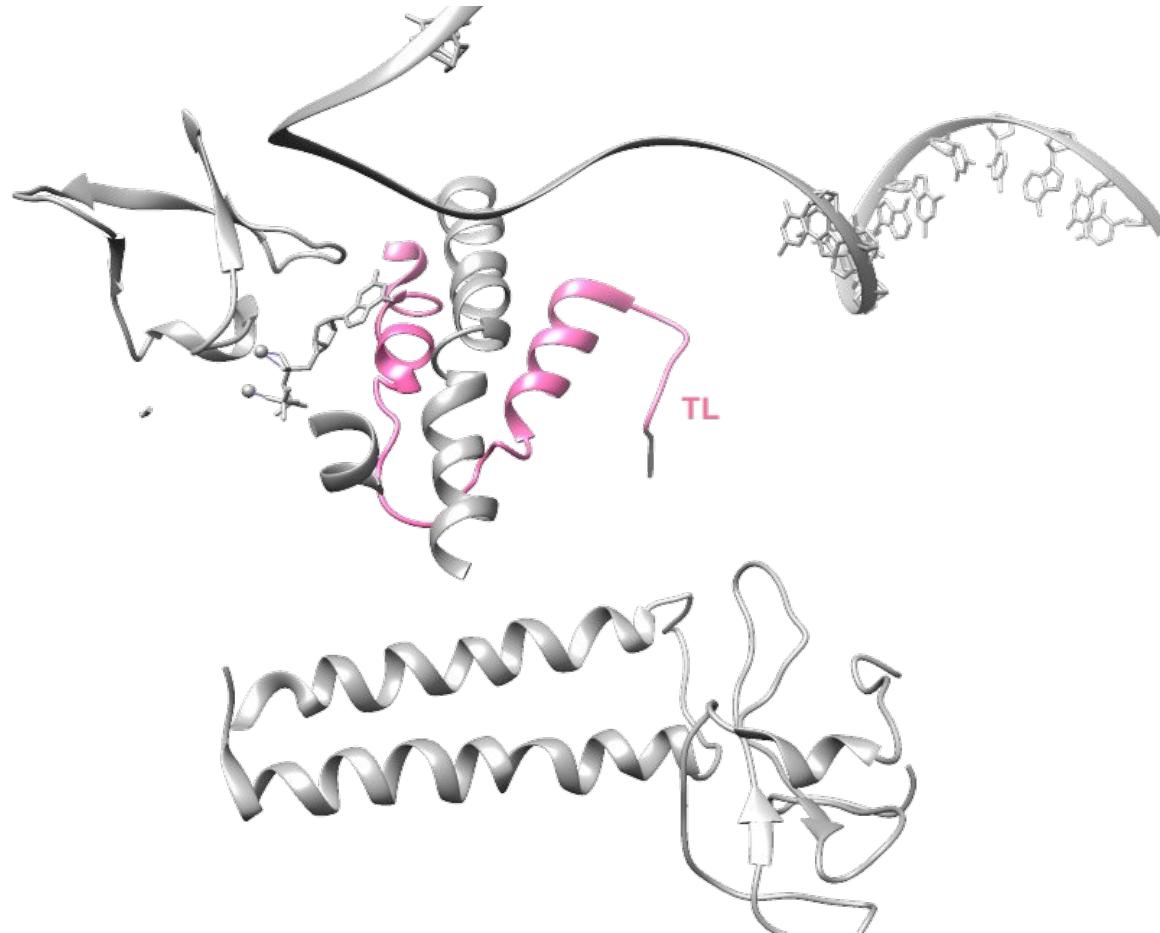
# ELONGATION CYCLE



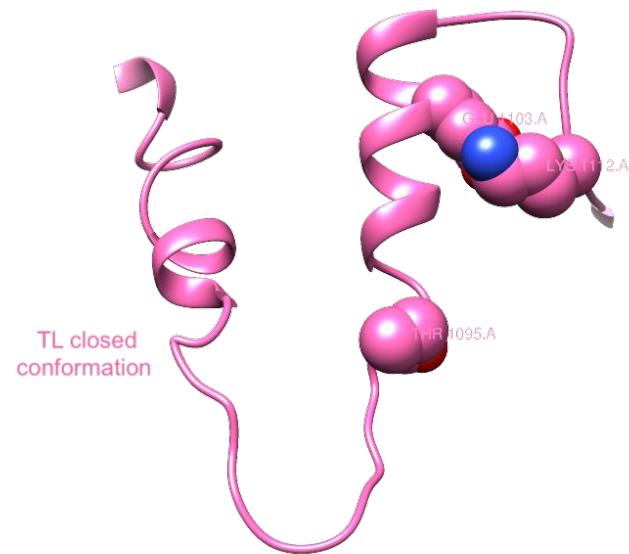
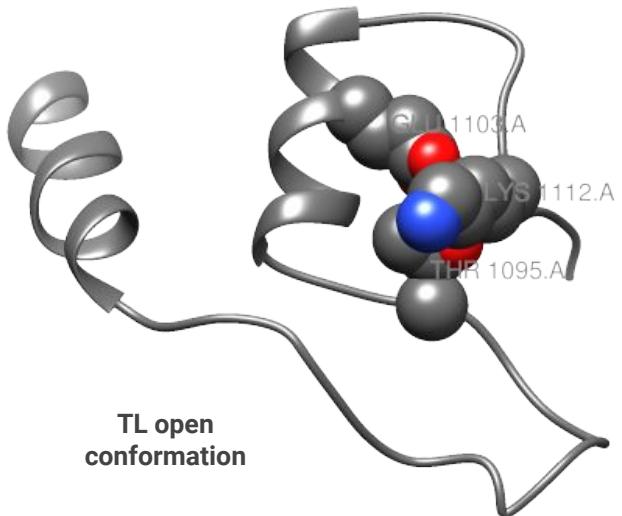
## CATALYSIS: Active site



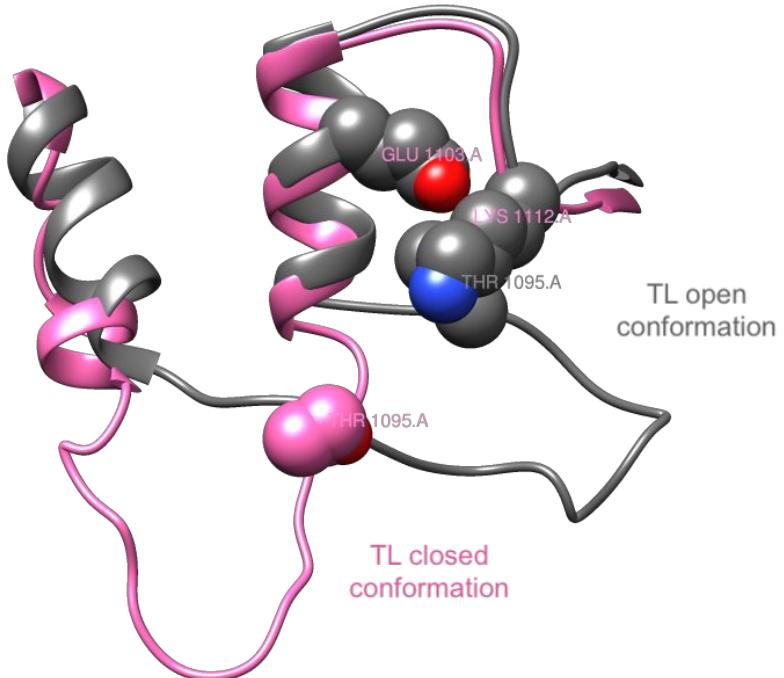
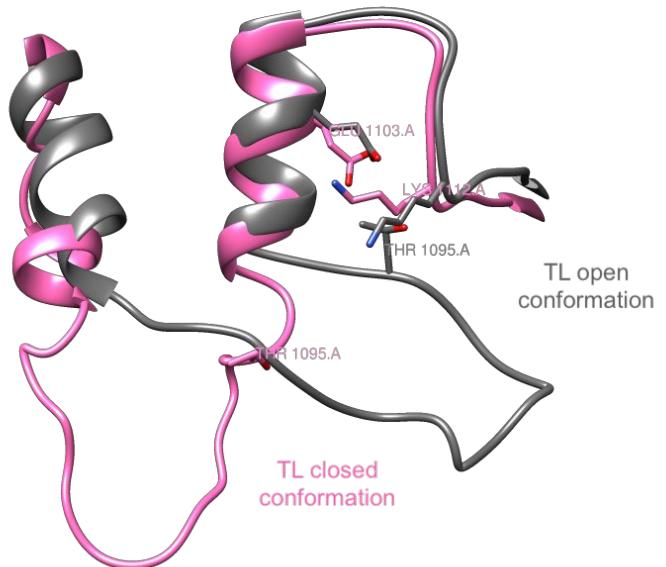
## CATALYSIS: Trigger loop (TL) mobility



# CATALYSIS: Trigger loop (TL) mobility



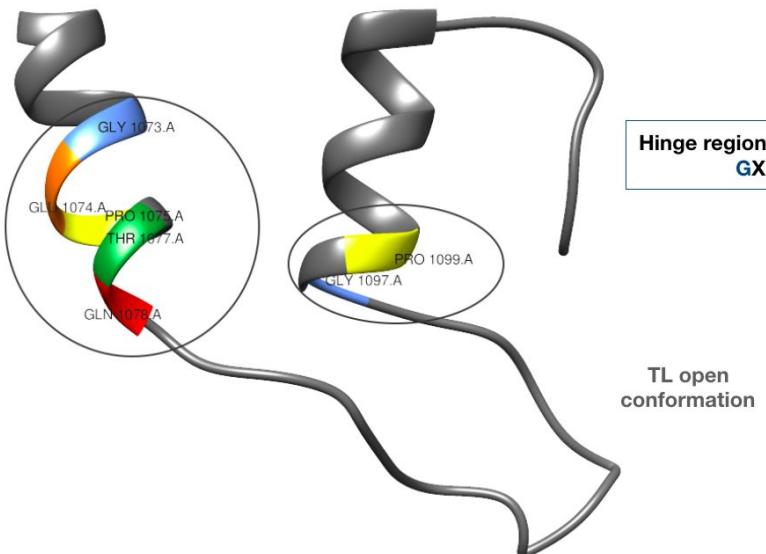
# CATALYSIS: TL mobility (superimposition)



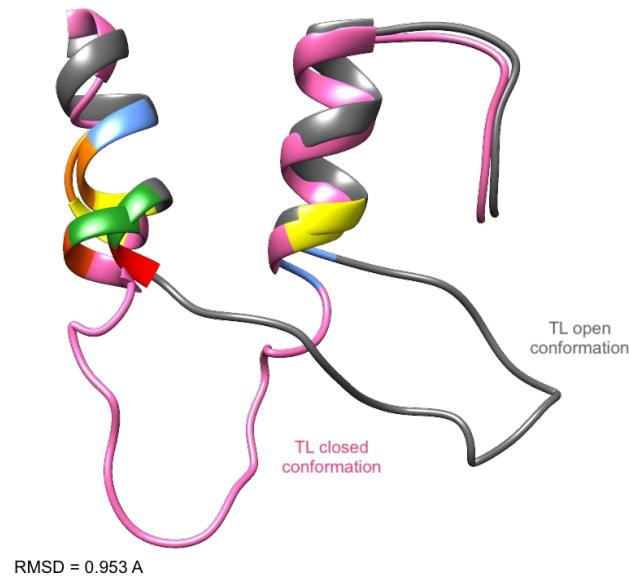
RMSD = 0.953 Å

# CATALYSIS: Hinge regions allow trigger loop mobility

Hinge region motif (H1):  
**GEPXTQ**



Hinge region motif (H2):  
**GXP**

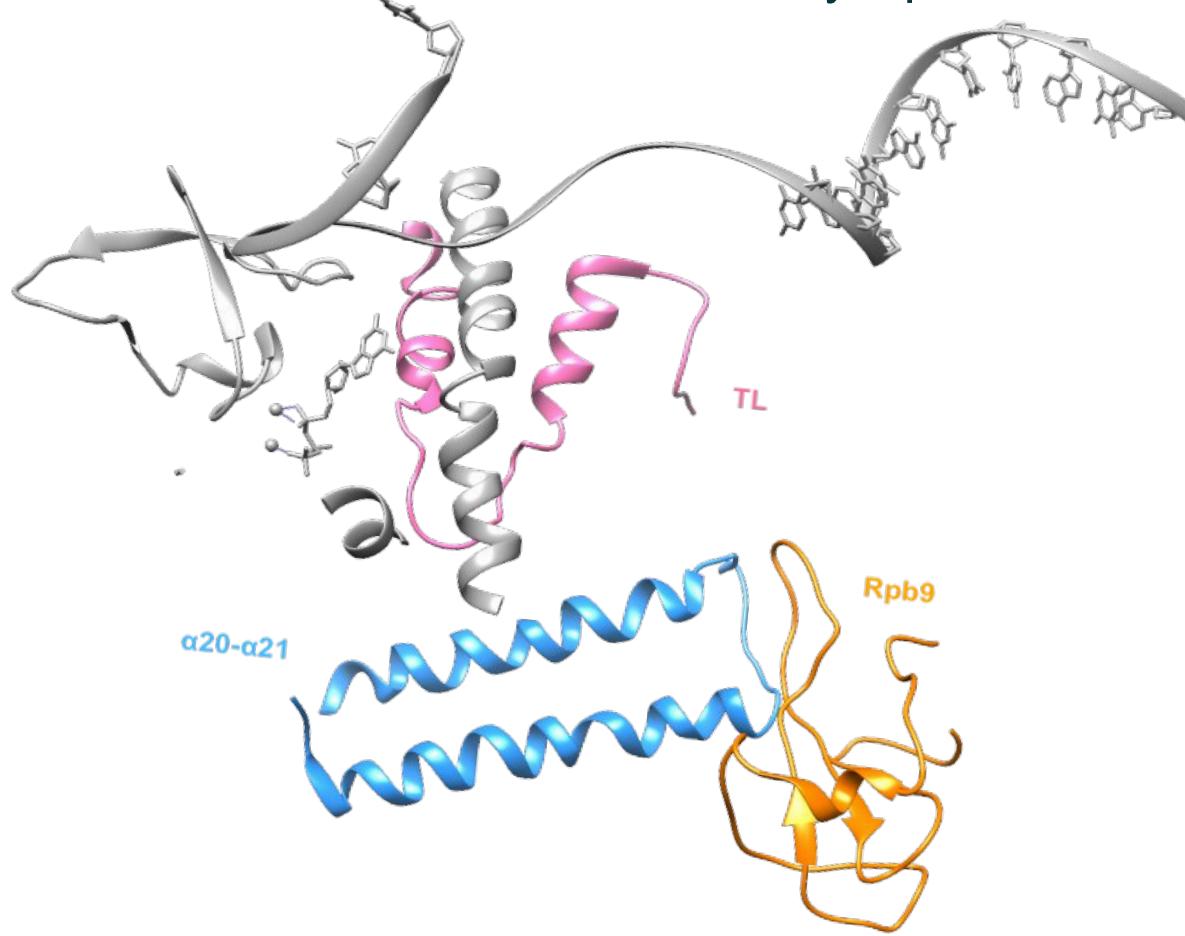


## CATALYSIS: residues involved in TL movement are conserved

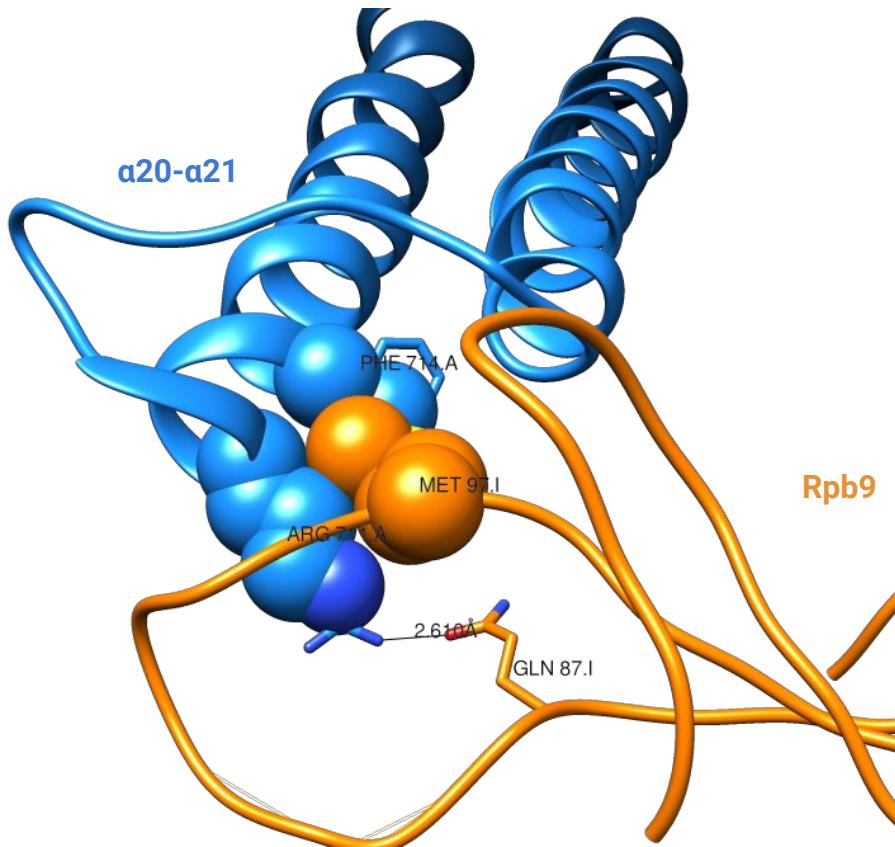
Rpb1 (TL)

	Hinge region (H1)	1095	Hinge region (H2)	1103	1112
S. POMBE	AAQSI <b>GEPATQ</b> MTLNTFHYAGVSSKNVTL		<b>GVR</b> RLKEI	LNVAKNI	KTPSLT
DROSOPHILA	AAQSL <b>GEPATQ</b> MTLNTFHAGVSSKNVTL		<b>GVR</b> RLKEI	IINISKKP	KAPSLT
MOUSE	AAQSL <b>GEPATQ</b> MTLNTFHYAGVSAKNVTL		<b>GVR</b> RLKEI	INISKKP	KTPSLT
HUMAN	AAQSL <b>GEPATQ</b> MTLNTFHYAGVSAKNVTL		<b>GVR</b> RLKEI	INISKKP	KTPSLT
C. ELEGAN	AAQSL <b>GEPATQ</b> MTLNTFHYAGVSAKNVTL		<b>GVR</b> RLKEI	IINVSKTL	KTPSLT
D. DISCOIDEUM	AAQSI <b>GEPATQ</b> MTLNTFHYAGVSSKNVTL		<b>GVR</b> RLKEI	IINIAKQV	KTPSLT
A. THALIANA	AAQSI <b>GEPATQ</b> MTLNTFHYAGVSAKNVTL		<b>GVR</b> RLREI	INVAKRI	KTPSLS
E. COLI	AAQSI <b>GEPGTQ</b> LTMR <sup>T</sup> FHIGGAASRAAAE		SSI	QVKNKGSIKLSNV	KSVVN
S. CEREVISIAE	AAQSI <b>GEPATQ</b> MTLNTFHAGVASKKVTS		<b>GVR</b> RLKEI	LNVAKNM	KTPSLT
#=GC RF	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX		XXXXXX	XXXXXX	XXXXXX

## CATALYSIS: Stabilization of the TL movement by Rpb9 and $\alpha$ 20- $\alpha$ 21



## CATALYSIS: Rpb9 stabilizes $\alpha$ 20 and $\alpha$ 21 from Rpb1



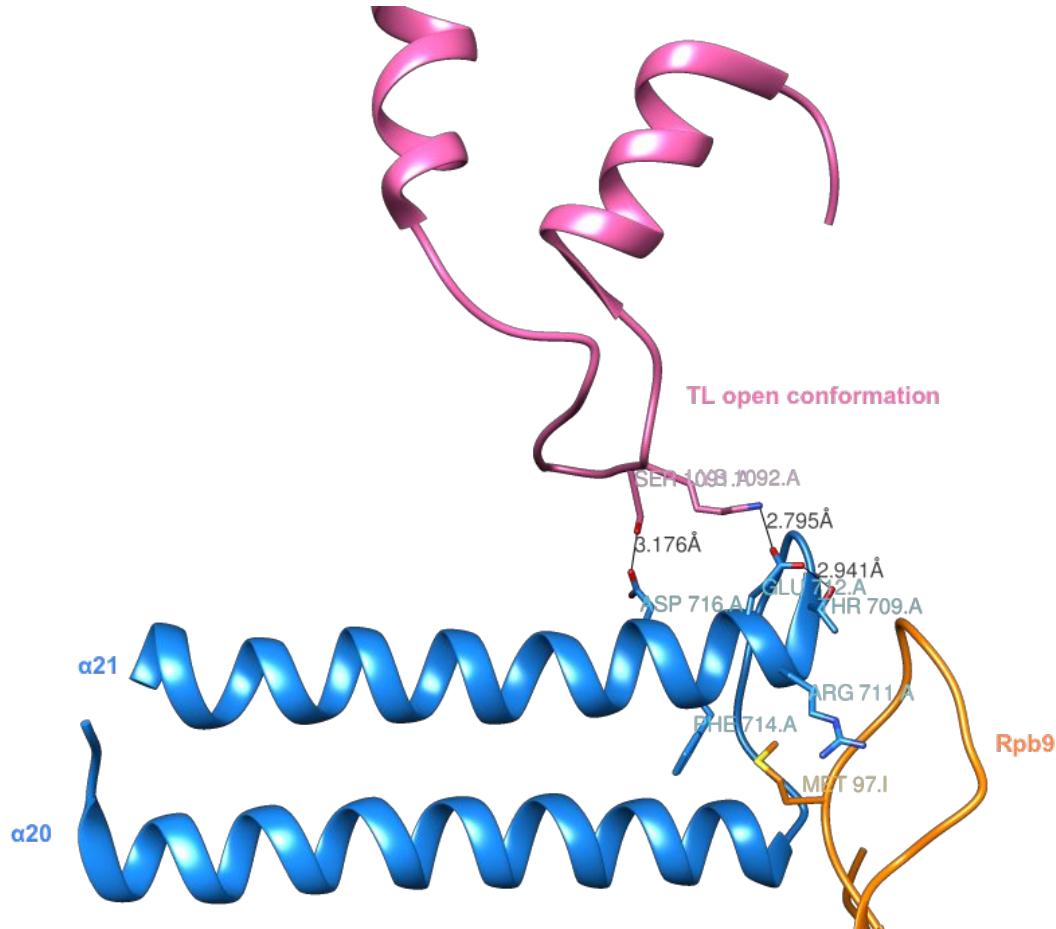
Rpb9

HUMAN	KITHEVDELTQIIADVSQDPTLPRTEDHPCQKCGHKEAVFFQSHSARAEDAMRLYYVCTA
PIG	KITHEVDELTQIIADVSQDPTLPRTEDHPCQKCGHKEAVFFQSHSARAEDAMRLYYVCTA
MOUSE	KITHEVDELTQIIADVSQDPTLPRTEDHPCQKCGHKEAVFFQSHSARAEDAMRLYYVCTA
BOVIN	KITHEVDELTQIIADVSQDPTLPRTEDHPCQKCGHKEAVFFQSHSARAEDAMRLYYVCTA
DROSOPHILA	KIMHEIDELTHIVPDVISDPTLPRTEDHACPKCSHREAVFFQAQTRRAEEEAMRLYYVCTN
S. POMBE	ELQSSNVENTTVSHDASTDPTLPR-SDKECPRCHQHEAVFYQTHSRRGDTMMTLIYVCVH
S. CEREVISIAE	ELITNIGETAGVQDIGSDPTLPR-SDRECPKCHSRENVFFQSQQRRKDTSMLVFFVCLS

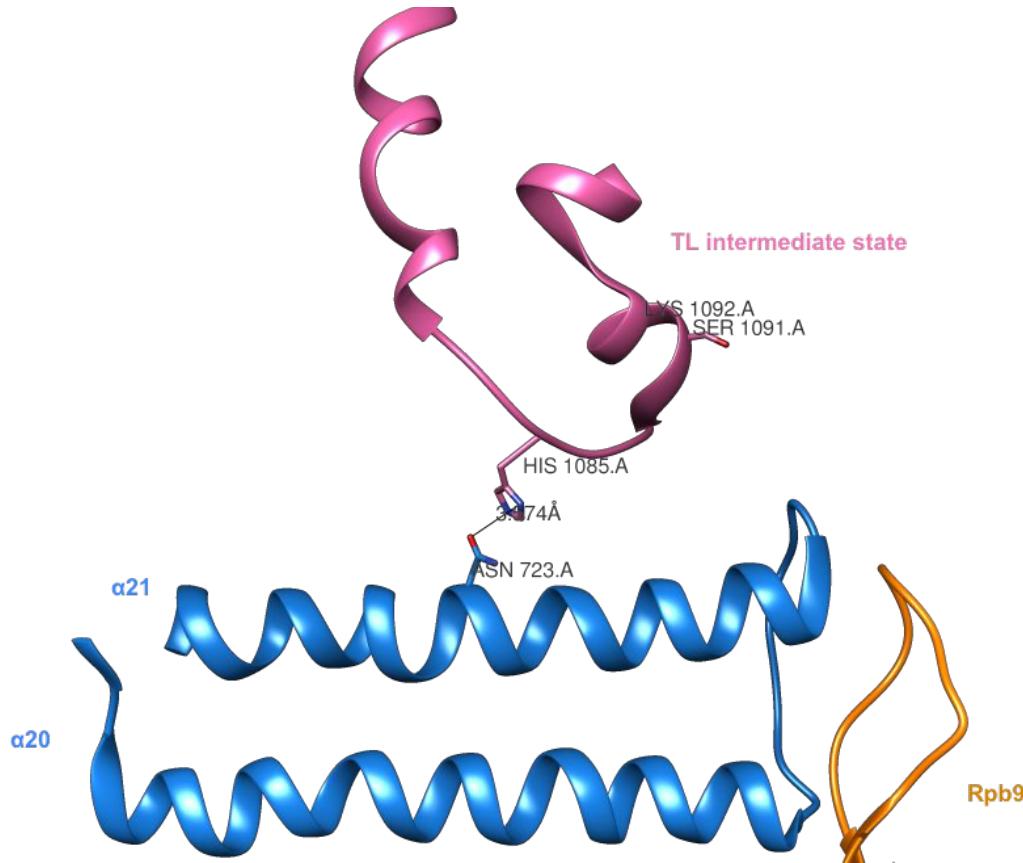
## Rpb1

S.POMBE	evtrtvkearrqvaecIQDAQH-NRLKPEPGMTLRESFEAKVSRILNQAR
D.DISCOIDEUM	kvltissaknqvkelIIKAQN-KQFECQPGKSVIETFEQKVNVQVLNKAR
C.ELEGANS	dinqtirkakqdvvdvIEKAHN-DDLEPTPGNTLRQTFENKVNQILNDAR
MOUSE	dinqtikkakqdvievIEKAHN-NELEPTPGNTLRQTFENQVNRLNDAR
HUMAN	dinqtikkakqdvievIEKAHN-NELEPTPGNTLRQTFENQVNRLNDAR
DROSOPHILA	eiqqaikkakddvinvIQKAHN-MELEPTPGNTLRQTFENKVNRLNDAR
ARCHEA	rieahlrnaearvdqlIEAYEN-GELEPLPGRSLEETLEMKIMQVLGEAR
S.CEREVISIAE	eitetiaeakkvldvTKEAQA-NLLTAKHGMTLRESFEDNVVRFLNEAR
#=GC RF	.....XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

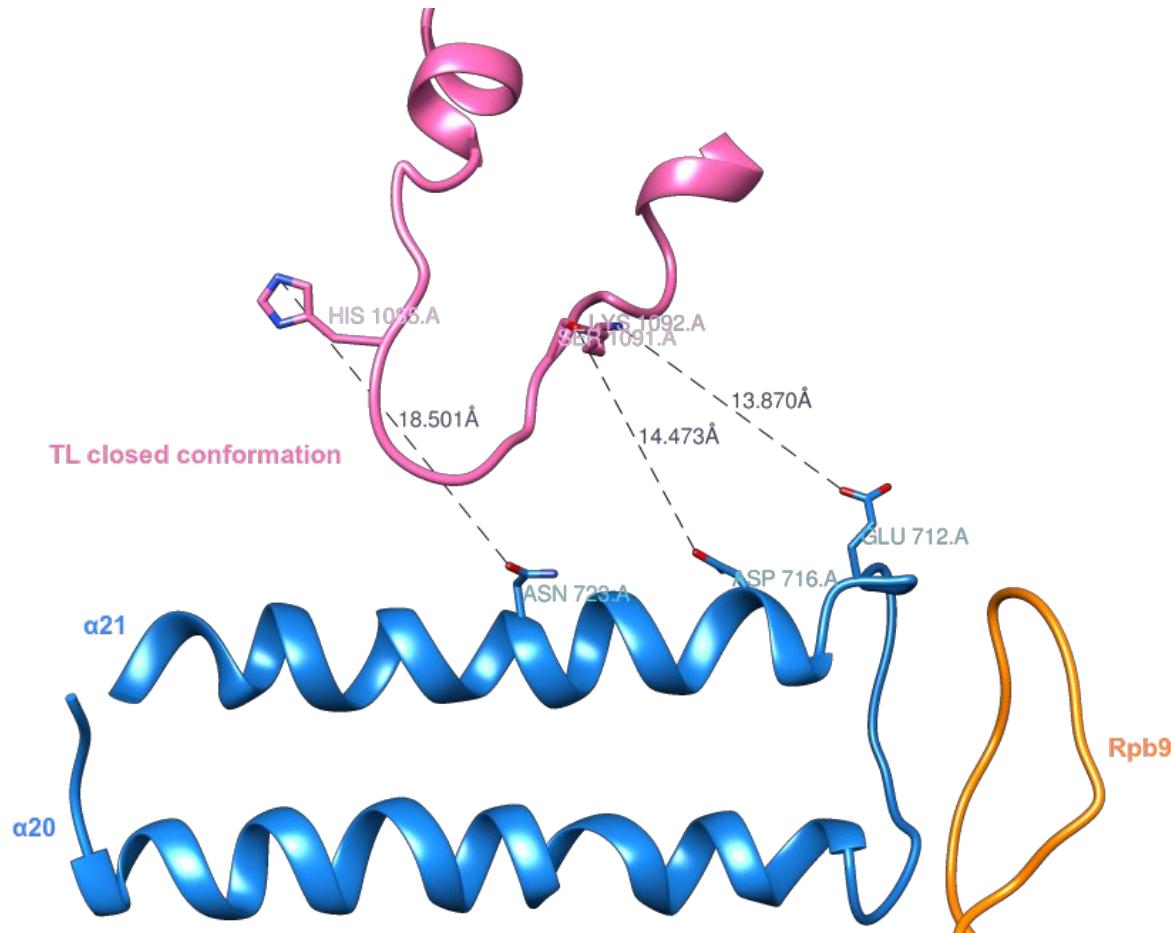
## CATALYSIS: $\alpha$ 20 and $\alpha$ 21 stabilize TL movement



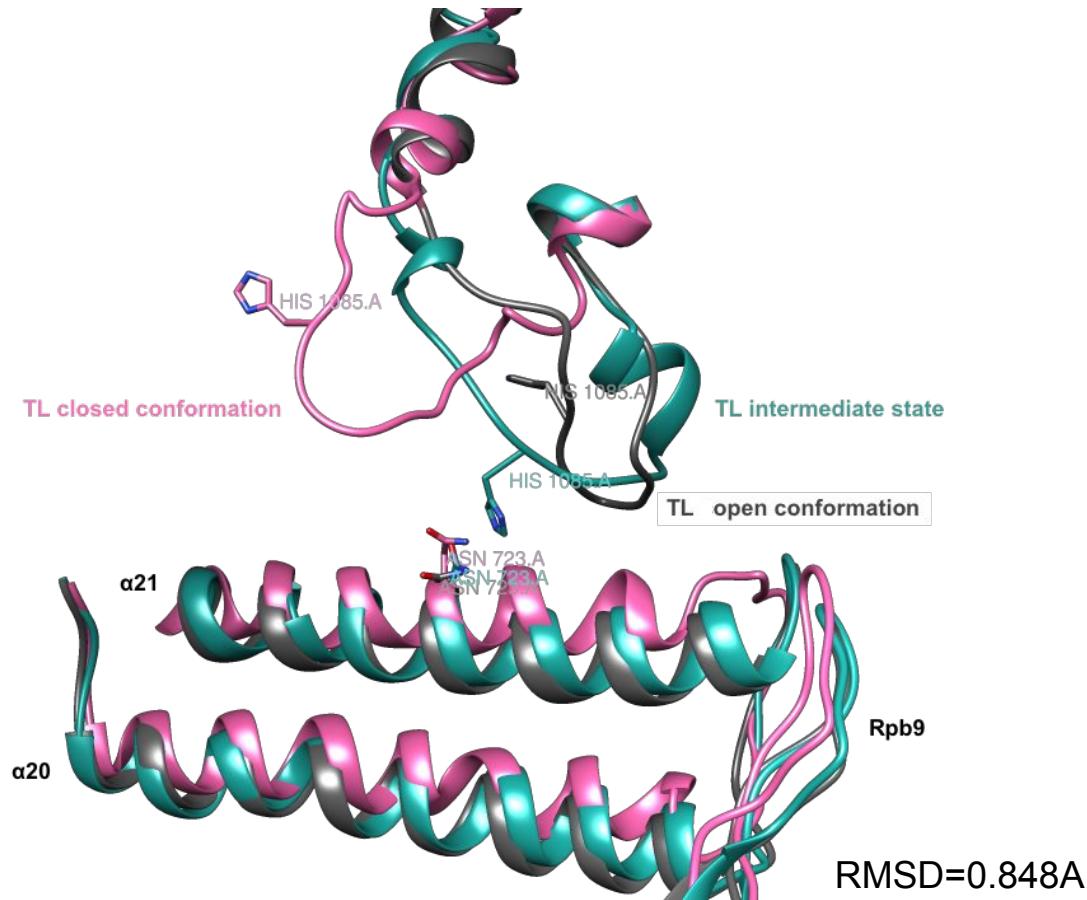
## CATALYSIS: $\alpha$ 20 and $\alpha$ 21 stabilize TL movement



## CATALYSIS: $\alpha$ 20 and $\alpha$ 21 stabilize TL movement



## CATALYSIS: $\alpha$ 20 and $\alpha$ 21 stabilize TL movement (superimposition)



# CATALYSIS: conservation of residues that stabilize TL movement

## Rpb1 (TL)

		1085	1091	1092	
S. POMBE	AAQSIGEPATQMTLNTF	HY	AGVSSK	NVTLGVPR	
DROSOPHILA	AAQSLGEPATQMTLNTF	HF	AGVSSK	NVTLGVPR	
MOUSE	AAQSLGEPATQMTLNTF	HY	AGVSAK	NVTLGVPR	
HUMAN	AAQSLGEPATQMTLNTF	HY	AGVSAK	NVTLGVPR	
C. ELEGAN	AAQSLGEPATQMTLNTF	HY	AGVSAK	NVTLGVPR	
D. DISCOIDEUM	AAQSIGEPA	TQMTLNTF	HY	AGVSAK	NVTLGVPR
A. THALIANA	AAQSIGEPA	TQMTLNTF	HY	AGVSAK	NVTLGVPR
E. COLI	AAQSIGEPA	TQMTLNTF	HY	AGVSAK	NVTLGVPR
S. CEREVISIAE	AAQSIGEPA	TQMTLNTF	HF	AGVASK	KVTSGVPR
#=GC RF	XXXXXXXXXXXXXX	XXXXXXXXXXXXXX	XXXXXXXXXXXXXX	XXXXXXXXXXXXXX	XXXXXXXXXXXXXX

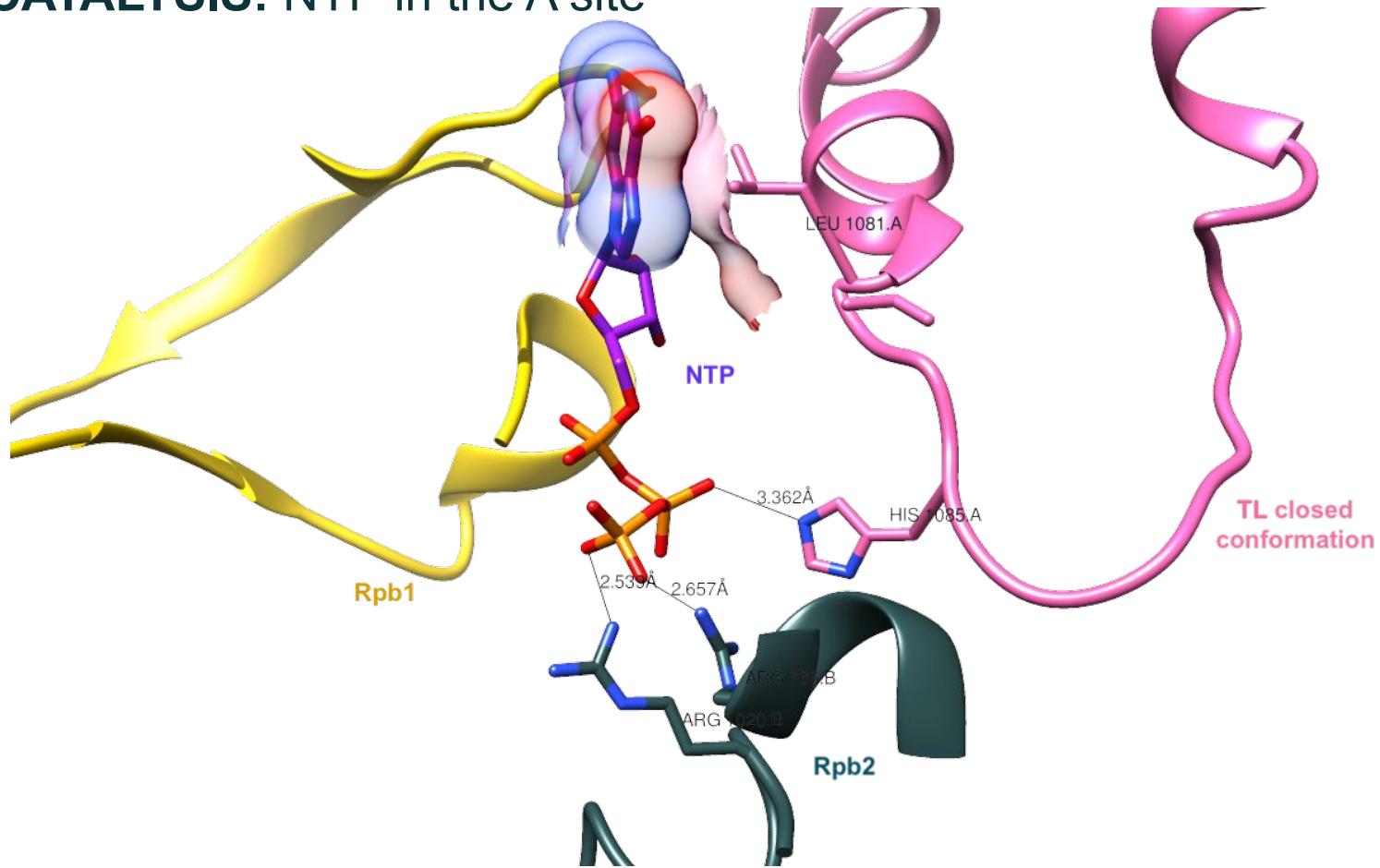
## Rpb1 (α20-α21)

		712	716	723
S. POMBE	evtrtvkearrqvaec	I	Q	D
D. DISCOIDEUM	I	Q	D	A
C. ELEGANS	K	V	T	I
MOUSE	T	I	S	S
HUMAN	D	I	S	S
DROSOPHILA	I	Q	A	K
ARCHEA	Q	A	K	K
S. CEREVISIAE	A	K	K	V
#=GC RF	.....	XXXXXXXXXXXXXX	XXXXXXXXXXXXXX	XXXXXXXXXXXXXX

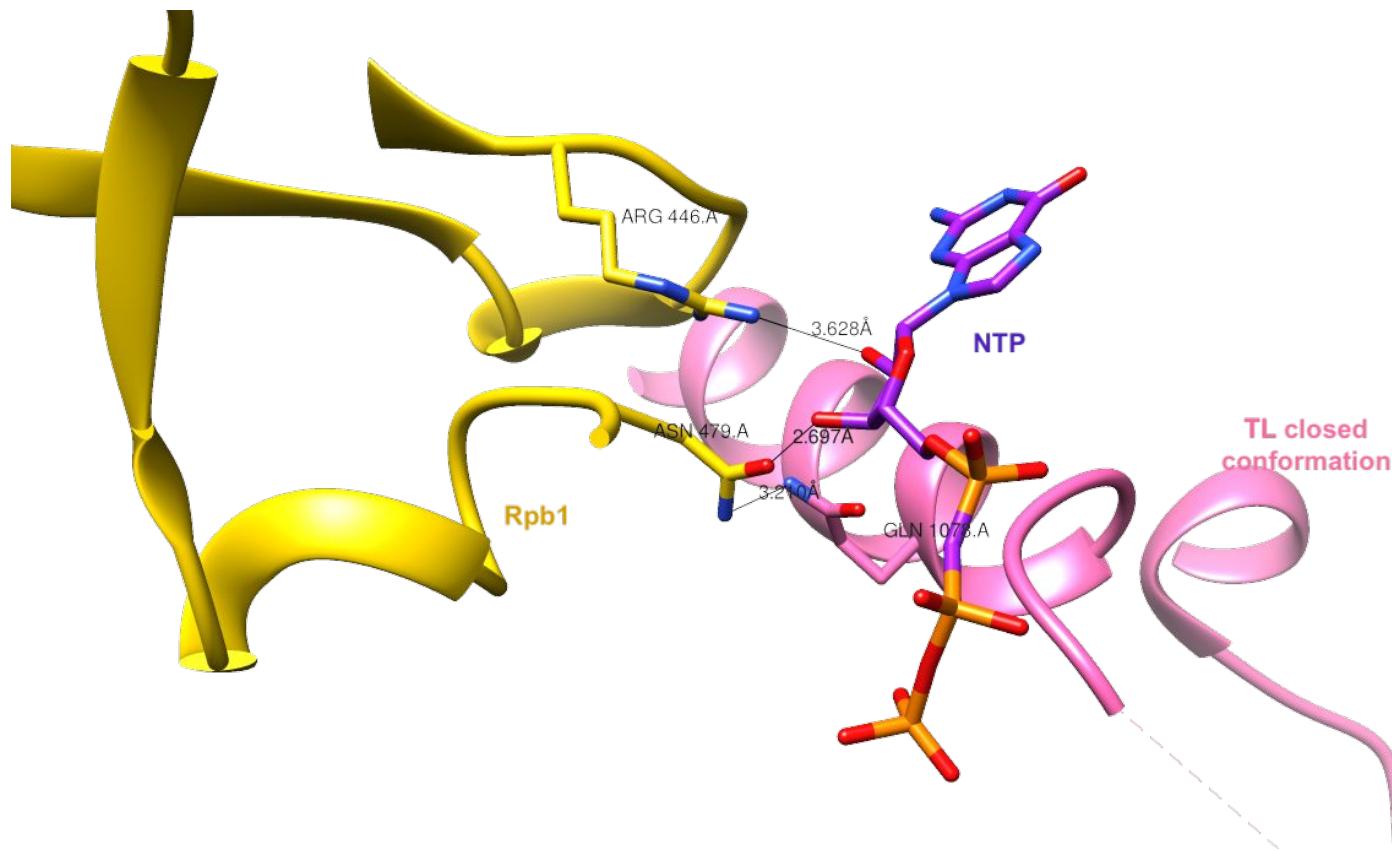
## CATALYSIS: NTP in the A site



## CATALYSIS: NTP in the A site



## CATALYSIS: NTP in the A site



# CATALYSIS: Residues that contact the NTP are conserved

Rpb1 (TL)

1078 1081 1085

S. POMBE	AAQSIGEPATQMTLNTFHAGVSSKNVTLGVPRLKEILNVAKNIKTPSLT
DROSOPHILA	AAQSLGEPATQMTLNTFHAGVSSKNVTLGVPRLKEIINISKPKAPS LT
MOUSE	AAQSLGEPATQMTLNTFHAGVSAKNVTLGVPRLKELINISKPKTPSLT
HUMAN	AAQSLGEPATQMTLNTFHAGVSAKNVTLGVPRLKELINISKPKTPSLT
C. ELEGAN	AAQSLGEPATQMTLNTFHAGVSAKNVTLGVPRLKEIINVSCTLKTPSLT
D. DISCOIDEUM	AAQSIGEPATQMTLNTFHAGVSSKNVTLGVPRLKEIINIAKQVKTPSLT
A. THALIANA	AAQSIGEPATQMTLNTFHAGVSAKNVTLGVPRLREIINVAKRICKTPSL S
E. COLI	AAQSIGEPGTQLTMRTFHIGGAASRAAAESSIQVKNKGSIKLSNVKSVVN
S. CEREVISIAE	AAQSIGEPATQMTLNTFHAGVASKKVTSGVPRLKEILNVAKNMKTPSLT
#=GC RF	XX

Rpb1

446

Rpb1

479

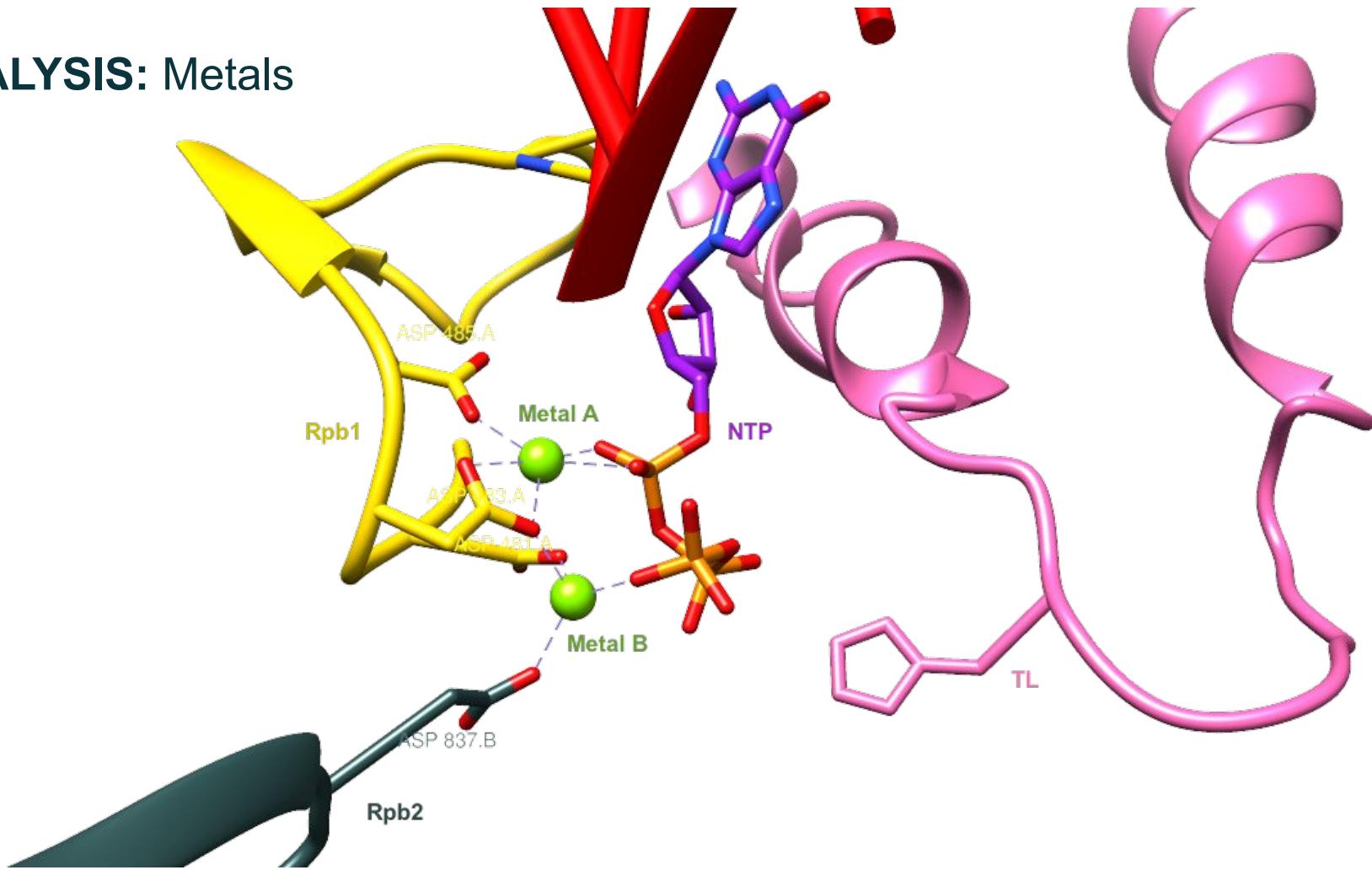
S. POMBE	----RVERHIRDGDVVIFNRQPS	S. POMBE	SPYNAFDGDEMNMH'
D. DISCOIDEUM	----KVERHINDGDVVFIFNRQPS	D. DISCOIDEUM	SPYNAFDGDEMNLH'
C. ELEGANS	----RVERHMKDGDIVIFNRQPT	C. ELEGANS	SPYNAFDGDEMNLH
MOUSE	----KVERHMCMDGDIVIFNRQPT	MOUSE	TPYNAFDGDEMNLH
HUMAN	----KVERHMCMDGDIVIFNRQPT	HUMAN	TPYNAFDGDEMNLH
DROSOPHILA	----KVERHLRDDDVFIFNRQPT	DROSOPHILA	SPYNAFDGDEMNLH'
A. THALIANA	----KVERHLQDGDFVLFLNRFQPS	A. THALIANA	SPYNAFDGDEMNMH'
E. COLI	----DILDEVIREHPVLLNRAPT	E. COLI	AAYNAFDGDDQMAVH'
S. CEREVISIAE	----KVERHIMDNDPVLFNRFQPS	S. CEREVISIAE	SPYNAFDGDEMNLH'
#=GC RF	XXXXXXXXXXXXXXXXXXXX	#=GC RF	XXXXXXXXXXXXXX

**CATALYSIS:** Residues that contact the NTP are conserved

Rpb2	766
CANDIDA_YEAST_	HH-----ATTFTHCEIHPNSMILGVAASIIPFPDHNQSPRNTYQSAMGKQA
S.POMBE	PH-----VHAWTHCEIHPAMILGILASIIPFPDHNQSPRNTYQSAMGKQA
HUMAN	AY-----CSTYTHCEIHPNSMILGVCASIIPFPDHNQSPRNTYQSAMGKQA
D.DISCOIDEUM	QI-----VHTYTHCEIHPNSMILGICCSIIIPFPDHNQSPRNTYQAAMGKQA
C.ELEGANS	GY-----CDTHTHCEIHPAMILGVCASIIPFPDHNQSPRNTYQSAMGKQA
DROSOPHILA	AY-----CTTYTHCEIHPAMILGVCASIIPFPDHNQSPRNTYQSAMGKQA
E.COLI	GESSLFSRDQVDYMDVSTQQVVSVGASLIPFLEHDDANRALMGANMQRQA
S.CEREVISIAE	HH-----ATTFTHCEIHPNSMILGVAASIIPFPDHNQSPRNTYQSAMGKQA

Rpb2	1020
DROSOPHILA	LGPTYYQRLKHMVDDKIHSRARGPVQILVRQPMEGRARDGGLRFGEMERD
MOUSE	IGPTYYQRLKHMVDDKIHSRARGPIQILNRQPMEGRSRDGGLRFGEMERD
HUMAN	IGPTYYQRLKHMVDDKIHSRARGPIQILNRQPMEGRSRDGGLRFGEMERD
C. ELEGANS	FGPTYYQRLKHMVDDKIHSRARGPIQMMNRQPMEGRARDGGLRFGEMERD
S. POMBE	LGPTYYQRLKHLVDDKIHARARGPVQILTRQPVEGRSRDGGLRFGEMERD
A. THALIANA	LGPTYYQRLKHMVDDKIHSRGRGPVQILTRQPAEGRSRDGGLRFGEMERD
E. COLI	VGYMYMLKLNHLVDDKMHARSTGSYSLVTQQPLGGKAQFGGQRFGEMEVW
D. DISCOIDEUM	IGPTYYQRLKHMVDDKIHSRSRGPVQILTRQPVEGRSRDGGLRFGEMERD
S. CEREVISIAE	FGPTYYQRLRHMVDDKIHARARGPMQLTRQPVEGRSRDGGLRFGEMERD

## CATALYSIS: Metals



## CATALYSIS: Residues that contact Mg<sup>2+</sup> are conserved

Rpb1  
S.POMBE  
D.DISCOIDEUM  
C.ELEGANS  
MOUSE  
HUMAN  
DROSOPHILA  
A.THALIANA  
E.COLI  
S.CEREVIAE  
#=GC RF

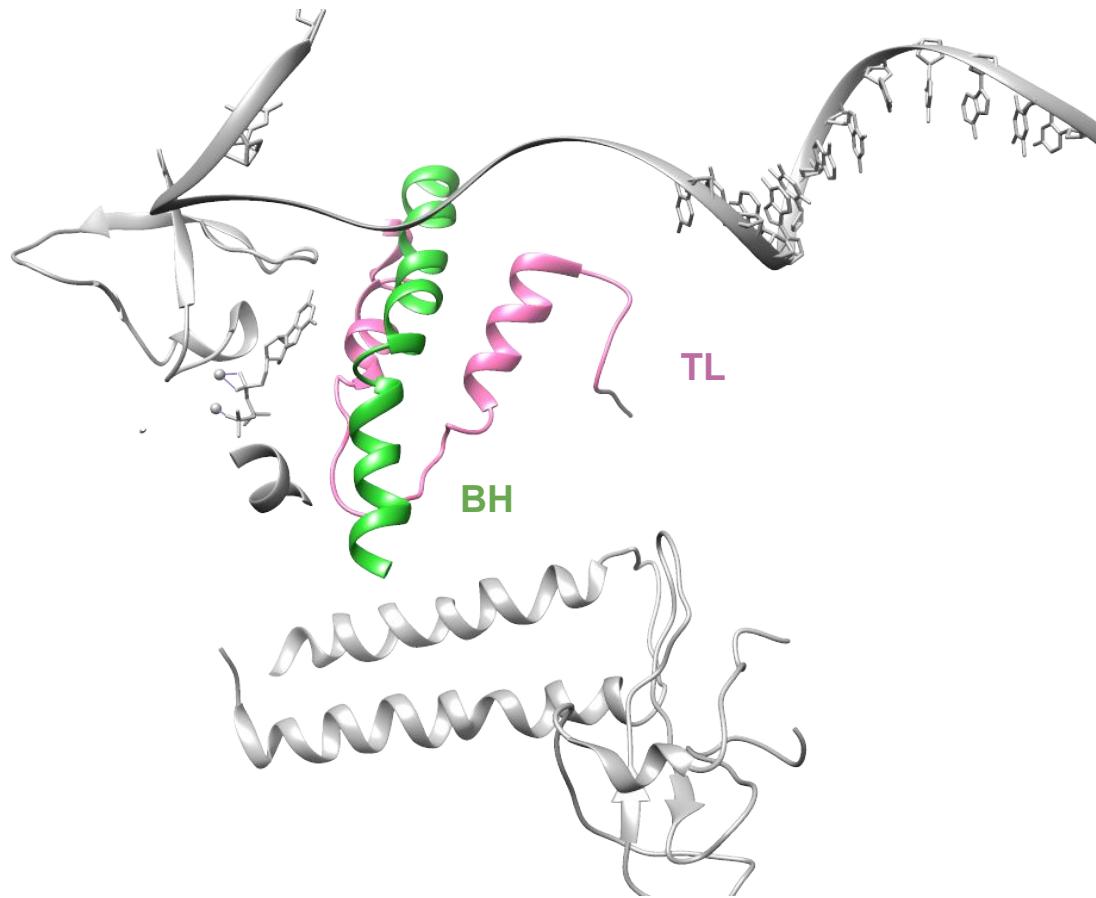
481 483 485  
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SPYNADFDGDEMNLHVPQTLETRAEVIEIMMPrqivspqsnrpvmgivq  
SPYNADFDGDEMNLHLPQSLETTRAEEIIAMPrqlitpqankpvmgivq  
TPYNADFDGDEMNLHLPQSLETTRAEIQEELAMVprmivtpqsnrpvmgivq  
TPYNADFDGDEMNLHLPQSLETTRAEIQEELAMVprmivtpqsnrpvmgivq  
SPYNADFDGDEMNLHVPQSMETRAEVENIHTprqiitpqankpvmgivq  
SPYNADFDGDEMNMHVPQSFETRAEVLELMMVpkcivspqanrpvmgivq  
AAYNAFDGQMAHVPLTLEAQLEARALMMStnnilspangeipiivpsq  
SPYNADFDGDEMNLHVPQSEETRAELSQLCAVplqivspqsnkpcmgivq  
xxxxxxxxxxxxxxxxxxxxxxxxxxxxx.....

Rpb2  
CANDIDA\_YEAST\_  
S.POMBE  
HUMAN  
D.DISCOIDEUM  
C.ELEGANS  
DROSOPHILA  
E.COLI  
S.CEREVIAE

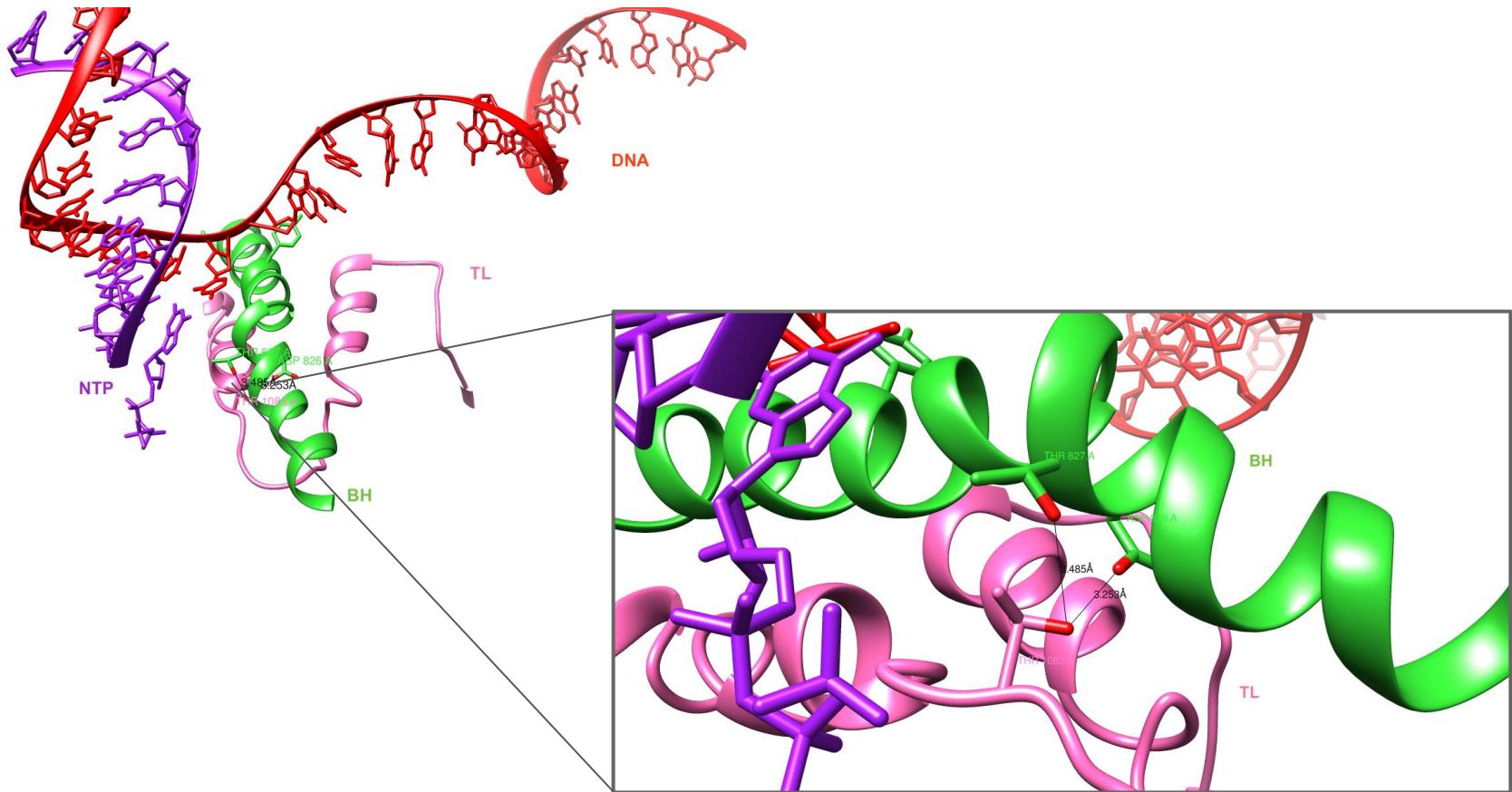
837  
-----AMEYLKFRELPAQNAIVAIACYSQYNQEDSMIMNQSSI  
-----SMEYLKFRELPAQNAIVAILCYSGYNQEDSIIMNQASI  
-----SMEYLRFRELPAQINSIVAIASYTQYNQEDSVIMNRSAV  
-----SMEYLFRELPAQNCVVAIACYSQYNQEDSVILNQSAI  
-----SMEYLRFNELPAGINAIVAILSYSQYNQEDSVIMNNSAI  
-----SMEYLRFRELPAQINSIVAILCYTQYNQEDSVILNASAV  
GEPVERGDVLADGPSTDLGELALGQNMRVAFMPWNGYNFEDSILVSERVV  
-----AMEYLKFRELPAQNAIVAIACYSQYNQEDSMIMNQSSI

: \*\*. \* \* \*\*: :.\*\*\* \*\*\*::: :

## TRANSLOCATION: key role of bridge helix (BH)



# TRANSLOCATION: BH and TL interaction



# TRANSLOCATION: conservation of residues involved in BH and TL interaction

## Rpb1 (TL)

S. POMBE  
DROSOPHILA  
MOUSE  
HUMAN  
C. ELEGAN  
D. DISCOIDEUM  
A. THALIANA  
E. COLI  
S. CEREVISIAE  
#=GC RF

1083

AAQSIGEPATQMTLNTFHYAGVSSKNVTLGVPRLKEILNVAKNIKTPSLT  
AAQSLGEPATQMTLNTFHFAGVSSKNVTLGVPRLKEIINISKKPKAPS LT  
AAQSLGEPATQMTLNTFHYAGVSAKNVTLGVPRLKELINISKKPKTPSLT  
AAQSLGEPATQMTLNTFHYAGVSAKNVTLGVPRLKELINISKKPKTPSLT  
AAQSLGEPATQMTLNTFHYAGVSAKNVTLGVPRLKEIINVSKTLKTPSLT  
AAQSIGEPATQMTLNTFHYAGVSSKNVTLGVPRLKEIINIAKQVKTPSLT  
AAQSIGEPATQMTLNTFHYAGVSAKNVTLGVPRLREIINVAKRIKTPSL S  
AAQSIGEPGTQLTMRTFHIGGAASRAAAESSIQVKNKGSIKLSNVKSVVN  
AAQSIGEPATQMTLNTFHFAGVASKVVTSGVPRLKEILNVAKNMKTPSLT  
XX

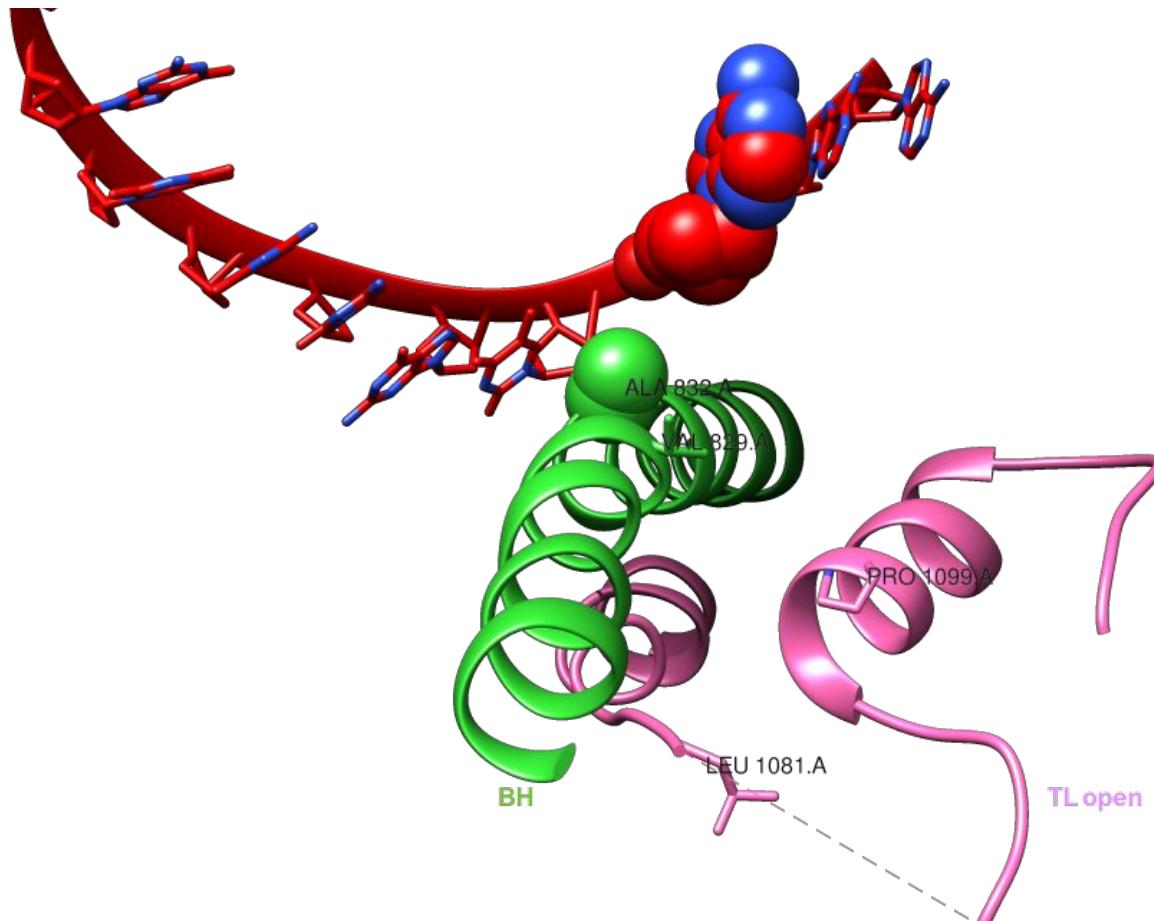
## BH (Rpb1)

S. POMBE  
DROSOPHILA  
MOUSE  
HUMAN  
C. ELEGAN  
D. DISCOIDEUM  
A. THALIANA  
E. COLI  
S. CEREVISIAE  
#=GC RF

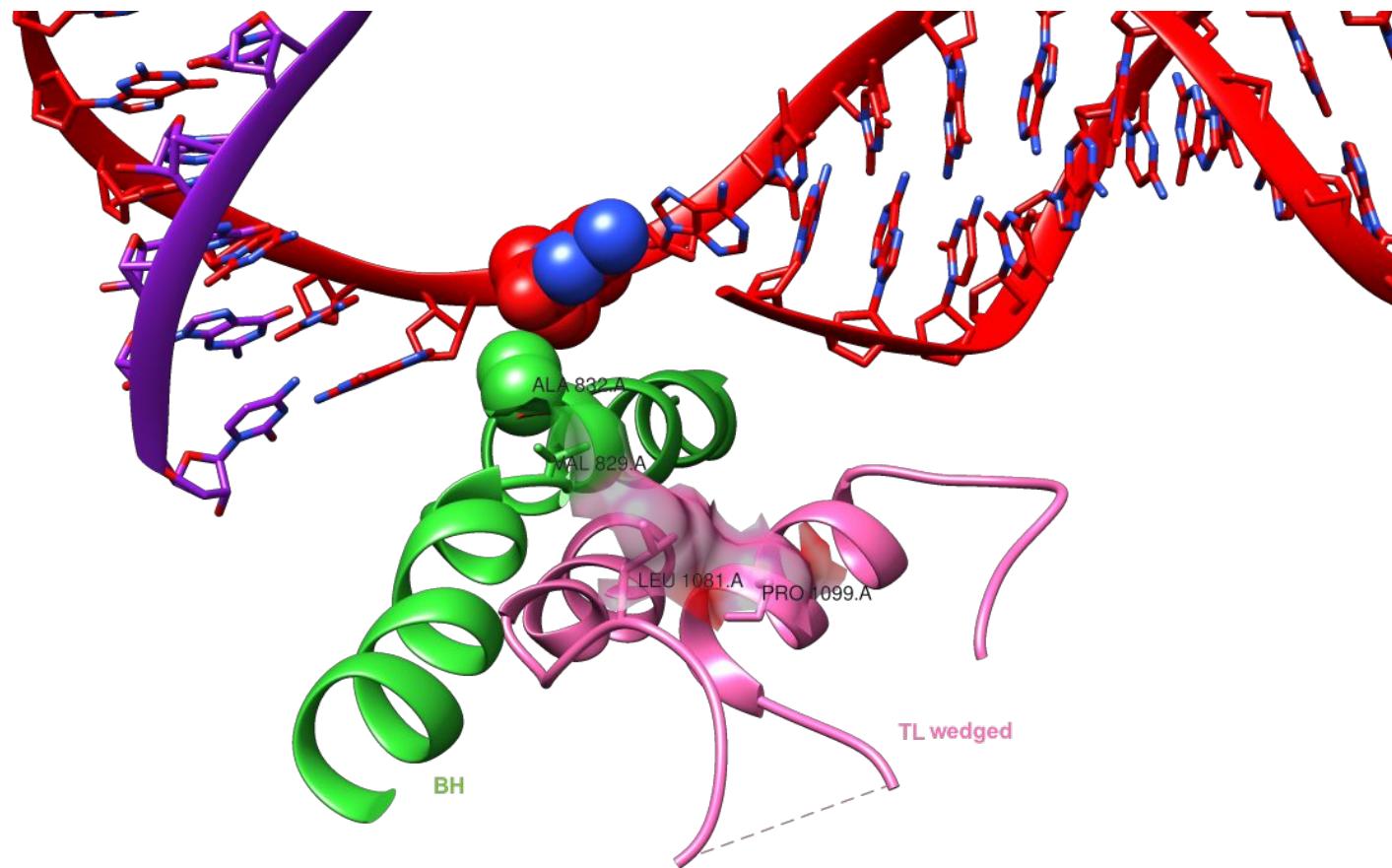
826-827

gfkrytlphfpkddspesrgfiensylrGLTPQEFFFHAMAGREGLIDT  
gfrkrtlphfikddygpesrgfvensylaGLTPSEFYFHAMGGREGLIDT  
gfkhrtlphfikddygpesrgfvensylaGLTPTEFFFHAMGGREGLIDT  
gfkhrtlphfikddygpesrgfvensylaGLTPTEFFFHAMGGREGLIDT  
gfrhrtlphfikddygpeskgfvensylaGLTPSEFFFHAMGGREGLIDT  
gfqsrtlphftkddygpesrgfvensylrGLTPQEFFFHAMGGREGLIDT  
gfdgrtlphftkddygpesrgfvensylrGLTPQEFFFHAMGGREGLIDT  
irqlagmrglma kpdgsi etpit anfre GLNVLQYFISTHGARKGLADT  
.....---PQEFFFHAMGGREGLIDT  
.....XXXXXXXXXXXXXXXXXXXX

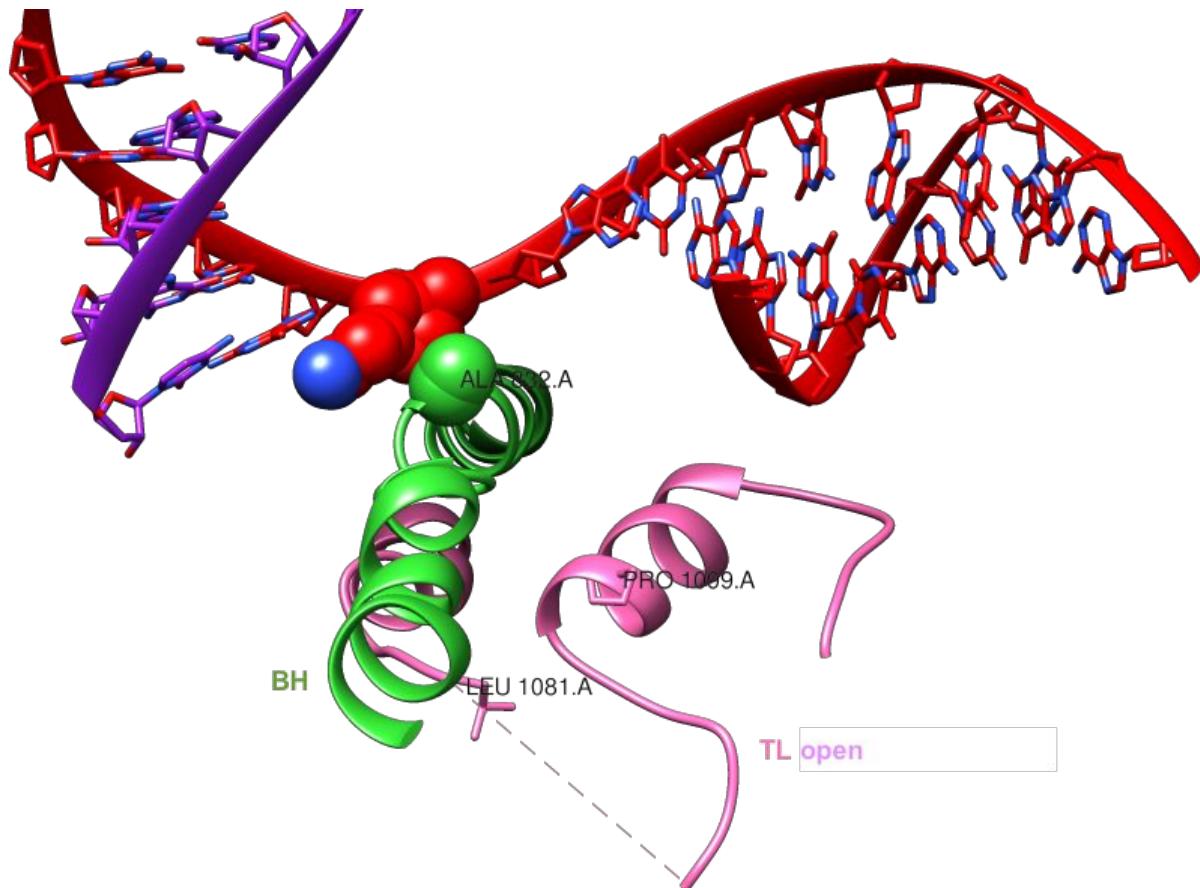
## TRANSLOCATION: Pre-translocation



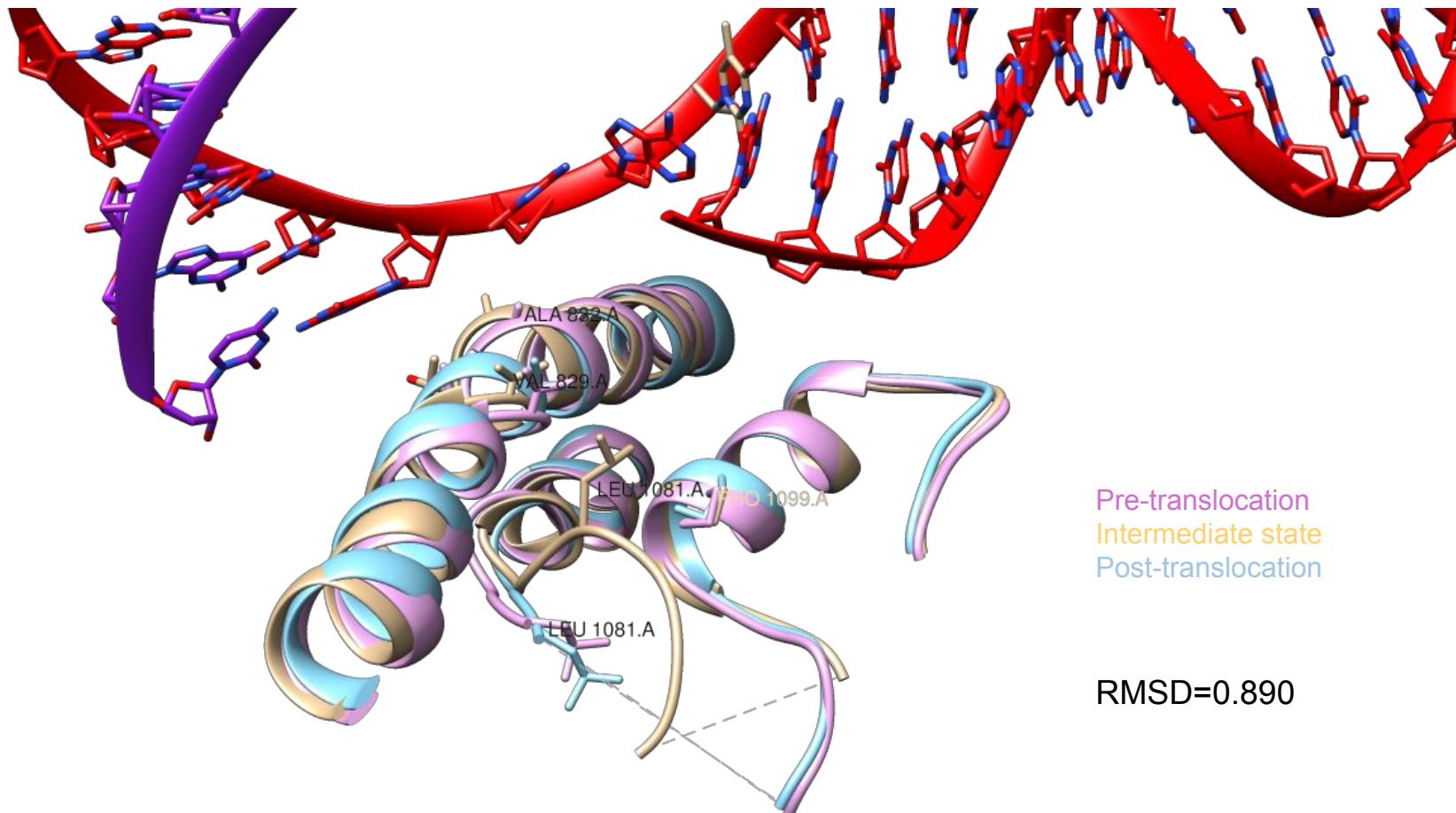
## TRANSLOCATION: Intermediate state



# TRANSLOCATION: Post-translocation



## TRANSLOCATION: Superimposition of the three states



# TRANSLOCATION: conservation of key residues

## BH (Rpb1)

S. POMBE  
DROSOPHILA  
MOUSE  
HUMAN  
C. ELEGAN  
D. DISCOIDEUM  
A. THALIANA  
E. COLI  
S. CEREVISIAE  
#=GC RF

829 832

GLTPQEFFFHAMAGREGLIDTAVKTAETGYIQRRLVKAMEDVMVRYDGTVRNAMGDI  
GLTPSEFYFHAMGGREGLIDTAVKTAETGYIQRRRIKAMESVMVNYDGTVRNSVGQL  
GLTPTEFFFHAMGGREGLIDTAVKTAETGYIQRRRIKSMESVMVKYDATVRNSINQV  
GLTPTEFFFHAMGGREGLIDTAVKTAETGYIQRRRIKSMESVMVKYDATVRNSINQV  
GLTPSEFFFHAMGGREGLIDTAVKTAETGYIQRRRIKAMESVMVNYDGTVRNSLAQM  
GLTPQEFFFHAMGGREGLIDTAVKTSSETGYIQRRLVKAMEDVSIKYDATVRNSLGDV  
GLTPQEFFFHAMGGREGLIDTAVKTSETGYIQRRLVKAMEDIMVKYDGTVRNSLGDV  
GLNVLQYFISTHGARKGLADTALKTANSGYLTRRLVDVAQDLVTTEDDCGTHEGIMM  
---PQEFFFHAMGGREGLIDTAVKTAETGYIQRRLVKALEDIMVHYDNTTRNSLGNV  
XX

## Rpb1 (TL)

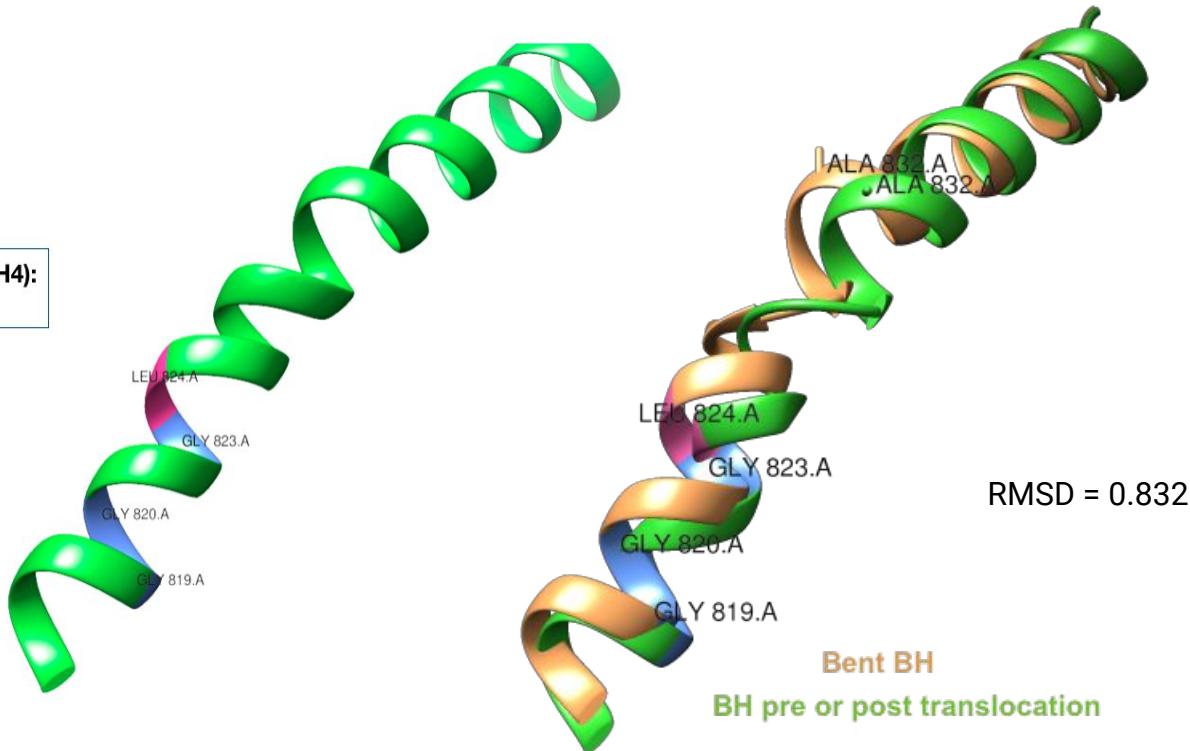
S. POMBE  
DROSOPHILA  
MOUSE  
HUMAN  
C. ELEGAN  
D. DISCOIDEUM  
A. THALIANA  
E. COLI  
S. CEREVISIAE  
#=GC RF

1081 1099

AAQSIGEPATQMTLNTFHYAGVSSKNVTLGVPRLKEILNVAKNIKTPSLT  
AAQSLGEPATQMTLNTFHAGVSSKNVTLGVPRLKEIINISKKPKAPSLT  
AAQSLGEPATQMTLNTFHYAGVSAKNVTLGVPRLKELINISKKPKTPSLT  
AAQSLGEPATQMTLNTFHYAGVSAKNVTLGVPRLKELINISKKPKTPSLT  
AAQSLGEPATQMTLNTFHYAGVSAKNVTLGVPRLKEIINVSKTLKTPSLT  
AAQSIGEPATQMTLNTFHYAGVSSKNVTLGVPRLKEIINIAKQVKTPSLT  
AAQSIGEPATQMTLNTFHYAGVSAKNVTLGVPRLREIINVAKRKTPSLS  
AAQSIGEPTQLMTRTFHIGGAASRAAAESSIQVKNKGSIKLSNVKSVN  
AAQSIGEPATQMTLNTFHAGVASKVTSVPRLKEILNVAKNMKTPSLT  
XX

# TRANSLOCATION: Hinge regions of BH

Hinge region motif (H3/H4):  
**GGXGL**



# TRANSLOCATION: Hinge regions of BH

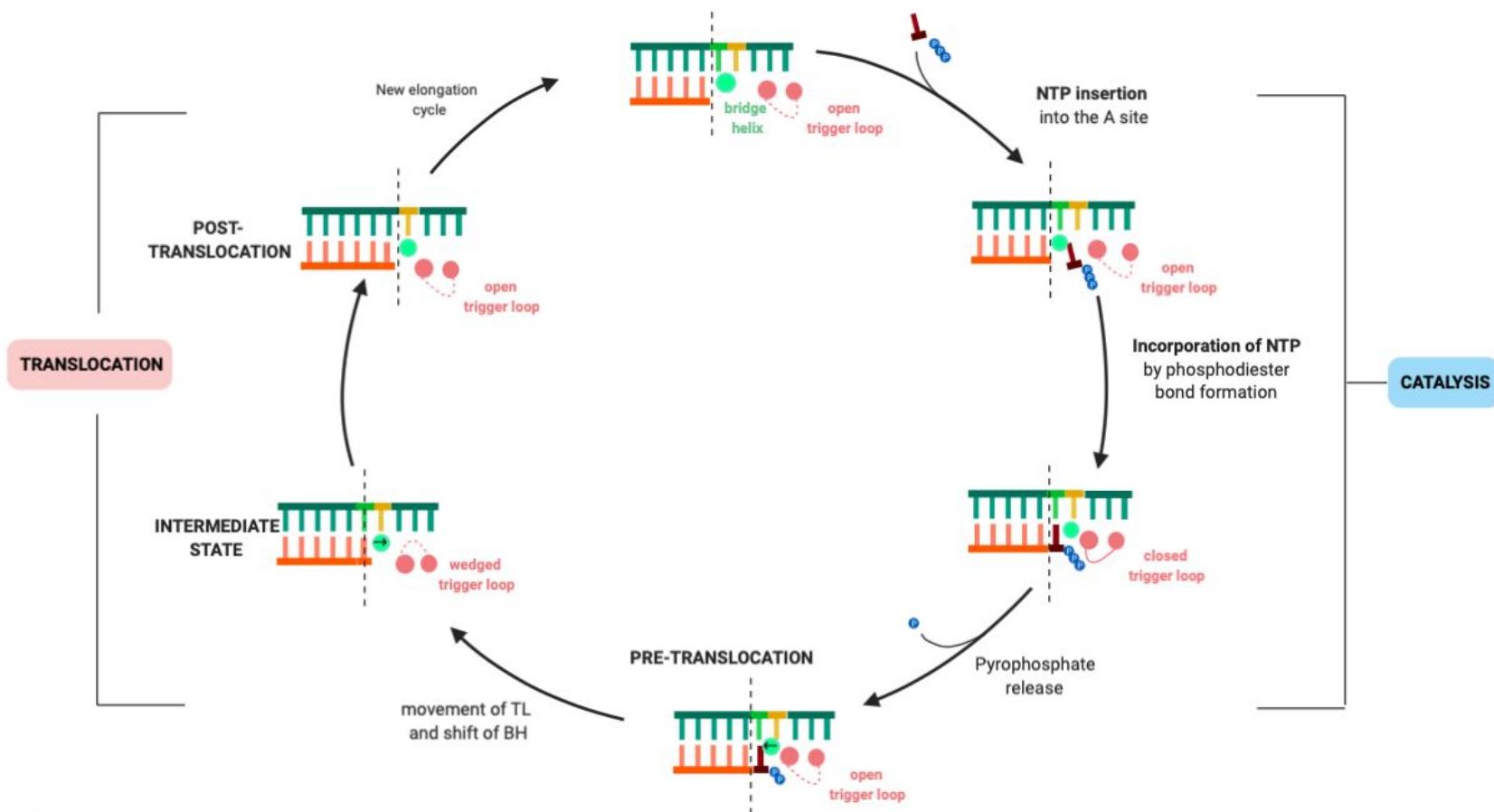
## BH (Rpb1)

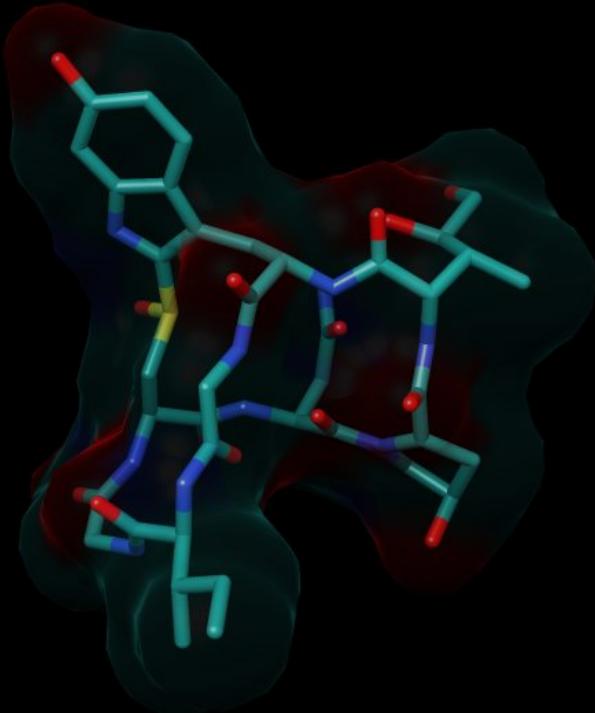
S. POMBE  
DROSOPHILA  
MOUSE  
HUMAN  
C. ELEGAN  
D. DISCOIDEUM  
A. THALIANA  
E. COLI  
S. CEREVISIAE  
#=GC RF

819-820 823-824

GLTPQEFFFHAMAGREGLIDTAVKAETGYIQRRLVKAMEDVMVRYDGTVRNAMGDI  
GLTPSEFYFHAMGGREGLIDTAVKAETGYIQRRLIKAMESVMVNYDGTVRNSVGQL  
GLTPTEFFFHAMGGREGLIDTAVKAETGYIQRRLIKSMESVMVKYDATVRNSINQV  
GLTPTEFFFHAMGGREGLIDTAVKAETGYIQRRLIKSMESVMVKYDATVRNSINQV  
GLTPSEFFFHAMGGREGLIDTAVKAETGYIQRRLIKAMESVMVNYDGTVRNSLAQM  
GLTPQEFFFHAMGGREGLIDTAVKTSETGYIQRRLVKAMEDVSIKYDATVRNSLGDV  
GLTPQEFFFHAMGGREGLIDTAVKTSETGYIQRRLVKAMEDIMVKYDGTVRNSLGDV  
GLNVLQYFISTHGARKGLADTALKTANSGLTRRLVDVAQDLVVTEDDCGTHEGIMM  
---PQEFFFHAMGGREGLIDTAVKAETGYIQRRLVKALEDIMVHYDNTTRNSLGNV  
XX

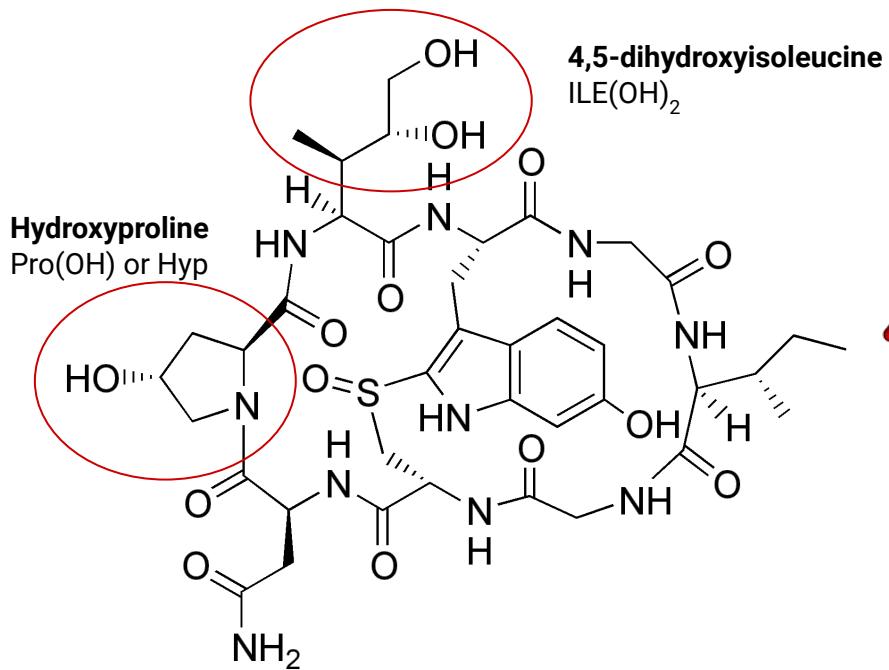
# ELONGATION CYCLE: summary





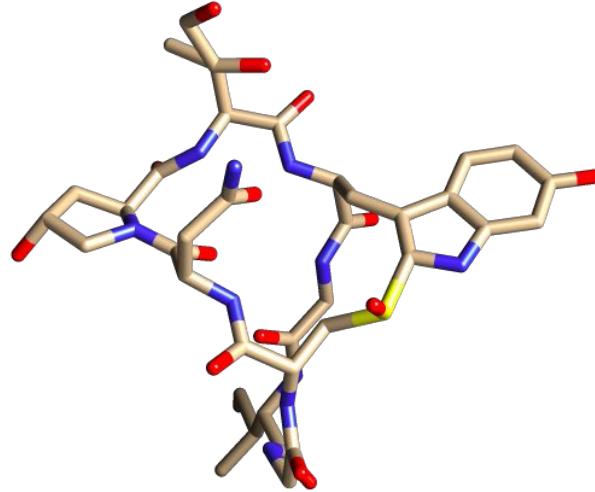
INHIBITOR:  $\alpha$ -AMANITIN

# INHIBITOR: $\alpha$ -amanitin



4,5-dihydroxyisoleucine  
ILE(OH)<sub>2</sub>

Hydroxyproline  
Pro(OH) or Hyp

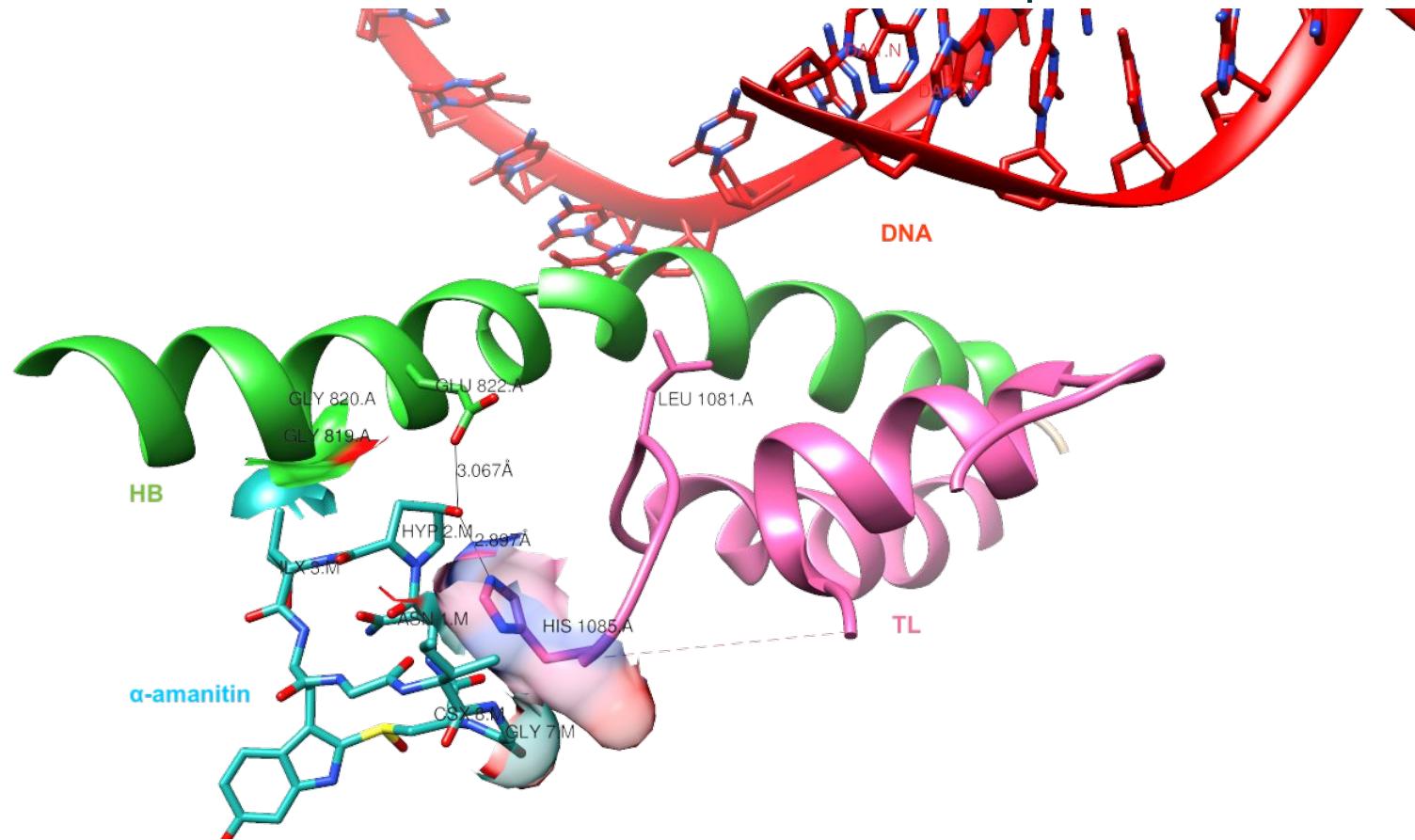


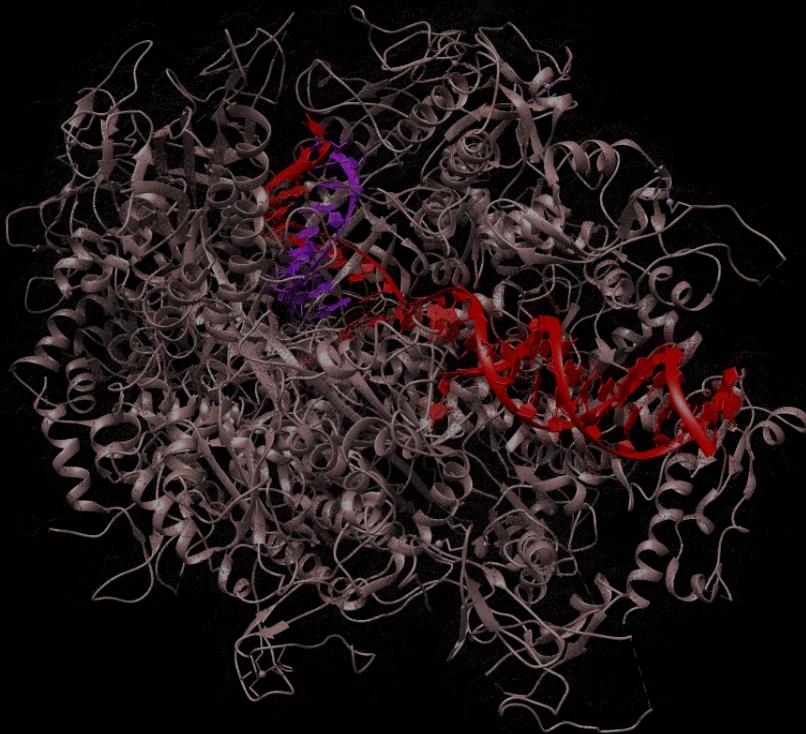
Death cap  
(*A. phalloides*)



Destroying angel  
(*A. bisporigera*)

# INHIBITOR: $\alpha$ -amanitin binds to the active site of RNA pol II

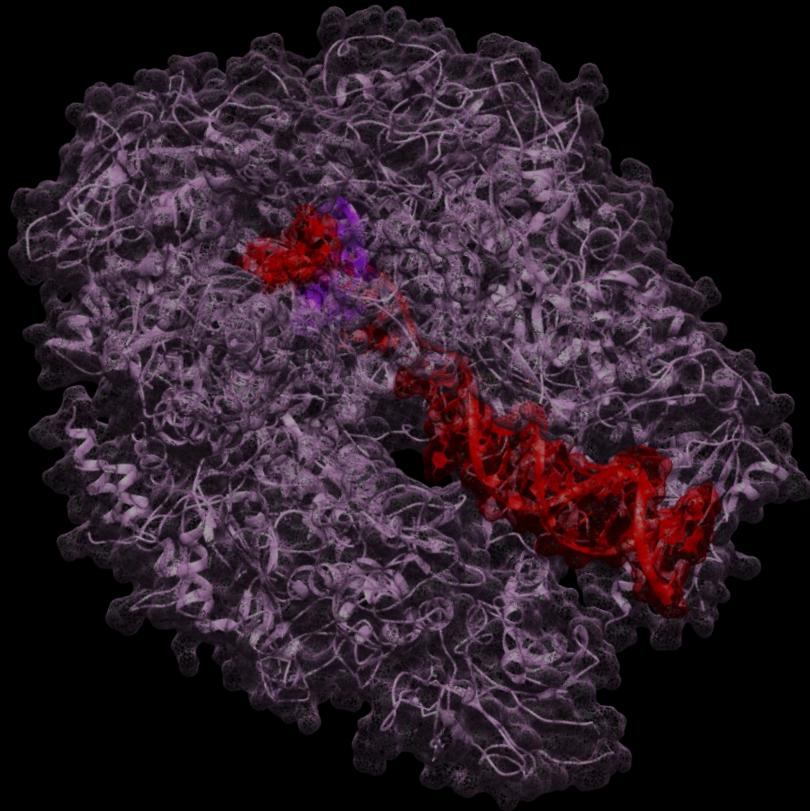




## CONCLUSIONS

# CONCLUSIONS

1. Transcription is an event that is **conserved** among all species and cell types.
2. There are different RNA polymerases but in eukaryotes, only **RNA polymerase II** transcribes protein coding genes.
3. RNA polymerase II is a multisubunit complex: subunits **Rpb1**, **Rpb2** and **Rpb9** form and/or stabilize the active site of the enzyme.
4. The **elongation cycle** is the process in which NTPs are added to the new RNA strand. It is divided in two phases: **catalysis** and **translocation**.
5. In **catalysis**, the **TL** has to **move** from open conformation to close conformation. In the active site, the **NTP** establishes interactions with the **TL**, other residues from **Rpb1** and **Rpb2**.
6. In **translocation**, the **TL** moves from closed to open conformation and adopts an intermediate **wedged state**. The **BH** bends to move the next DNA nucleotide towards the active site.
7. **Amanitin** inhibits transcription by **blocking the TL** in the **wedged state** and not allowing translocation.
8. **Sequence alignments** show high conservation in key residues involved in transcription.



THANK YOU

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## MULTIPLE CHOICE QUESTIONS

1. Which of the following structures is formed when the trigger loop (TL) and the bridge helix (BH) are together?
  - a. TIM barrel
  - b. Rossmann folding
  - c. Polymerase like folding
  - d. Three helix bundle**
  - e. Greek key
  
2. Select all the correct statements:
  1. Rpb2 is the largest subunit of RNA polymerase II
  2. Rpb1 and Rpb2 share similar SCOP information
  3. Bridge helix and trigger loop can be found in subunit Rpb9
  4. RNA polymerase II is composed by 12 subunits
    - a) 1, 2, 3
    - b) 1, 3
    - c) 2, 4**
    - d) 4
    - e) 1, 2, 3, 4

# MULTIPLE CHOICE QUESTIONS

- 1. In RNA polymerase II, catalysis and translocation:**
  - a. Require coordination of the main two structures involved in the processes (bridge helix and trigger loop).**
  - b. They are independent processes so can happen simultaneously.
  - c. In translocation, the NTP is incorporated to the RNA.
  - d. Translocation is different from catalysis because it does not involve any structures from the active site.
  - e. All of the above.
  
- 2. Select the correct statements about the metals of active site**
  - a. Both metals are always present in the active site
  - b. Metal A is always present while metal B is present only when NTP is in the active site.
  - c. These metals are both zinc ions.
  - d. More polymerases have 2 metals in the active site
    - a) 1,2,3
    - b) 1,3
    - c) 2,4**
    - d) 4
    - e) 1,2,3,4

# MULTIPLE CHOICE QUESTIONS

1. When the NTP is incorporated into the forming RNA strand:
  - a. Trigger loop is in its open conformation
  - b. Trigger loop is in its closed conformation**
  - c. Trigger loop is in its wedged conformation
  - d. TL can have all the different conformations
  - e. None of the statements above are correct
  
2. Select the false statement:
  - a. Rpb9 subunit contacts directly with TL**
  - b. Rpb9 subunit modulates the TL movement
  - c. alfa20-alfa21 from Rpb1 are important for the mobility of the TL
  - d. The TL mobility is allowed by the hinge regions which provide flexibility to the structure
  - e. alfa20-alfa21 interact with Rpb9 by VDW and hydrogen bond interactions.

## MULTIPLE CHOICE QUESTIONS

1. In the wedged conformation of the trigger loop (TL), translocation:
  - a. It's over.
  - b. It's being carried out.**
  - c. It has not started yet.
  - d. All of the above.
  - e. None is correct.
  
2. In translocation of DNA to begin another elongation cycle:
  - a. The nucleotide is pulled to the active site by a hydrogen bond.
  - b. The DNA moves independently to arrive to the active site.
  - c. The DNA is translocated by a transcription factor.
  - d. When the bridge helix bends, it forms multiple hydrogen bonds with the following nucleotides.
  - e. When the bridge helix bends, an alanine can contact the dNTP through VDW interactions and can push it to the active site.**

## MULTIPLE CHOICE QUESTIONS

**1. About the hinge regions of the bridge helix:**

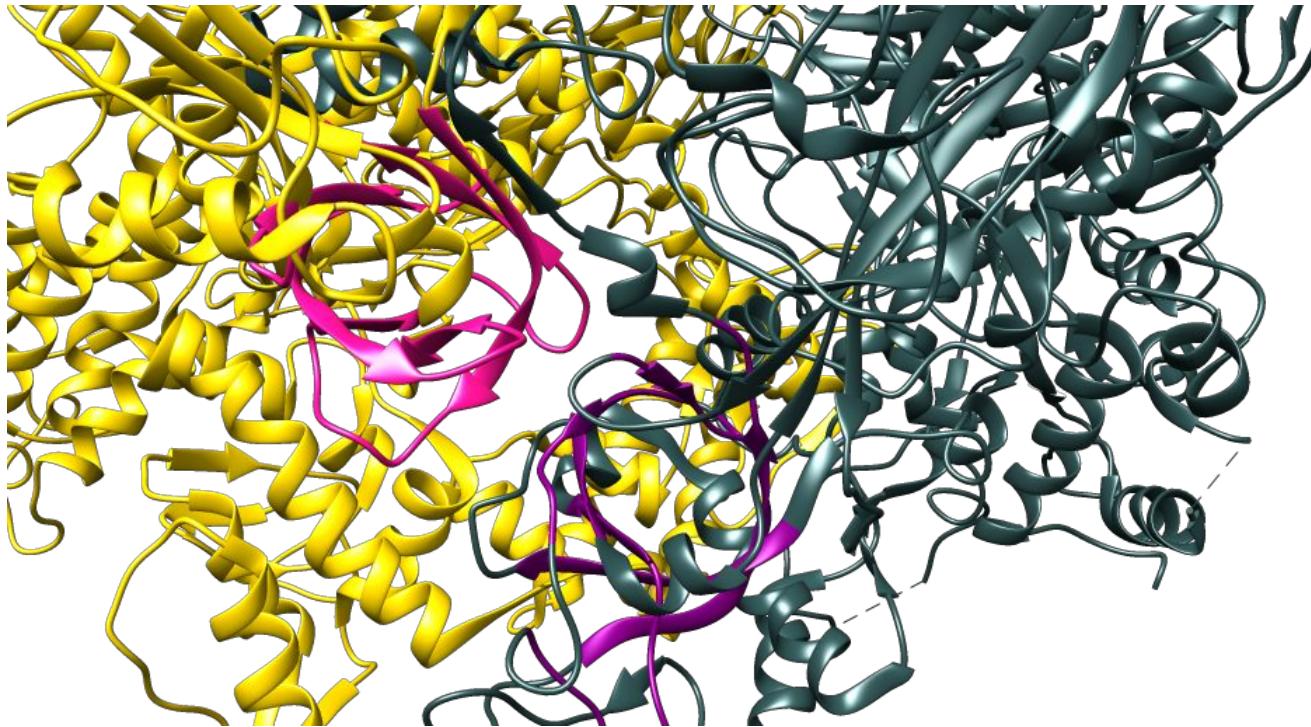
- a. They are regions that provide flexibility to the structure.
- b. Their sequence is highly conserved among species.
- c. Their residues cannot make hydrogen bonds.
- d. They allow the bridge helix bending.
- e. All of the above.**

**2. About the inhibitor alpha-amanitin:**

- a. Alpha-amanitin binds to the enzyme through the modified amino acids hydroxyproline and dihydroxyisoleucine.
- b. Trigger loop movement is blocked when alpha-amanitin interacts with the enzyme
- c. The two above are correct**
- d. Alpha-amanitin binds to the surface of the enzyme and causes a conformational change that blocks transcription
- e. All the above are correct

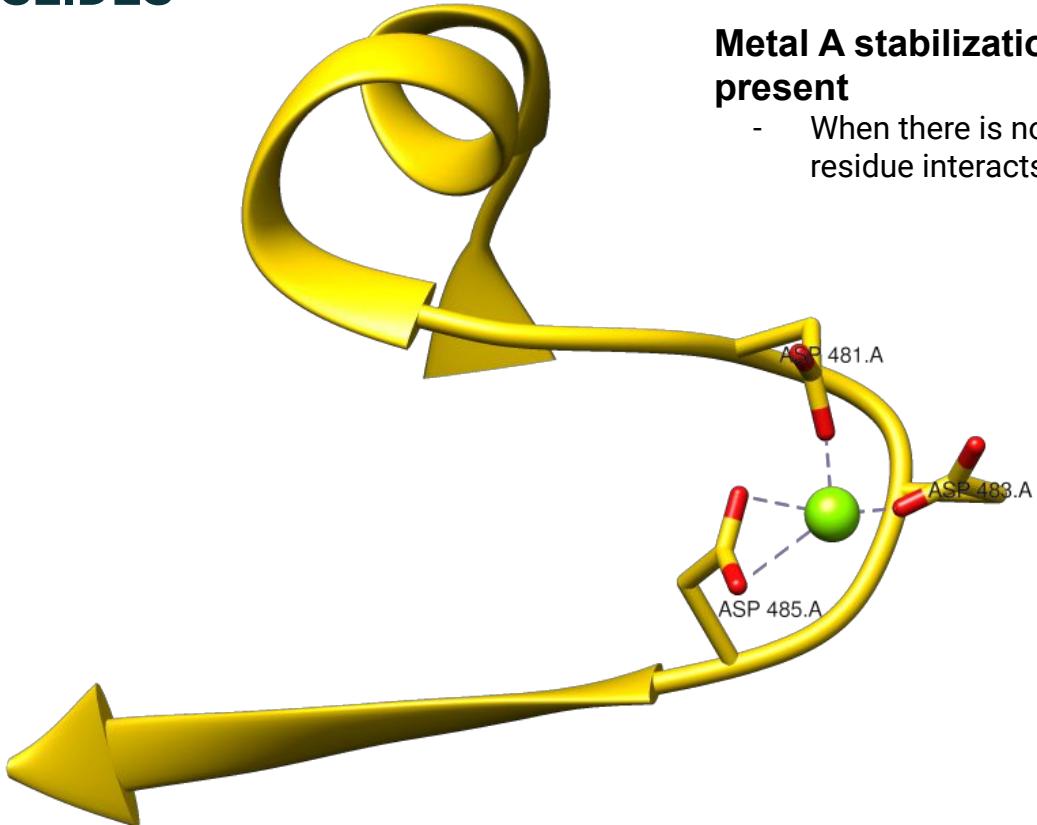
# EXTRA SLIDES

## EXTRA SLIDES



**Double psi barrel**  
A different view.

## EXTRA SLIDES

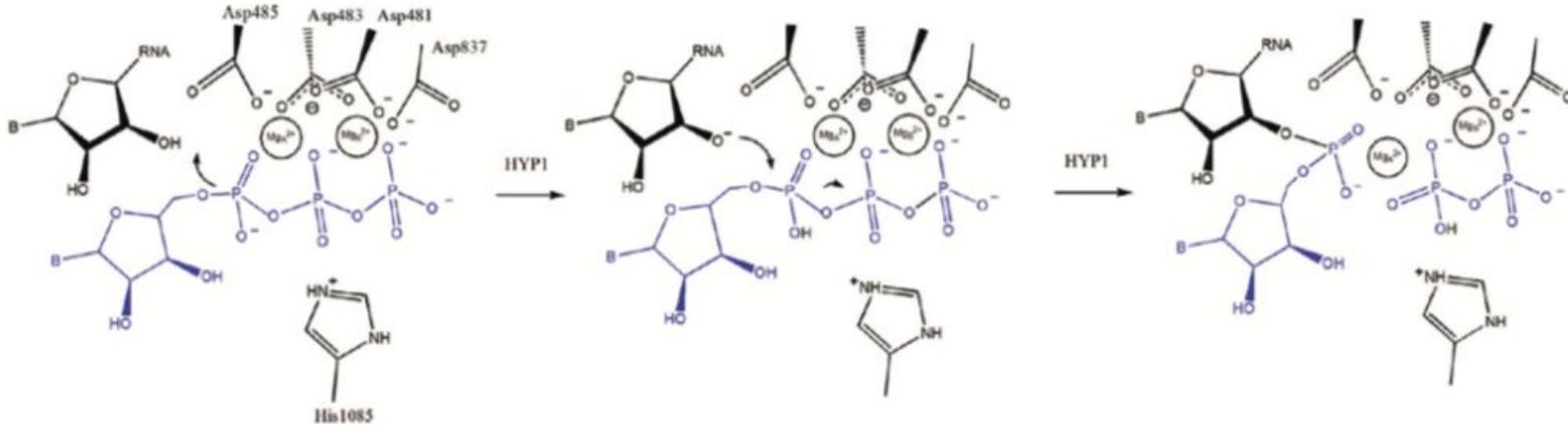
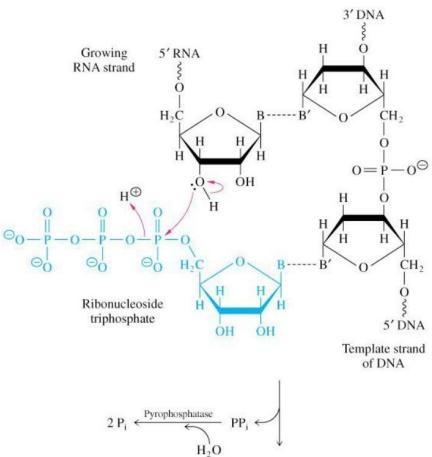


### Metal A stabilization when NTP is not present

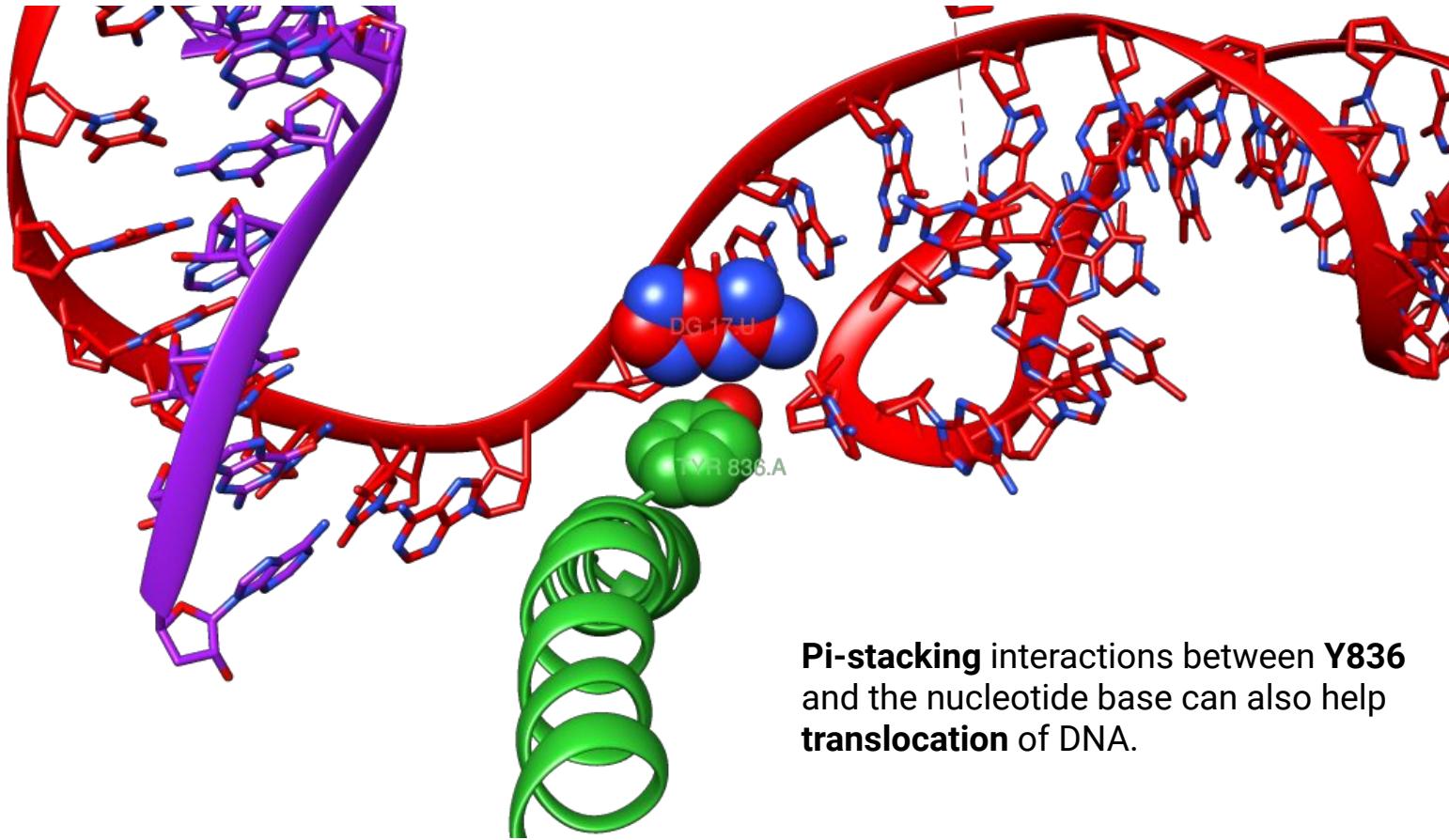
- When there is no NTP, a new aspartic acid residue interacts with the magnesium atom.

# EXTRA SLIDES

- RNA polymerase reaction

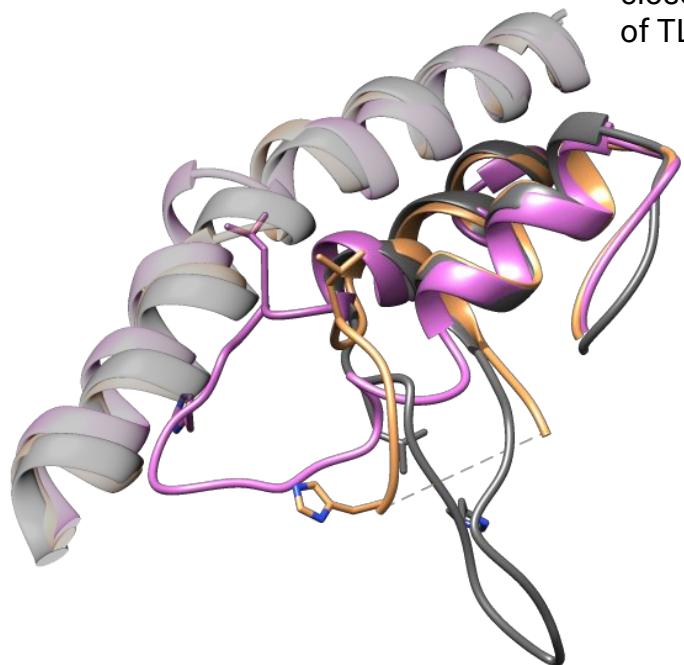


## EXTRA SLIDES



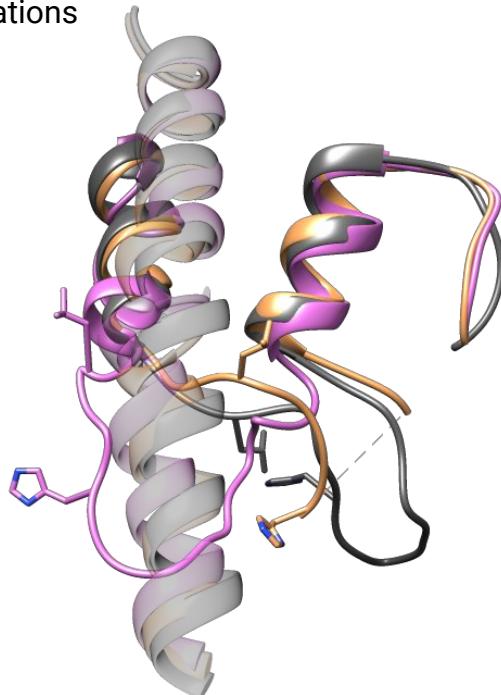
## EXTRA SLIDES

**TL movement during translocation.** A closer look to the different conformations of TL in translocation.

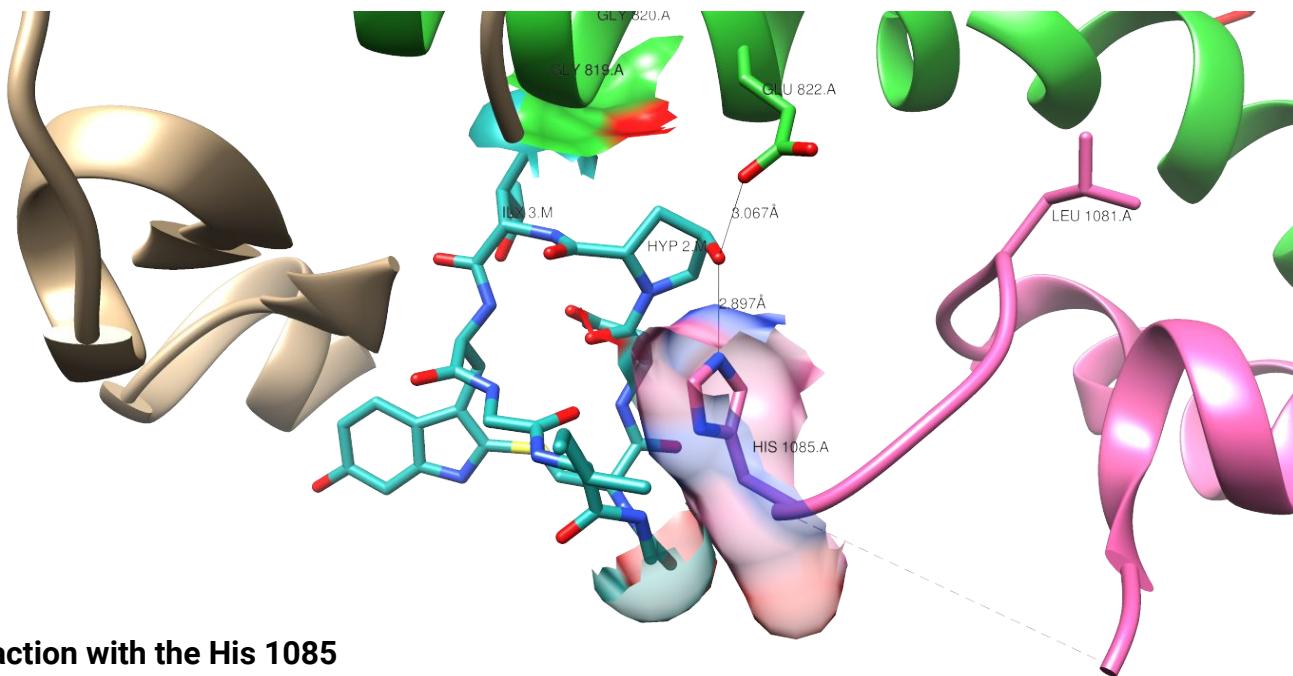


TL closed  
TL wedged  
TL open

RMSD= 0.826 Å



## EXTRA SLIDES

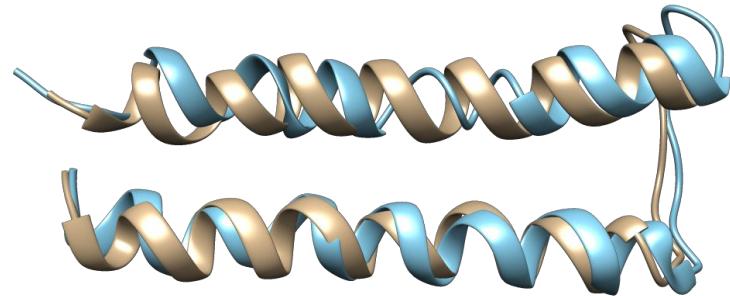


### Amanitin interaction with the His 1085

Interaction of the OH of the hydroxyProline with:

- the NH of the His
- the NH<sub>2</sub> of the Asp

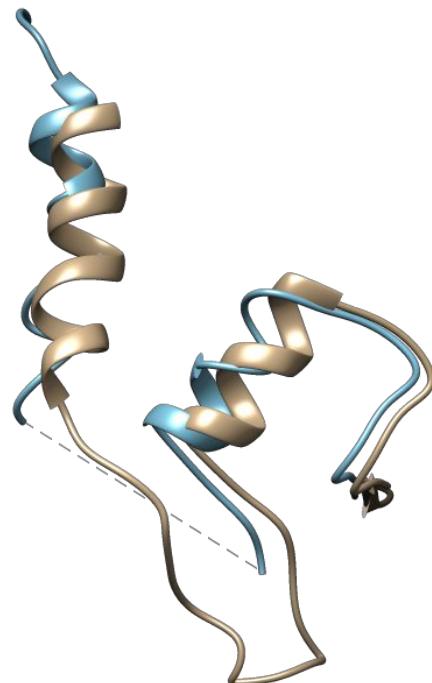
## EXTRA SLIDES: superimposition human/S.cerevisiae



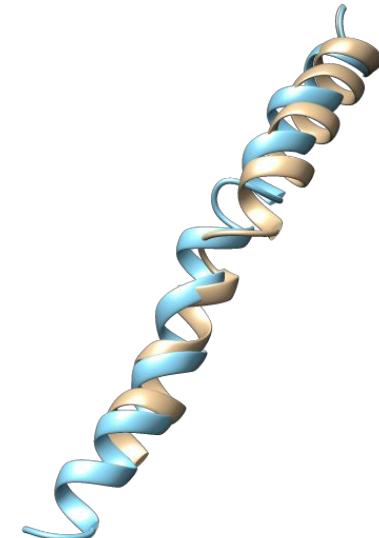
alfa20-21

RMSD=1.288 Å

Human  
*S.cerevisiae*



Trigger Loop

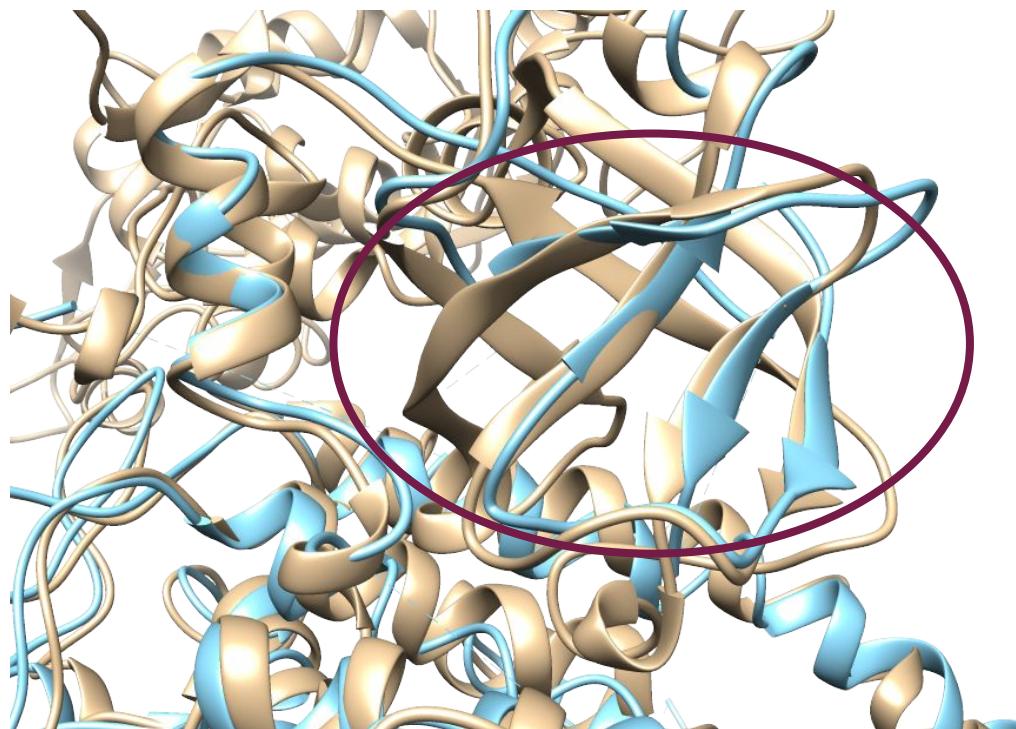


Bridge Helix

## EXTRA SLIDES: superimposition human/S.cerevisiae



## EXTRA SLIDES: superimposition human/S.cerevisiae



Human  
S.cerevisiae

RMSD = 1.288A

Human pdb is cut, some beta sheet are not present, it may be due to the crystallization process