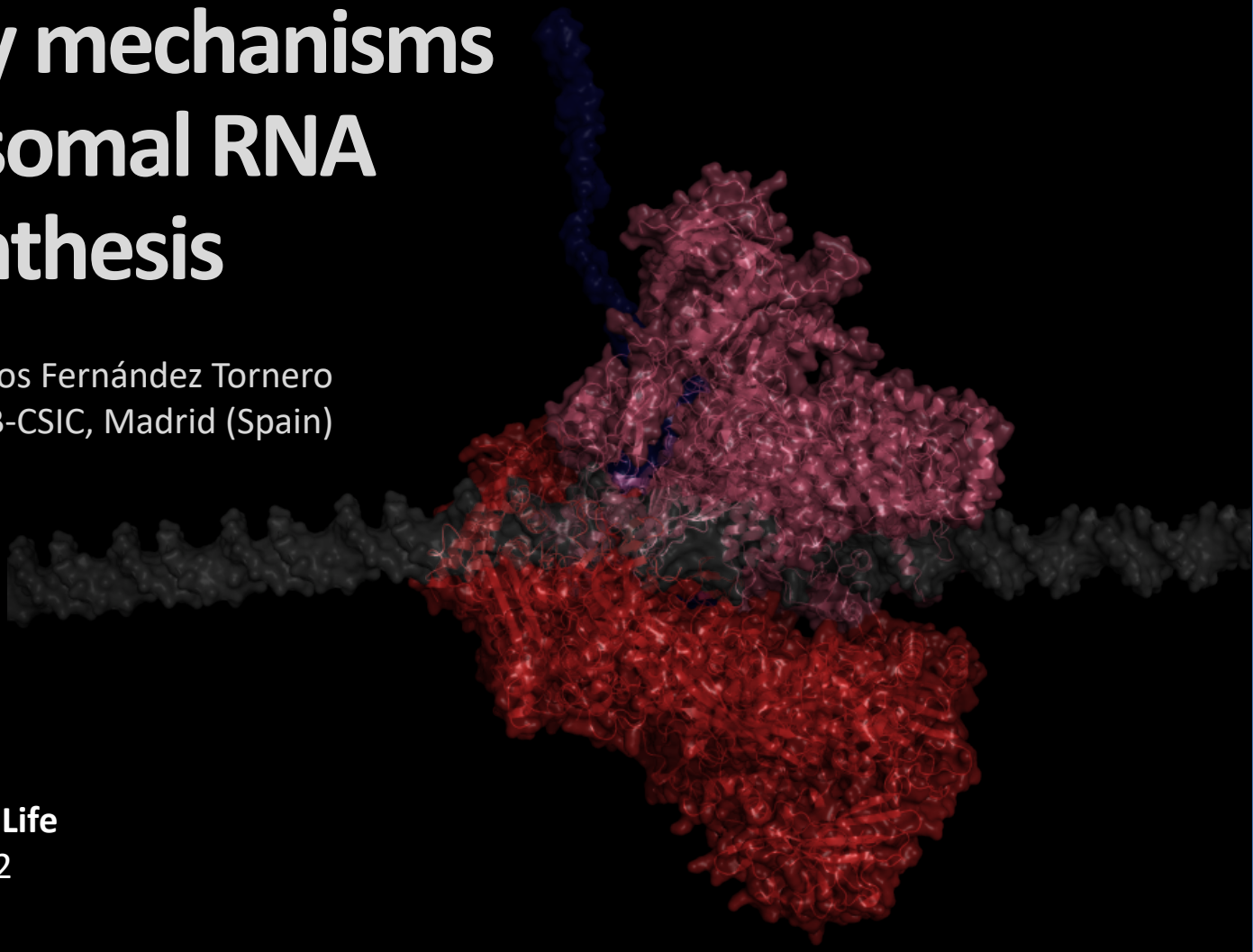
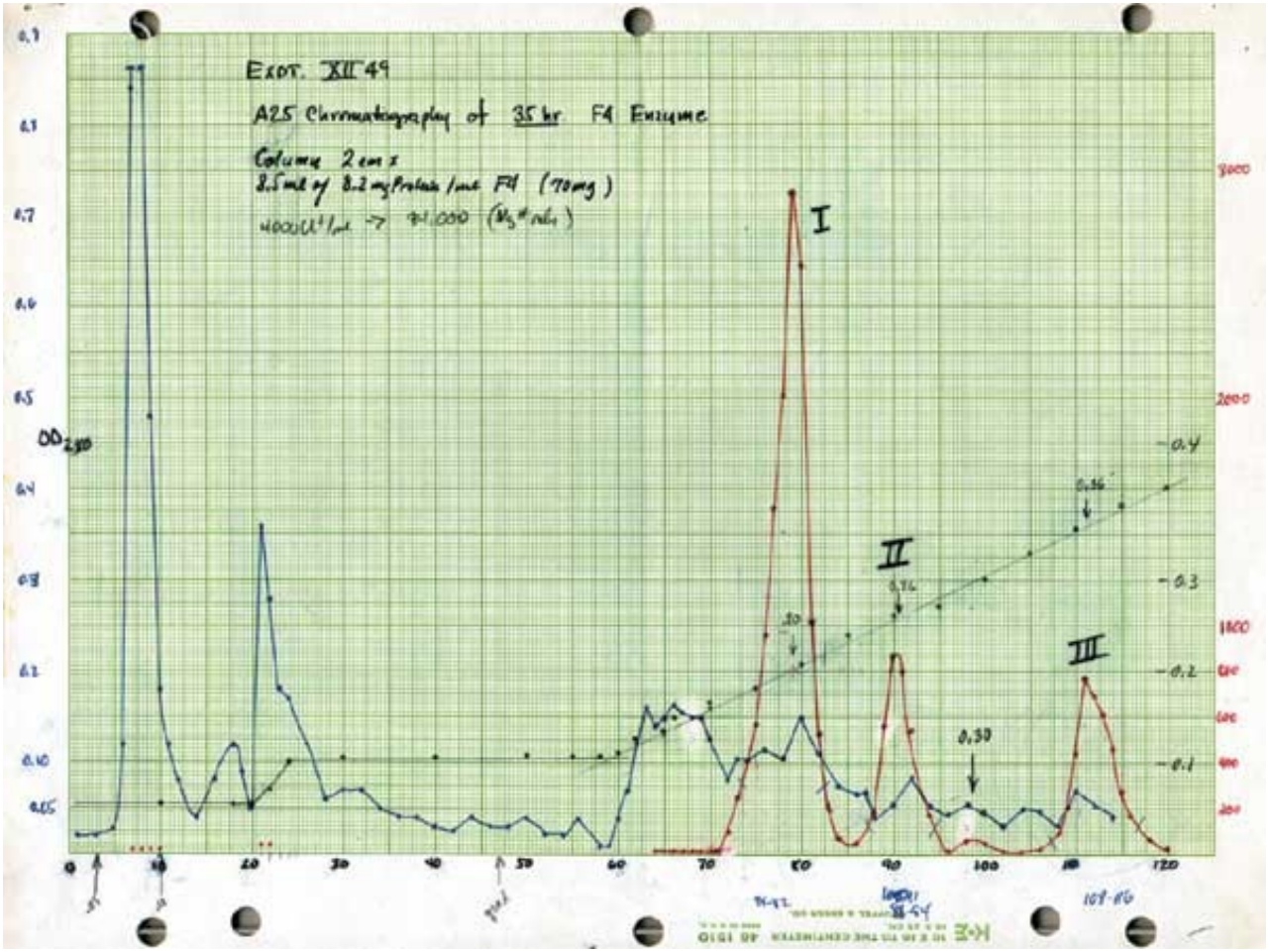
Regulatory mechanisms in ribosomal RNA synthesis

Carlos Fernández Tornero
CIB-CSIC, Madrid (Spain)



LifeHub – Synthetic Life
Madrid - 10.03.2022

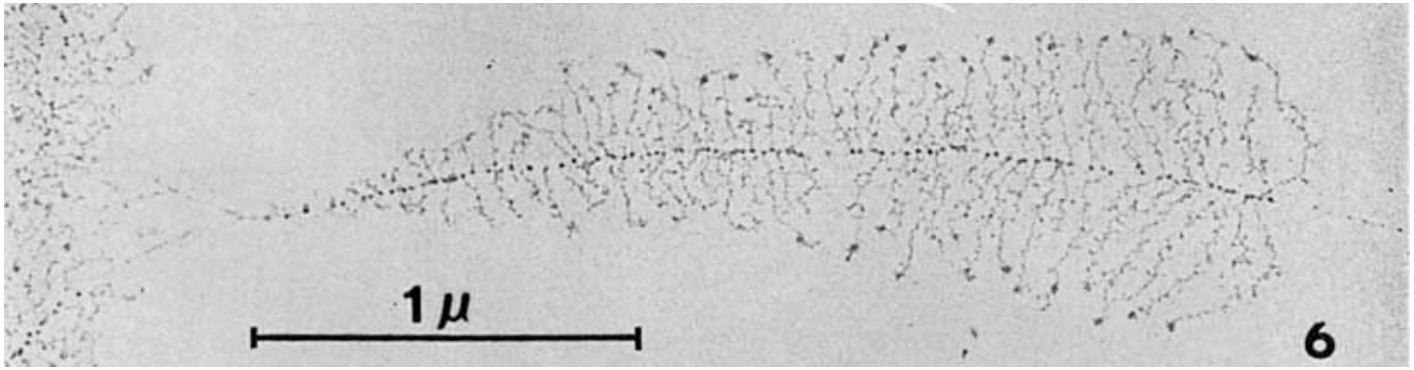
Eukaryotic RNA polymerases



Roeder & Rutter (1969)
Nature **224**:234

Plants – Pol IV + Pol V for regulatory RNAs

Pol I and cell biology



Miller & Beatty (1969)
Science **164**:955

- A single transcript (pre-rRNA) that matures into 18S, 5.8S, 25S/28S

- rDNA gene copies: 150 in yeast (chr XII), 300 in human, 4000 in pea

- Gene size: 6.9 kb in yeast, 13 kb in human

- Transcriptional activity: Pol I (60%) > Pol III (20%) > Pol II (20%)

- RNA content: rRNA (85%) > tRNA (12%) > mRNA (3%)

Master of housekeeping genes

Pol I and disease

Cancer

Cancer Cell
Article

Cell PRESS

Inhibition of RNA Polymerase I as a Therapeutic Strategy to Promote Cancer-Specific Activation of p53

Megan J. Bywater,[1,3] Gretchen Poortinga,[1,6] Elaine Sanij,[1,4] Nadine Hein,[1] Abigail Peck,[1] Carleen Cullinane,[1] Meaghan Wall,[7] Leonie Cluse,[1] Denis Drygin,[8] Kenna Anderes,[8] Nanni Huser,[8] Chris Proffitt,[8] Joshua Bliesath,[8] Mustapha Haddach,[8] Michael K. Schwaebe,[8] David M. Ryckman,[8] William G. Rice,[8] Clemens Schmitt,[9,10] Scott W. Lowe,[11] Ricky W. Johnstone,[1,3,4] Richard B. Pearson,[1,3,5,12] Grant A. McArthur,[1,2,3,5,14] and Ross D. Hannan[1,2,3,5,12,13,*]

cells

MDPI

Review

Targeting the RNA Polymerase I Transcription for Cancer Therapy Comes of Age

Rita Ferreira [1,*], John S. Schneekloth Jr. [2], Konstantin I. Panov [1,3], Katherine M. Hannan [1,4] and Ross D. Hannan [1,4,5,6,7]

Specific Pol I Inhibitors	CX-3543	No	Dissociation of Nucleolin-rDNA G-quadruplex complexes	Inhibition—Undetermined mechanism	Yes	Clinical trial phase II	[55]
	CX-5461	No	Disruption of interaction between SL-1 and Pol I at the rRNA promoter	Inhibition of transcription initiation	Yes	Clinical trial phase II	[56]
	BMH-21	No	Degradation of RPA194 and displacement of RRN3	Inhibition of transcription elongation	No	No	[57]

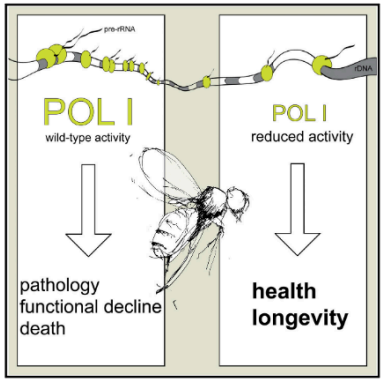
Aging

Report

Cell Reports

Partial Inhibition of RNA Polymerase I Promotes Animal Health and Longevity

Graphical Abstract



Authors

Guillermo Martínez Corrales, Danny Filer, Katharina C. Wenz, ..., Yodit Feseha, Susan J. Broughton, Nazif Alic

Correspondence

n.alic@ucl.ac.uk

In Brief

RNA polymerase I is a conserved eukaryotic enzyme that transcribes a single gene to generate precursor ribosomal RNA. Martínez Corrales et al. show that reducing the activity of this polymerase, even only in subsets of adult cells, can promote broad health benefits and longevity in the animal model *Drosophila melanogaster*.

Ribosomopathies

Biochimica et Biophysica Acta 1829 (2013) 342–360

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagrm

ELSEVIER

BBA Gene Regulatory Mechanisms

Review

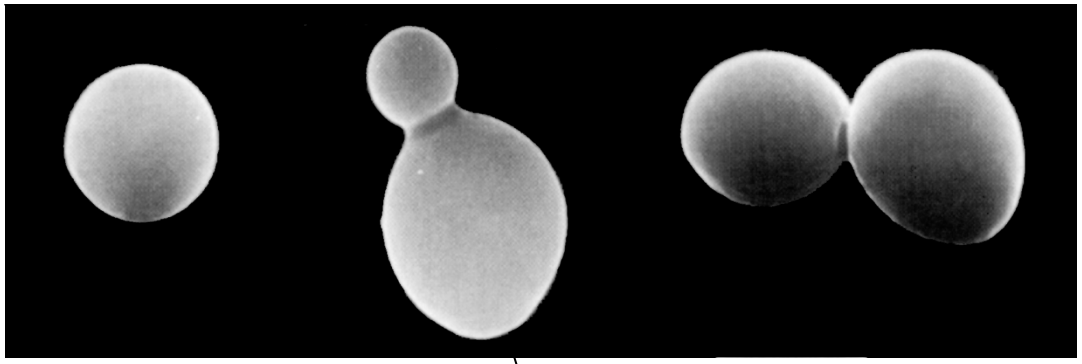
Dysregulation of RNA polymerase I transcription during disease☆

K.M. Hannan [a], E. Sanij [a,b], L.I. Rothblum [c], R.D. Hannan [a,d,e,f,g,*,1], R.B. Pearson [a,d,e,f,1]

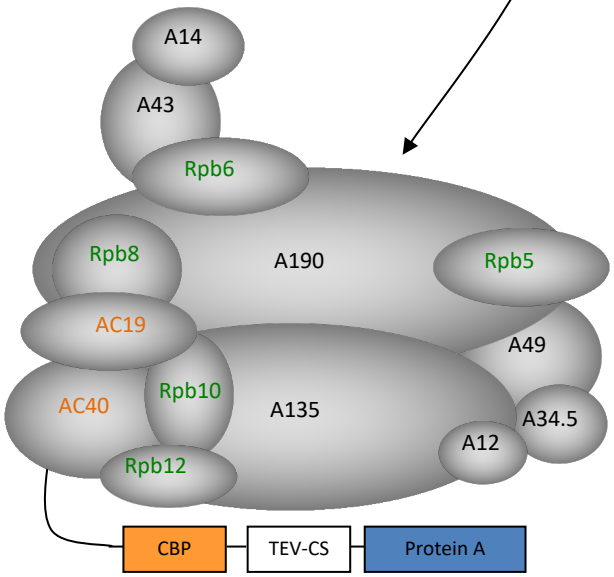
CrossMark

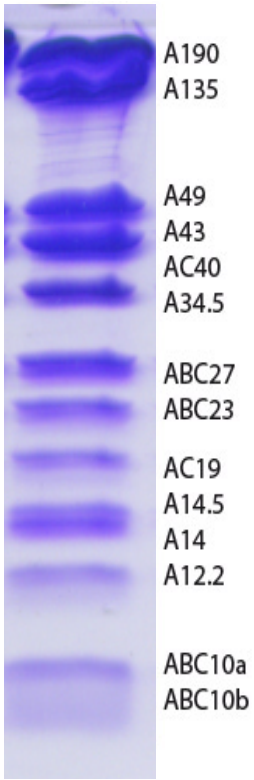
Acrofacial Dysostosis (AD)
Treacher Collins syndrome (TCS)
Hypomyelinating Leukodystrophy (HLD)

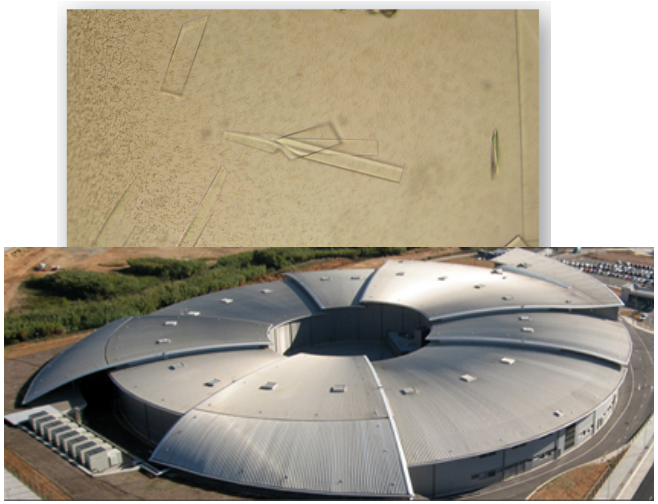
Purification and crystallization



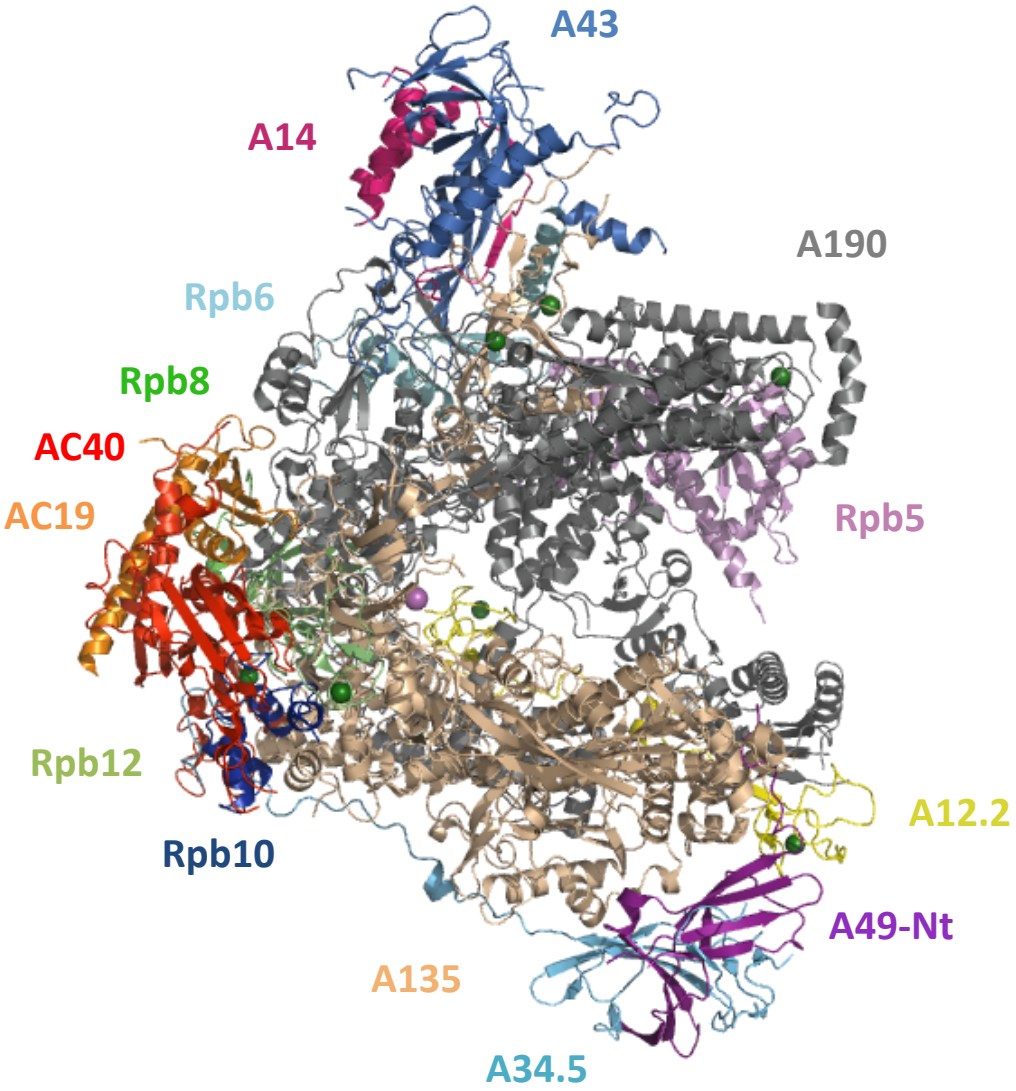
Pol I
14 subunits
600 kDa
5,000 residues
80,000 atoms







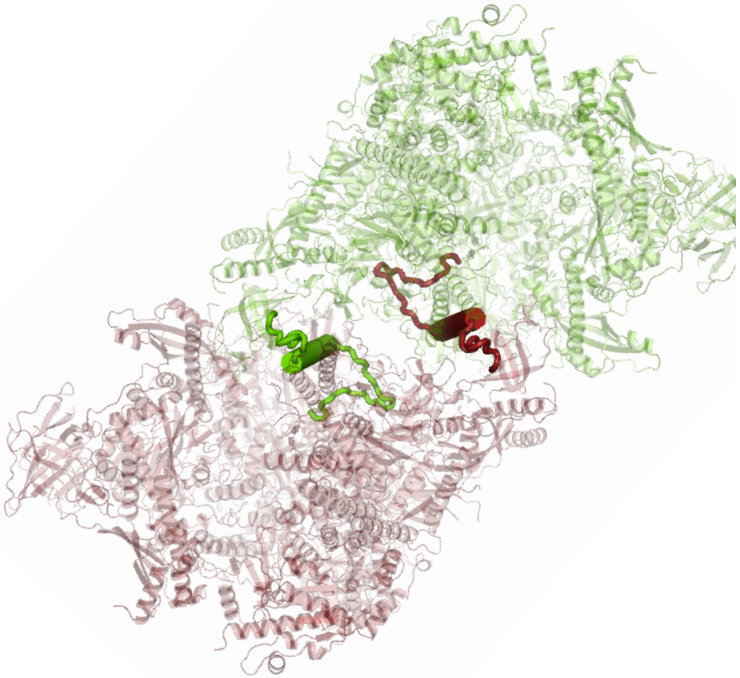
Crystal structure of yeast Pol I at 3 Å resolution



Fernández-Tornero*… Müller* (2013) *Nature* **502**:644

Structural transitions in the RNA polymerase I activation process

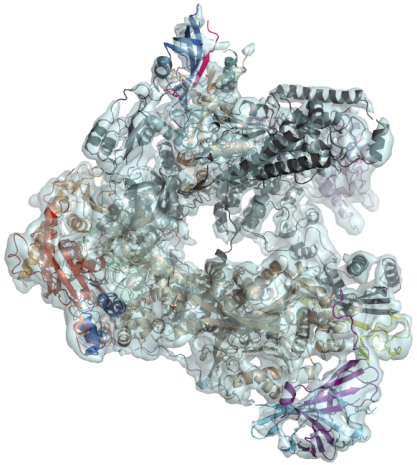
Pol I homodimer
(Inactive/Latent)



X-ray – 3.0 Å

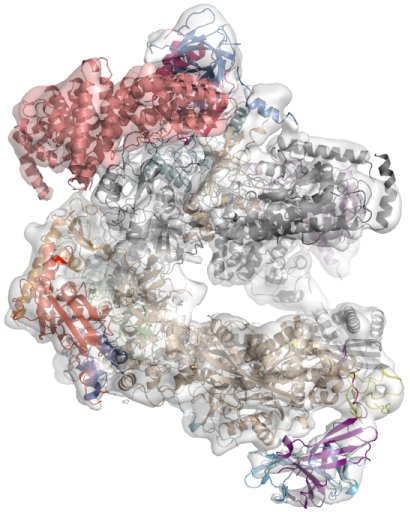
Fernández-Tornero*… Müller* (2013) *Nature* **502**:644

Pol I monomer
(Pre-activated)



Cryo-EM – 4.9 Å

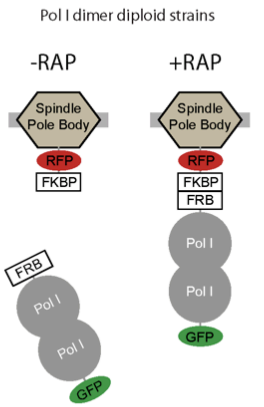
Pol I–Rrn3 complex
(Activated)

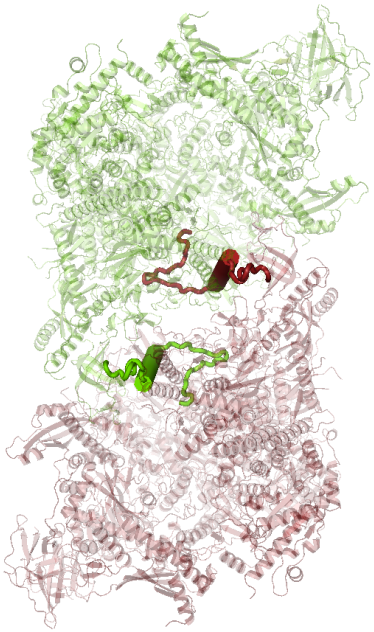


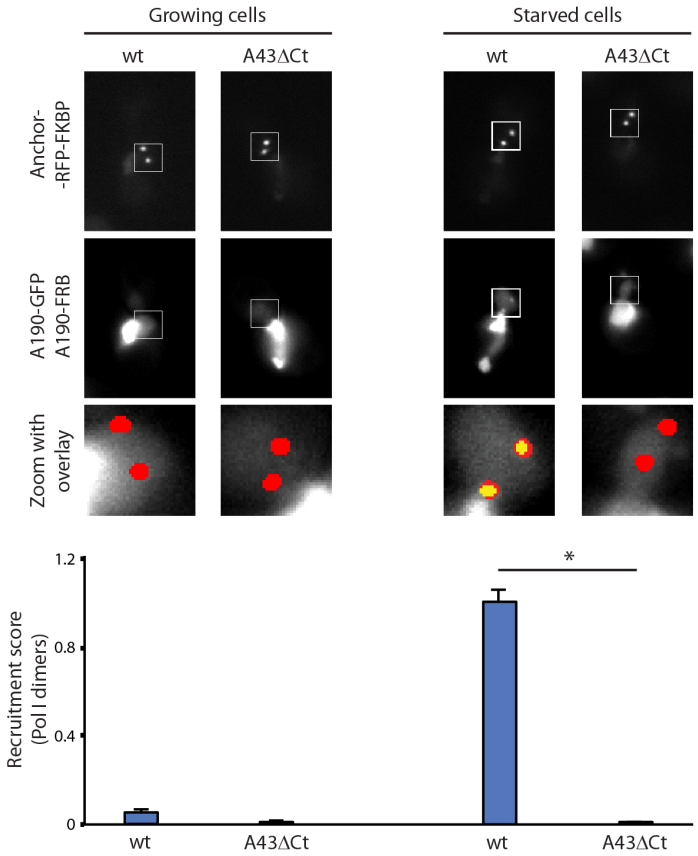
Cryo-EM – 7.7 Å

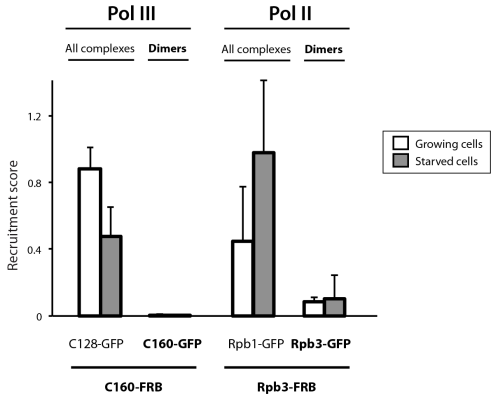
Torreira… Calvo*, Fernández-Tornero* (2017) *eLife* **6**:e20832

In vivo formation of Pol I homodimers



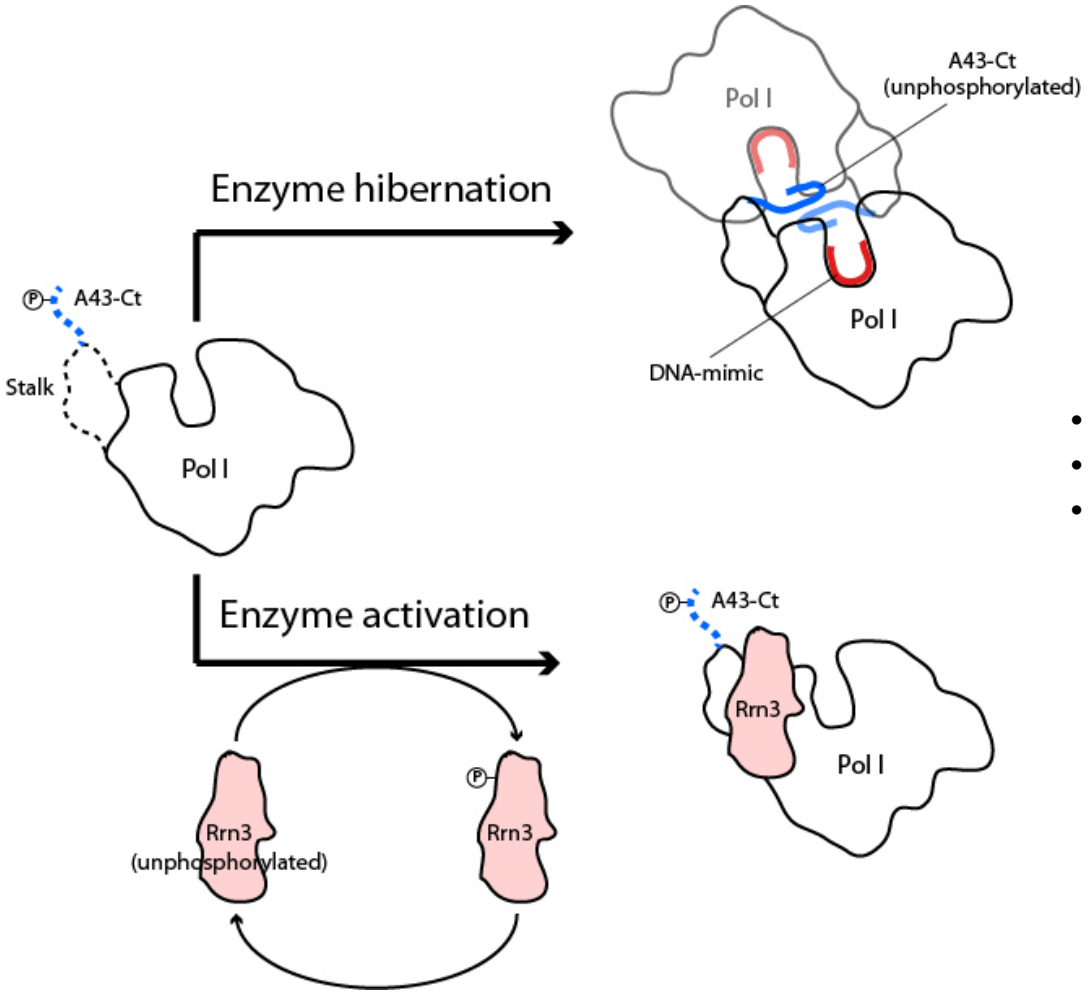






Torreira… Calvo*, Fernández-Tornero* (2017) *eLife* **6**:e20832

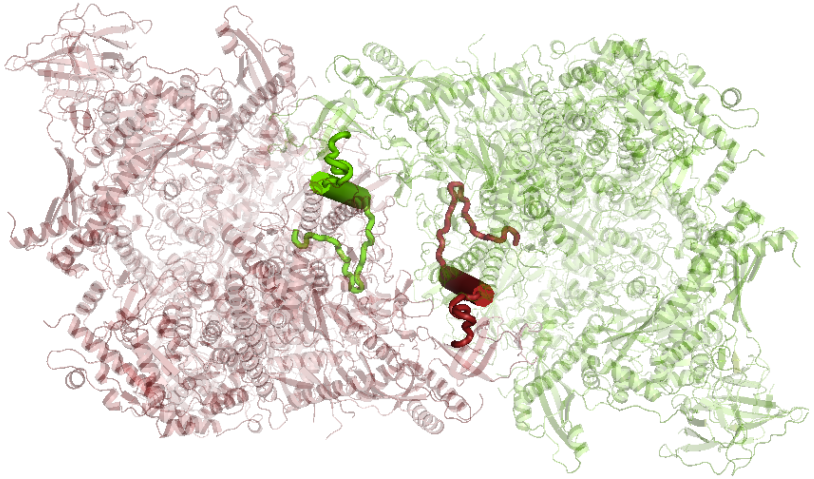
Pol I hibernation and activation



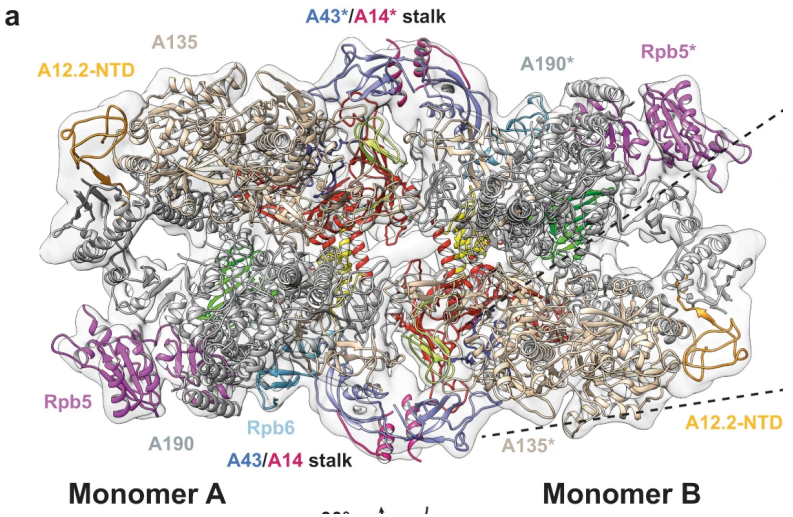
- Downregulation of rDNA transcription
- Storage & protection from degradation
- Easy reactivation

Fernández-Tornero (2018) *Transcription* **9**:248

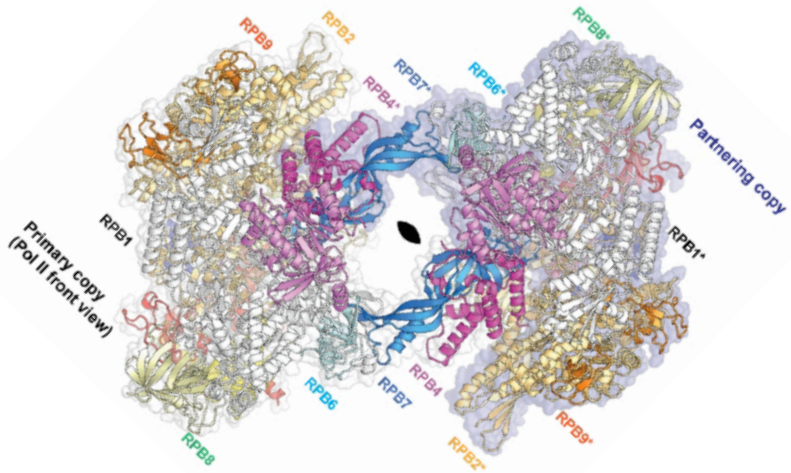
Conserved mechanism of hibernation by dimerization



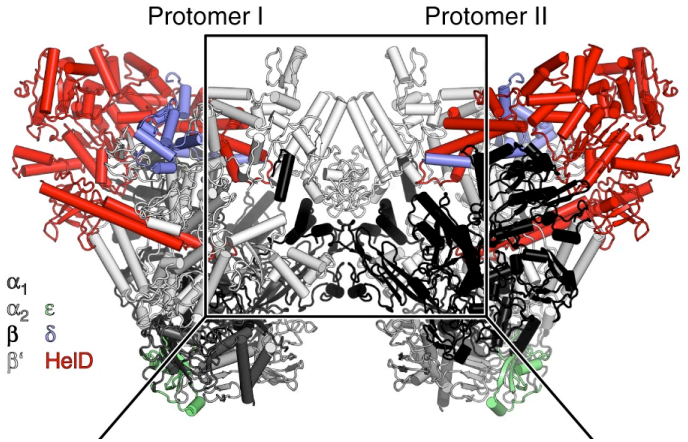
Fernández-Tornero*… Müller* (2013) *Nature* **502**:644



Heiss… Engel (2021) *Nat Comm* **12**:758

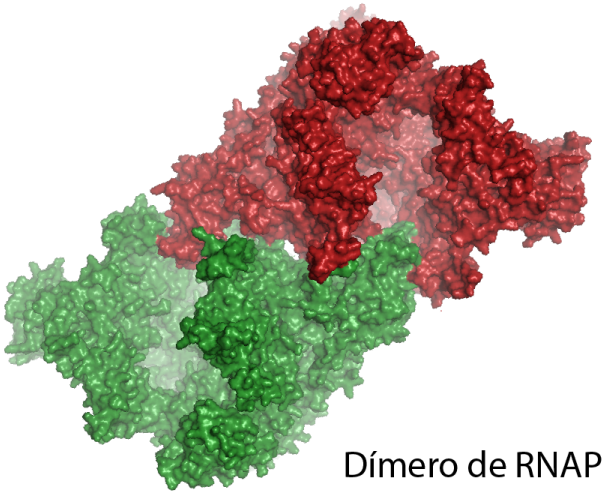


Aibara… Cramer (2021) *NAR* **49**:10747

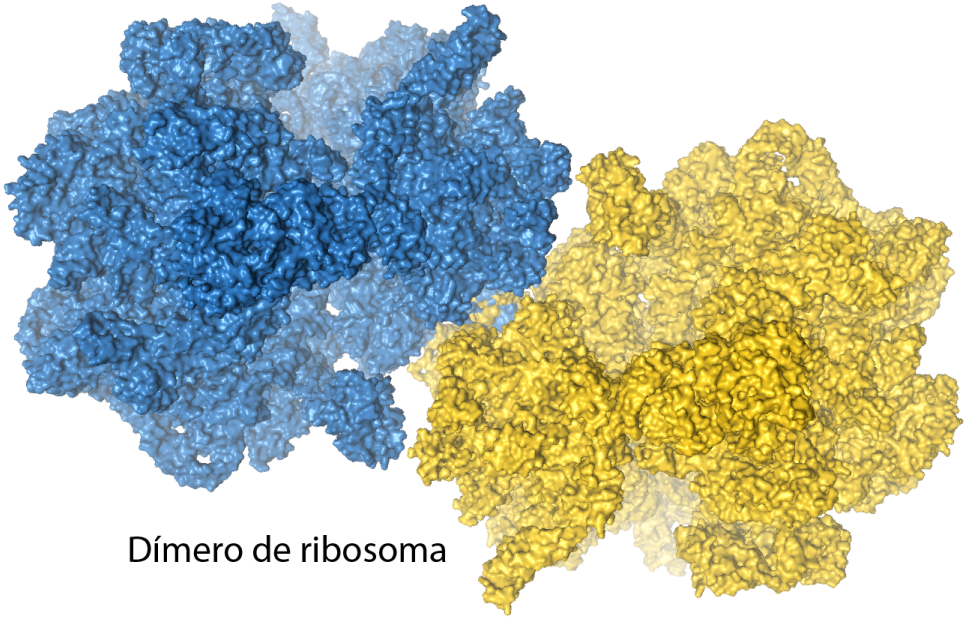


Pei… Wahl (2020) *Nat Comm* **11**:6418

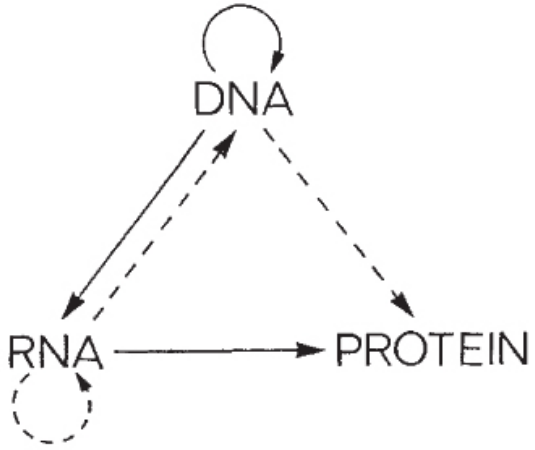
Molecular hibernation at the core of the Central Dogma



Dímero de RNAP



Dímero de ribosoma



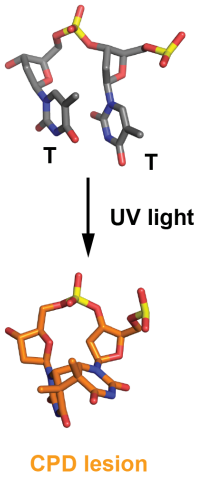
Crick (1970) *Nature* **227**: 561

https://sebbm.es/nuestros-cientificos/

1. **Stress conditions**:
    1. Inactivation of complex machineries
    2. Protection from degradation (storage)
    3. Down-regulation of gene expression
2. **Favourable conditions**:
    1. Rapid reactivation without re-synthesis
    2. Energy saving
    3. Fast reprograming of gene expression

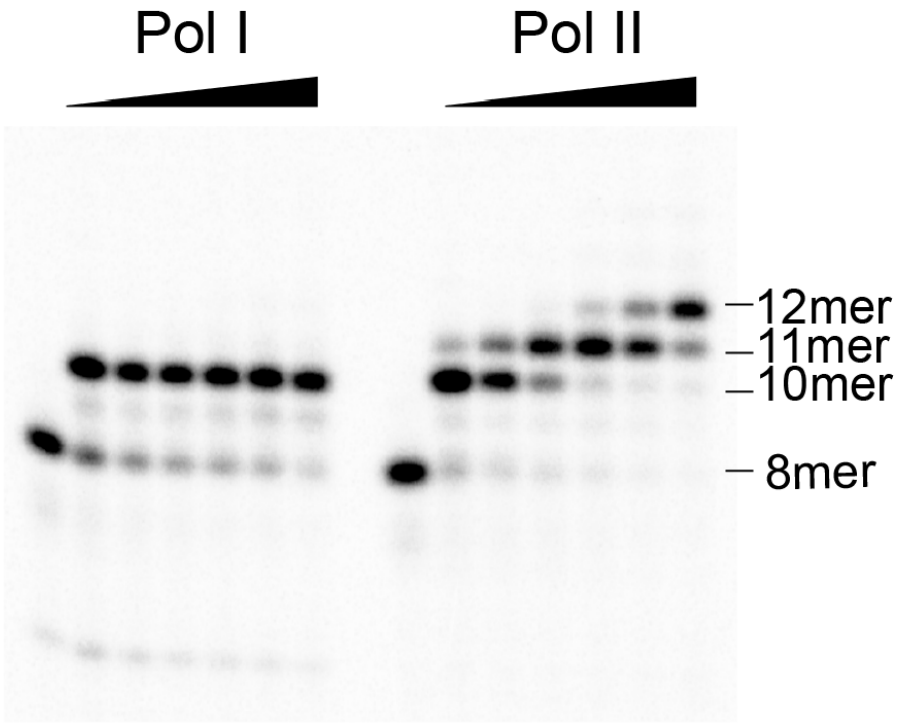
UV light harms DNA

Foto: Germán M. Bretones



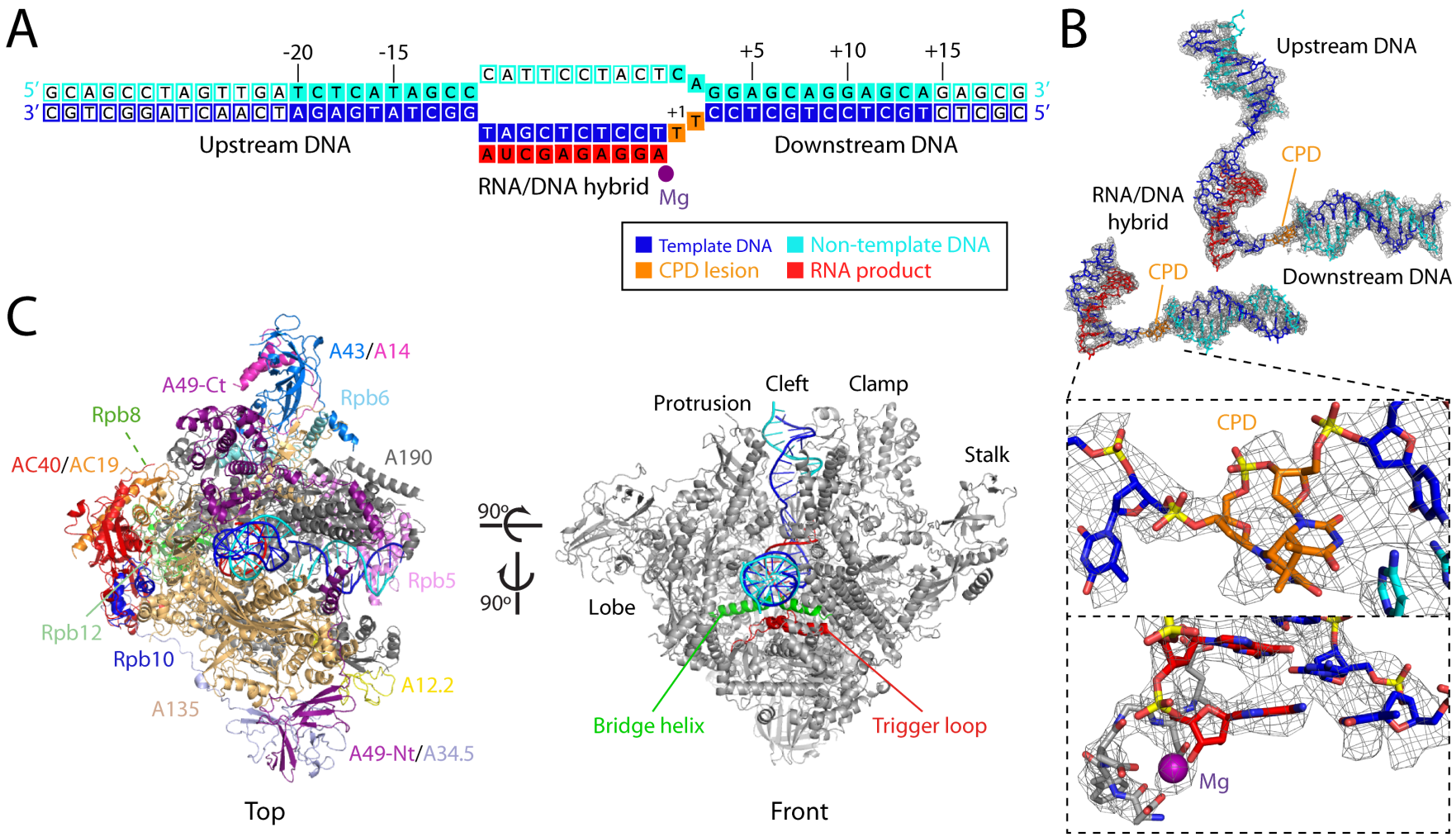
Pol I and Pol II handle CPD lesions differently

```
                                    TTTCATTCCTACTC
NTS:5′ GCAGCCTAGTTGATCTCATA                      AGGAGAAGGAGCAGAGCG
TS:3′ CGTCGGATCAACTAGAGTATCGGTAGCTCTCCTTCCTCTTCCTCGTCTCGC
          RNA(8mer):    *5′AUCGAGAG  TT=CPD
```



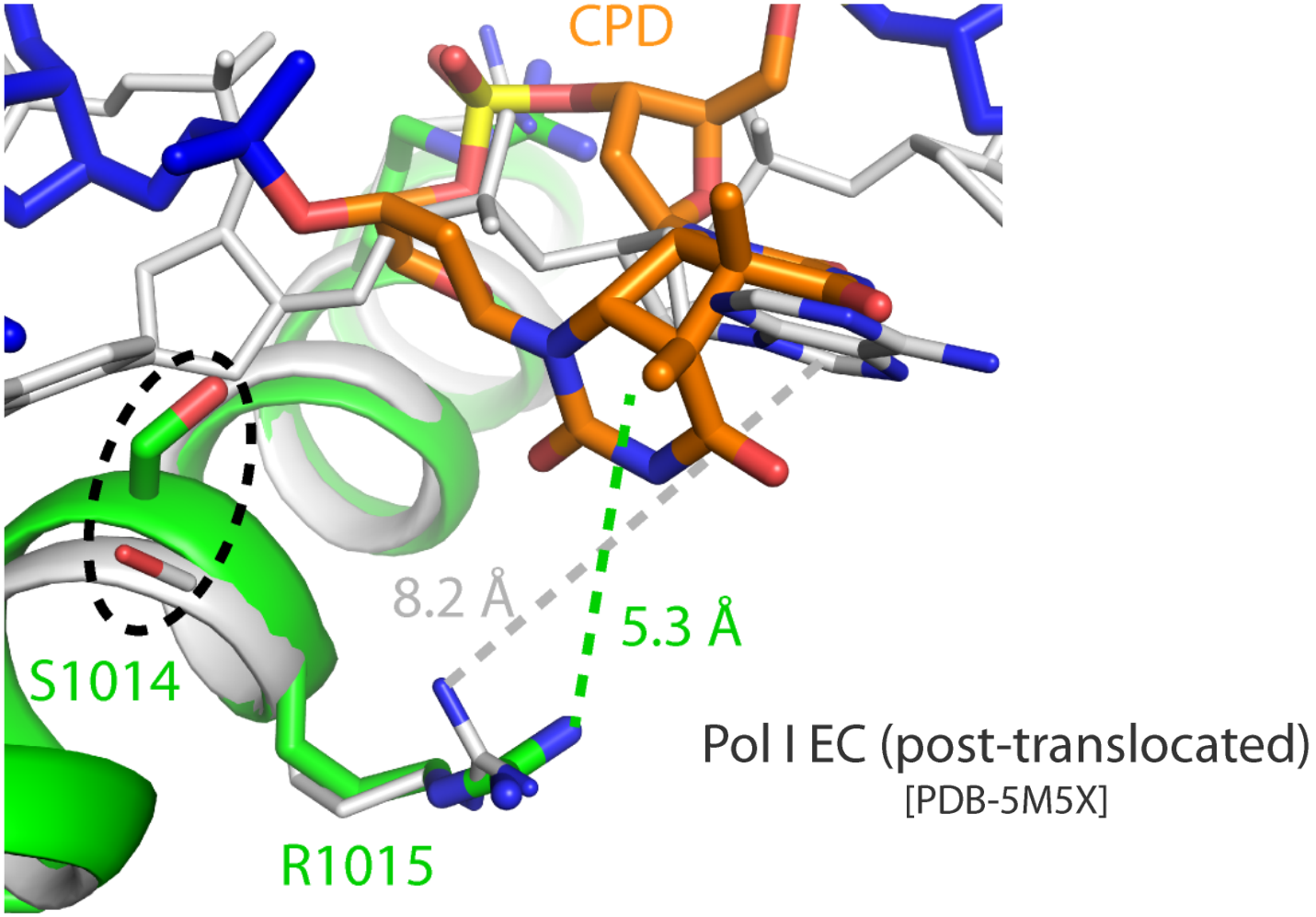
A dual mechanism enables firm Pol I stalling at CPD lesions

Pol I stalls when CPD approaches the bride helix

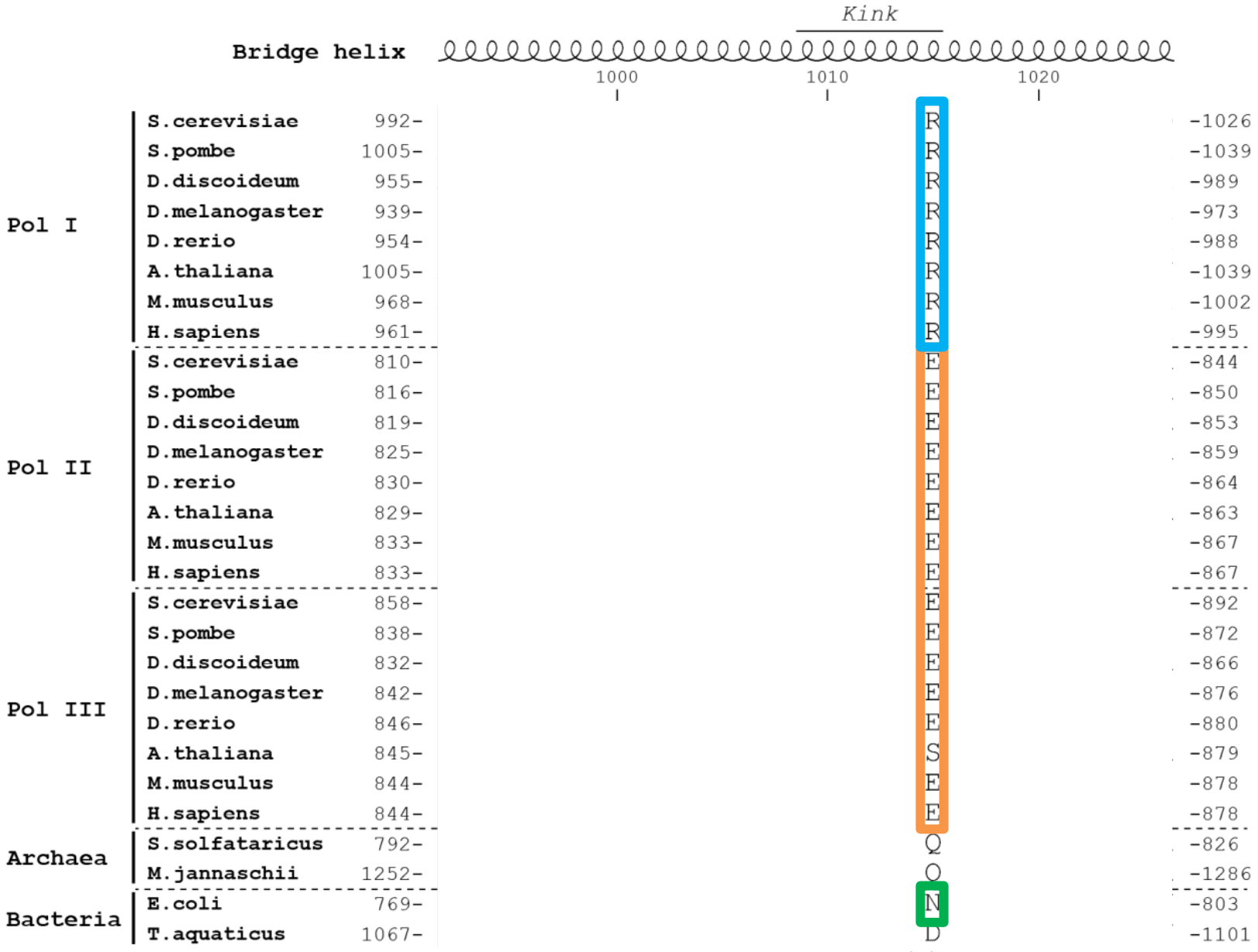


R1015 in A190 interacts with CPD damage

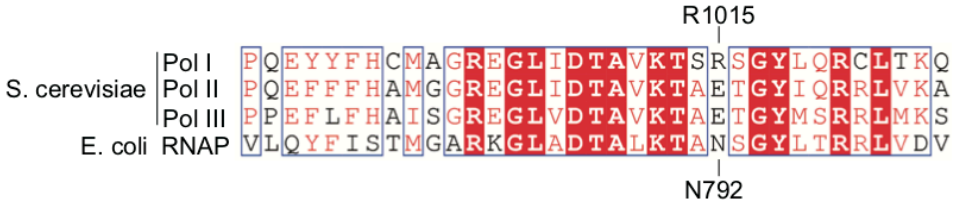
Active site

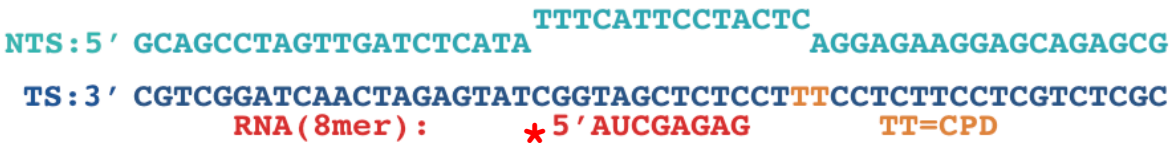


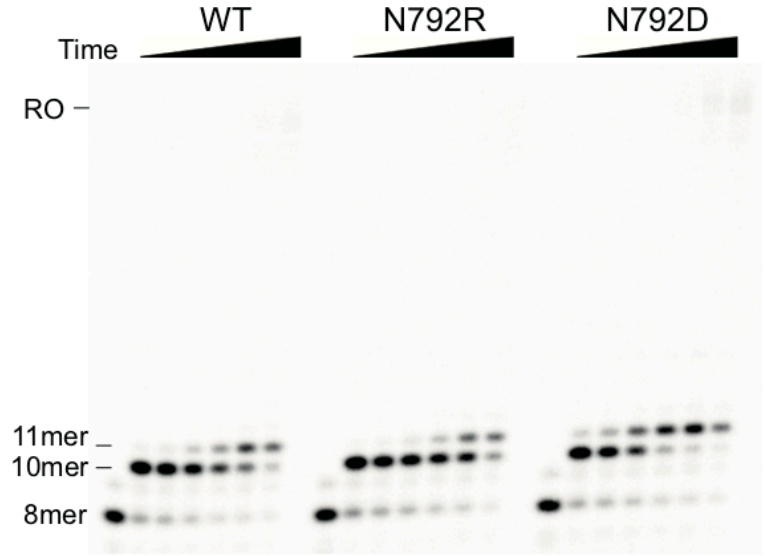
R1015 is unique in Pol I

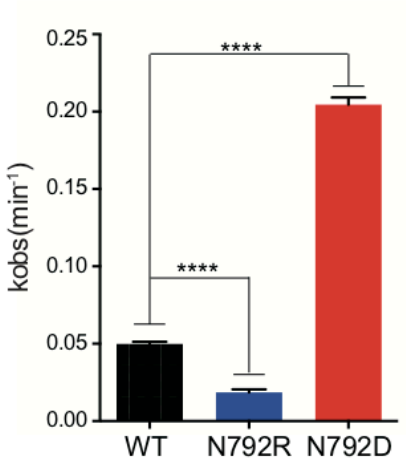


R1015 slows down bypass of CPD lesions





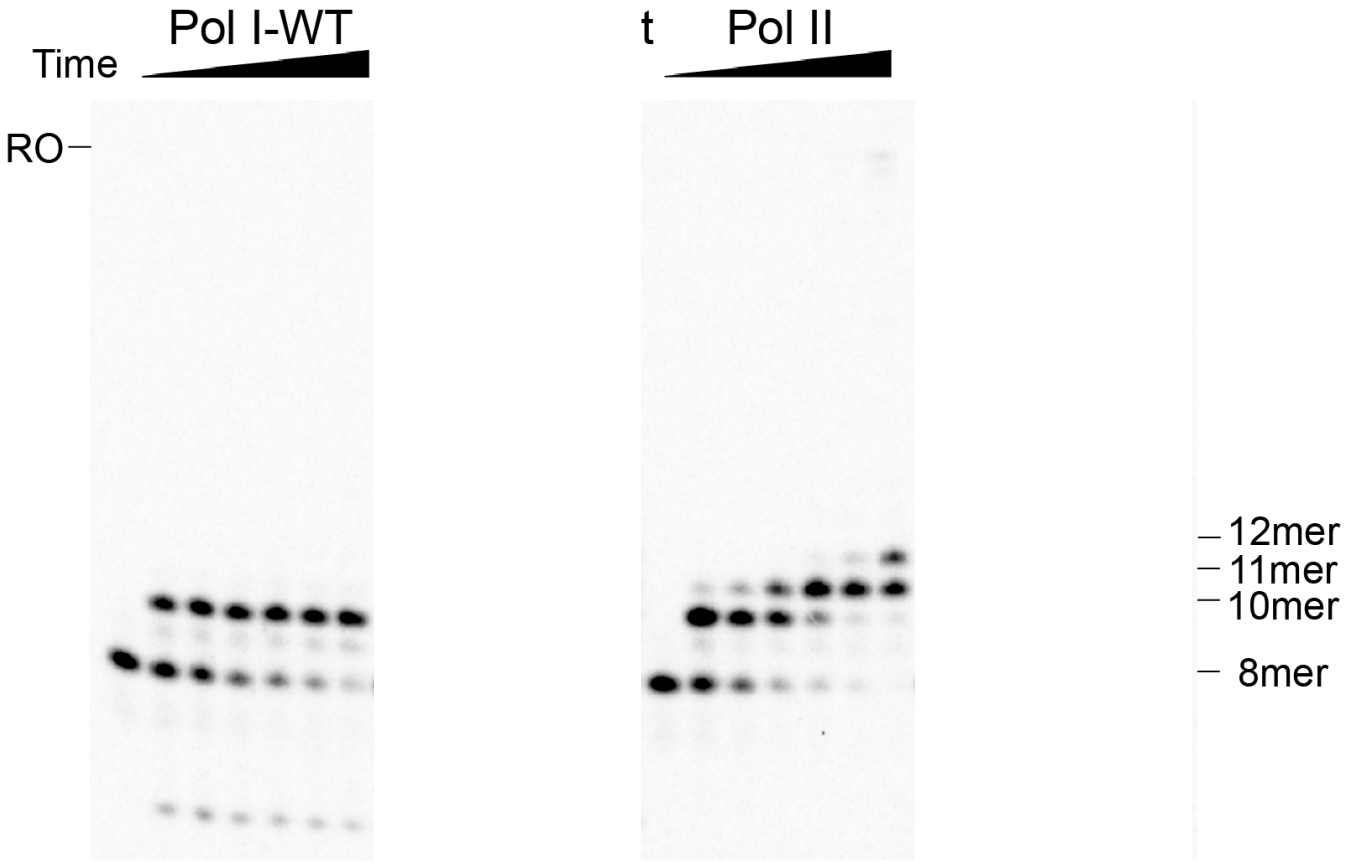




Intrinsic RNA cleavage in Pol I is critical for firm stalling

A12

```
                                        TTTCATTCCTACTC
NTS:5′ GCAGCCTAGTTGATCTCATA                            AGGAGAAGGAGCAGAGCG
TS:3′ CGTCGGATCAACTAGAGTATCGGTAGCTCTCCTTCCTCTTCCTCGTCTCGC
       RNA(8mer):     *5′AUCGAGAG   TT=CPD
```



Pol I efficiently detects UV light-induced DNA lesions

Foto: Germán M. Bretones

Pol I effciently stalls at CPD lesions

- Bridge helix R1015 slows bypass
- Intrinsic RNA cleavage activity

Cell protection against UV-light damage

Sanz-Murillo… Fernández-Tornero (2018) *PNAS* 115:8972

Conclusions

- Studying Pol I is of utmost interest to understand cell function
- Hibernation by dimerization is conserved in several RNA polymerases
- The mechanisms of DNA lesion detection by Pol I are unique
- Structural Biology data shed light on macromolecular function

Structure of Macromolecular Assemblies


Eva Torreira


Jaime Louro


Héctor Leal


Nicholas Taylor


Phong Nguyen

UC San Diego — Dong Wang

Turun yliopisto University of Turku — Georgyi Belogurov

ibfg — Olga Calvo

IRB Barcelona — Oriol Gallego

Margarita Salas — Germán Rivas

Margarita Salas — Rodrigo Bermejo

Margarita Salas — Core Facilities

ALBA, ESRF, Soleil, Diamond synchrotrons

Gobierno de España — Ministerio de Ciencia e Innovación

Agencia Estatal de Investigación

Agence Nationale de la Recherche — ANR

CSIC — Consejo Superior de Investigaciones Científicas

instruct Integrating Biology

Fundación Ramón Areces

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