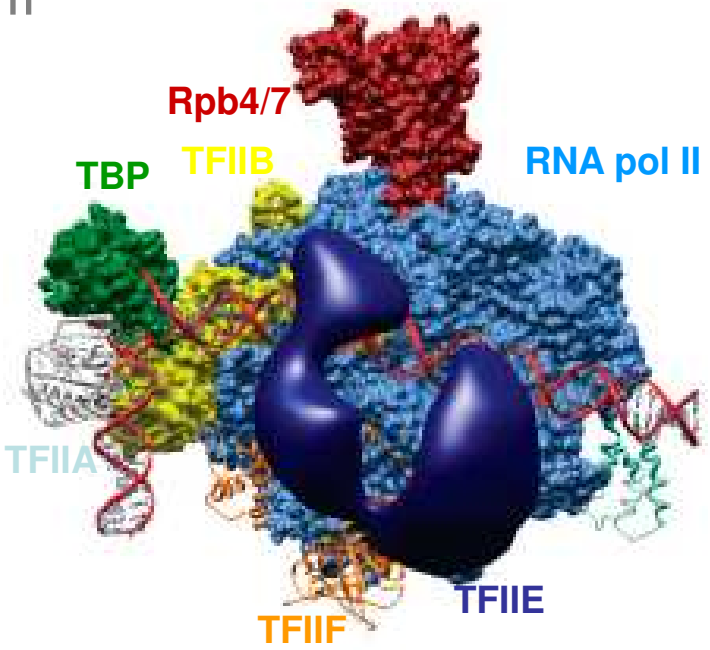
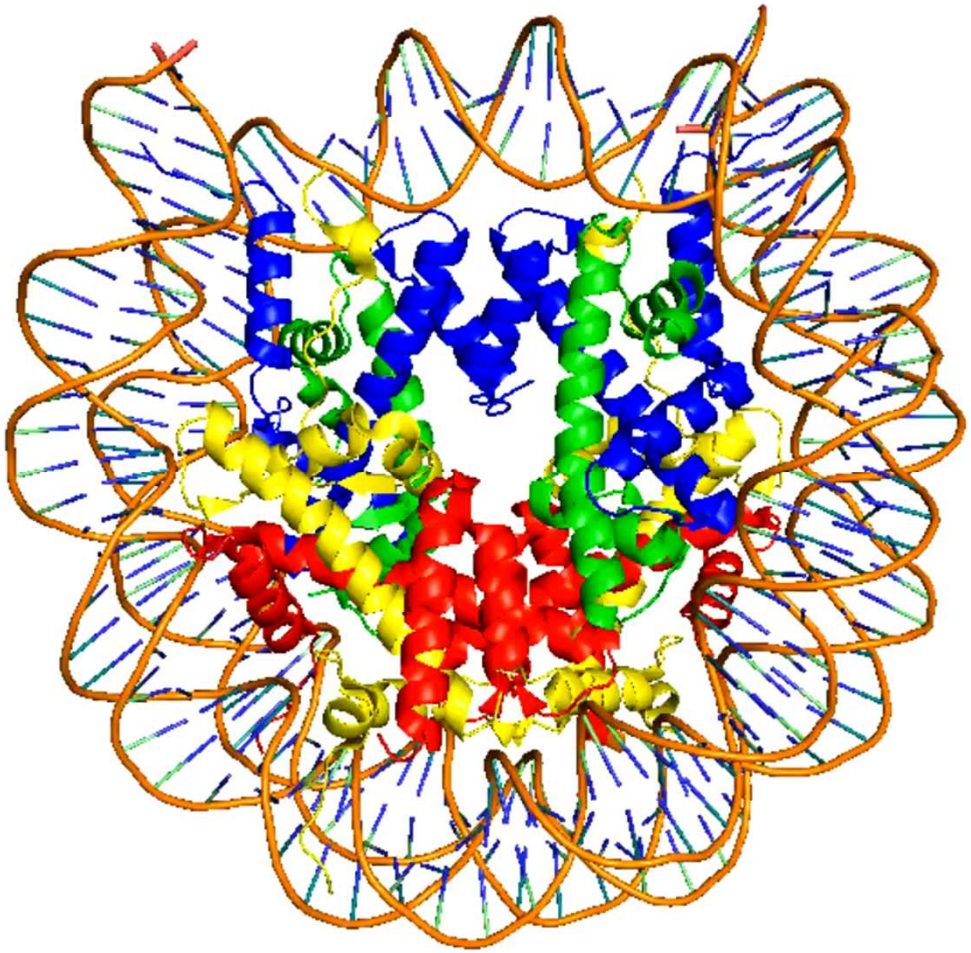
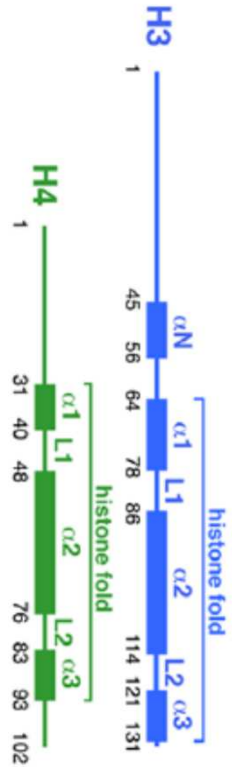
- **Zipper typ** – Jun-Fos
- **Helix-turn-helix** – H1, TALEN
- **Zinkový prst** – genome editing, GAL4
    - Velký žlábek DNA má velikost odpovídající rozměrům α-šroubovice a má exponované vazebné skupiny
    - Nejčastěji interaguje **Arg** (pozitivní náboj + vodíkové vazby)
- **Histon, HMG-box**
- **β-barrel**
- **β-hairpin/ribbon**
- **Smíšené α/β**



iniciace transkripce – skládání komplexů

- **histony** vážou DNA sekvenčně nespecificky
- histonové podjednotky (H2A, H2B, H3, H4) obsahují svazky 3-4 šroubovic skládaných proti sobě (**histon fold**)
- DNA se obtáčí kolem válcovitého oktameru (2x4 histony)
- šroubovice se vážou na cukrfosfátovou kostru DNA

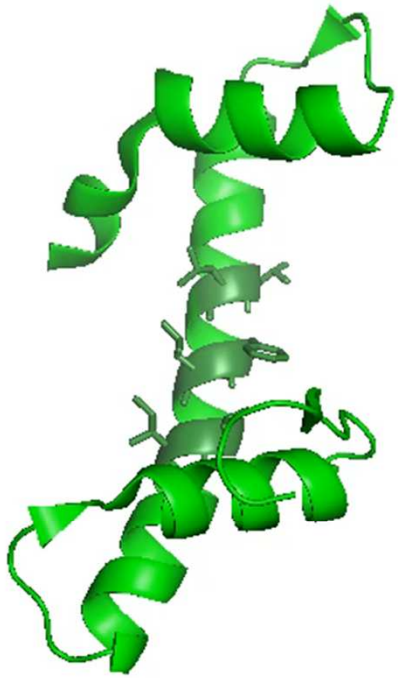




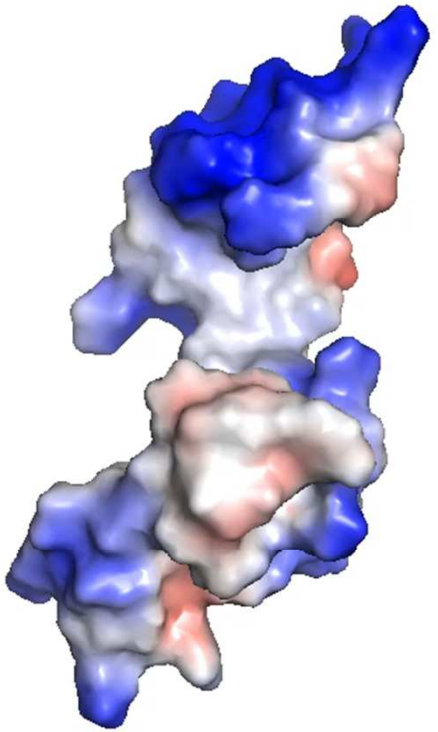
PDB: 3AFA

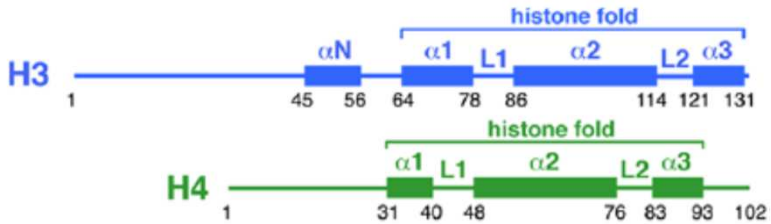
Histon fold H4

www.BANDICAM.com



www.BANDICAM.com

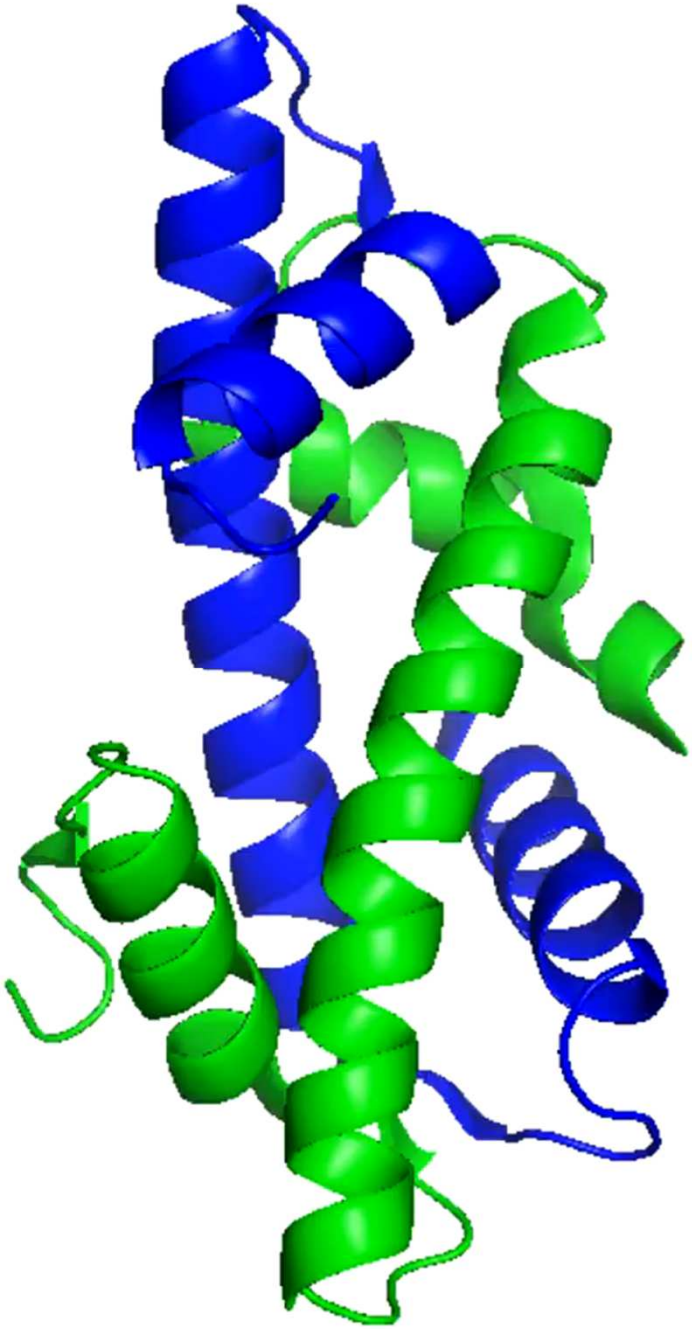


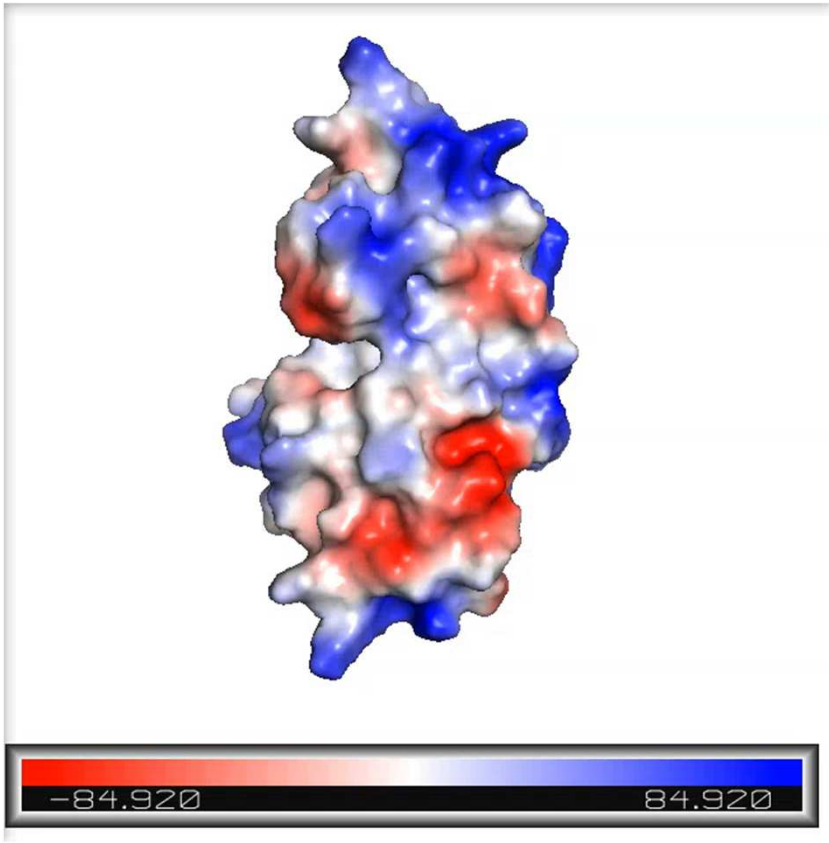


Povrch kapsy (kontakt) tvoří hydrofobní AMK

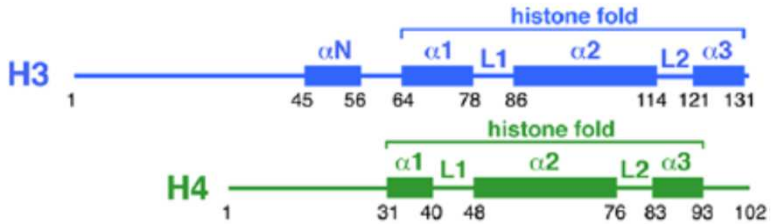
Histon fold

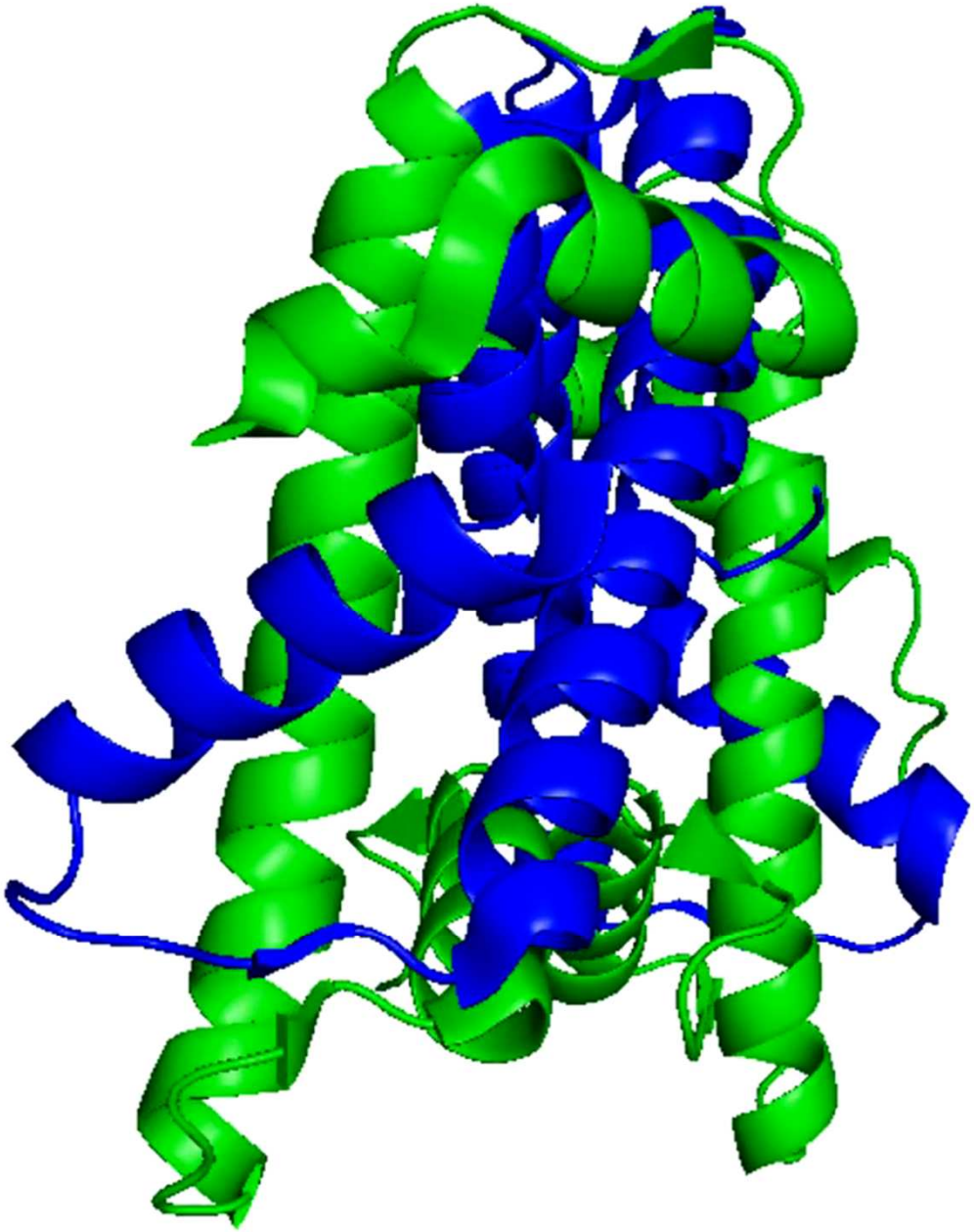
H3-H4 dimer



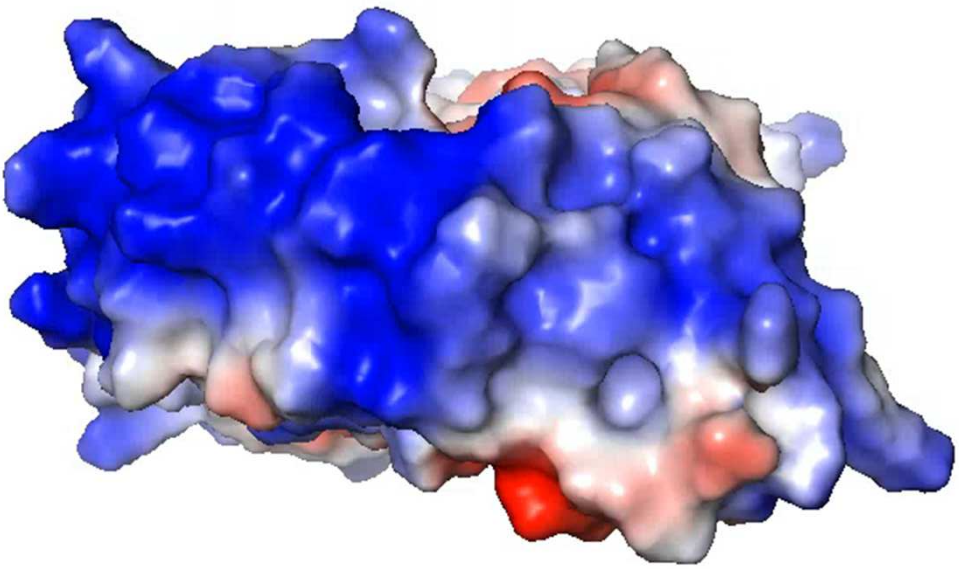


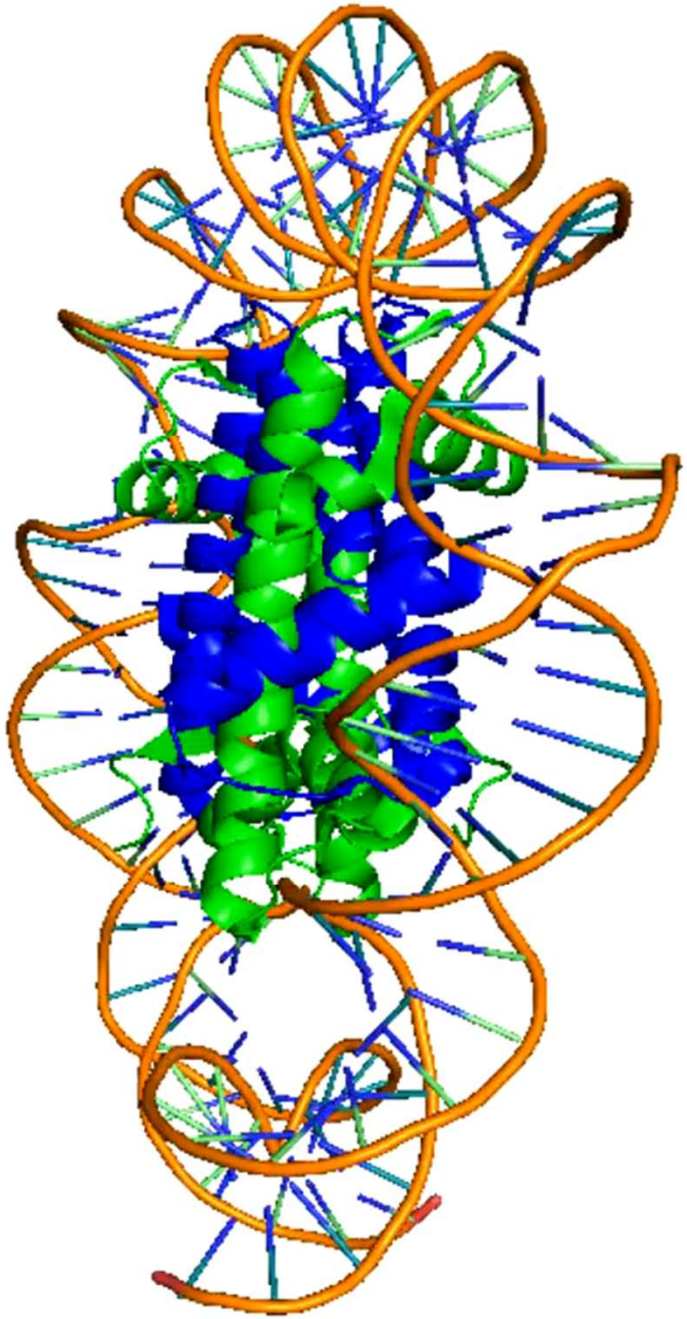
Povrch/kontakt tvoří lysiny a argininy



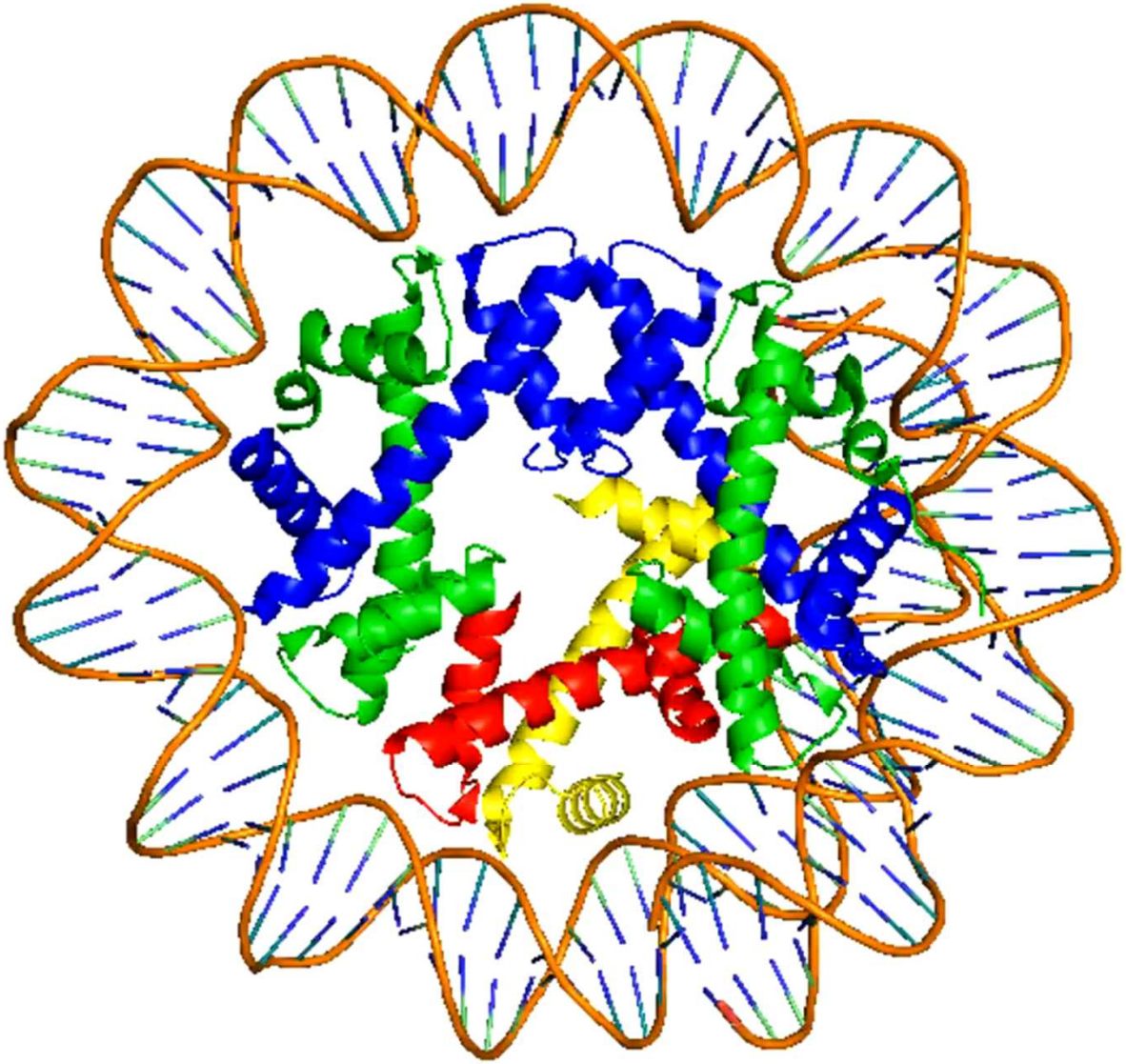


- H3-H3 interakce = $(H3\text{-}H4)_2$ tetramer

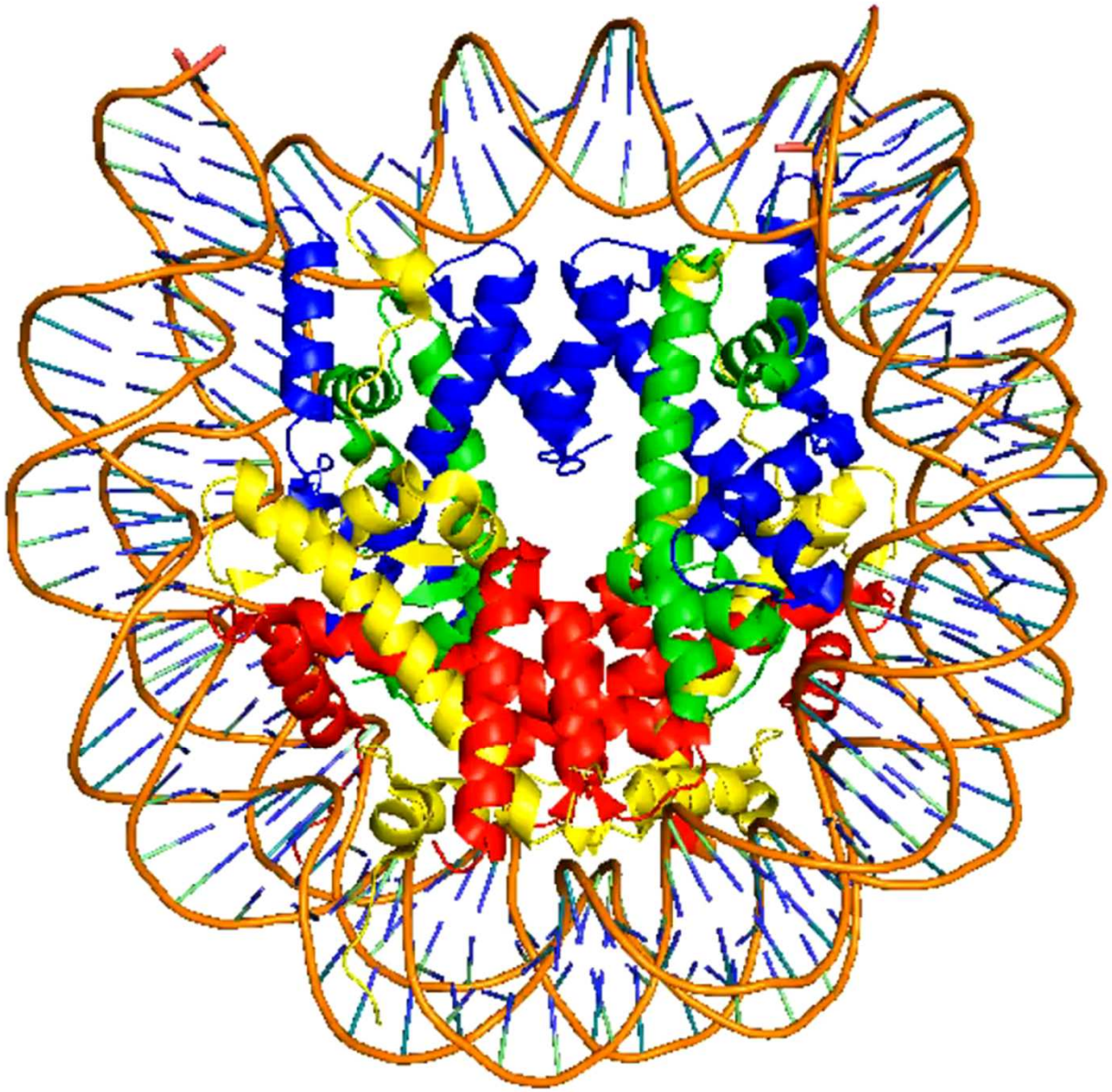




- H3-H3 interakce = $(H3\text{-}H4)_2$ tetramer – vazba na DNA



- H3-H3 interakce = $(H3\text{-}H4)_2$ tetramer
- dimery H2A-H2B (H2A – váže H4)



- H3-H3 interakce = $(H3\text{-}H4)_2$ tetramer
- dimery H2A-H2B (H2A – váže H4)

3AFA

Sestavování nukleozomu:

klíčová interakce mezi dvěma H3-H4 dimery je zprostředkována histonem H3

Dimery H2A-H2B se vážou následně z obou stran tetrameru $(H3\text{-}H4)_2$

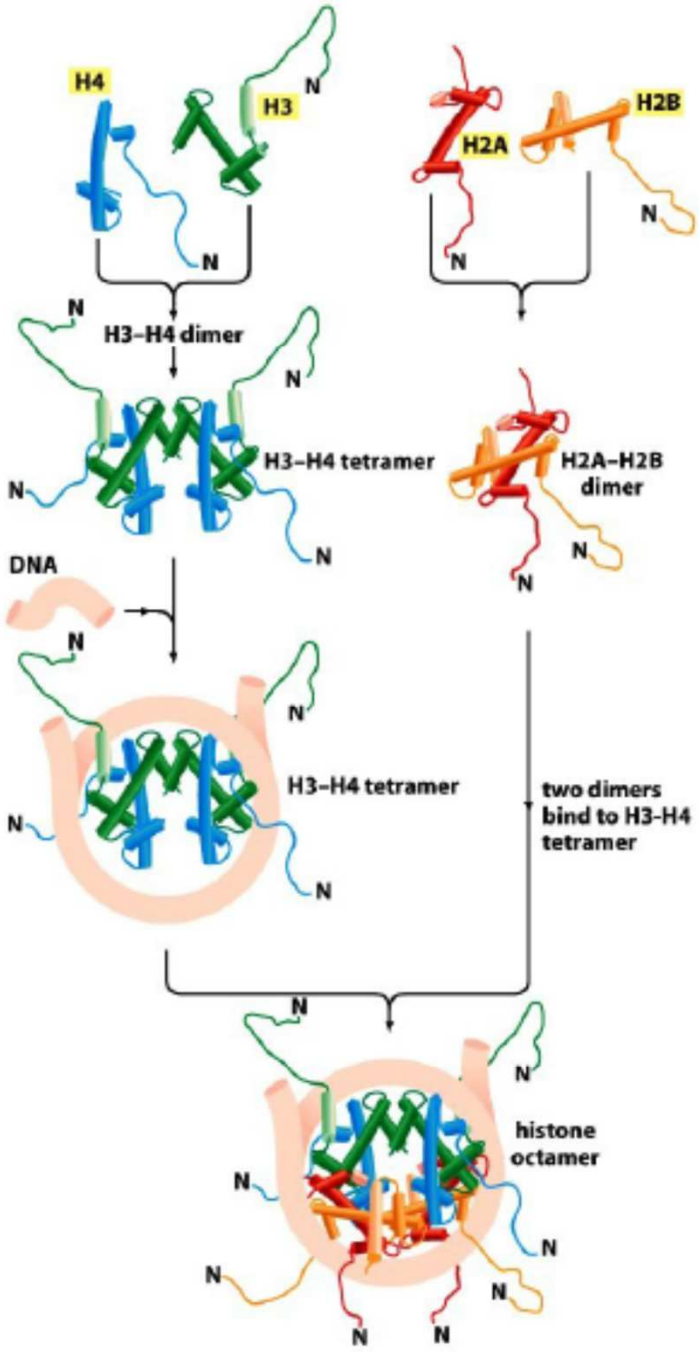
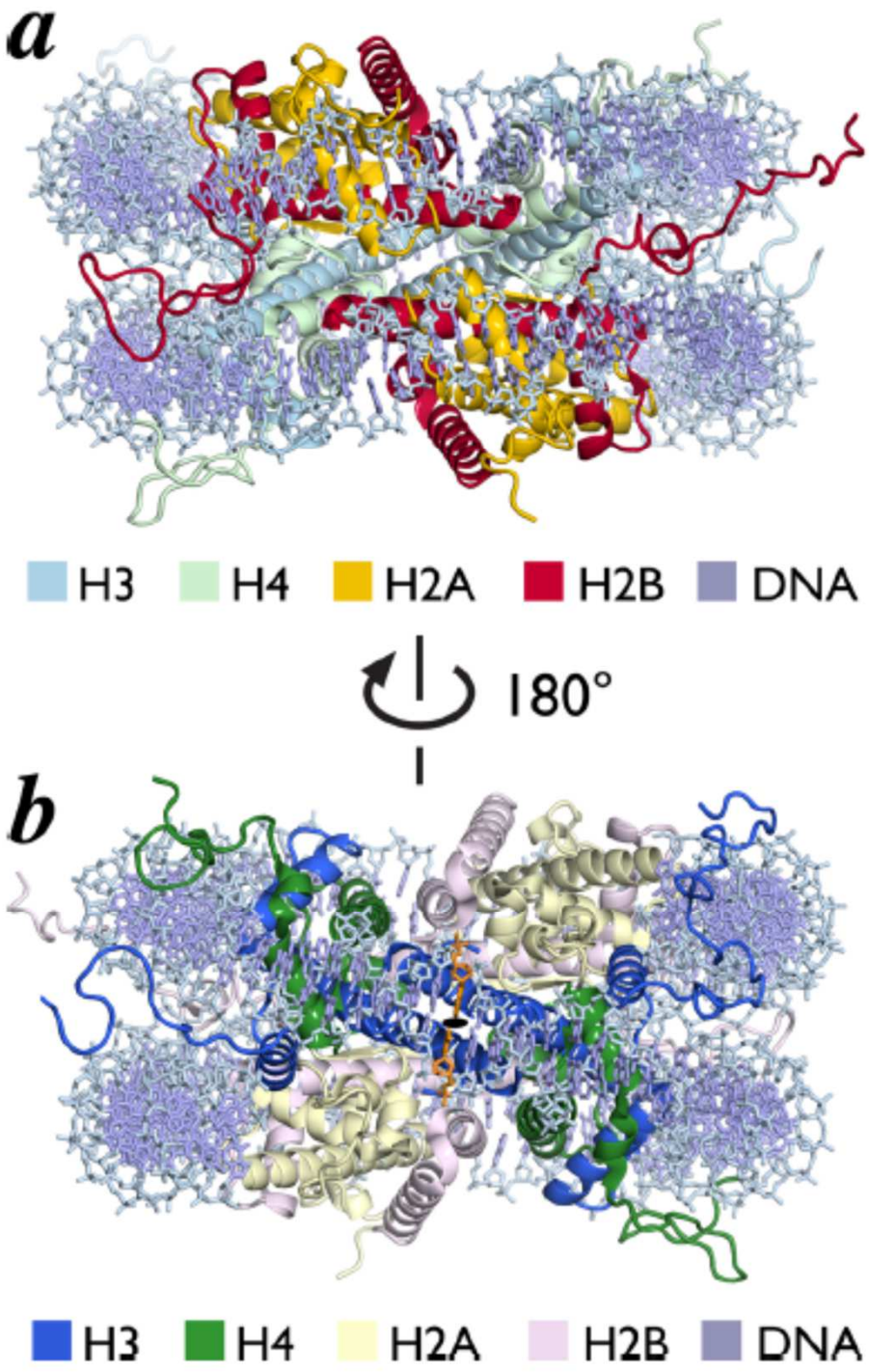
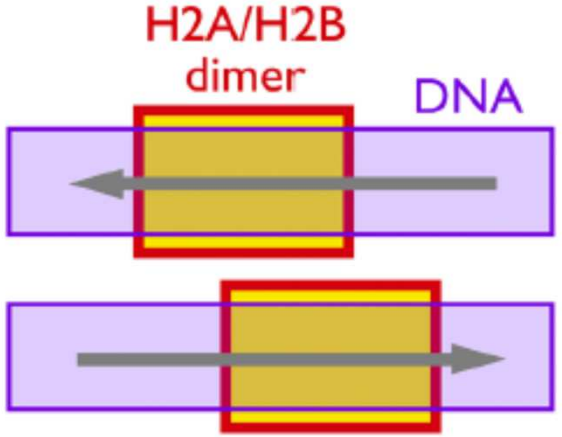
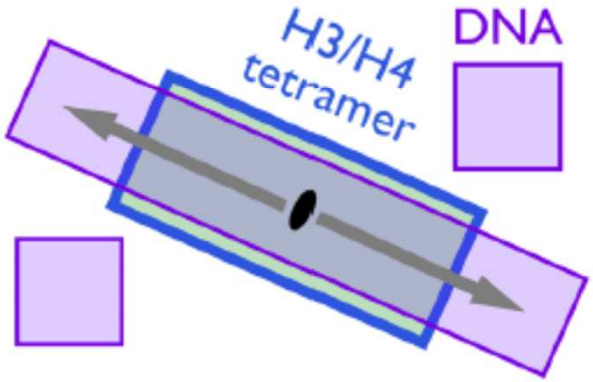


Figure 4-26 *Molecular Biology of the Cell* (© Garland Science 2008)





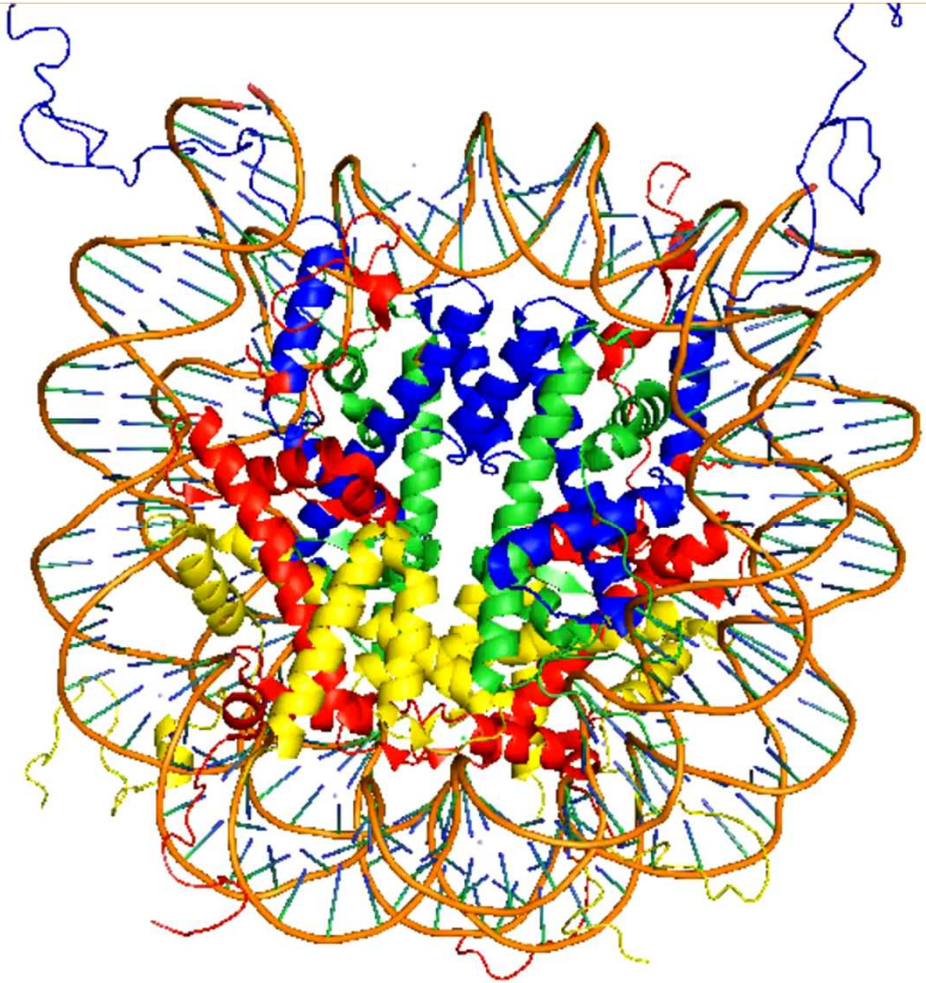
H2A/H2B váže dvě vlákna
H3/H4 váže jedno vlákno, ale silněji (více objímá – větší kontaktní povrch)

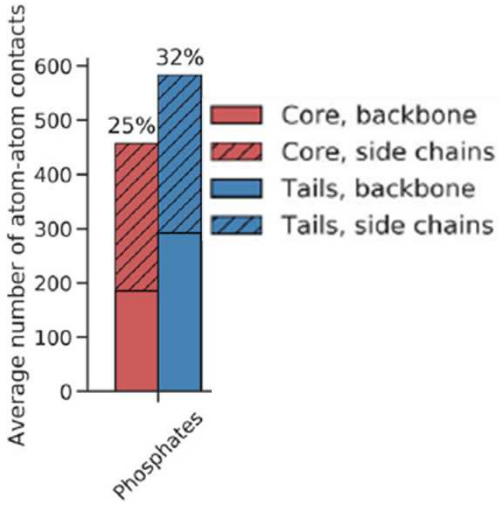


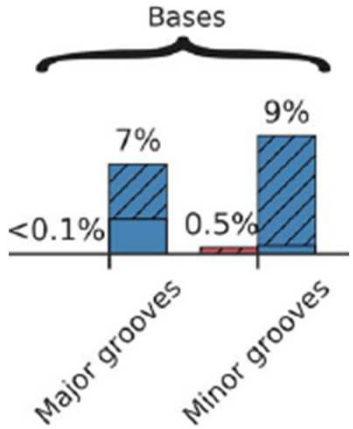
McGinty a Tan, Chem Rev, 2015

Konce histonů

- N- a C-konce histonů vybíhají z jádra nukleosomu a ovlivňují jeho vazbu
- konce vážou velký i malý žlábek

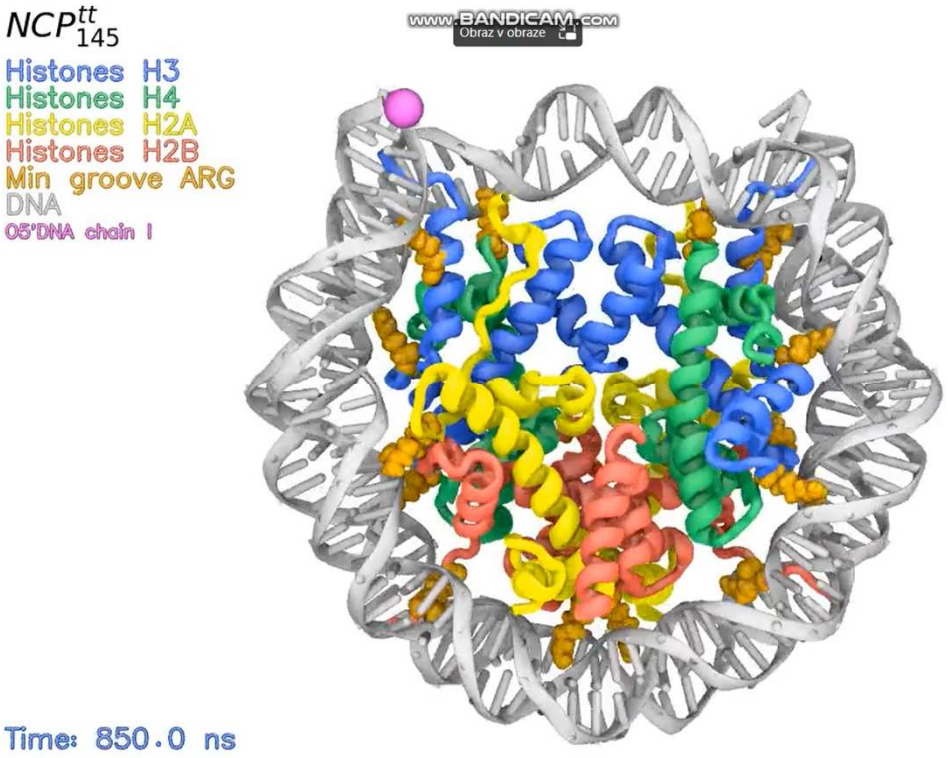






PDB: 1KX5

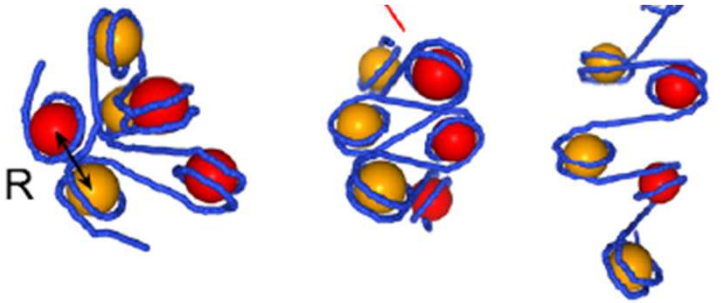
Armeev a spol, Nat Com, 2021



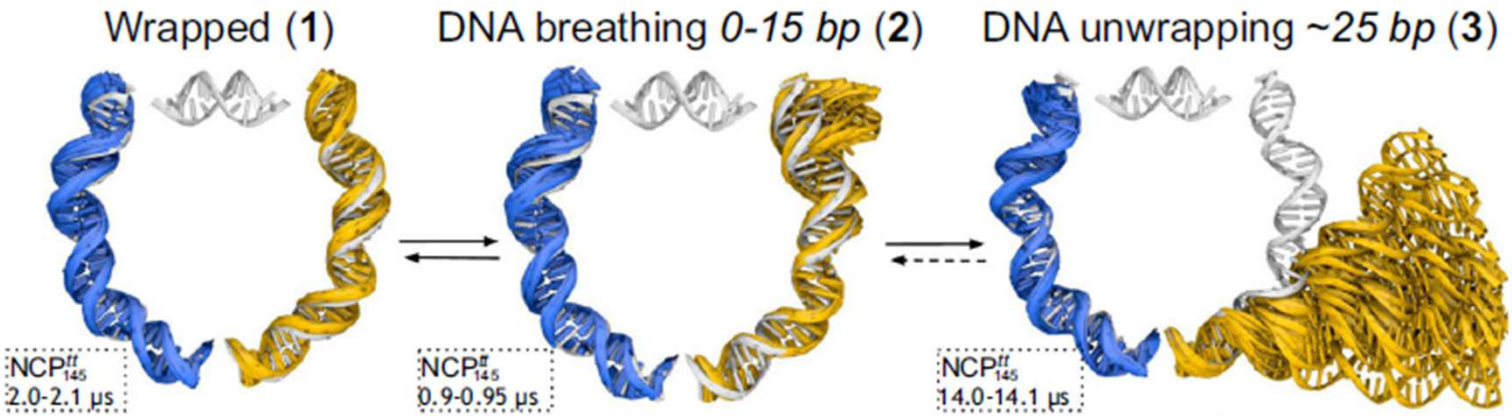
konce stabilizují komplex

MD: breathing - unwrapping (spíše se „odbalí“ při oslabení vazby konců)

i pouhý breathing má velký vliv na strukturu chromatinu

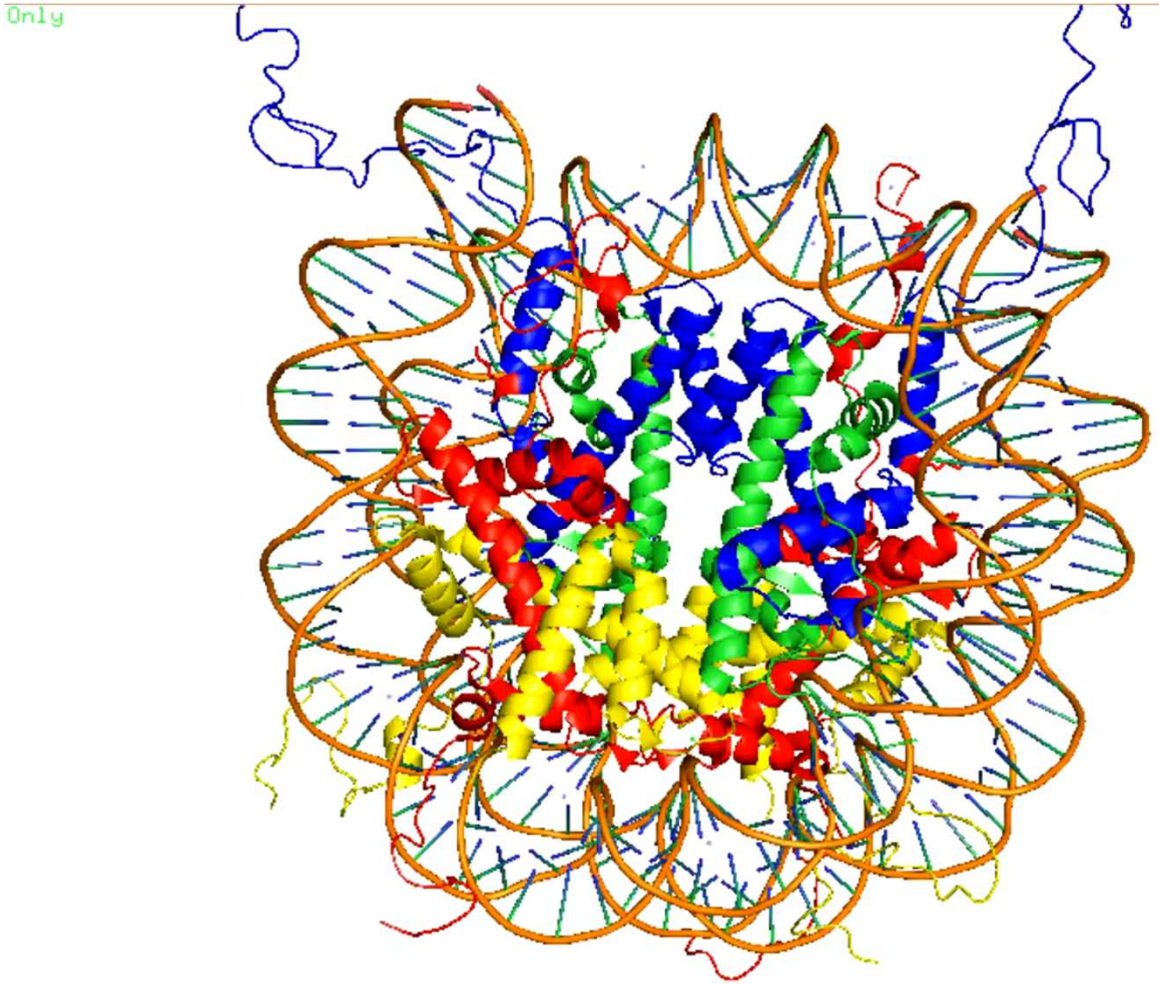


Armeev a spol, Nat Com, 2021

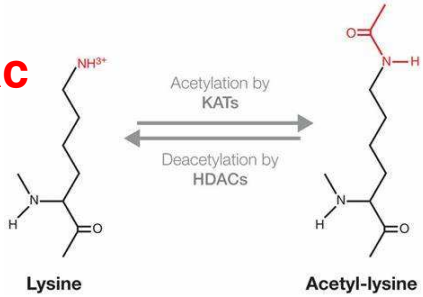


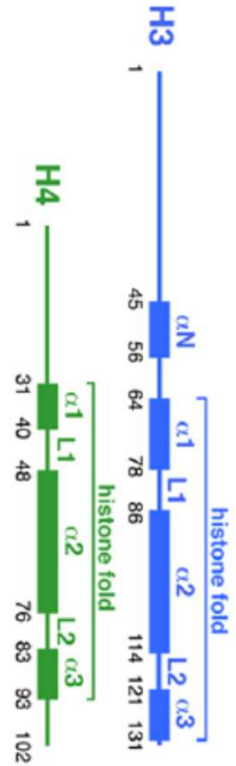
Konce histonů

- modifikace (např. acetylace) mohou oslabit interakce s DNA a rozvolnit nukleosom – mohou vázat jiné proteiny (např. bromodoména)



H3K56ac

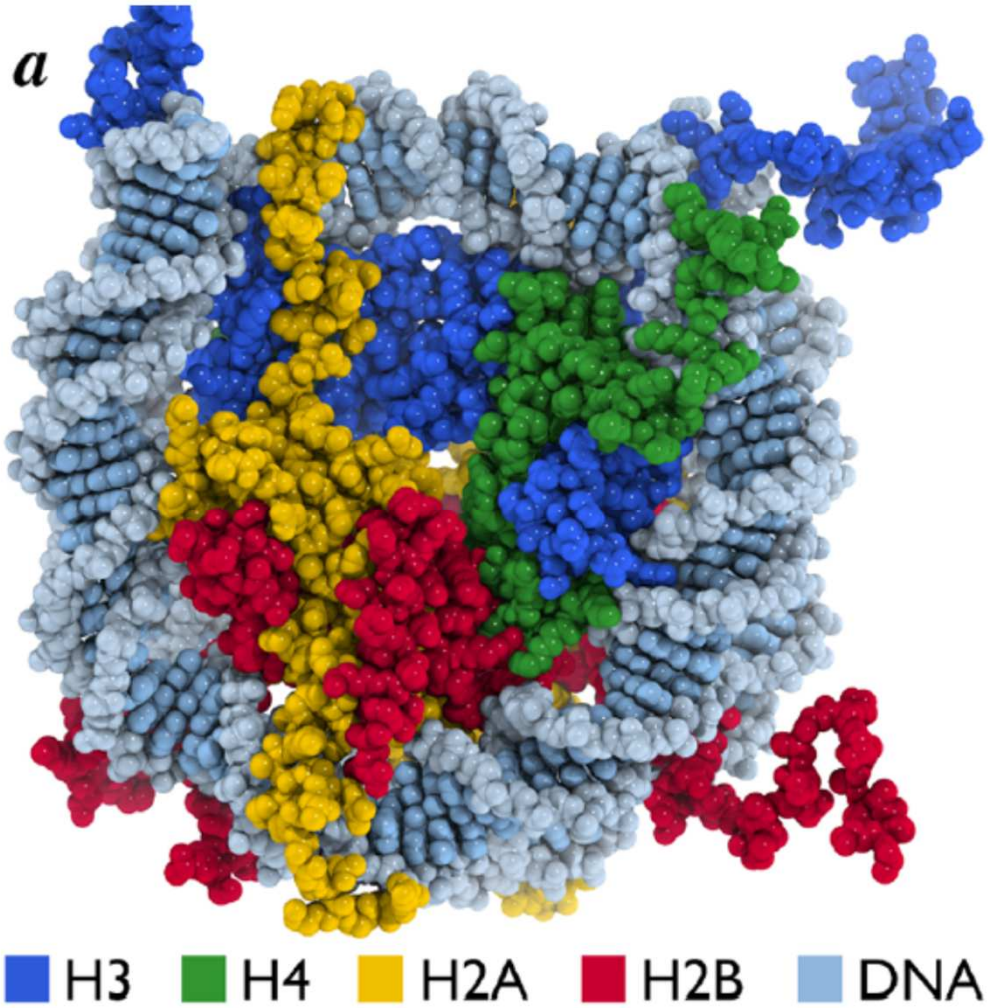




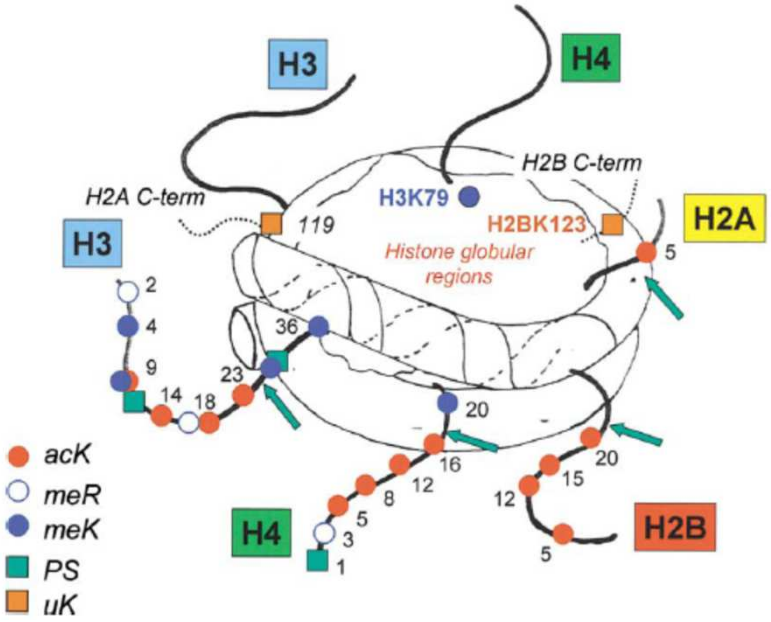
1KX5

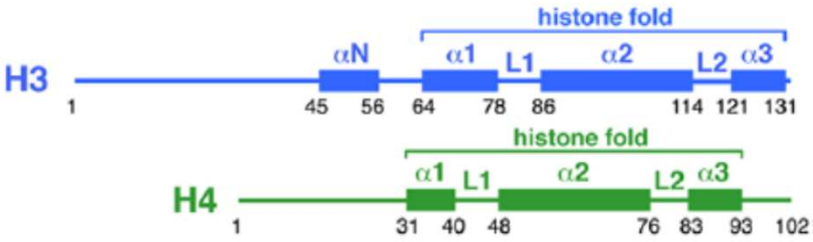
Konce histonů

- modifikace (např. acetylace) mohou oslabit interakce s DNA a rozvolnit nukleosom – mohou vázat jiné proteiny (např. bromodoména, chromodoména)



H3K56ac

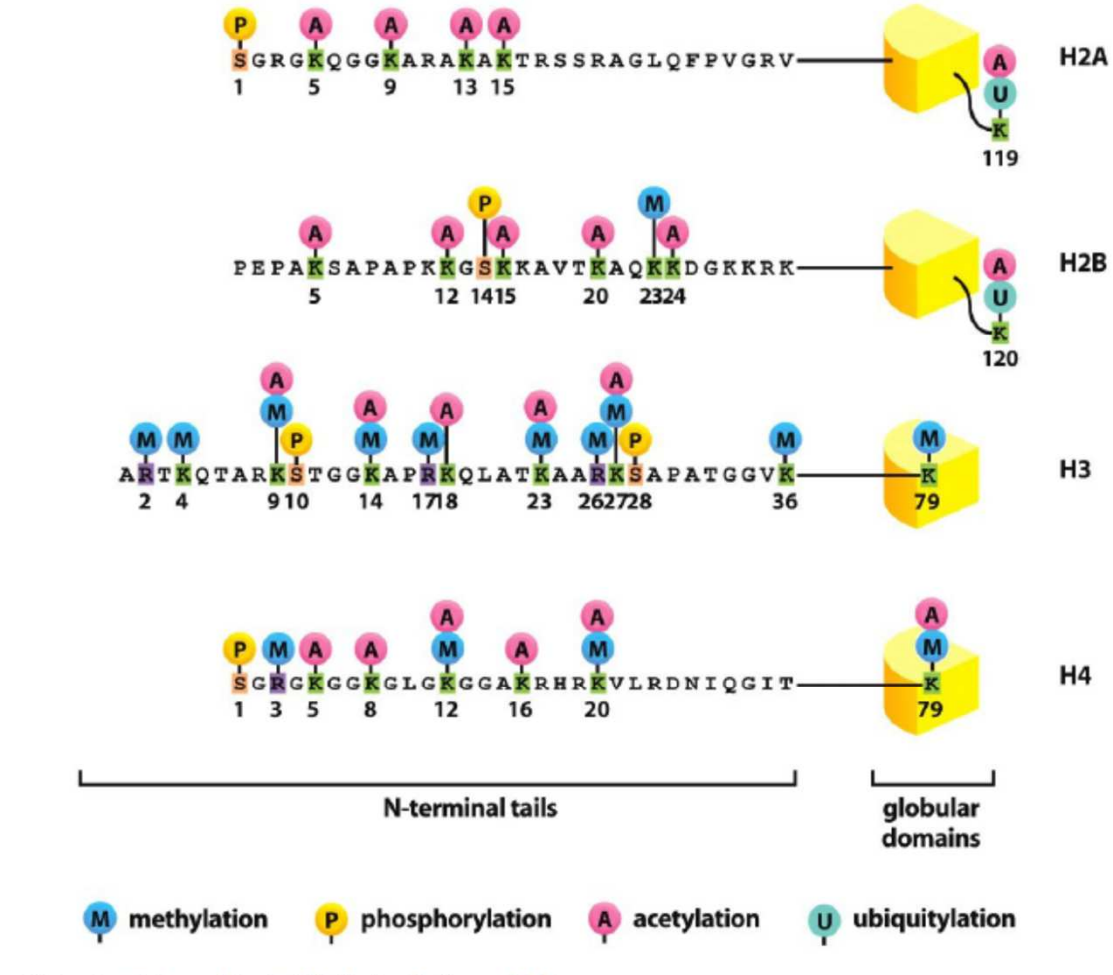




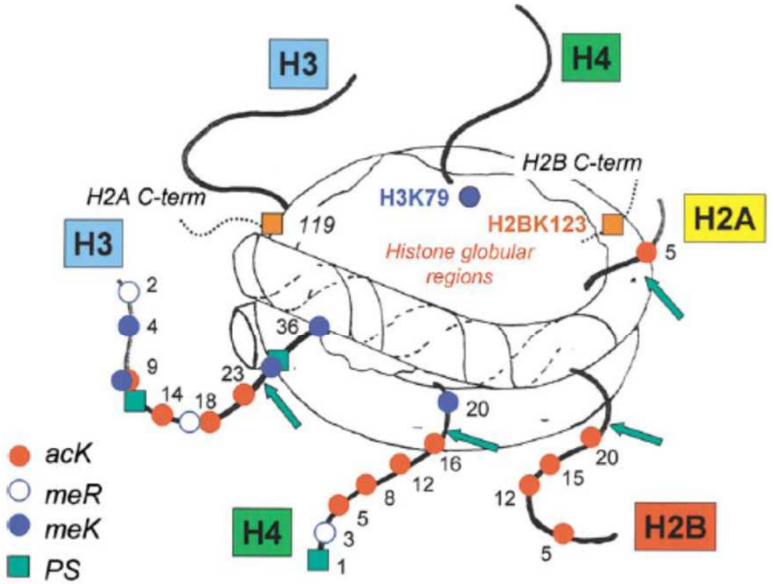
Modifikace histonů

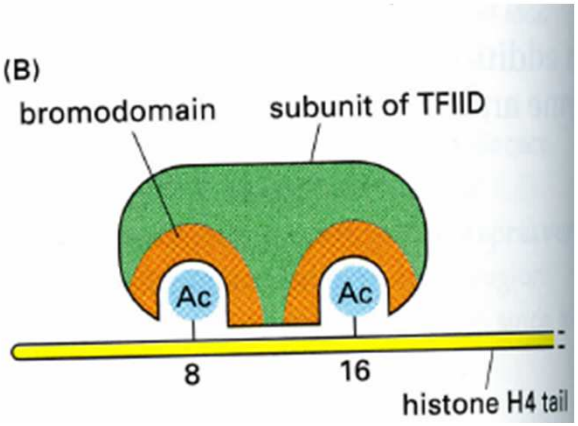
- např. acetylace lysinů (změna náboje) oslabí interakci s DNA a rozvolní vazbu oktameru (zpřístupní DNA pro TF, TBP … aktivaci transkripce)
- následně se TFIID váže na Ac-H4 (K8, K16) prostřednictvím bromodomény TAF1 proteinu (**reader/writer/eraser**)

H3K56ac



Molecular Biology of the Cell (© Garland Science 2008)



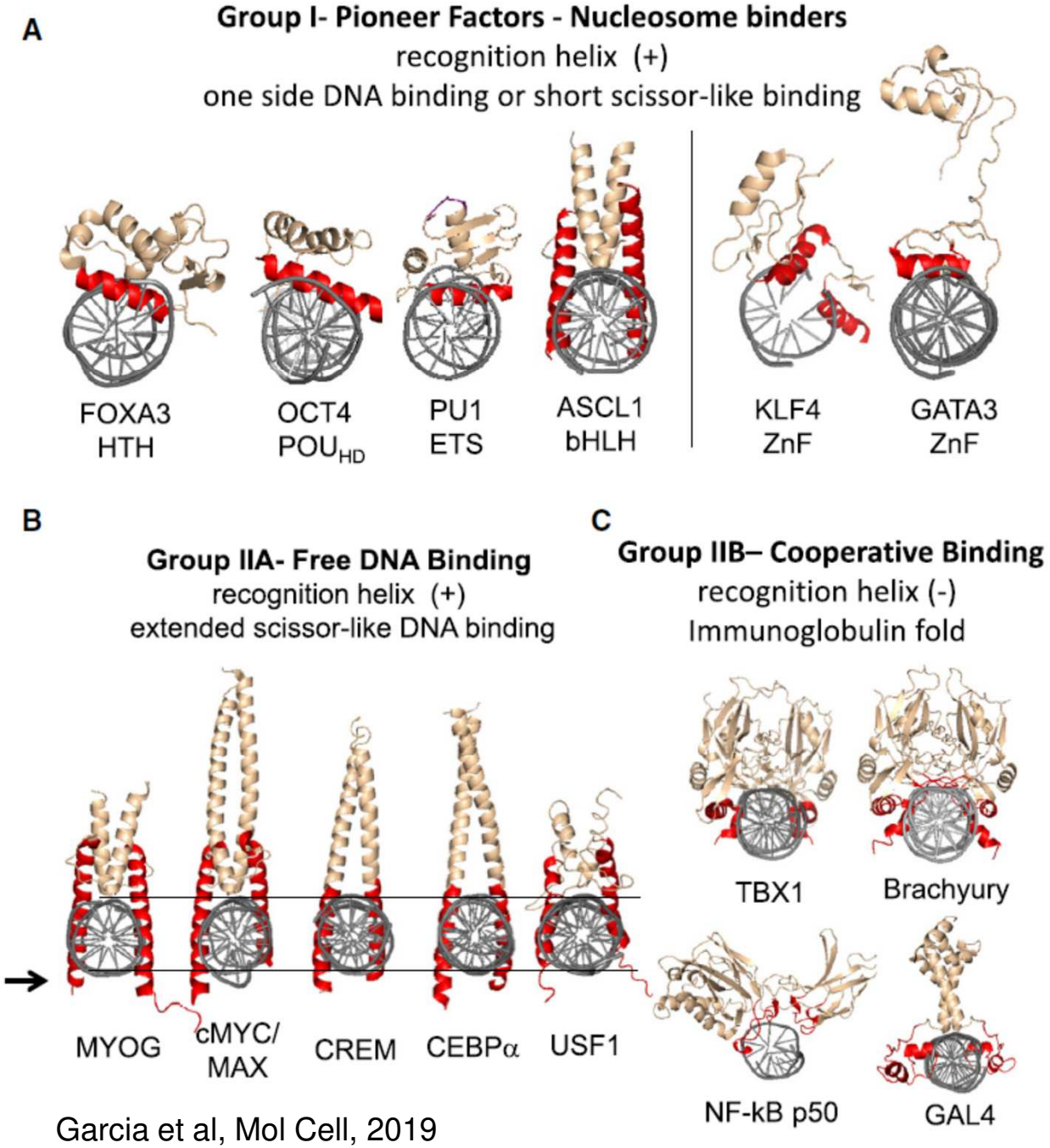


Pioneer TF

- přístupnost DNA v nukleosomu je zásadní pro aktivaci transkripce …

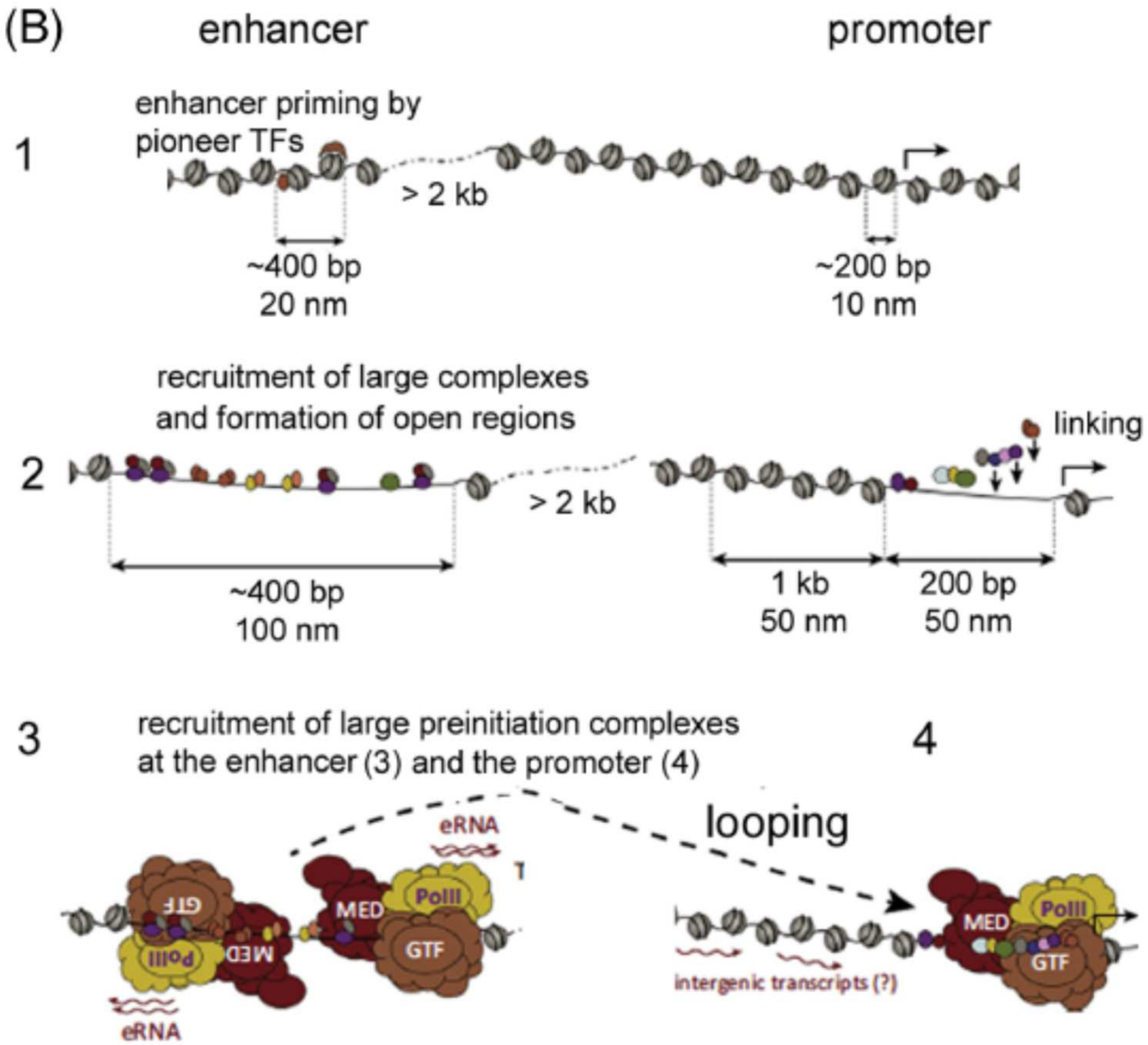
- pioneer TF se váže silně i v přítomnosti histonového oktameru (Group I) – krátké šroubovice

- Group II TF se vážou slaběji v přítomnosti histonového oktameru (ze sterických důvodů) – delší šroubovice a složitější vazebné motivy

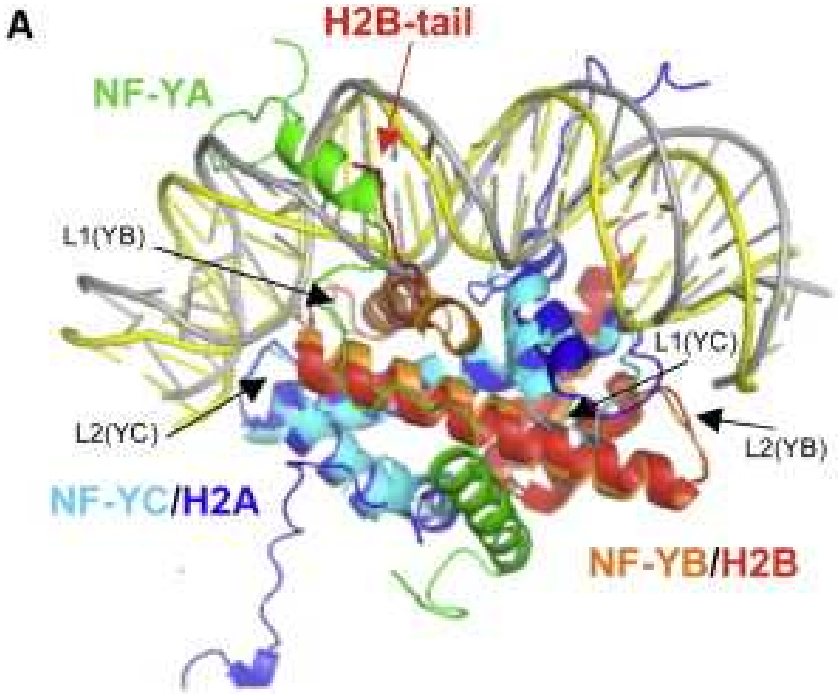


Garcia et al, Mol Cell, 2019

Pioneer TF se váže první a pomáhá dalším TF s vazbou …



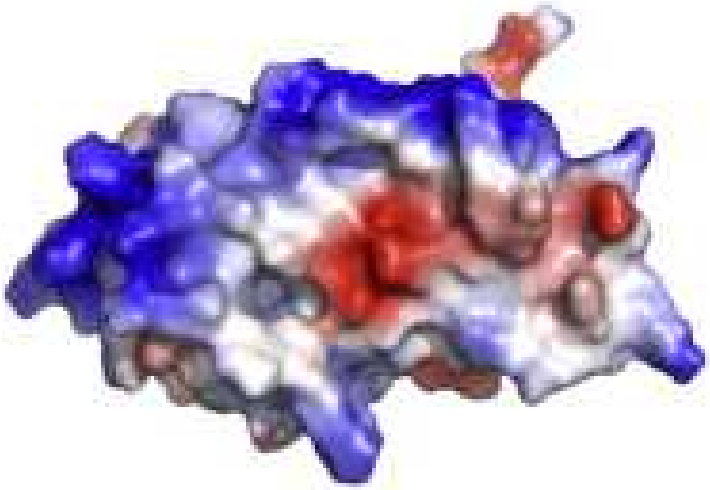
Cremer a Cremer, GCC, 2019



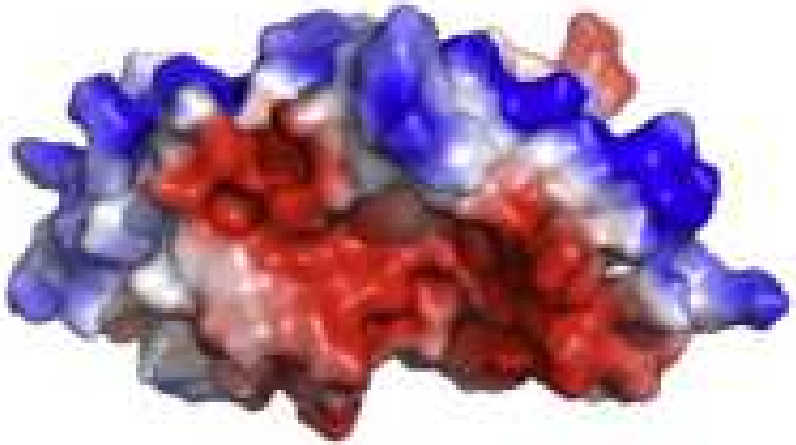
Transkripční faktory NF-Y

(podjednotky B a C) obsahují histon-fold a vážou se sekvenčně nespecificky na cukrfosfátovou kostru jako histony

- DNA se v důsledku vazby ohýbá podobně jako v nukleosomech
- NF-YA podjednotka rozeznává CCAAT sekvenci pomocí α-šroubovice (interkalace do MŽ)

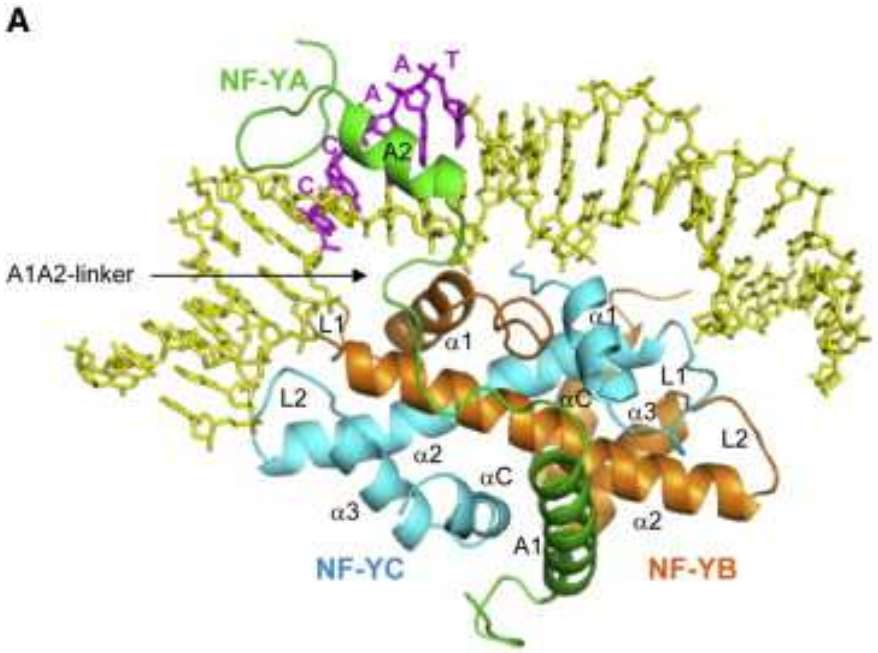


H2B/H2A

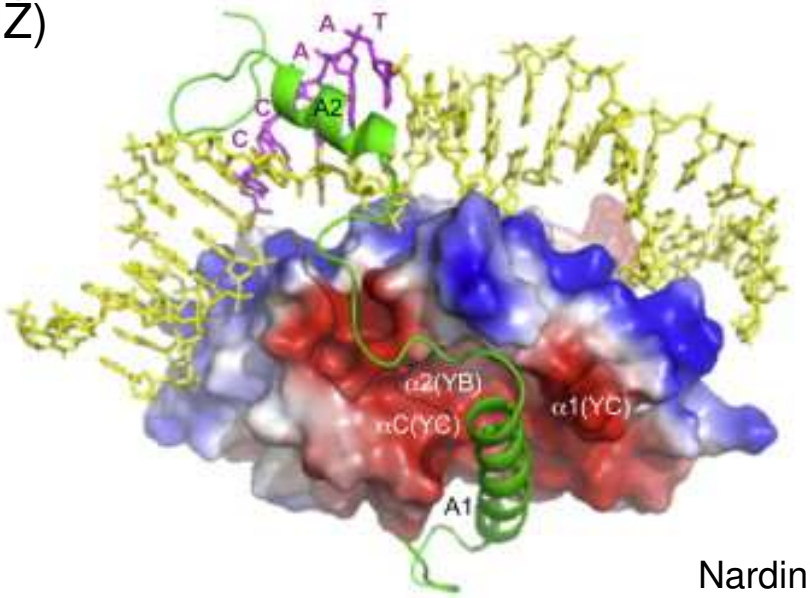


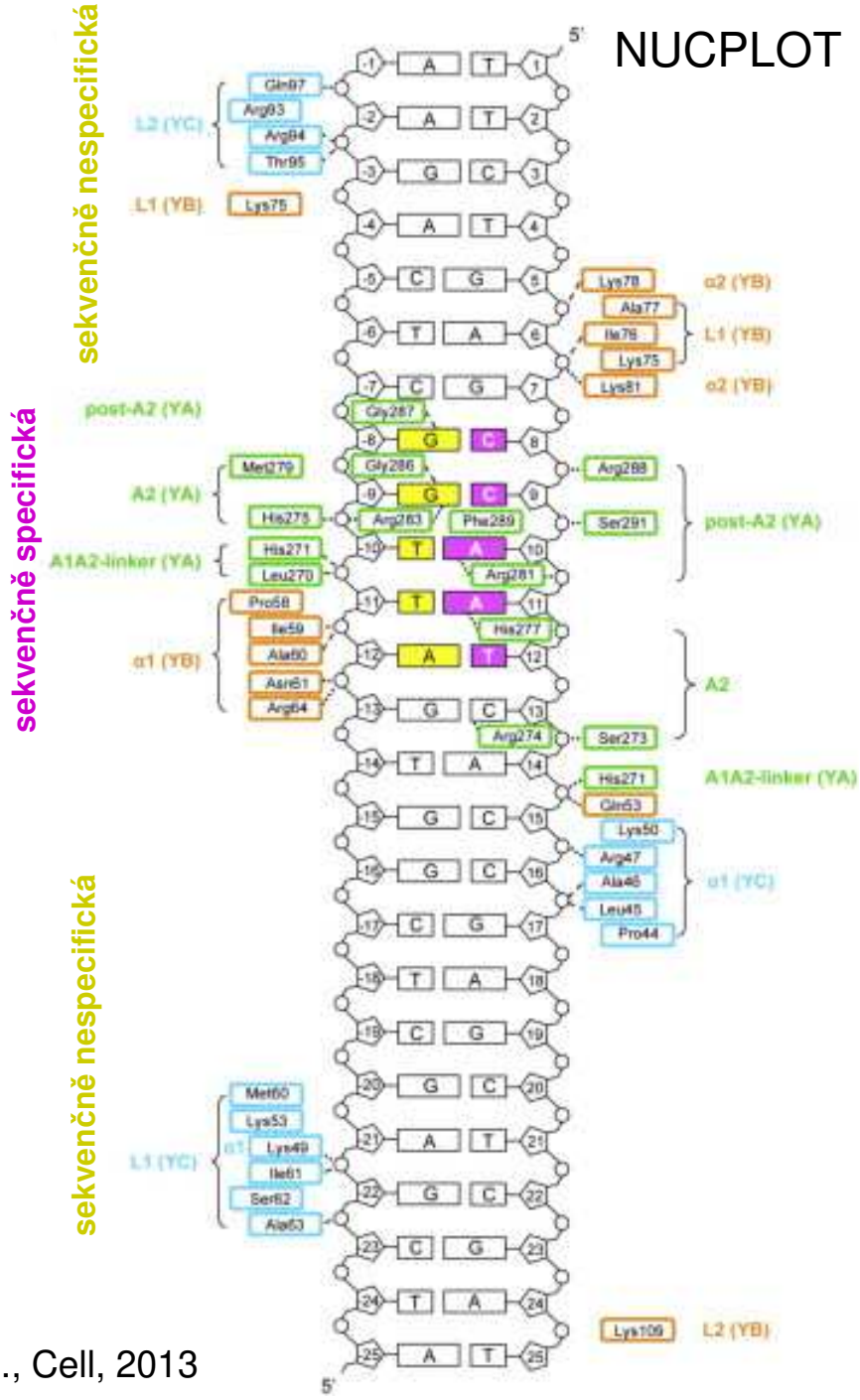
NF-YB/NF-YC

Nardini et al., Cell, 2013



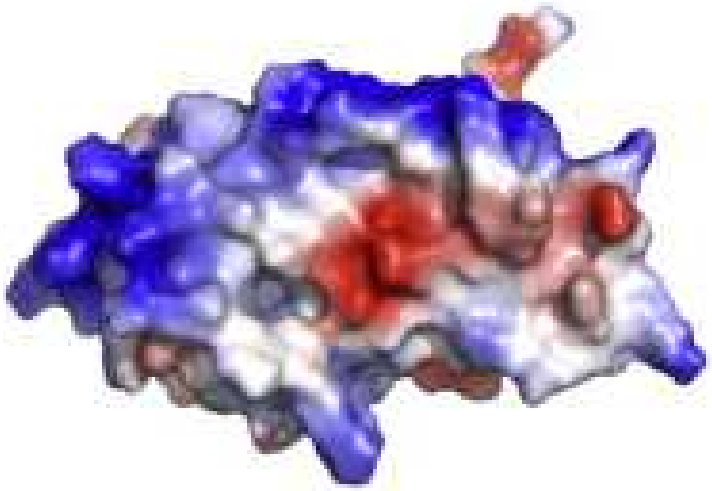
NF-YA podjednotka rozeznává CCAAT sekvenci pomocí α-šroubovice (interkalace do MŽ)



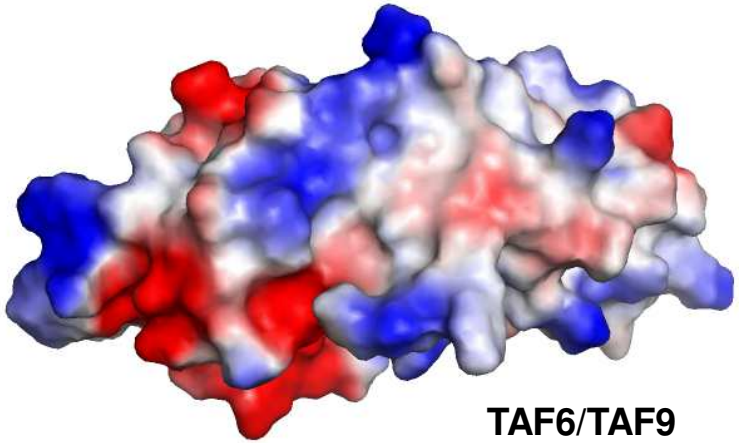


Nardini et al., Cell, 2013

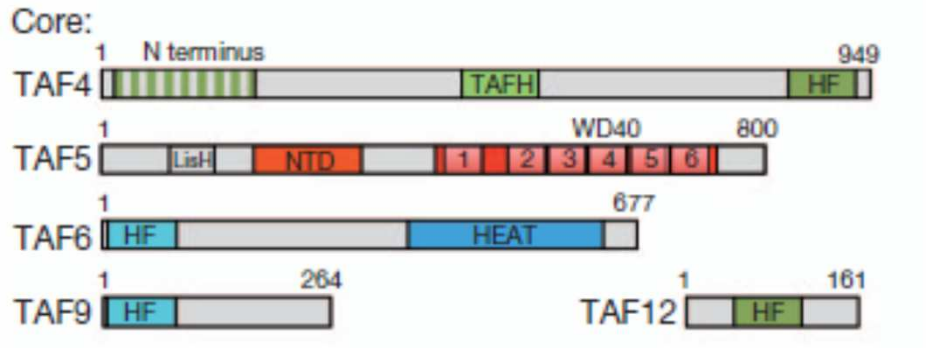
TBP–associated factors = TAF (podjednotky TFIID komplexu mají histon-fold (HF), ale nevytváří oktamery)

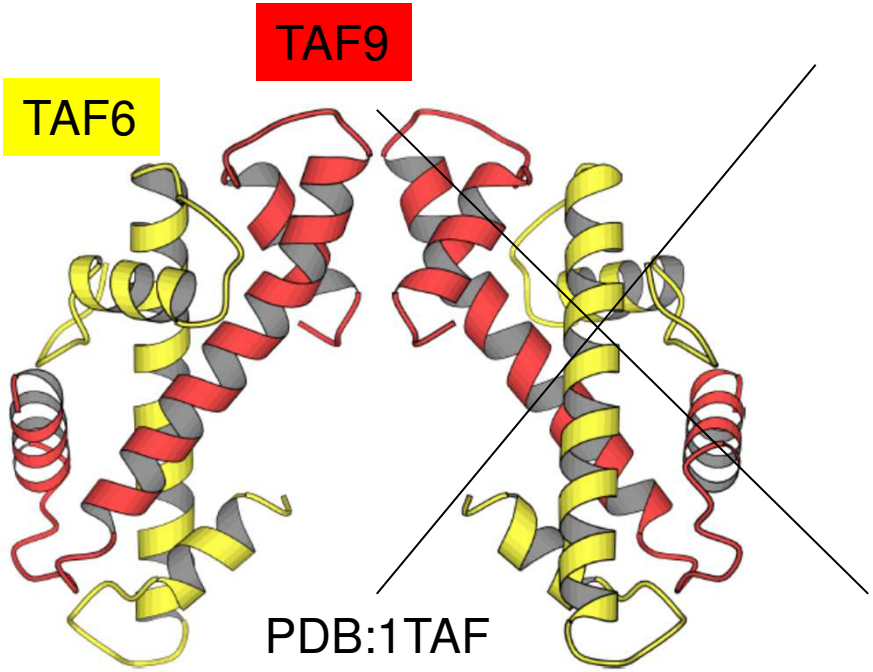


H2B/H2A



TAF6/TAF9

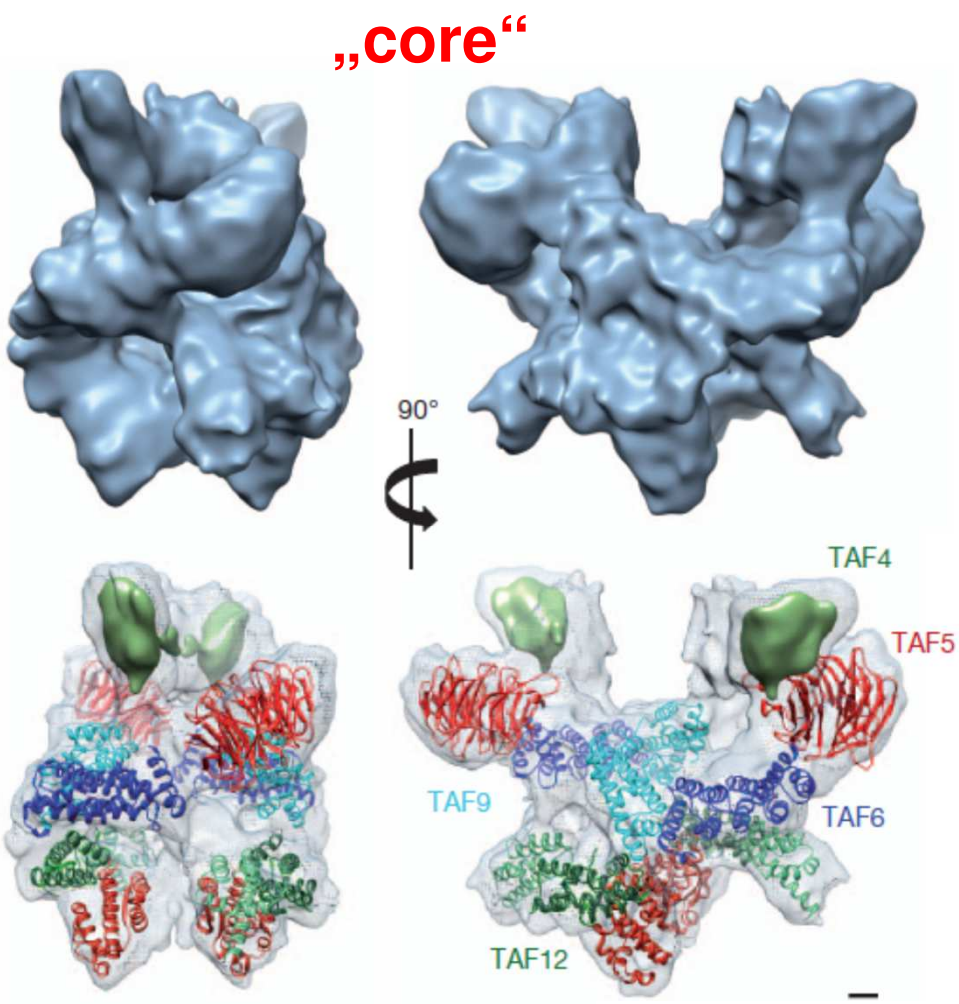


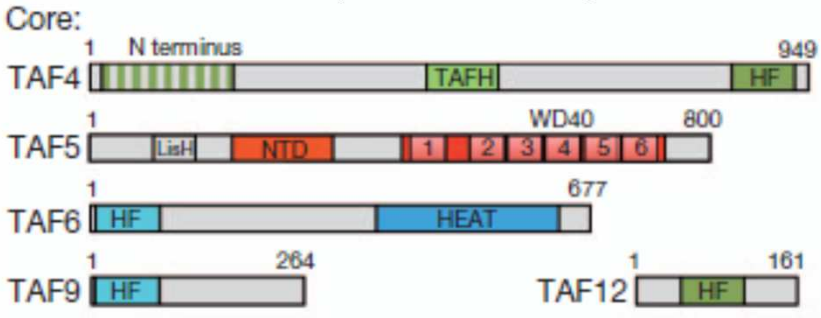


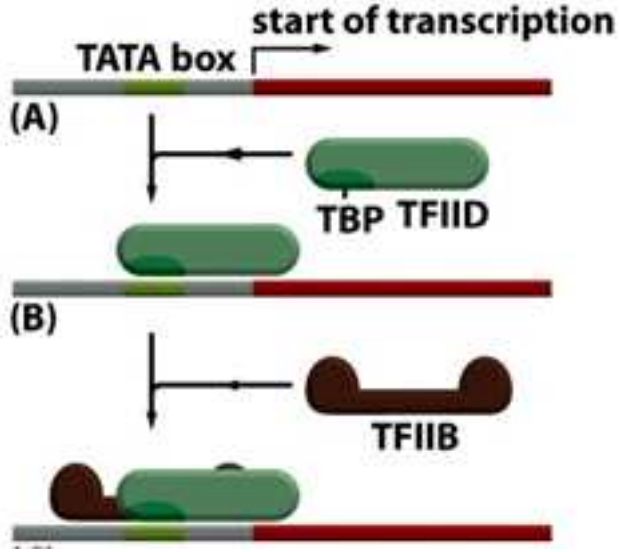
Histon-fold doména se neváže na DNA

Shao et al, MCB, 2005

- TAF4, 6, 9 a 12 obsahují histon-fold a jsou ve dvou kopiích (nikoli v dimerech) v „core“ části TFIID komplexu
- TFIID komplex a TBP jsou klíčové pro rozpoznání promotorů a poskytují scaffold/lešení pro sestavení transkripčního systému



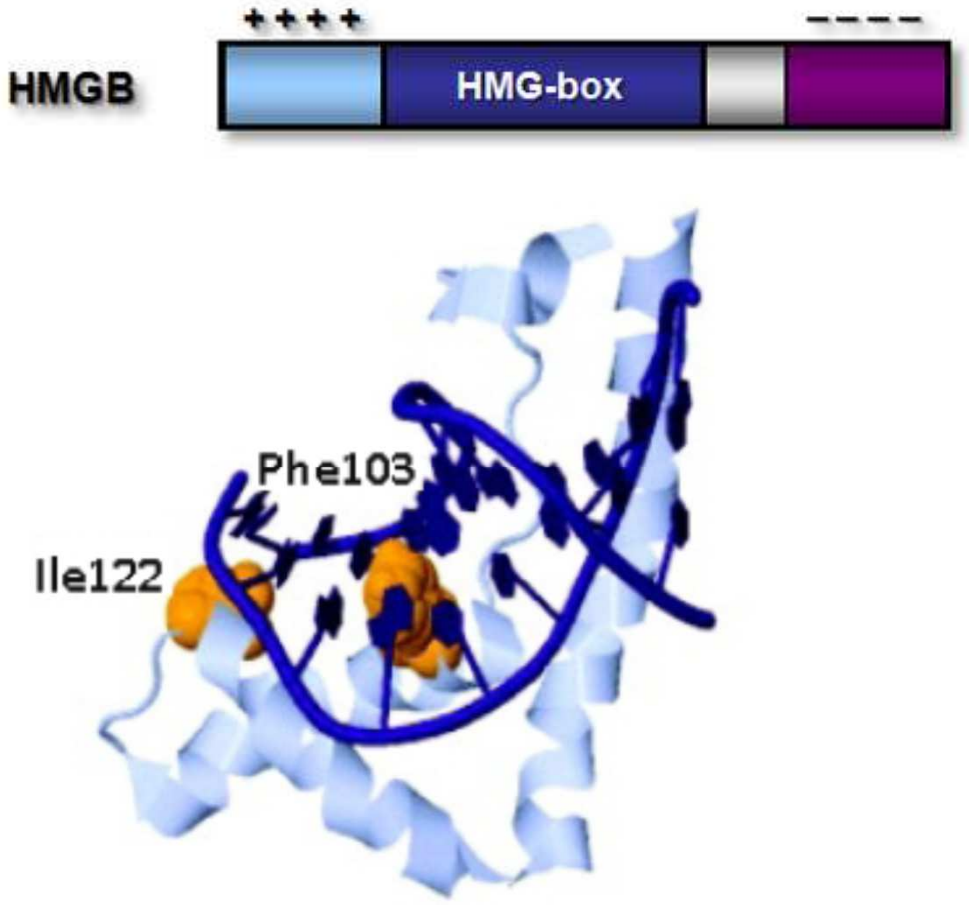




Bieniossek et al., Nature, 2013

spekulativní (viz Louder et al, Nature, 2016)

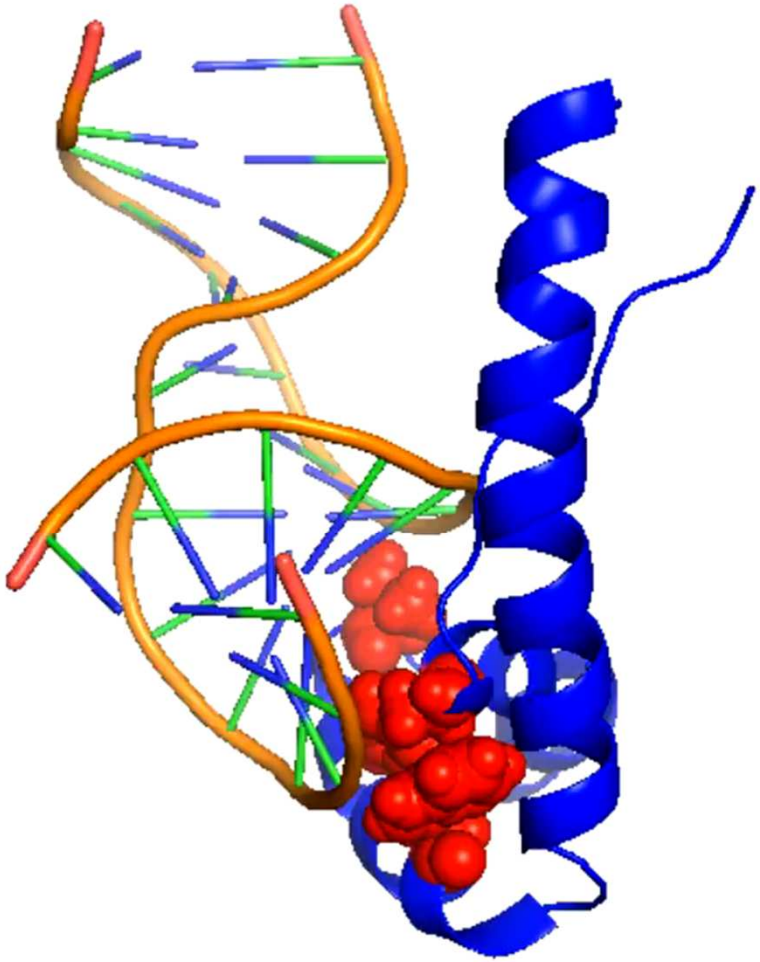
- **HMGB (HMG-box)** obsahuje 3 šroubovice uskupené ve tvaru L
- 1. a 2. šroubovice váže cukrfosfátovou kostru a báze v MŽ
- interkalace aromatických AMK způsobuje ohnutí DNA



- ohyb a rozvolnění párování dsDNA může napomáhat procesům replikace, transkripce a rekombinace/opravy poškozené DNA
- ohyb napomáhá přiblížení vzdálených míst nebo zpřístupňuje DNA pro transkripční faktory

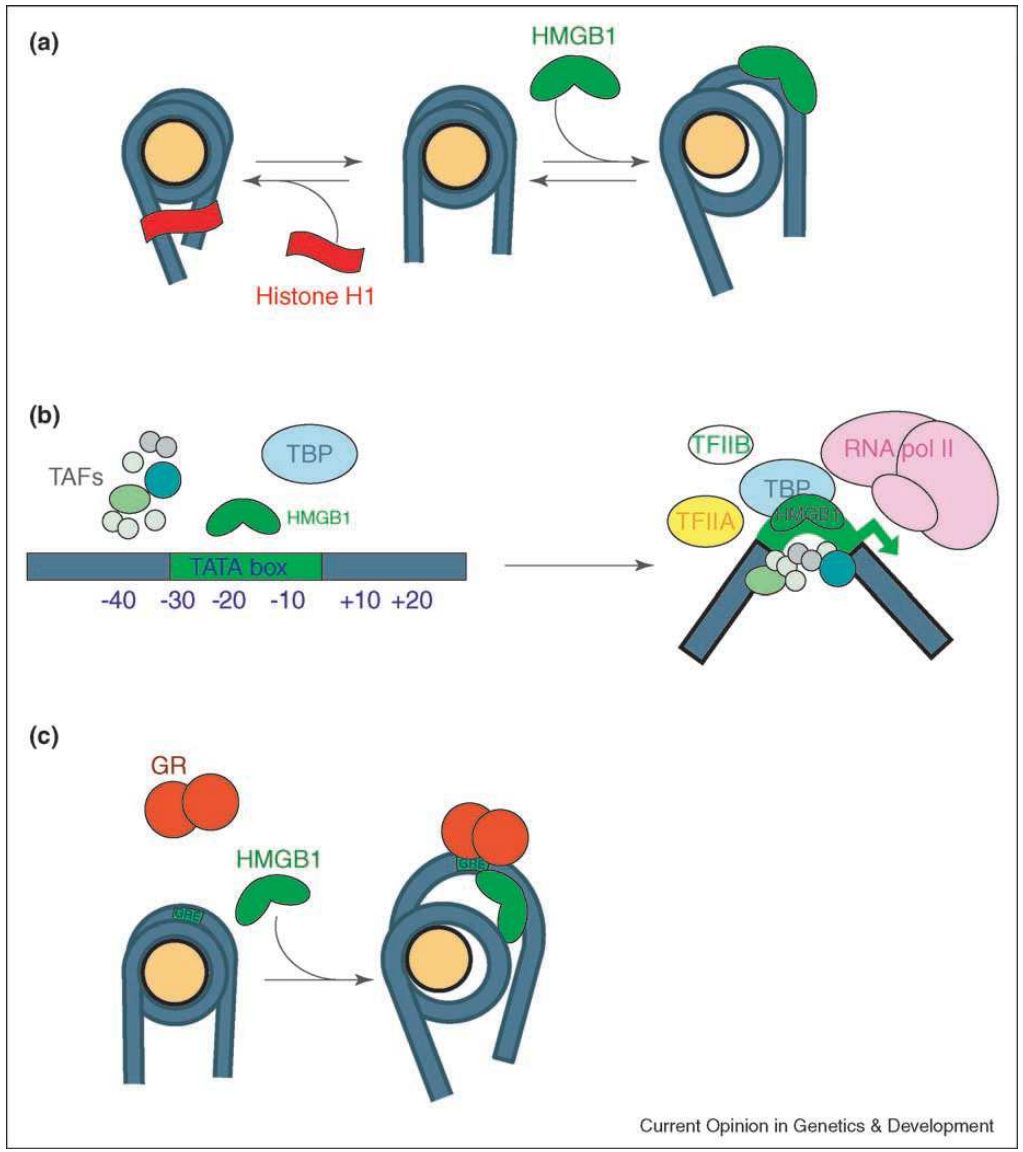
Štros, BBA, 2010

HMG-D (1E7J)



Aromatické aminokyseliny se interkalují do MŽ a vytváří kink

- 1. a 2. šroubovice váže cukrfosfátovou kostru a báze v MŽ
- interkalace Phe (aromatický kruh) způsobuje ohnutí DNA



- ohyb moduluje vazbu nukleosomu tj. napomáhá rozvolnění struktury chromatinu

- ohyb může pomáhat TBP a iniciaci transkripce

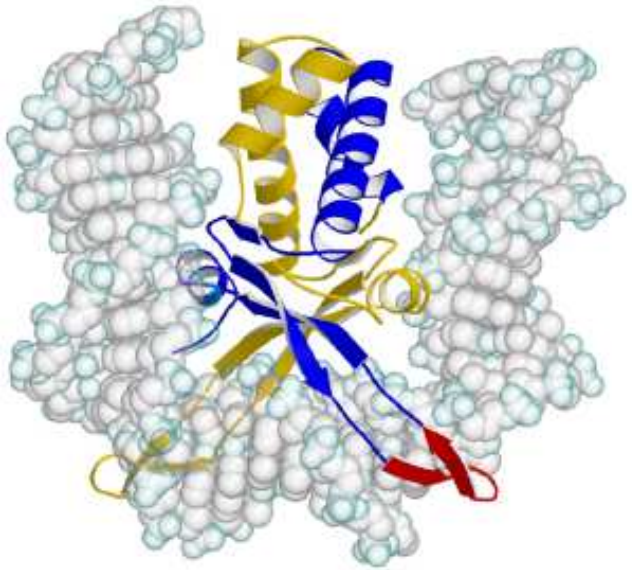
- pomáhá zpřístupnit DNA pro transkripční regulátory

Motivy DNA vazebných domén

- **Zipper typ**
  - Leucinový zip
  - Helix-loop-helix
- **Helix-otáčka-helix**
  - Winged helix
- **Zinkový prst**
  - ββα zinc-finger
  - Hormon-receptor
  - Loop-sheet-helix
  - Gal4
- **Histon, HMG-box**
- **β-hairpin/ribbon**
- **β- barrel**

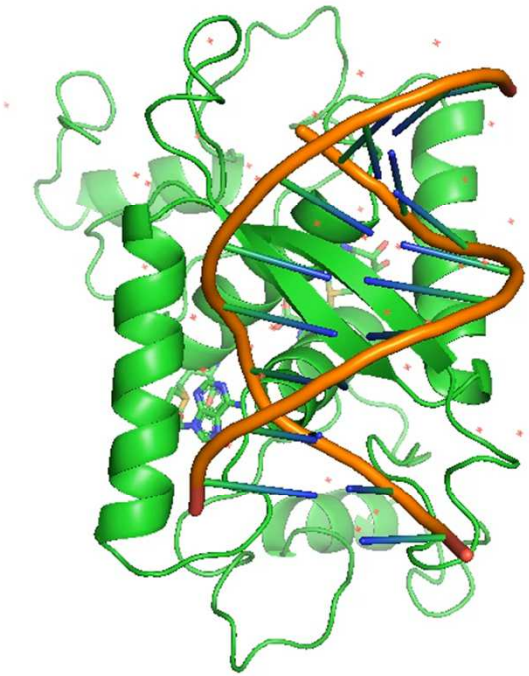
α-šroubovice

β-listy



33. Integration host factor (1ihf)

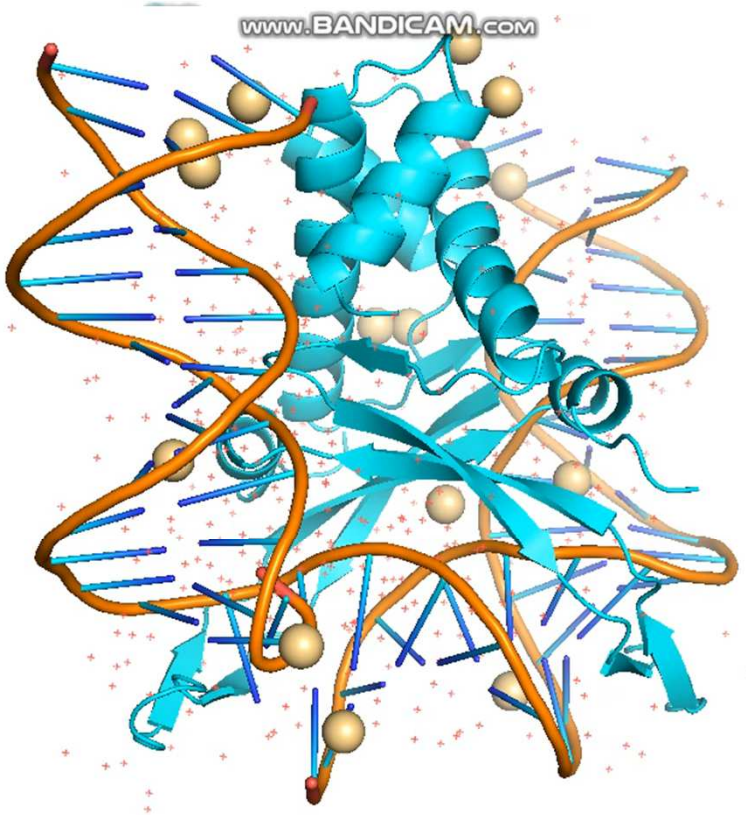
www.BANDICAM.com



31. Met repressor (1cma)

- pouze β-list nebo hairpin (vlásenka)
- represory, chromosomální proteiny (lešení pro strukturu DNA)
- vážou velký nebo malý žlábek

www.BANDICAM.com



33. Integration host factor (1ihf)

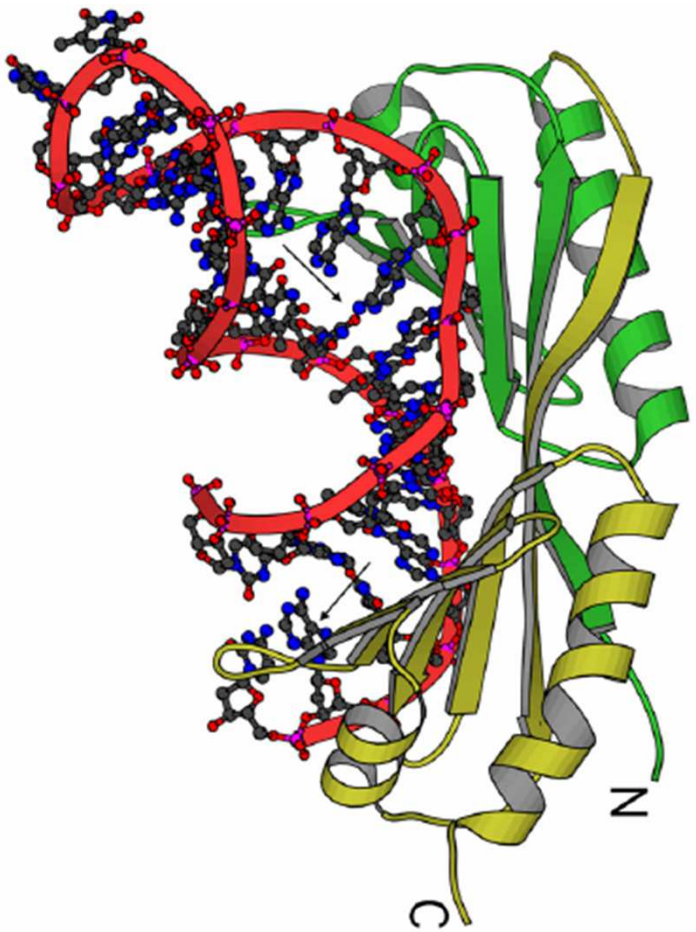
- β-hairpins lokalizovány podél malého žlábku
- inzerce Pro mezi baze - ohyb DNA

Motivy DNA vazebných domén

- **Zipper typ**
  - Leucinový zip
  - Helix-loop-helix
- **Helix-otáčka-helix**
  - Winged helix
- **Zinkový prst**
  - ββα zinc-finger
  - Hormon-receptor
  - Loop-sheet-helix
  - Gal4
- **Histon, HMG-box**
- **β-hairpin/ribbon**
- **β- barrel**

α-šroubovice

β-listy

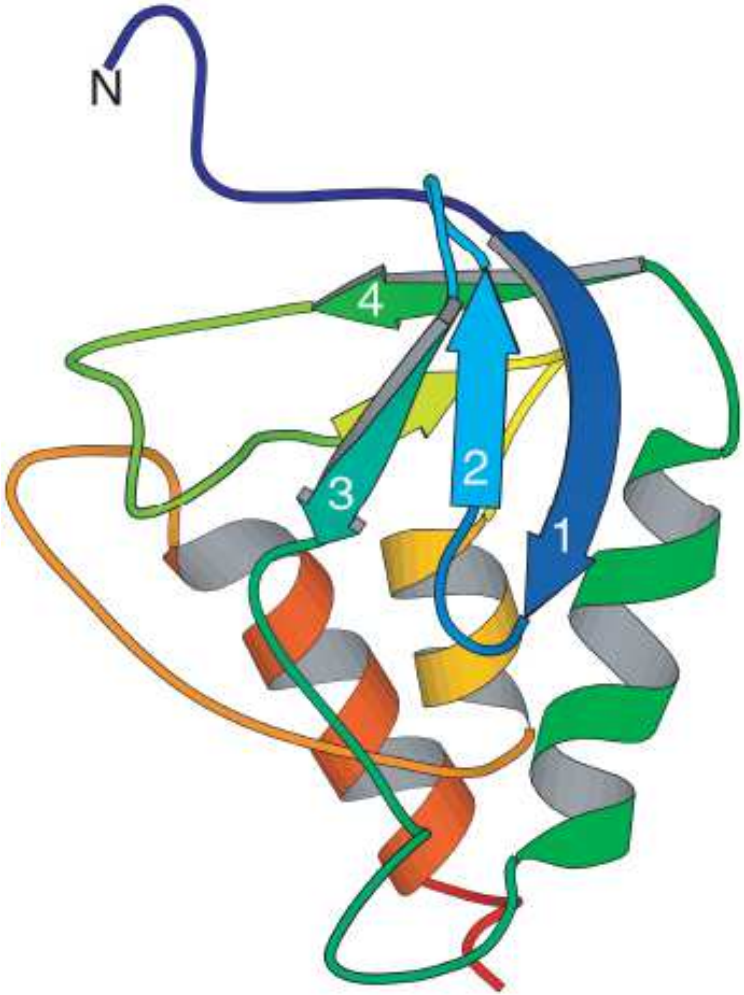


OB-fold (**o**ligonucleotide/oligosacharide **b**inding)

- 4-5 anti-paralelních β-listů (β-barel) zakončeno α-šroubovicí (kompaktní)
- vytváří úzkou jamku pro jednořetězcovou ssDNA (RNA, oligosacharidy)
- váže 2-5 nukleotidů (**báze, cukry i fosfáty**)
- SSB/RPA a TRF proteiny

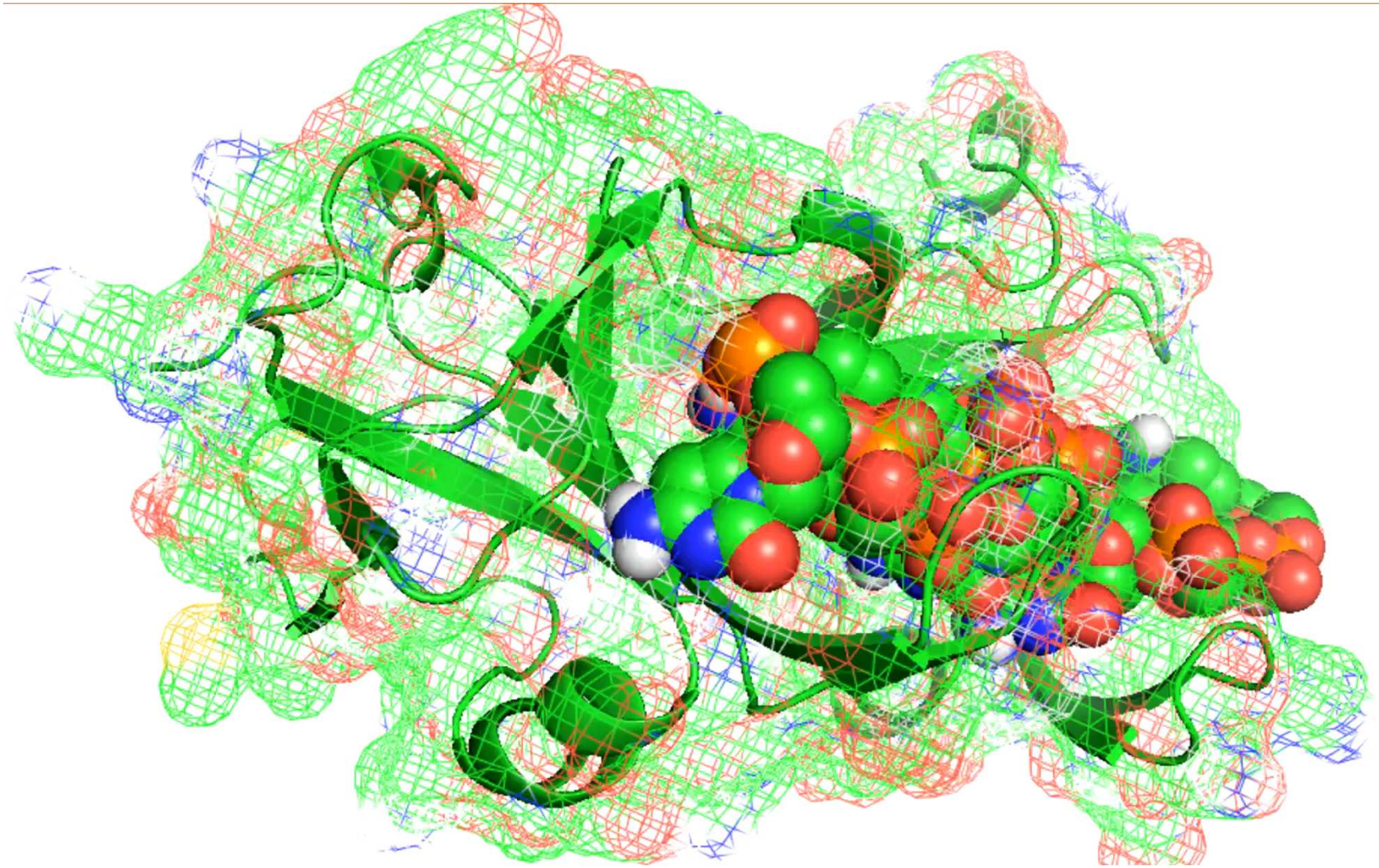
(replikace, HR, telomery ...

Dr. Šebesta)

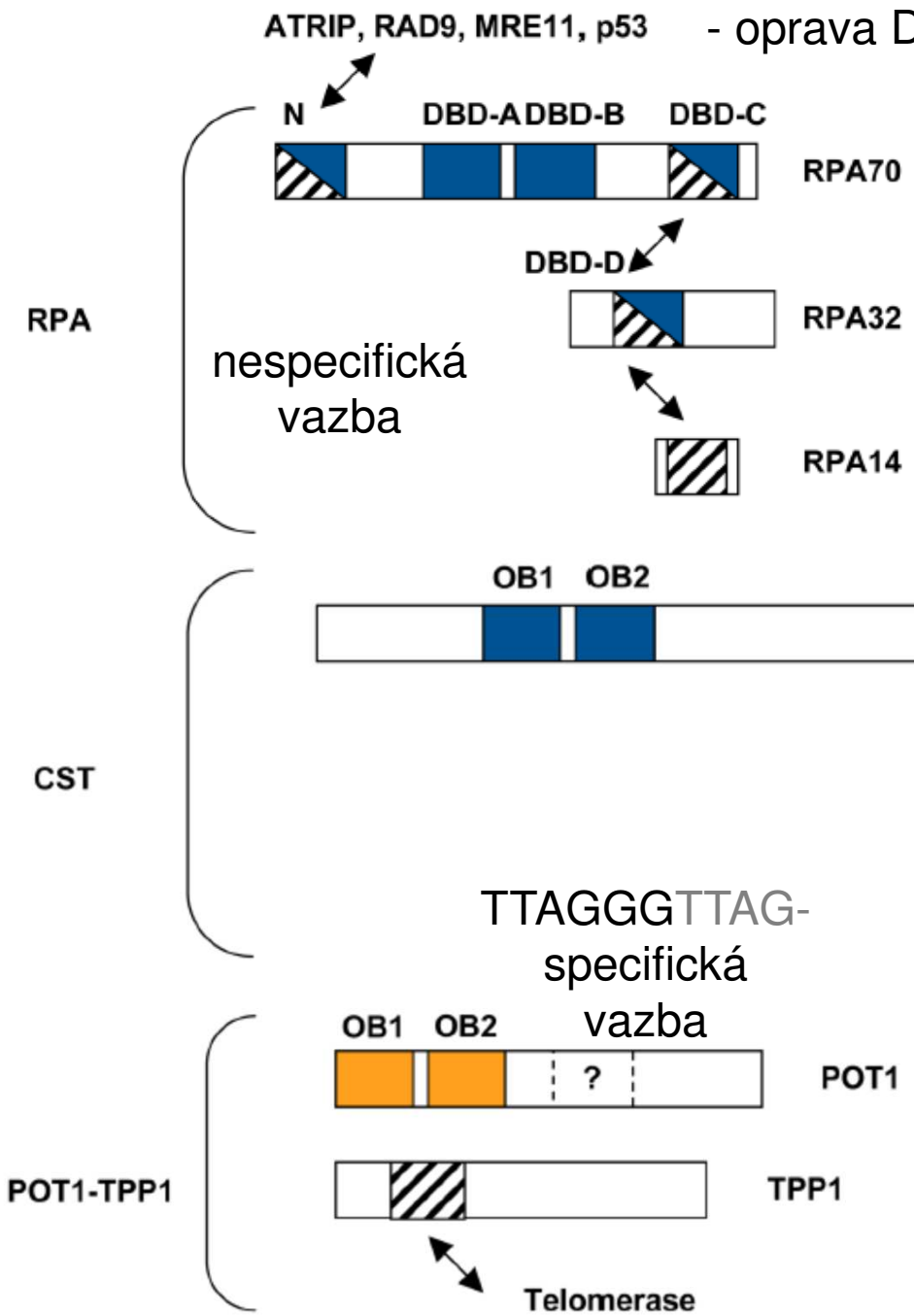


Liljas, kniha (2SNS)

OB-fold (oligonucleotide/oligosacharide binding, 1JMC)



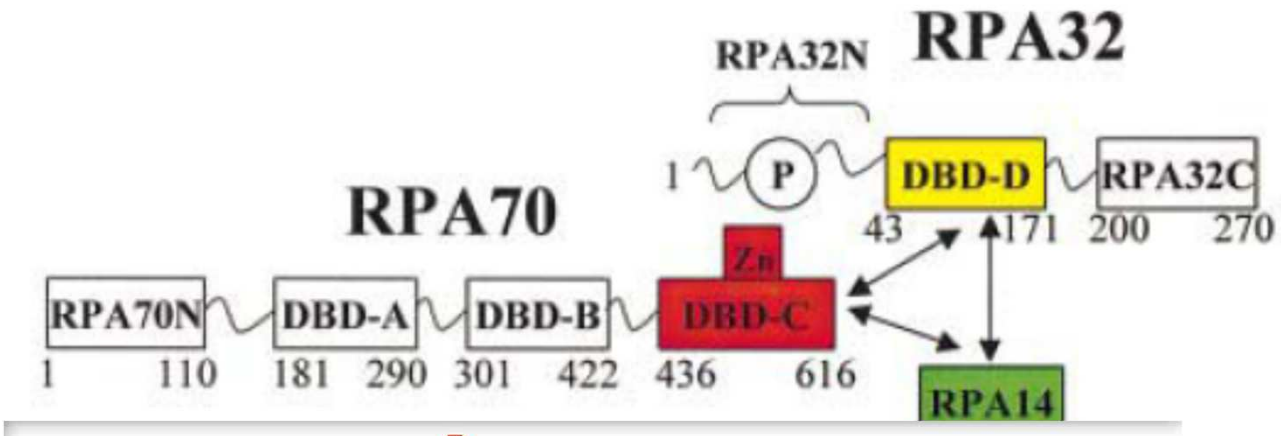
Více OB – RPA70 (A+B) vytváří prodlouženou lineární kapsu



- RPA váže a chrání ssDNA (oprava poškozené DNA)
- protein-proteinové interakce (integrita komplexu + interakce s dalšími proteiny/komplexy)

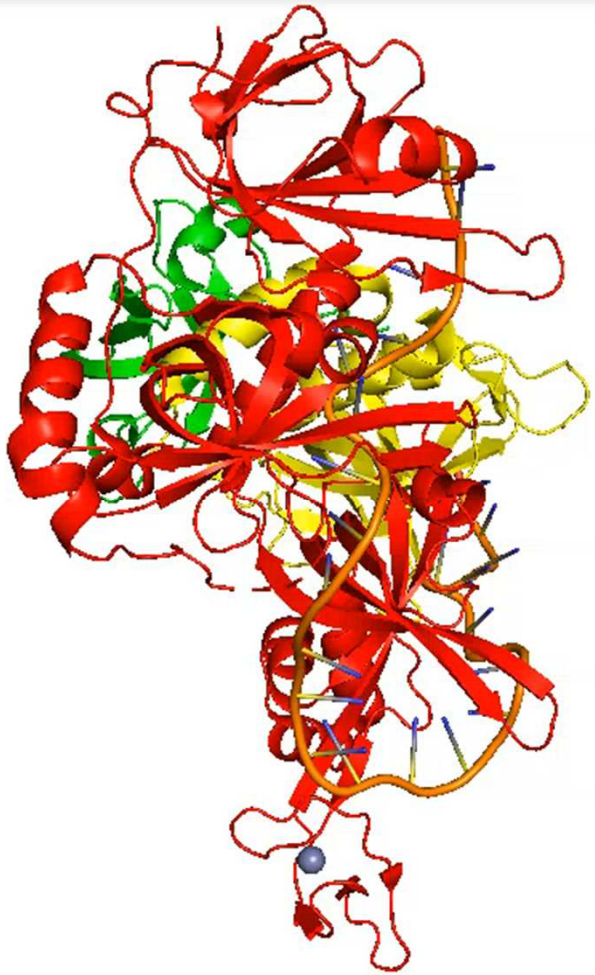
- část komplexu SHELTERIN – chrání telomery (aby nebyly považovány za DSB, reguluje přístup telomerásy)

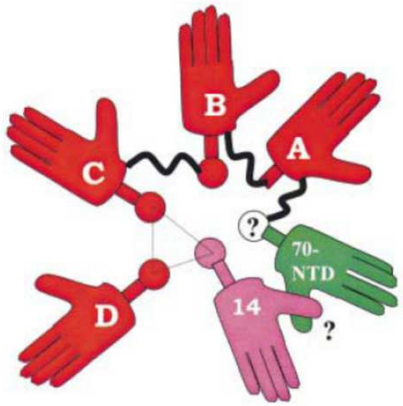
Flynn a Zou, CR in BMB, 2010



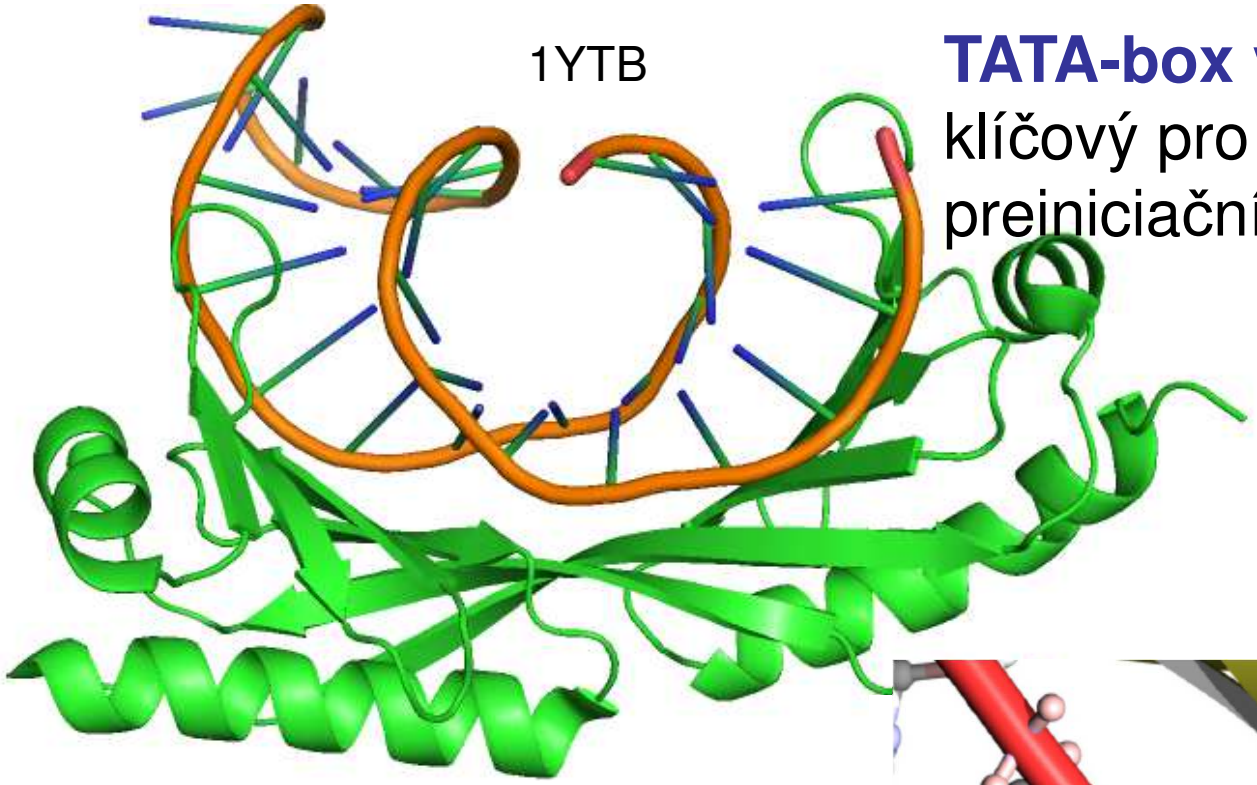
- protein-proteinové interakce (integrita komplexu)
- celý komplex ~30nt

RPA komplex
ssDNA: 4GNX

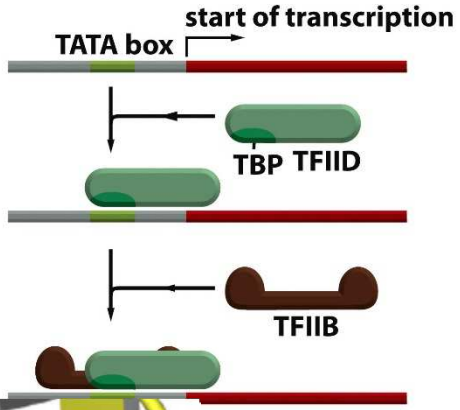




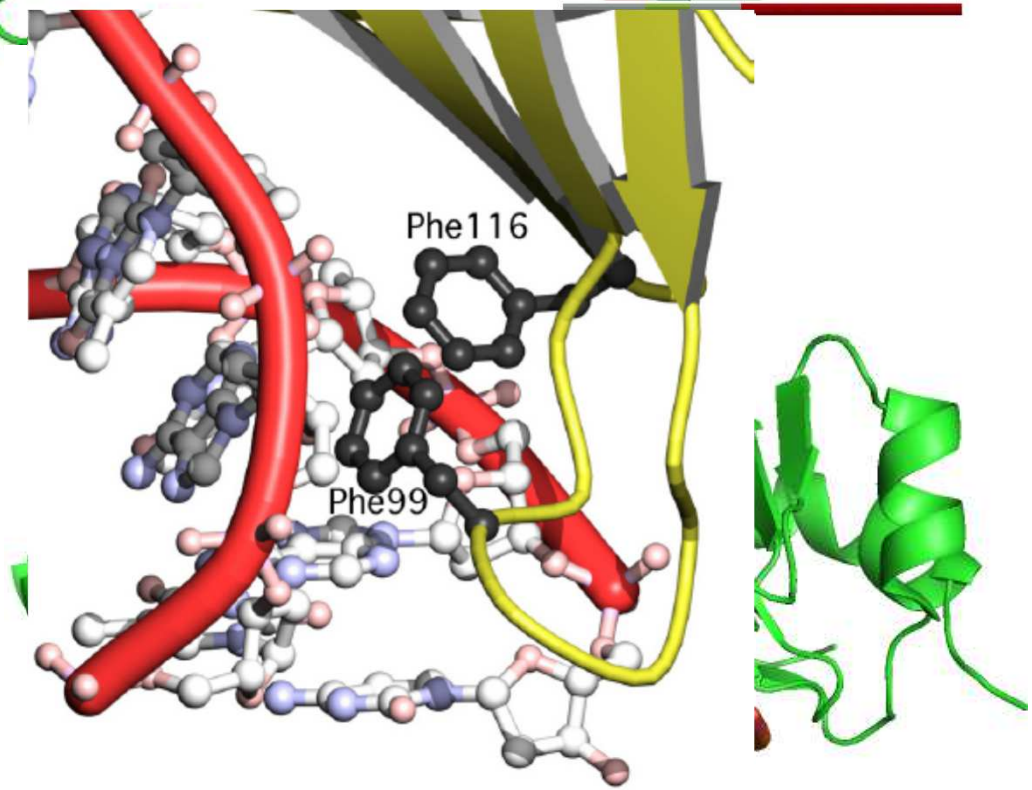
Bochkareva et al, EMBO J, 2002
Flynn a Zou, CR in BMB, 2010



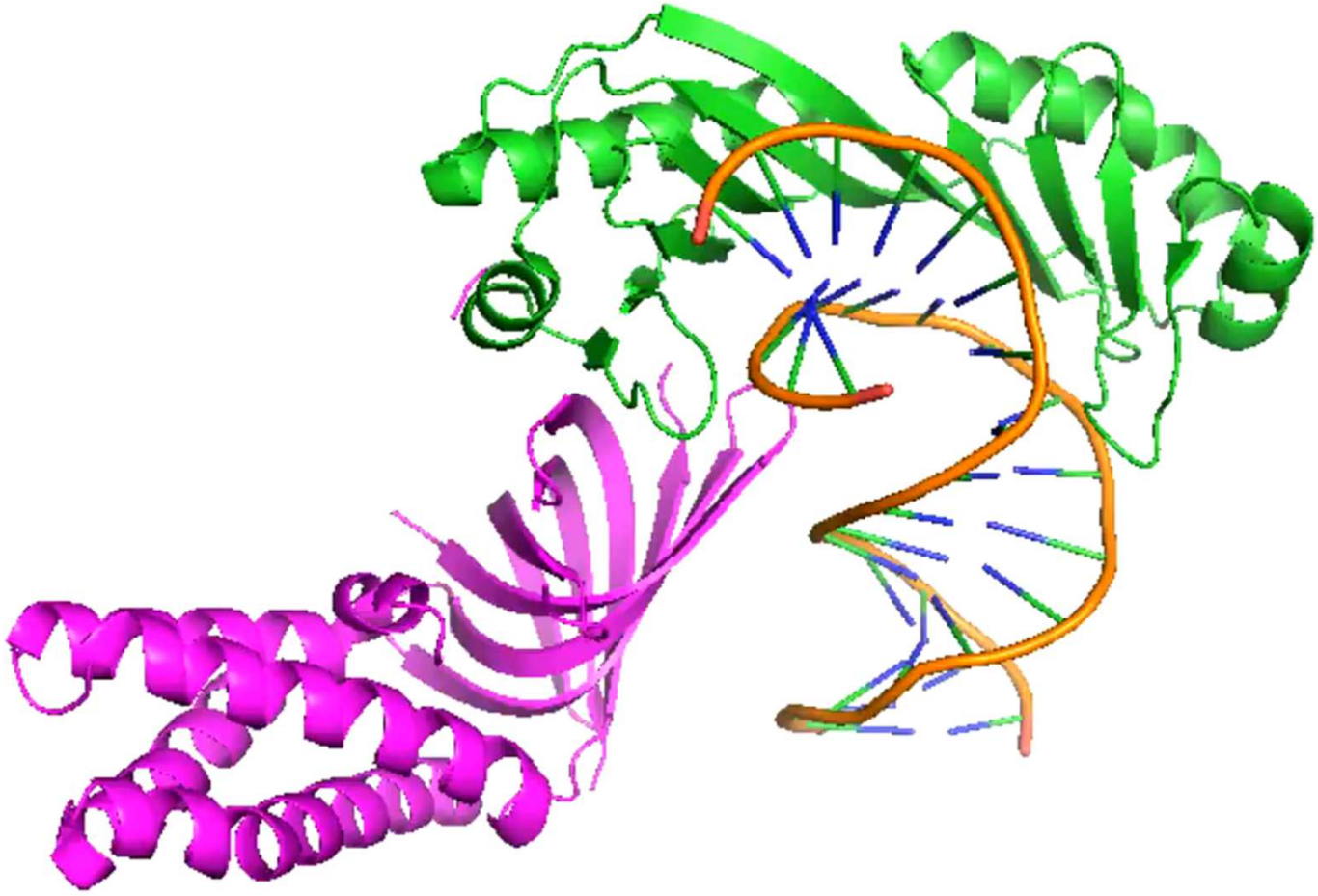
TATA-box vážící protein (TBP) – klíčový pro sestavení preiniciačního komplexu (PIC)



- 10 anti-paralelních β-listů pokrývá MŽ
- interkalují se mezi base postranní řetězce **Phe** (hydrofobní v.)
- vytvaří zlom **(kink) a** částečně rozplétá dsDNA

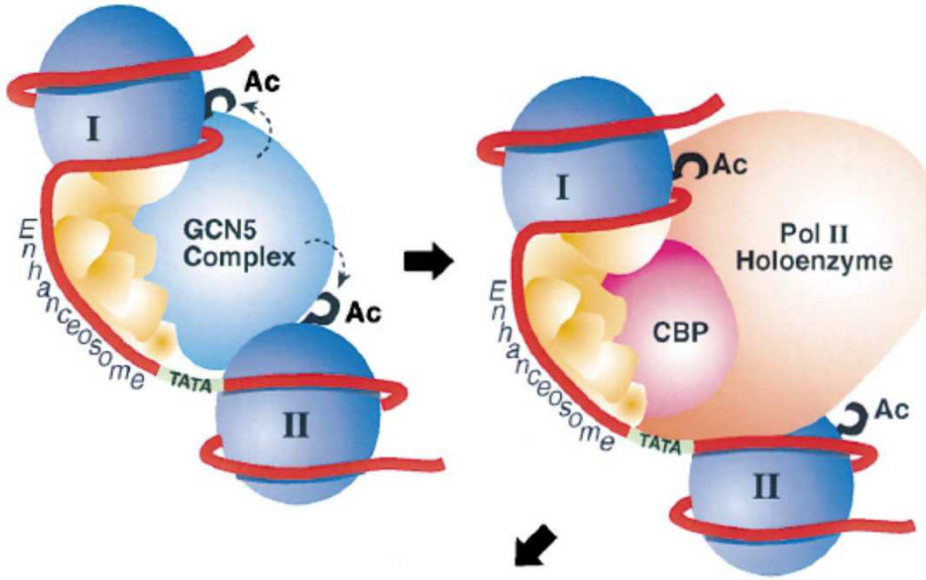


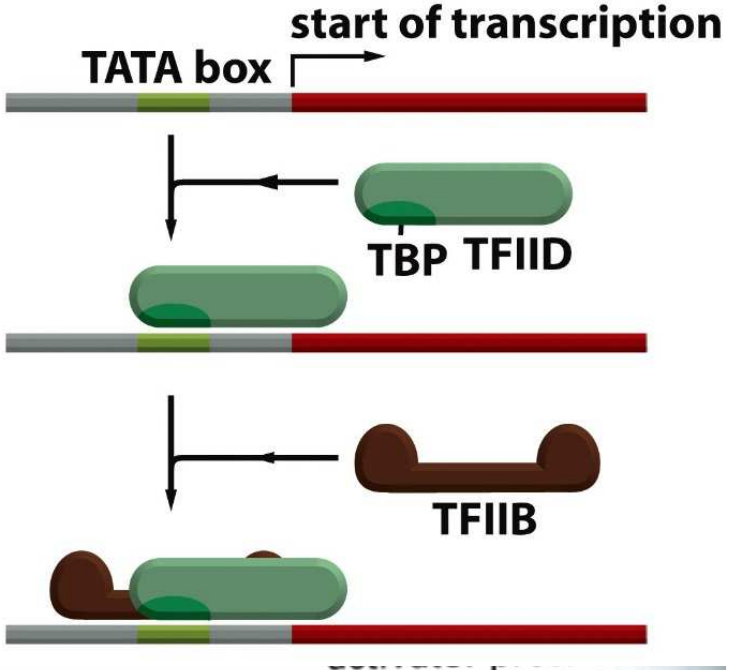
TBP a TFIIA (β-barrel – neovlivní DNA strukturu) 1NH2



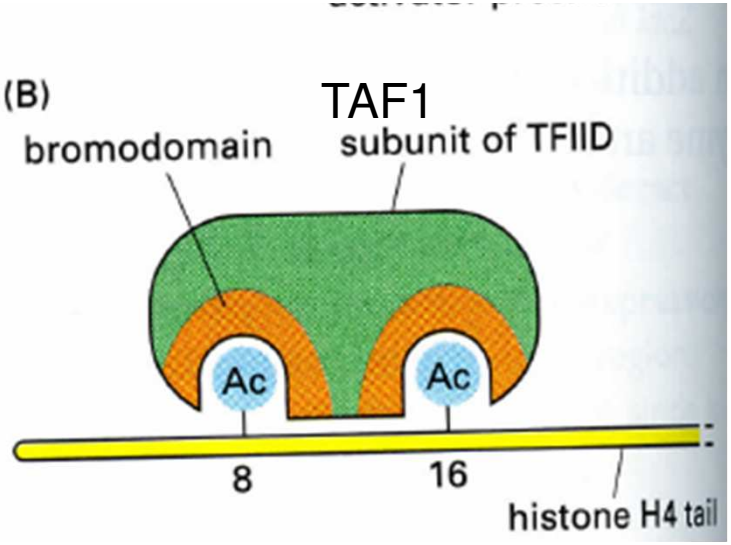
Transkripce

- Aktivace – specifické TF
- Iniciace – základní TF

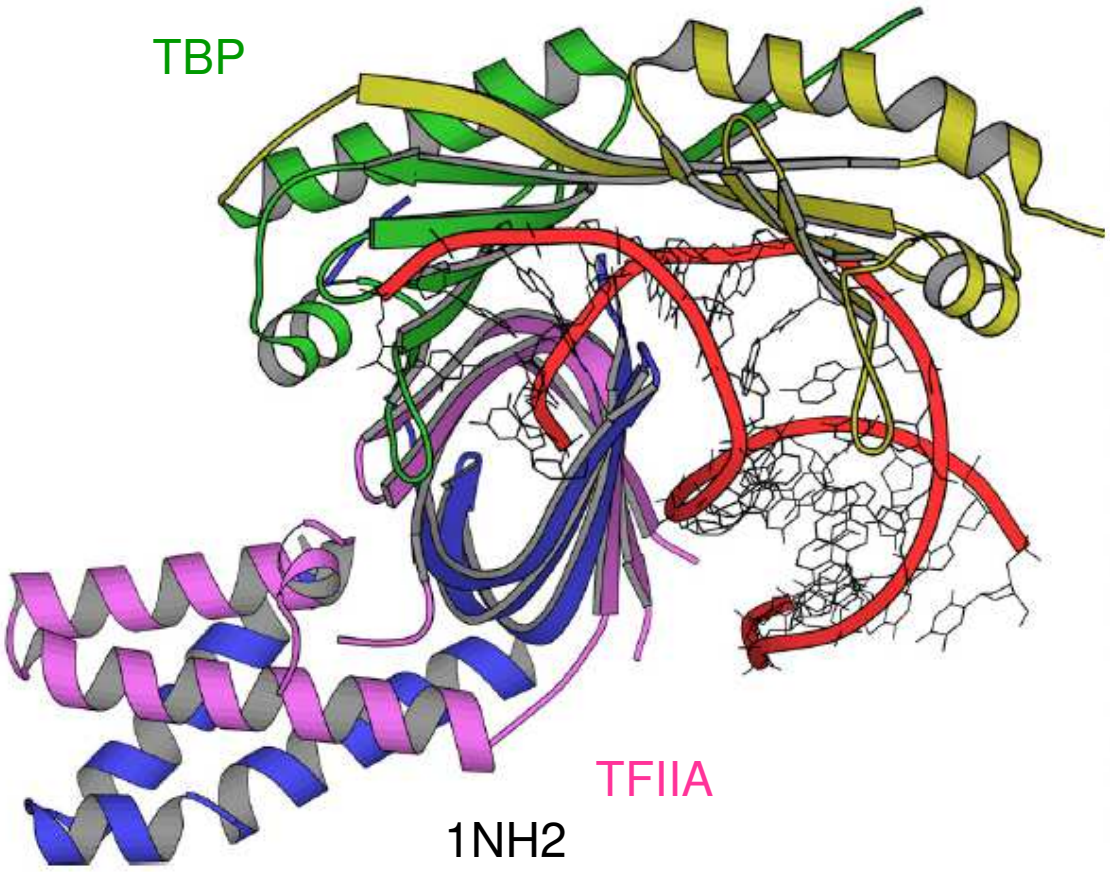


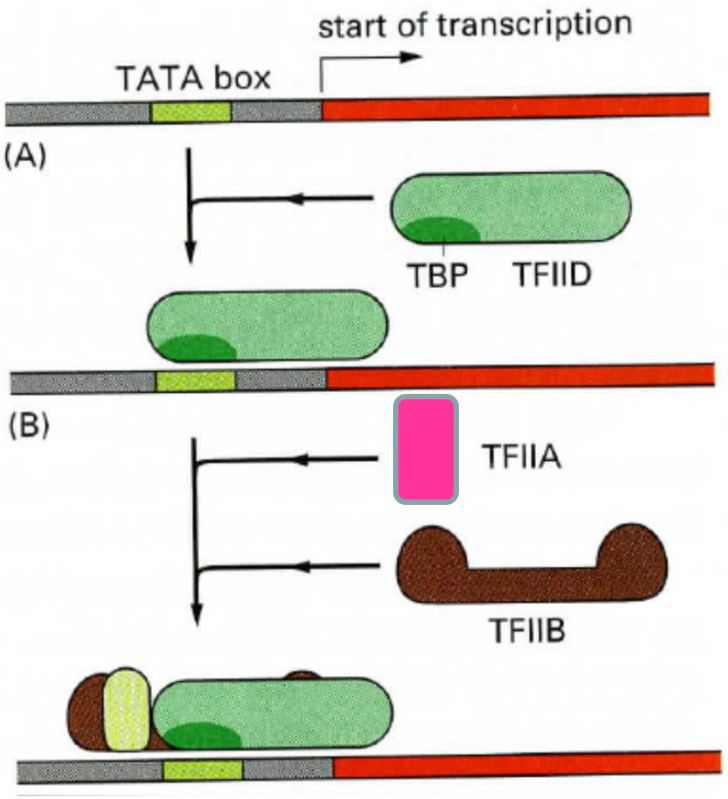


- aktivace (viz enhanceosom) – modulace chromatinu – iniciace (sestavení PIC)



- TBP (v rámci TFIID) nastavuje začátek transkripce na molekule DNA (zajišťuje správnou pozici TFIID)

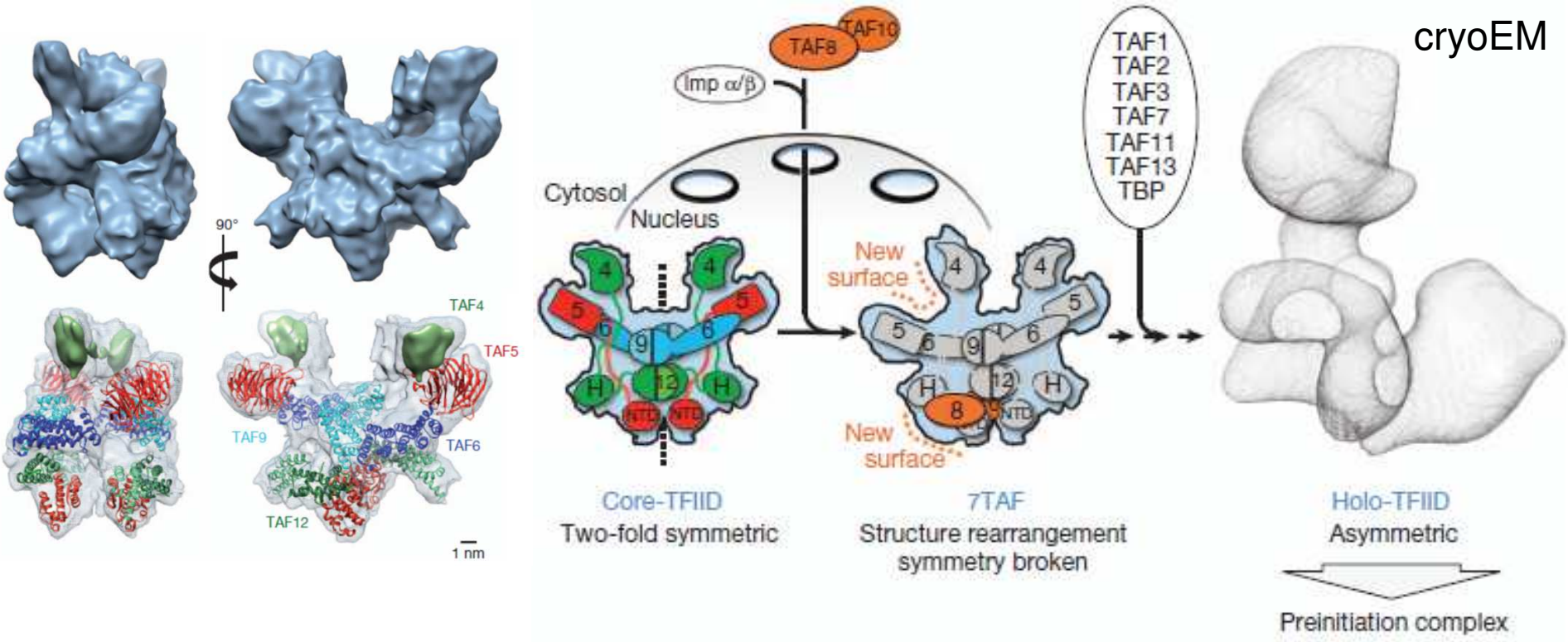




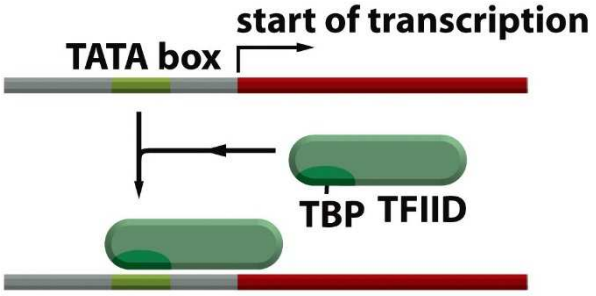
Liljas a spol.

- TFIID (1MDa, TAFs) komplex a TBP jsou klíčové pro rozpoznání promotorů a poskytují scaffold/lešení pro sestavení transkripčního systému (nukleace PIC)

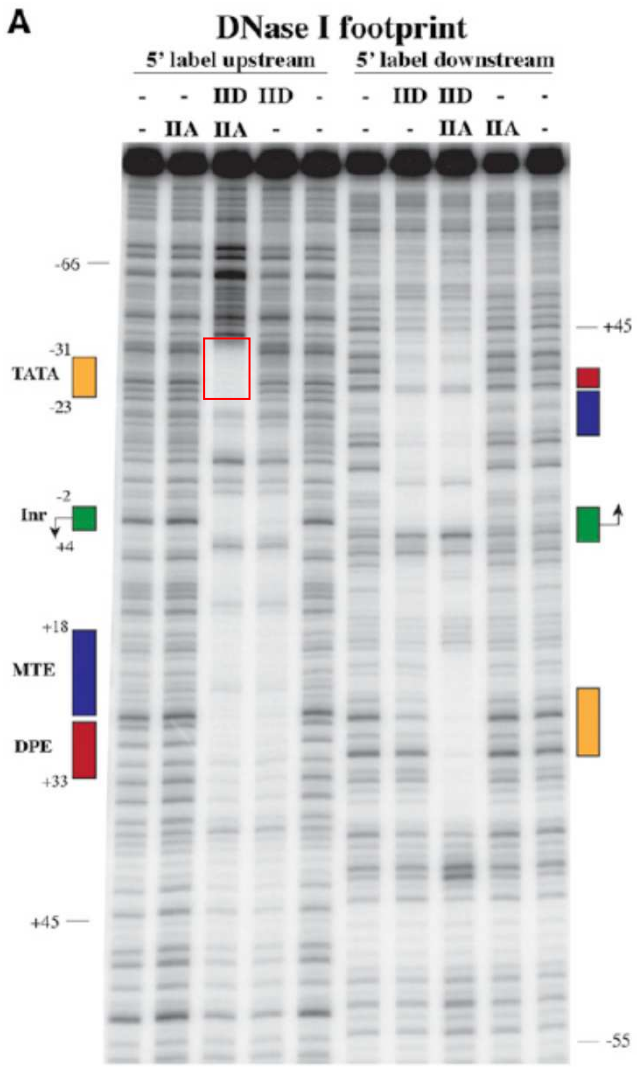
Bieniossek et al., Nature, 2013



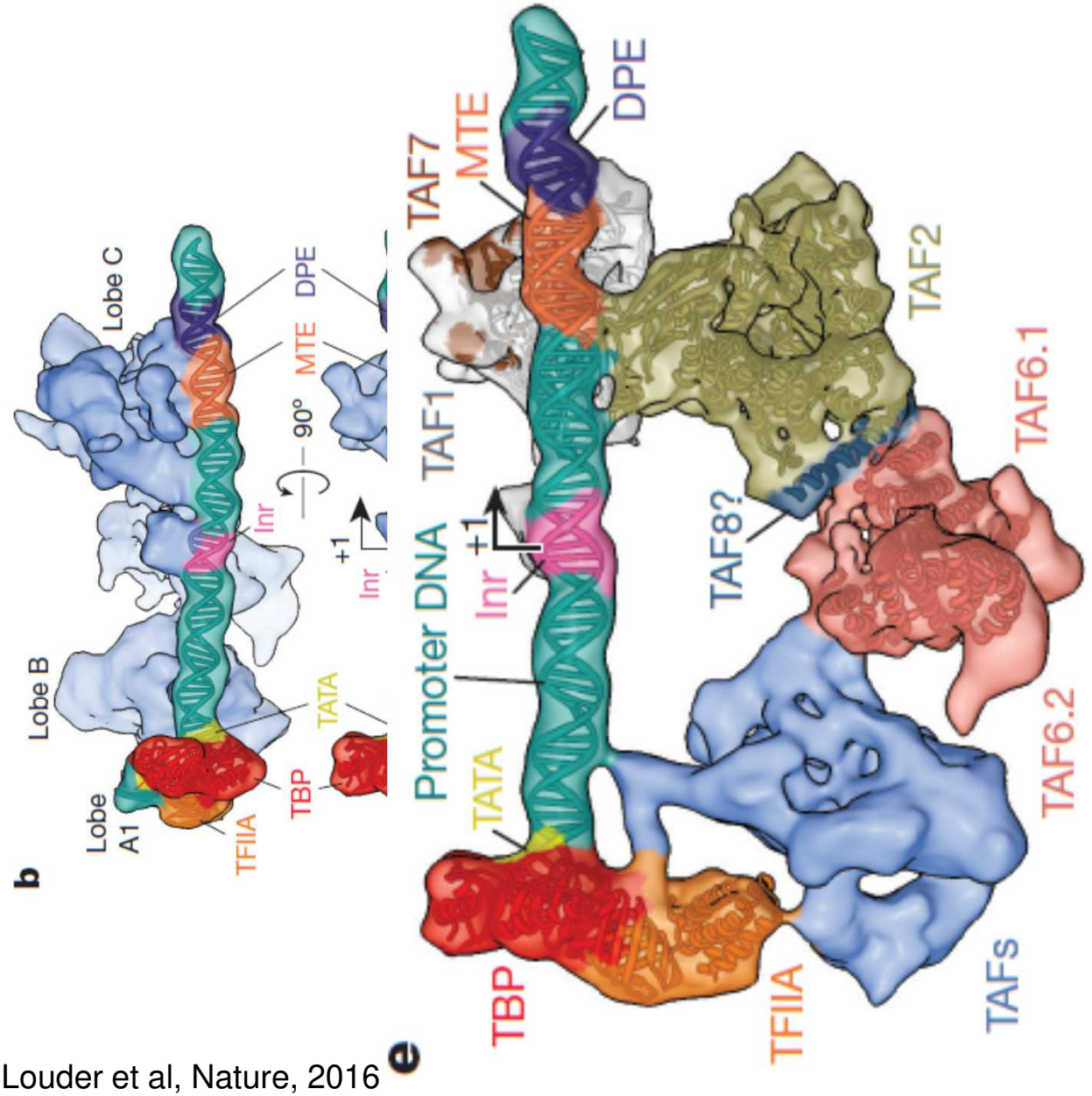
- jádro komplexu (symetrické) tvoří TAF4, 5, 6, 9 a 12 - po připojení všech podjednotek TFIID komplexu včetně TBP vzniká funkční (asymetrický) holokomplex (PIC)



- footprint analýza ukázala jak se TFIIA-TFIID/TBP komplex společně váže na DNA (TFIIA pomáhá vázat TATA box)

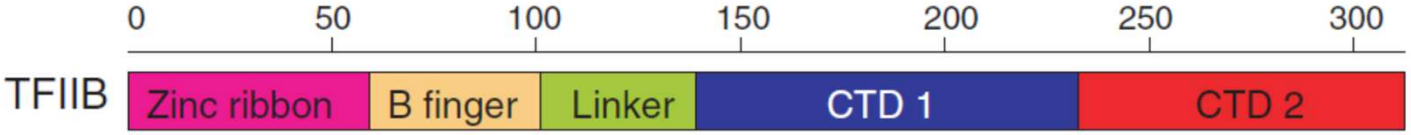


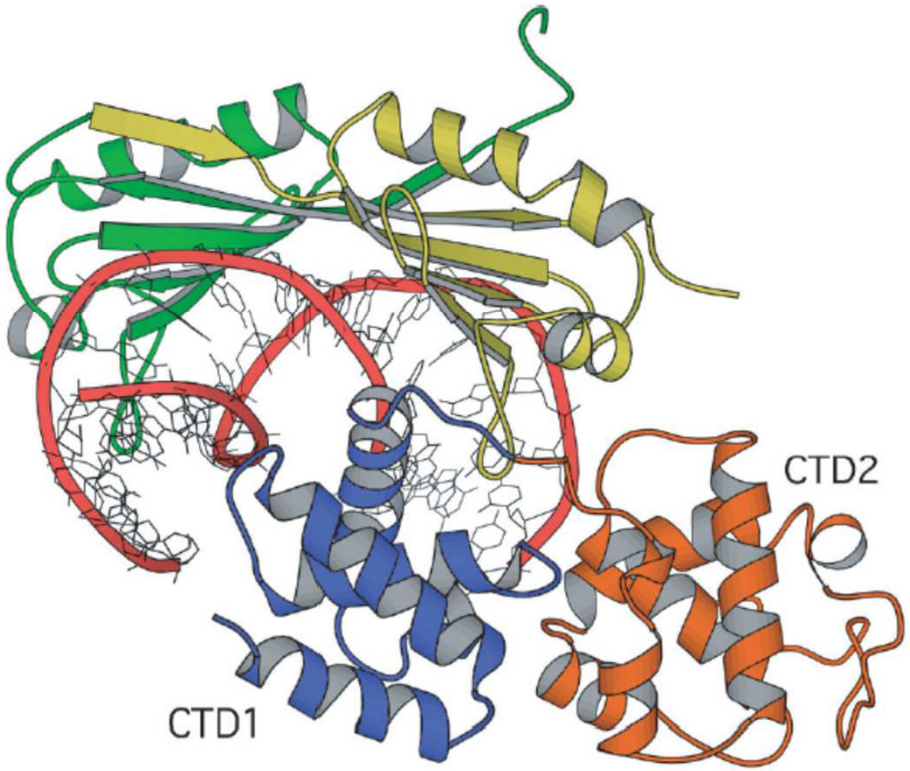
Cianfrocco et al, Cell, 2013

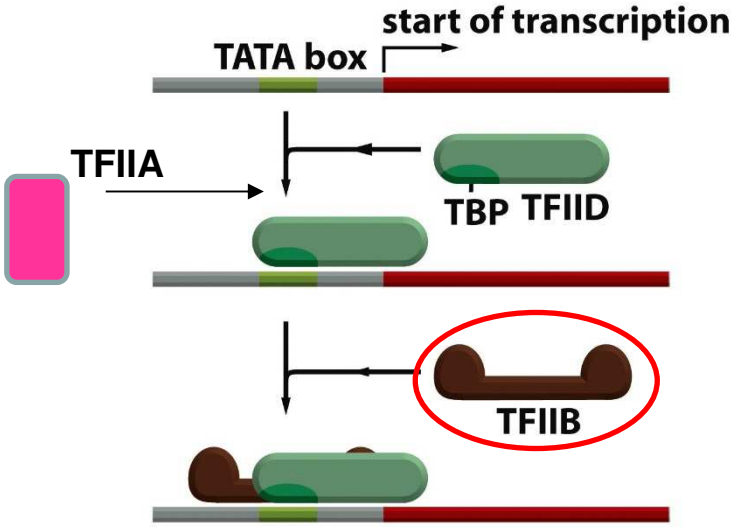


Louder et al, Nature, 2016

pro další postup transkripce je klíčový TFIIB – propojuje TFIID-TFIIA s RNApol II

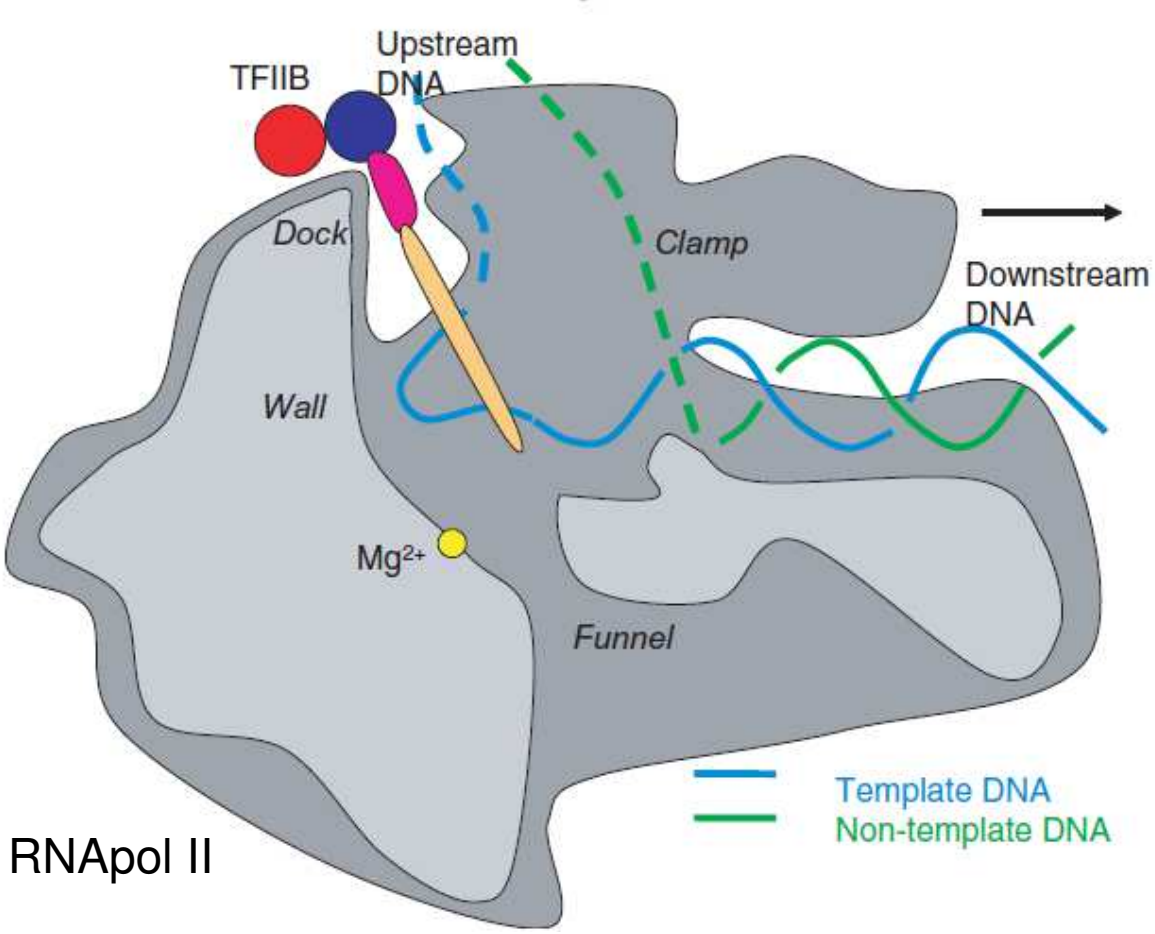




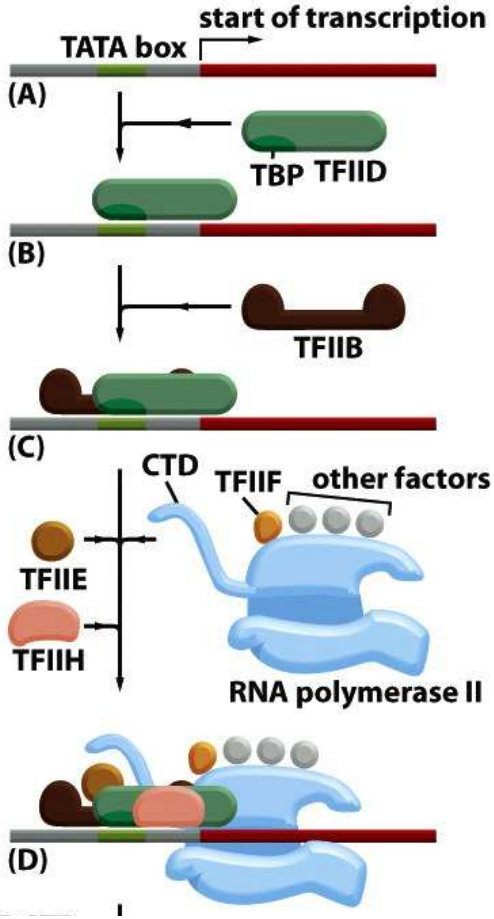


- TFIIB (C-konec = CTD1 a CTD2) váže TBP a 6-7bp up- a downstream od ohybu DNA přes cukrfosfátovou kostru (blízko TATA boxu)

- TFIIB (N-konec) váže RNA polymerasu II a orientuje ji vůči TFIID/TBP-DNA komplexu
- umístí DNA do aktivního centra/cleft RNA polymerásy
- vazba Zn-ribbon na Dock, B-finger/reader do RNA tunelu



RNApol II



- TFIIB (N-konec) váže RNA polymerasu II a orientuje ji vůči TFIID/TBP-DNA komplexu
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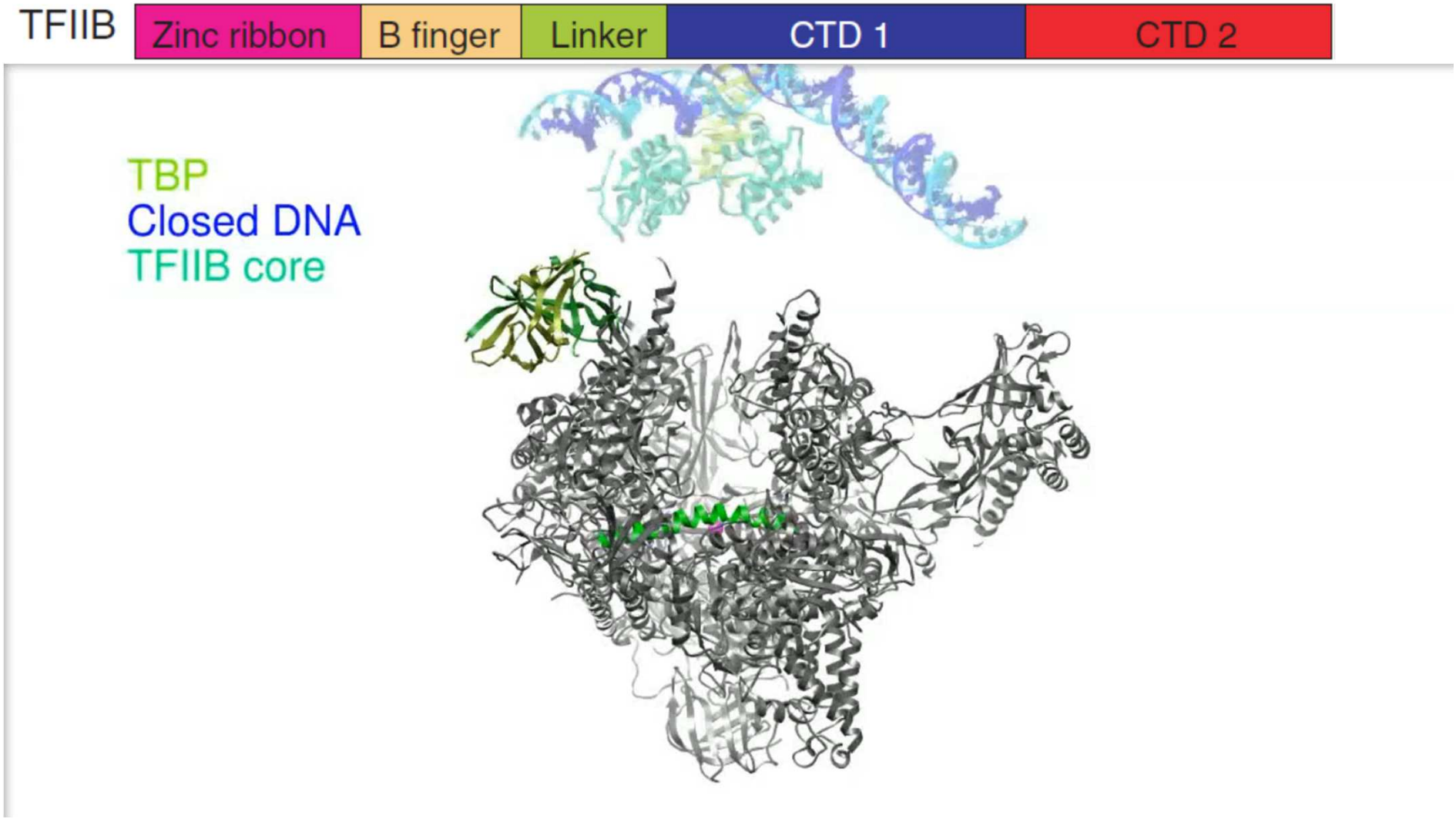
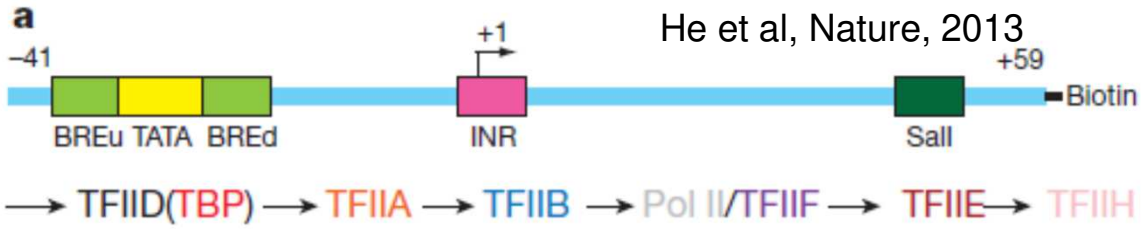
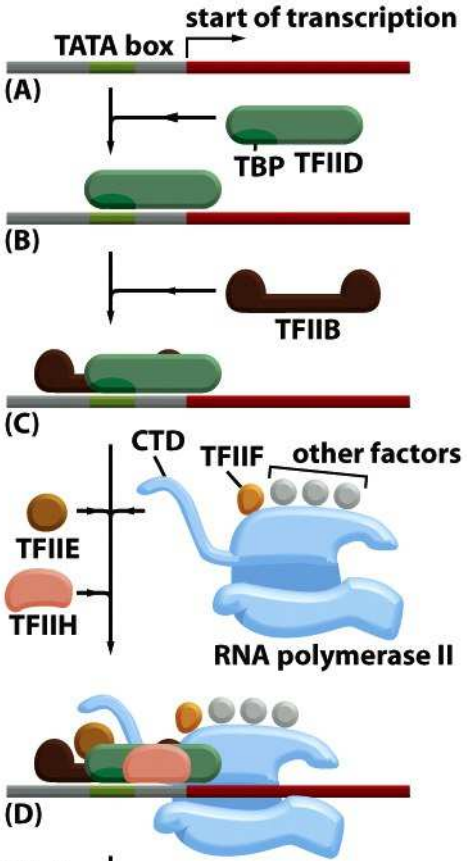


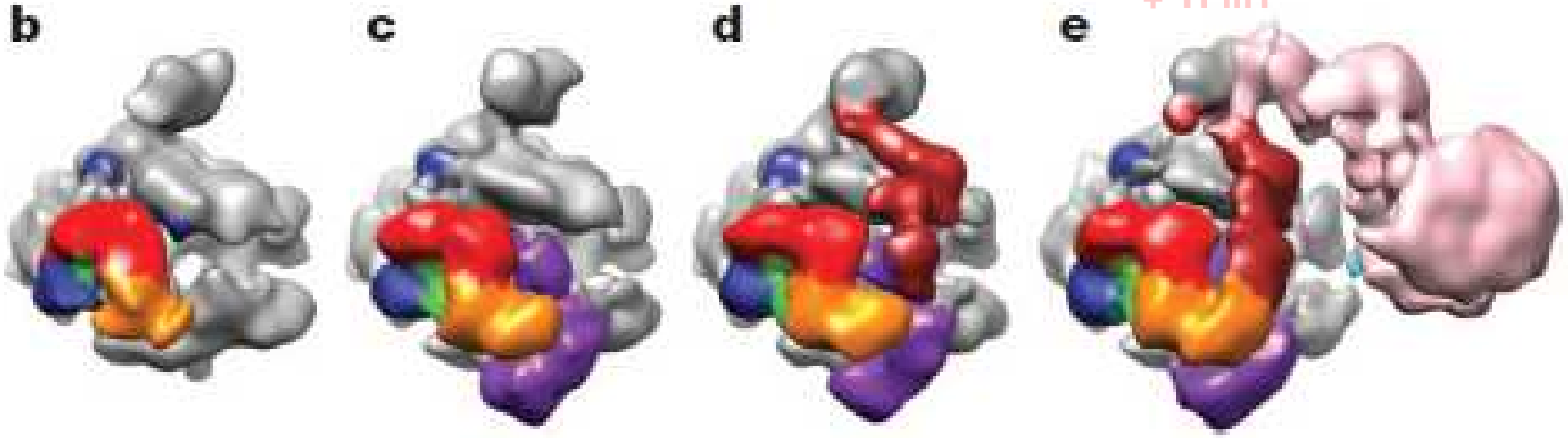
Rekonstrukce celého PIC

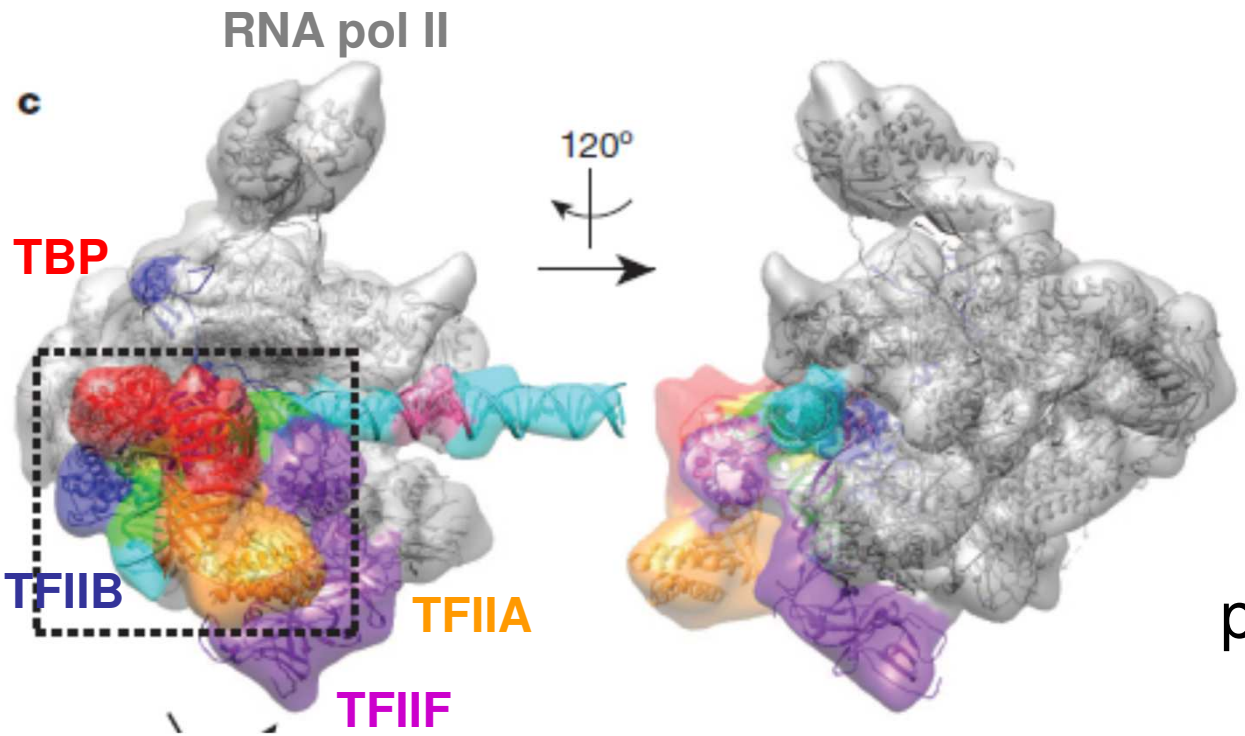
- biotin-DNA navázána na streptavidinové kuličky – na DNA nachytány *in vitro* sestavené komplexy – odštěpeno *Sal*I a cryoEM - postupně rekonstituován celý PIC



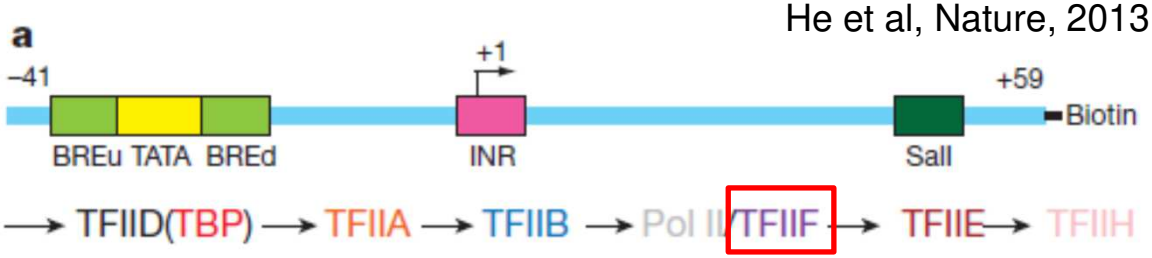
He et al, Nature, 2013

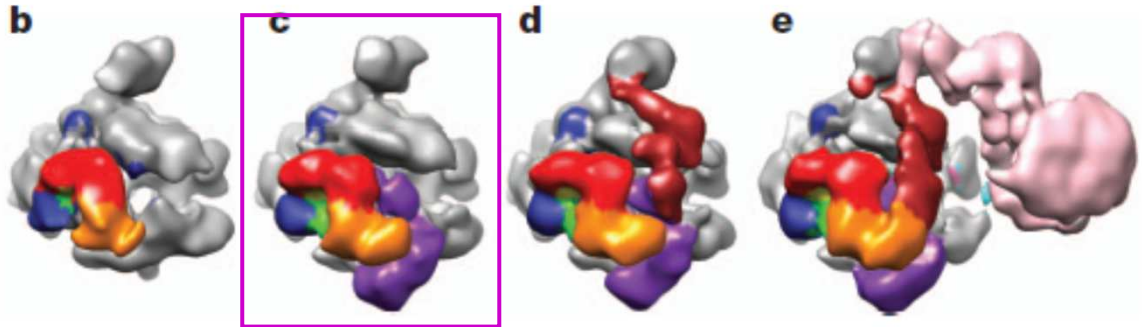




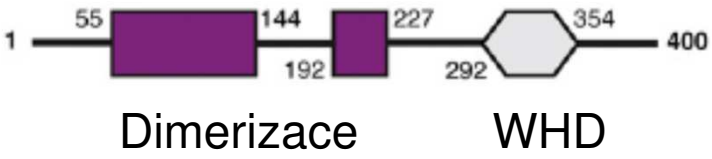


He et al, Nature, 2013

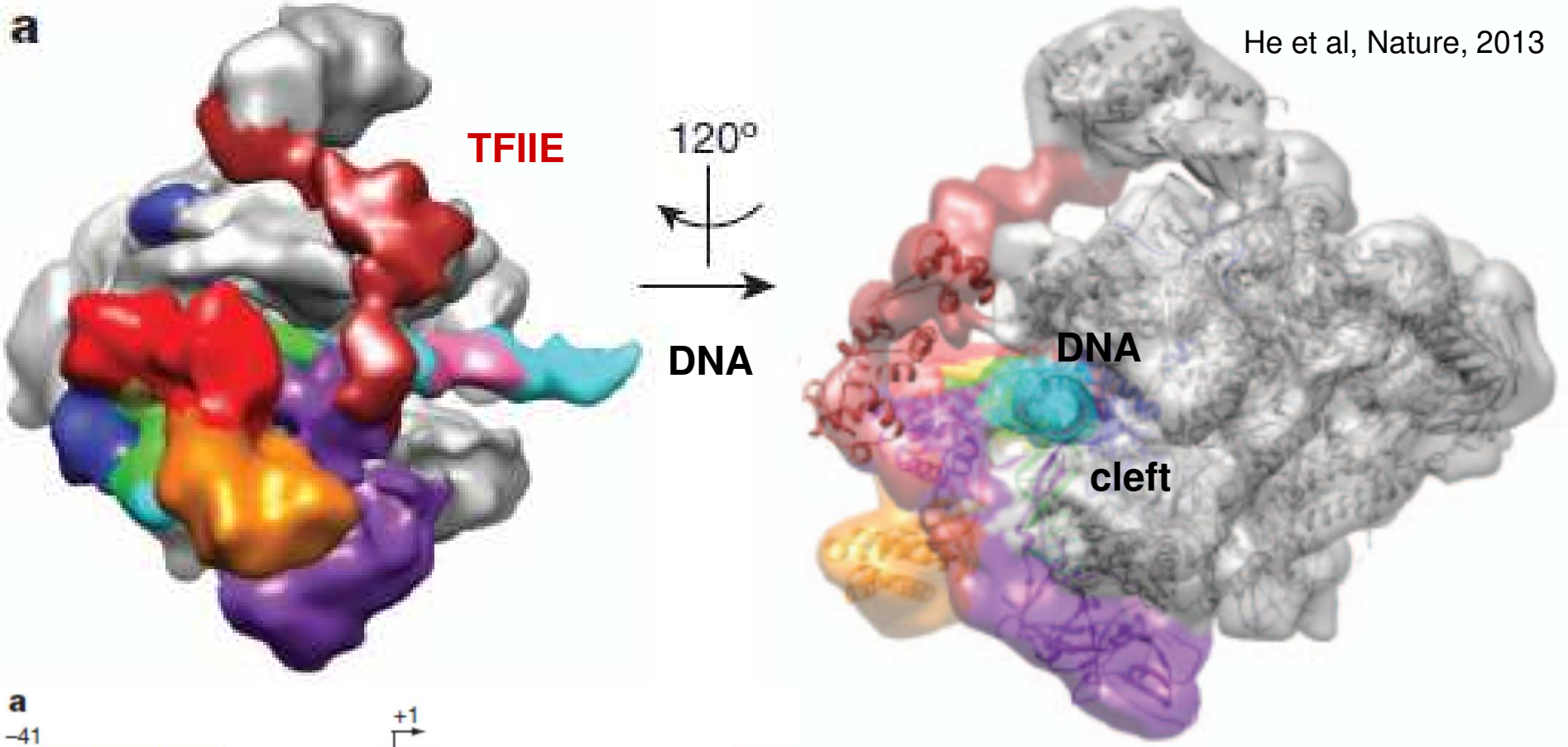




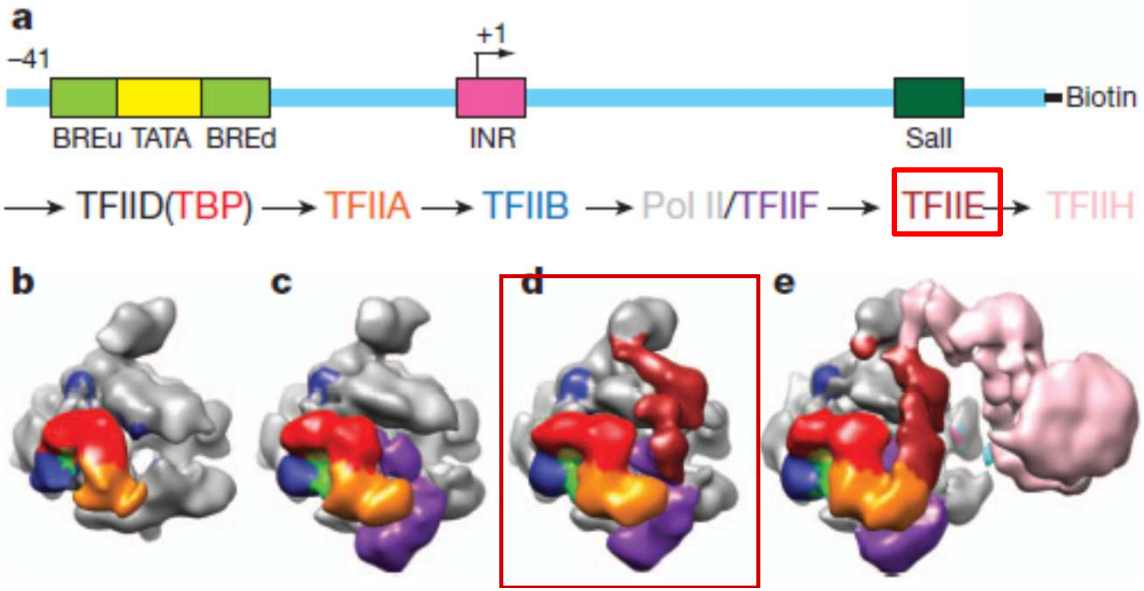
- **TFIIF** (navázaný na pol II) stabilizuje DNA v prohlubni/cleft pol II a pomáhá TFIIB s nastavením startu (WHD z RAP30 podjednotky váže přímo DNA: $BRE_{downstream}$)
- váže TFIIE a pomáhá tak stabilizaci komplexu



Vanini & Cramer, Mol Cell, 2012

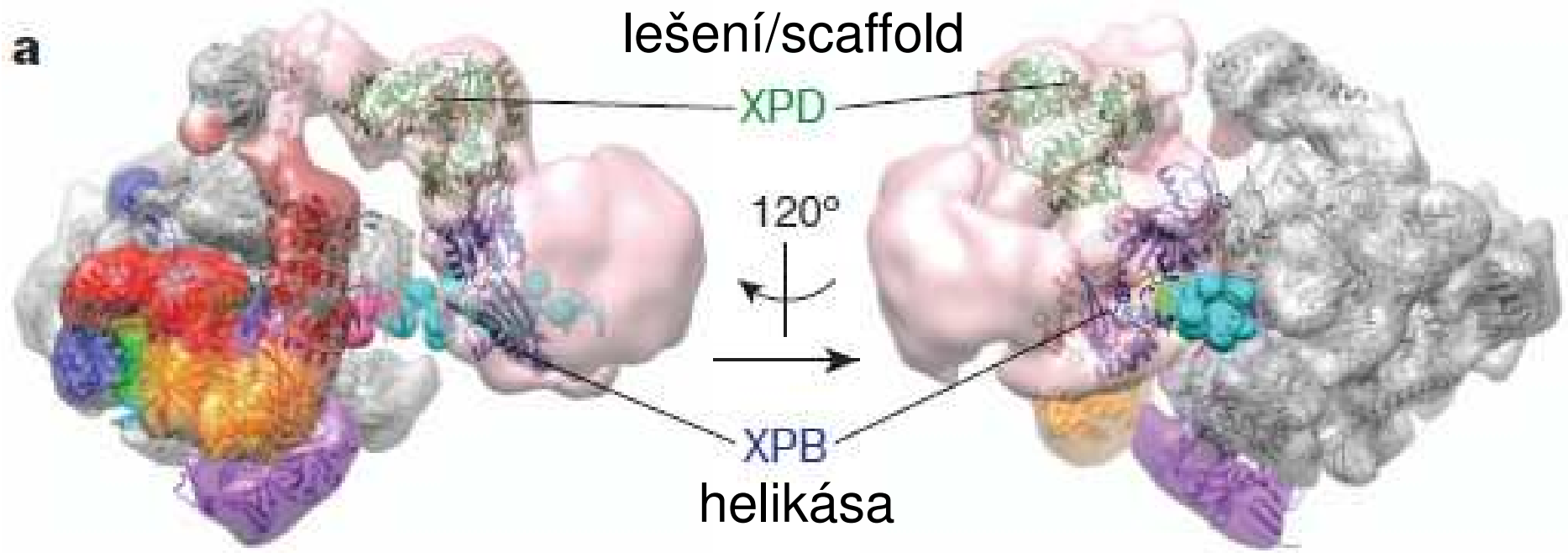


He et al, Nature, 2013

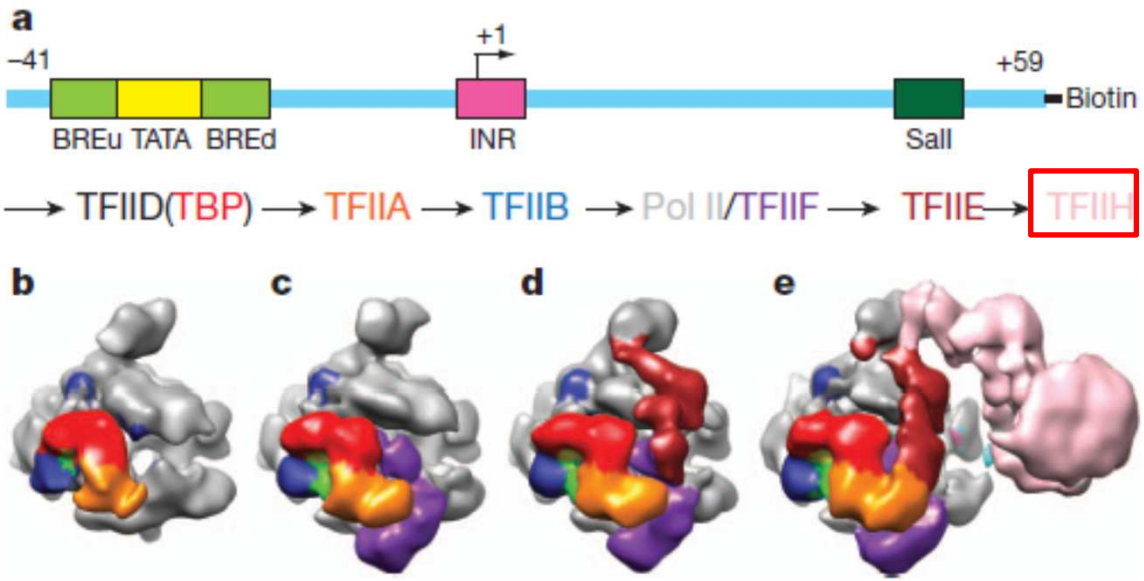


- TFIIF váže TFIIE a pomáhá tak stabilizaci komplexu
- tandem 4x WHD uzamyká DNA v RNApol II prohlubni

Vanini & Cramer, Mol Cell, 2012



- XPB a XPD regulují transkripci vs opravu DNA (NER)

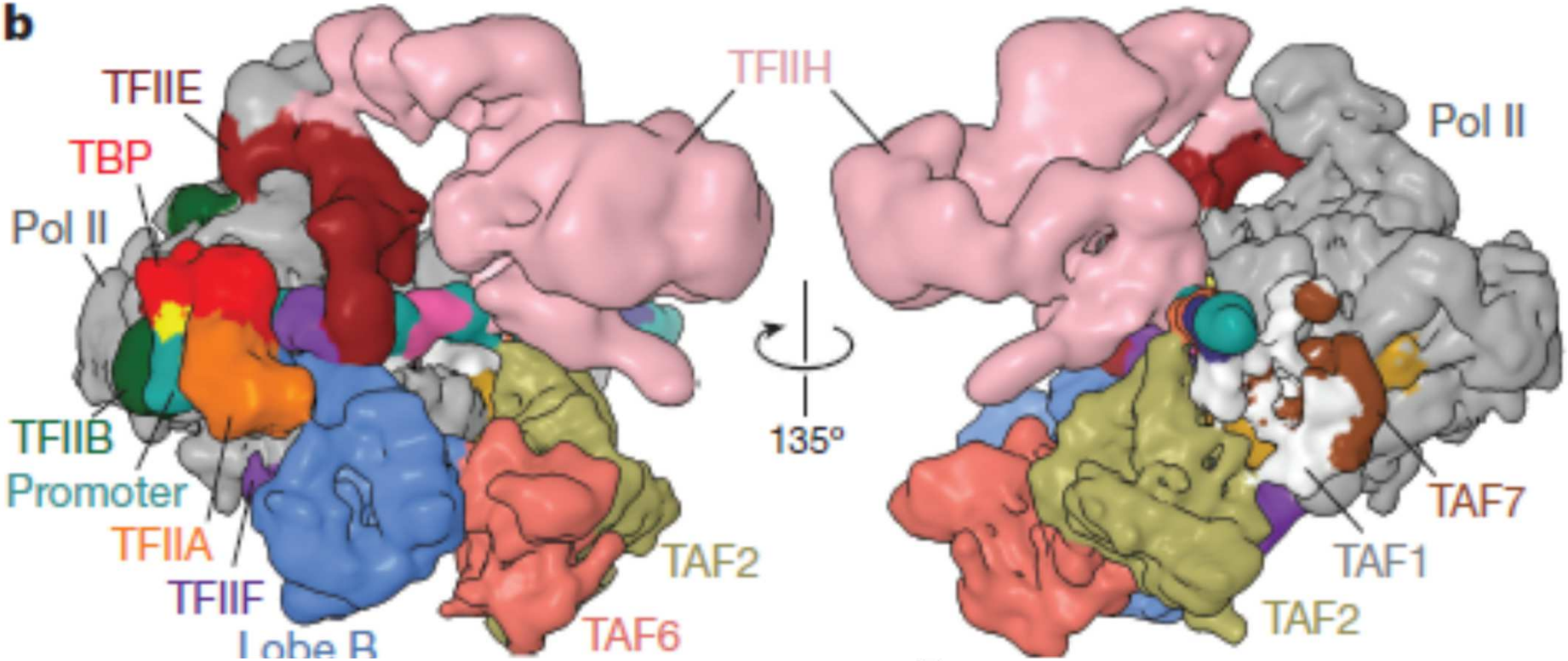


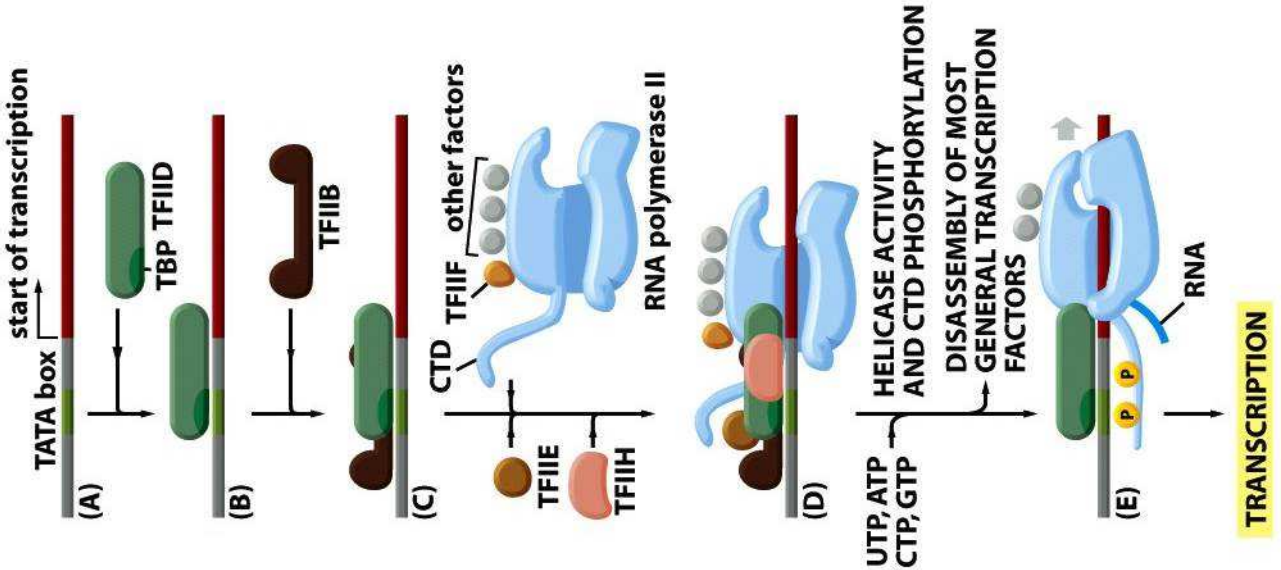
lidský PIC komplex
He et al, Nature, 2013

- **TFIIH** (10 podjednotek, 450KDa), podkomplex CDK7-cyclin H-MAT1 fosforyluje pol II (Rpb1)
- XPB v kontaktu s DNA rozvíjí dvoušroubovici

Kompletní PIC i s částí TFIID

Louder et al, Nature, 2016

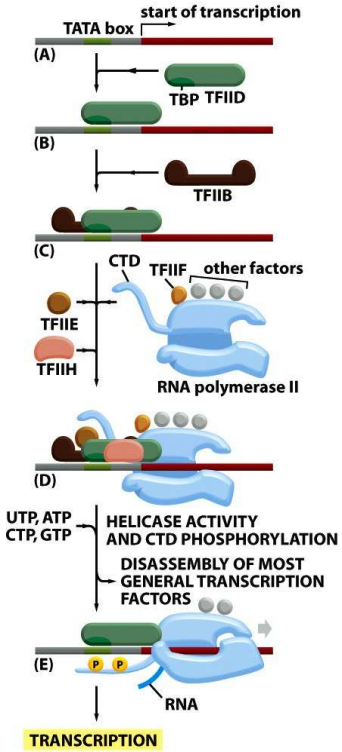




začátek transkripce - faktory

TABLE 1 Components of the human general transcription machinery

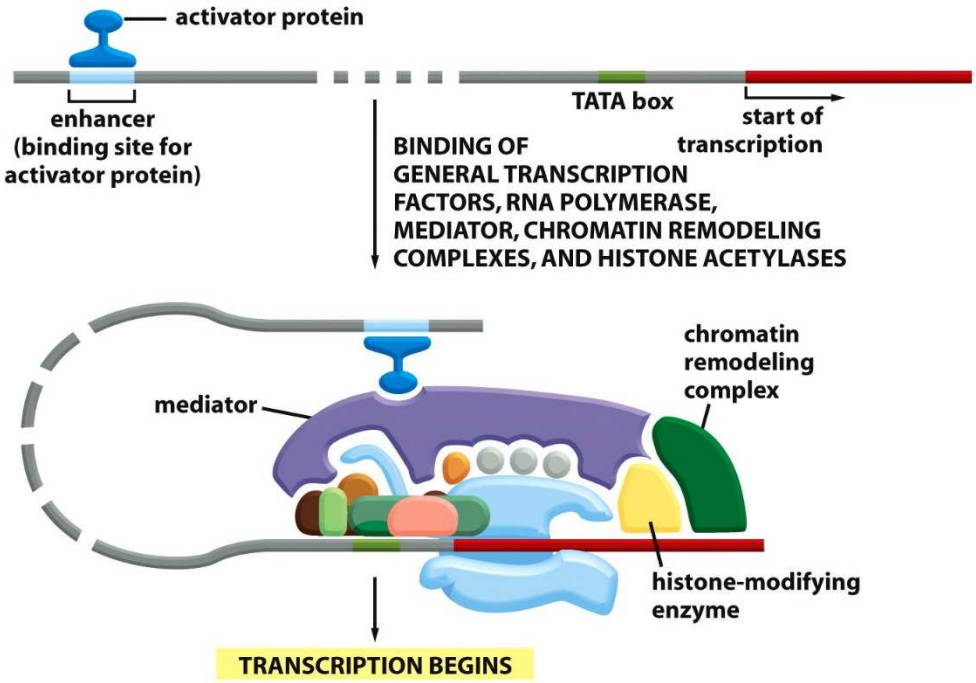
Factor	Protein composition	Function
TFIIA	p35 (α), p19 (β), and p12 (γ)	Antirepressor; stabilizes TBP-TATA complex; coactivator
TFIIB	p33	Start site selection; stabilize TBP-TATA complex; pol II/TFIIF recruitment
TFIID	TBP + TAFs (TAF1-TAF14)	Core promoter-binding factor Coactivator Protein kinase Ubiquitin-activating/conjugating activity Histone acetyltransferase
TFIIE	p56 (α) and p34 (β)	Recruits TFIIH Facilitates formation of an initiation-compentent pol II Involved in promoter clearance
TFIIF	RAP30 and RAP74	Binds pol II and facilitates pol II recruitment to the promoter Recruits TFIIE and TFIIH Functions with TFIIB and pol II in start site selection Facilitates pol II promoter escape Enhances the efficiency of pol II elongation
TFIIH	P89/XPB, p80/XPD, p62, p52, p44, p40/CDK7, p38/Cyclin H, p34, p32/MAT1, and p8/TFB5	ATPase activity for transcription initiation and promoter clearance Helicase activity for promoter opening Transcription-coupled nucleotide excision repair Kinase activity for phosphorylating pol II CTD E3 ubiquitin ligase activity
pol II	RPB1-RPB12	Transcription initiation, elongation, termination Recruitment of mRNA capping enzymes Transcription-coupled recruitment of splicing and 3′ end processing factors CTD phosphorylation, glycosylation, and ubiquitination

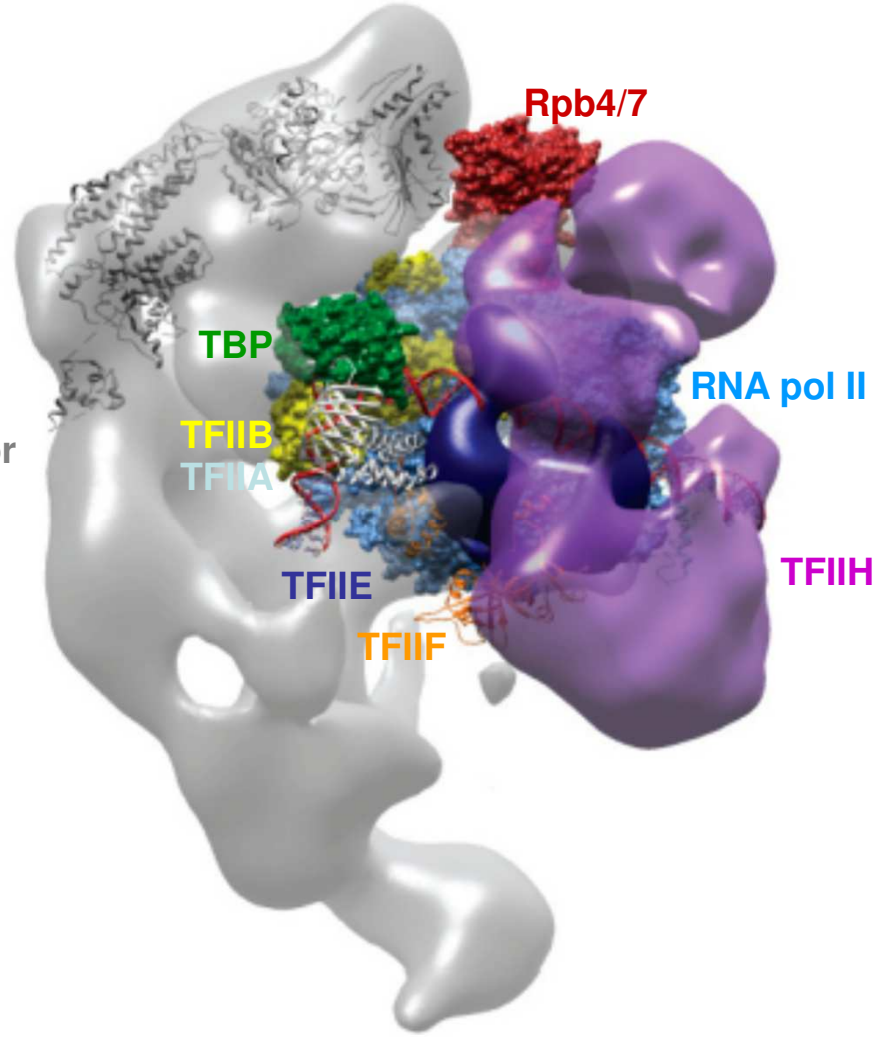


Thomas et al., CRiBMB, 2006

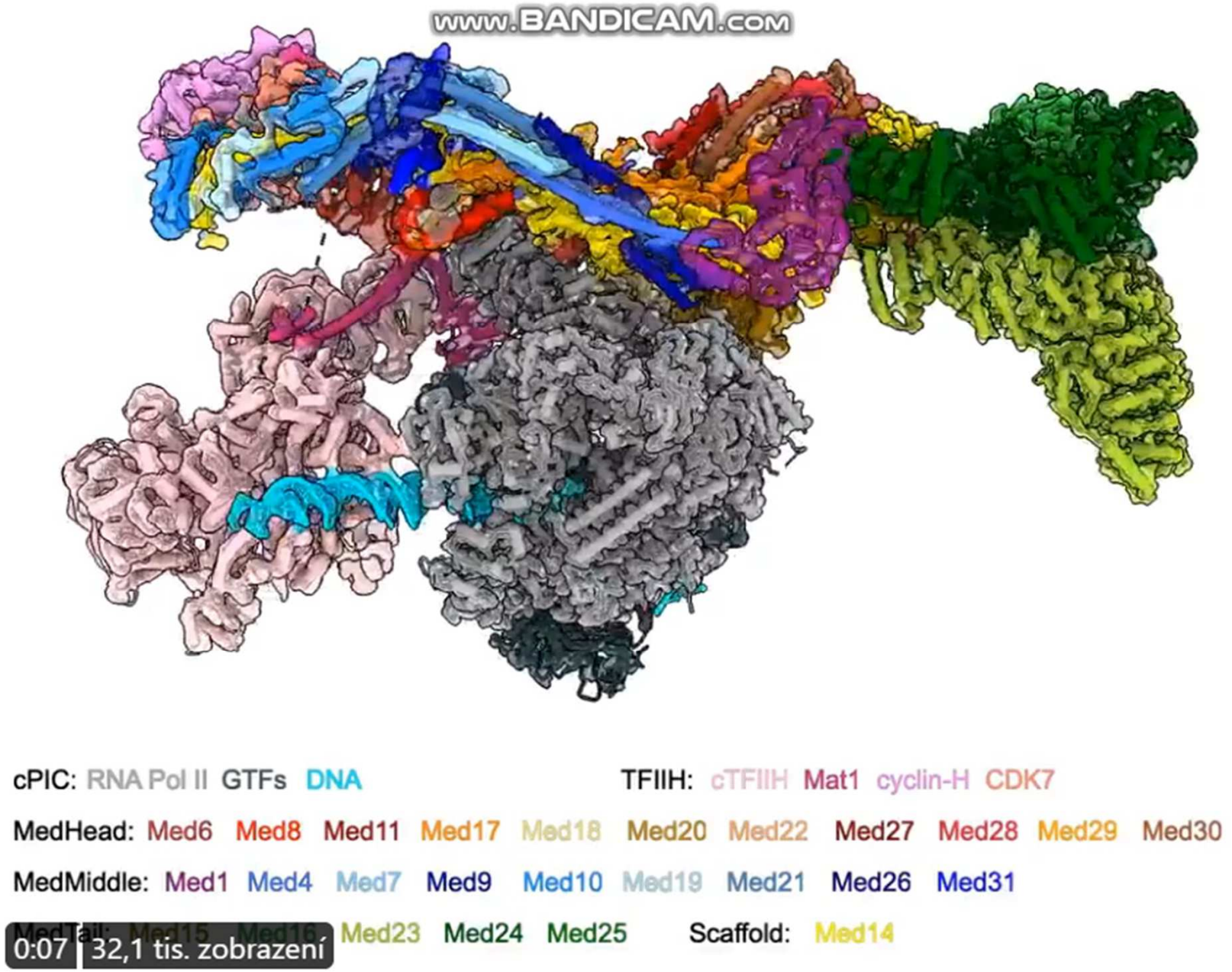
RPB1 CTD (heptapeptide YSPTSPS repeat) fosforylace

- mechanismus aktivace transkripce zahrnuje vazbu transkripčních aktivátorů, mediátoru (vytvoření transkripční smyčky) … uvolnění chromatinové struktury …



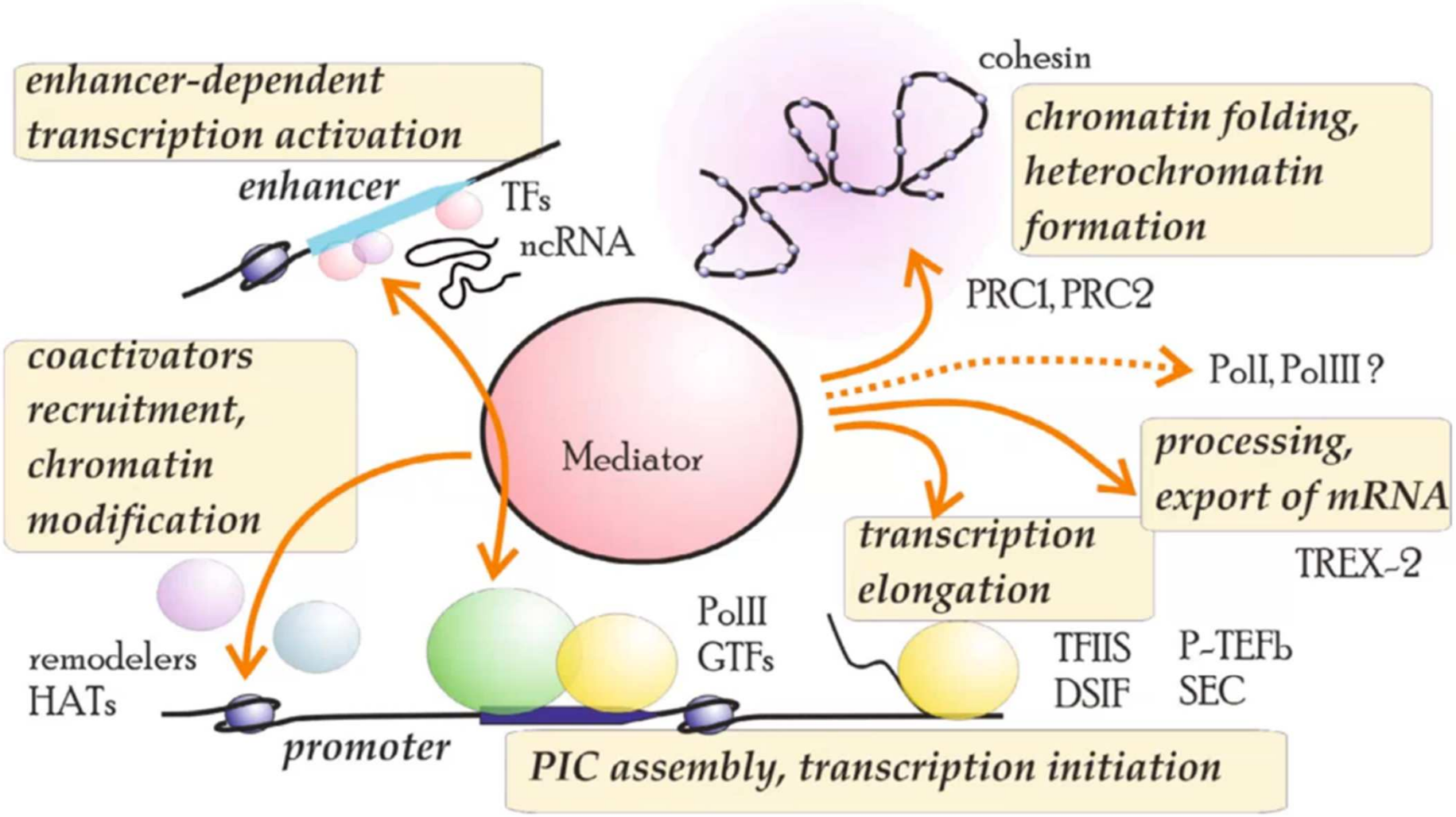


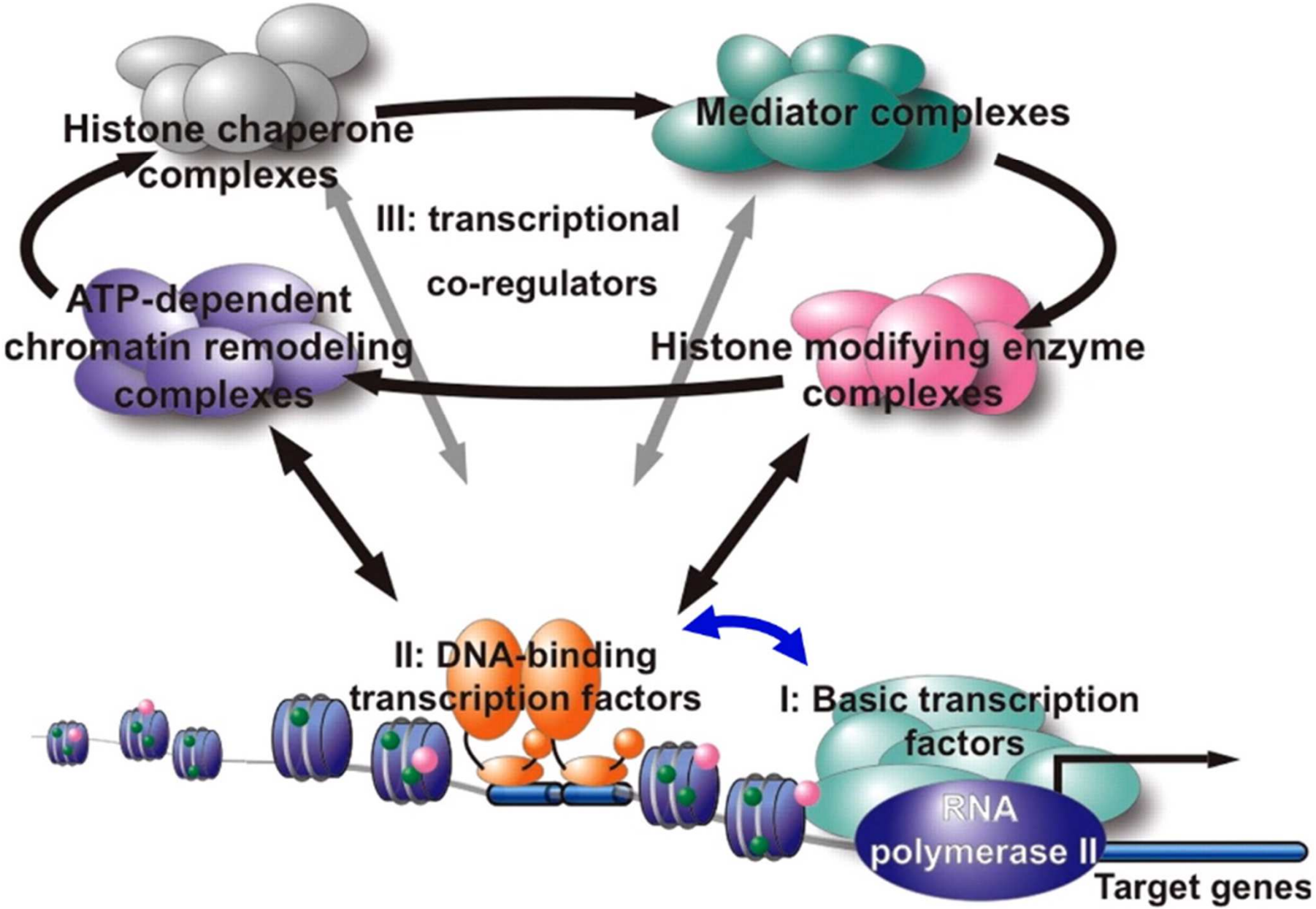
Gibbons et al, PNAS, 2012



Abdella et al, Science, 2021

Major functions of Mediator complex



Putlyaev et al. Biochem. 2018

TRANSCRIPTION