Ch 3 – L3.2

looping mechanisms

Mediator, as well as a number of **coactivator complexes**, are multi-subunit proteins.

Interaction of individual subunits with a series of Transcription Factors was demonstrated
(*CoIP + reconstruction of transcription in vitro*)

Mediator

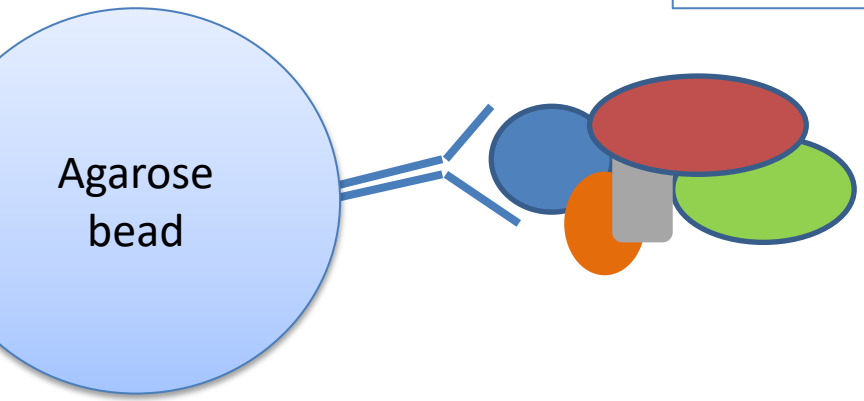
Cells or tissue

Nuclei

2000-2005

Nuclear extract (high salt)

Immunoprecipitation using anti-TF Ab (or other component)
or
using Tagged proteins and Ab against Tag (e.g. FLAG or HA epitope)



Mass spectrometry (identify)
Immunoblotting (validate)

Mediator structure worked out between 2000 and 2005

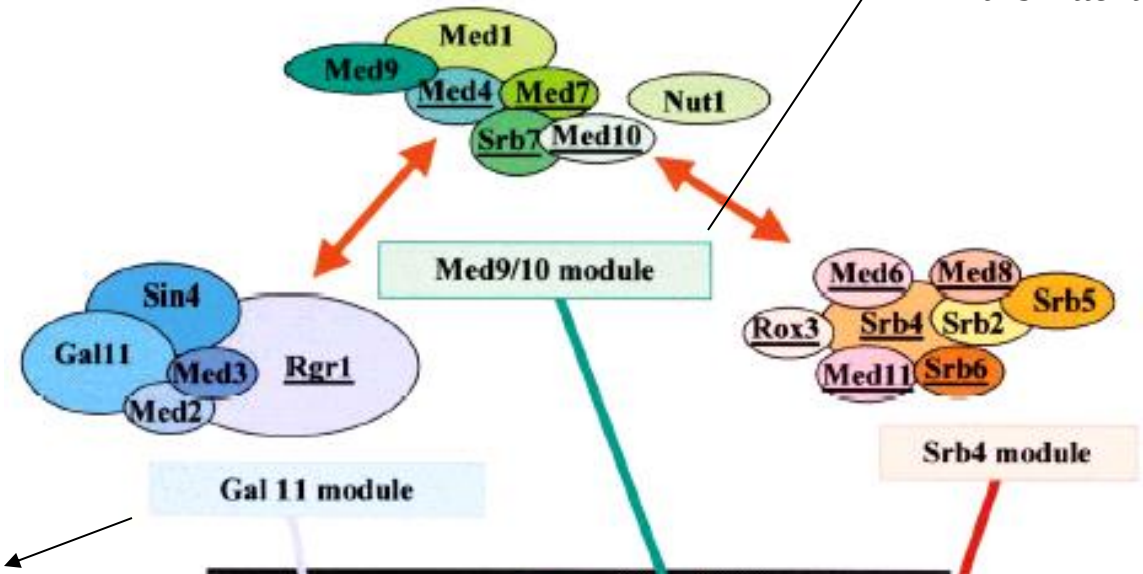
Subunit compositions of mediator complexes.

Different forms of "Mediator" Complex

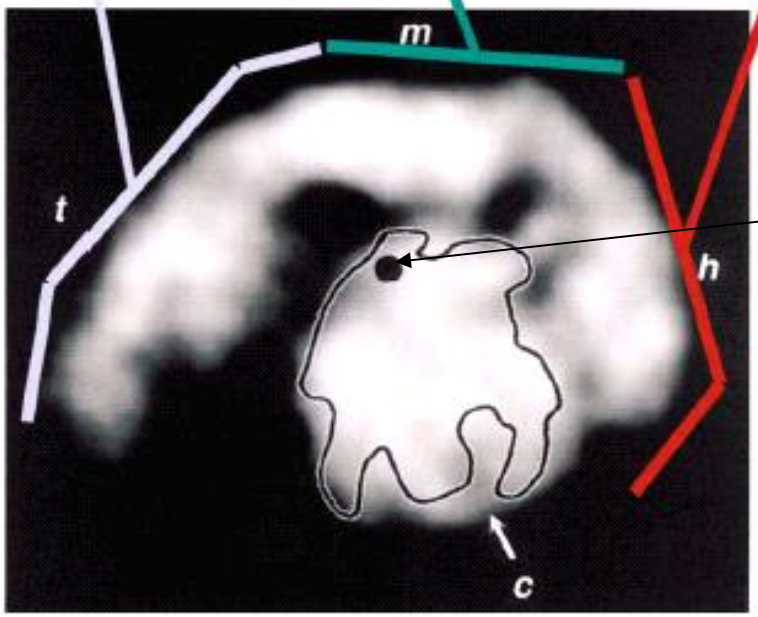
Unified subunit designation	DRIP Mediator-D	ARC Mediator-A	TRAP/ SMCC Mediator-T/S	PC2 Mediator-P	CRSP Mediator-C	NAT Mediator-N	hMediator Mediator-S	Murine Mediator Mediator-M	*S. cerevisiae*	*C. elegans*	*Drosophila*
		CBP/p300									
Med240	DRIP250	ARC250	TRAP240				ND				dTRAP240
Med230	DRIP240	ARC240	TRAP230			p230	ND	p160a	Nut1	Sop-1	dTRAP230
Med220	DRIP205	ARC205	TRAP220	(TRAP220)	CRSP200		ND	p160b	Gal11		dTRAP220
Med150	DRIP150	ARC150	TRAP170	TRAP170	CRSP150	p150	ND	Rgr1/p110	Rgr1		dTRAP170
Med130	DRIP130	ARC130	TRAP150	TRAP150b	CRSP130	p140/hSur2	hSur2			Sur-2	CG3695
Med105		ARC105/ TIG-1									
Med100	DRIP100	ARC100	TRAP100	TRAP100			ND		Sin4		dTRAP100
Med97	DRIP97		TRAP97			p95		Ring3/p96a	Srb4		
Med95	DRIP92	ARC92	TRAP95	TRAP95		p90	ND	p96b	Med1		dTRAP95
			TRAP93								
Med78	DRIP77	ARC77	TRAP80	TRAP80	CRSP77		ND	p78			dTRAP80
Med70	DRIP70-2	ARC70			CRSP70	p70	ND		Med2		
		ARC42		p37				p55	Pgd1/Hrs1		
Cdk8	(Cdk8)	(Cdk8)	hSrb10			p56/Cdk8	Cdk8		Srb10		dCdk8
Med36	DRIP36	ARC36		p36	CRSP34	p45	ND	p34	Med4		CG8609
Med34	DRIP34	ARC34	hMed7	hMed7	CRSP33	p37	Med7	Med7/p36	Med7	ceMed7	dMed7
						p36			Srb5		
Med33	DRIP33	ARC33	hMed6	(hMed6)		p33	ND	Med6/p32	Med6	ceMed6	CG9473
		ARC32	hTRF	hTRF			ND	TRF/p28a	Med8		
Cyclin C			hSrb11			p31/ Cyclin C	Cyclin C		Srb11		
						p30		p28b	Rox3		
						p23			Srb2		
			hSoh1	hSoh1		p22					
						p21			Med9/Cse2		
Med17	hSrb7		hSrb7	hSrb7		p17	ND	Srb7/p21	Srb7	ceSrb7	CG17397
Med10	hMed10		hNut2	hNut2		p14	ND		Med10/Nut2	ceMed10	dNut2
									Med11		
									Srb6		

Mediator

Interactcs with CTD
Both activator and repressor signal
Transmitter to Pol



Role in contacting
trans-activators



CTD

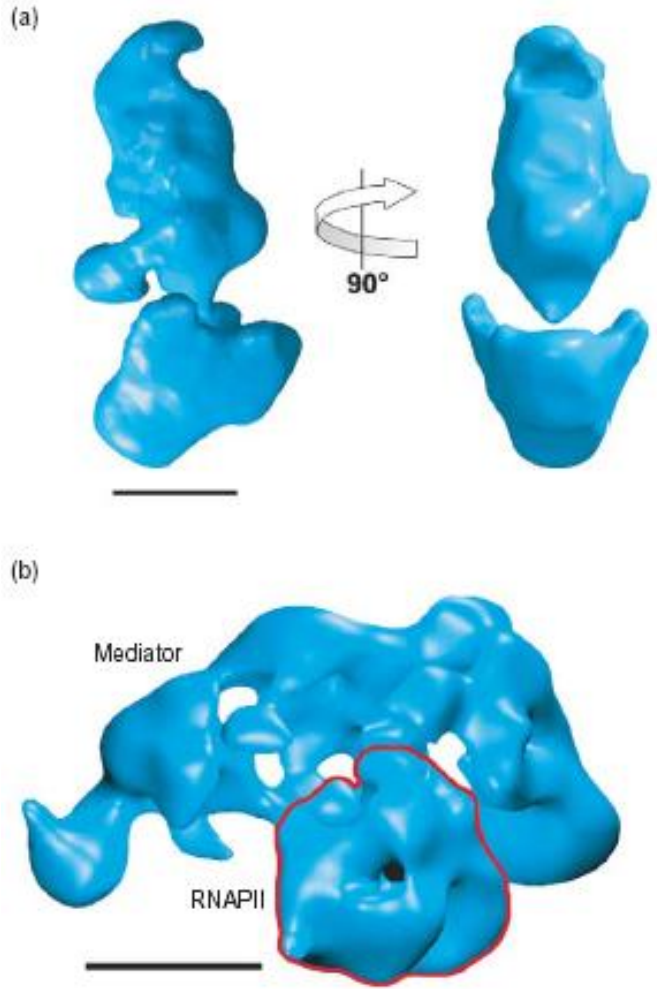


Figure 1. Structure of the yeast Mediator and holoenzyme complexes.

(a) A 3D reconstruction of the yeast Mediator structure was calculated from images of individual particles imaged in an electron microscope after preservation in stain. Mediator has a compact, roughly triangular shape. A large domain at the bottom is linked by a thin connection to the top portion of the structure. The resolution of the reconstruction is 35- A° , and the scale bar represents 100 A° .

(b) (b) Structure of the Mediator–RNA polymerase II holoenzyme complex calculated from electron microscope images of individual particles preserved in stain. Previous characterization of the polymerase and Mediator structures led to identification of the Mediator and RNA polymerase II (red outline) portions of the holoenzyme structure. In the holoenzyme, Mediator adopts an extended conformation, embracing the central polymerase density. The resolution of the reconstruction is w35 A° , and the scale bar represents 100 A° .

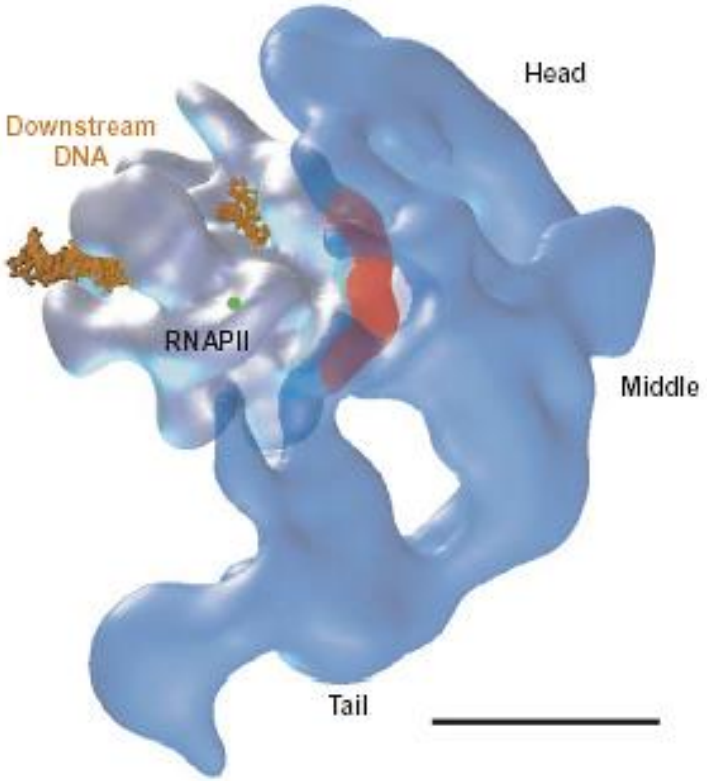


Figure 3. Interaction of Mediator and RNA polymerase II (RNAPII) in the holoenzyme complex. The precise orientation of RNAPII in the holoenzyme complex was established by 2D cross-correlation analysis between holoenzyme and RNAPII projections. The figure shows a cryoelectron microscopy reconstruction of polymerase fitted into the extended Mediator structure in the orientation determined by cross-correlation analysis. Multiple contacts between Mediator and RNAPII are established in the holoenzyme complex, involving mostly the head and middle domains, and distributed around the Rpb3–Rpb11 polymerase subunits (highlighted in red). The small green circle indicates the point where the carboxyterminal domain of Rpb1 (the largest polymerase subunit), crucial for Mediator polymerase interaction, emanates from the surface of the enzyme. The bacterial homolog of the Rpb3–Rpb11 complex, the a2 homodimer, is involved in transcription regulation in bacteria, suggesting a conservation between prokaryotes and eukaryotes of the RNA polymerase surface involved in regulation. The scale bar represents 100 A° .

Different forms of Mediator exist in different cell types/developmental stage and possibly gene context, depending on the kind of TFs bound.

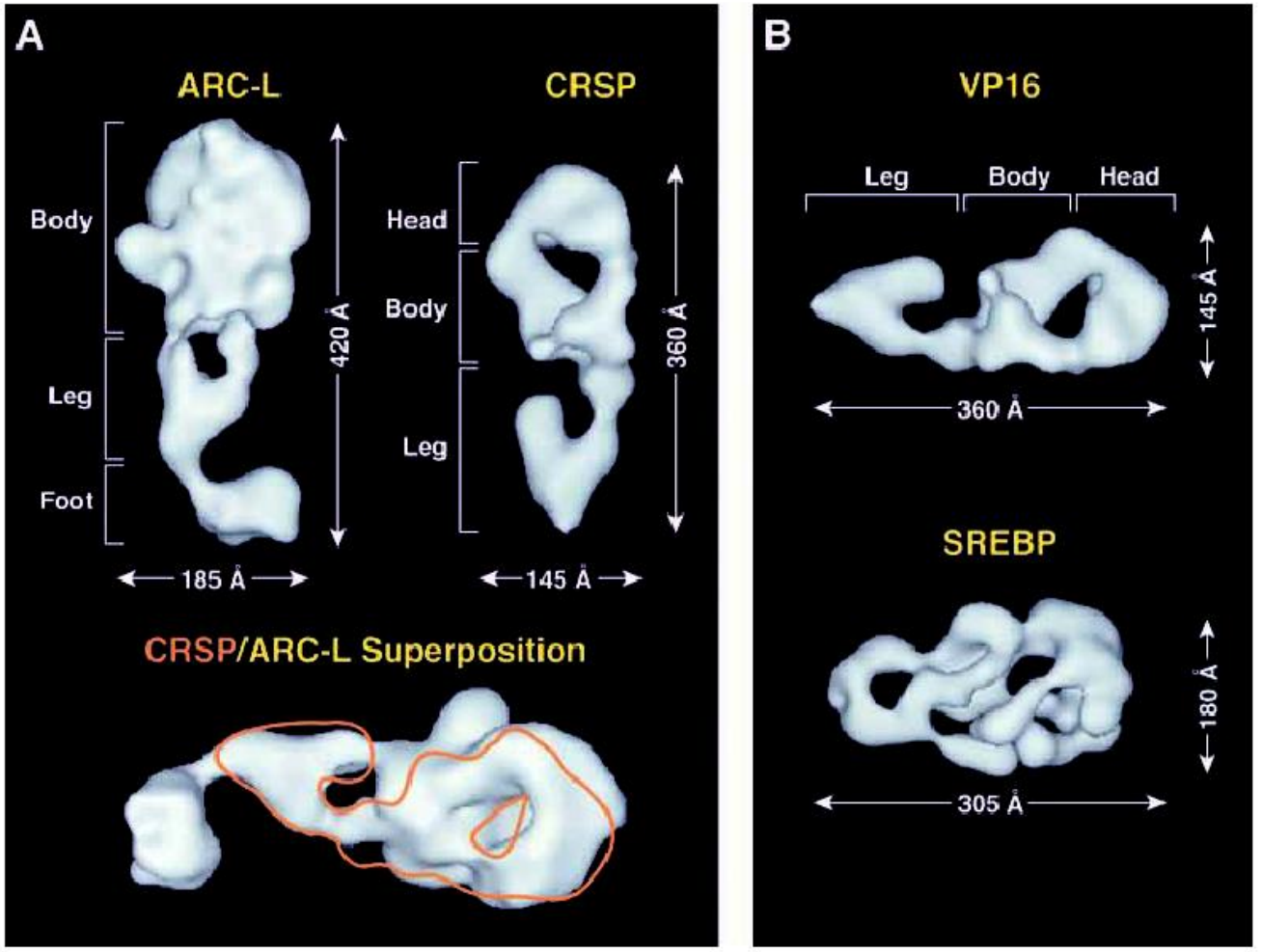
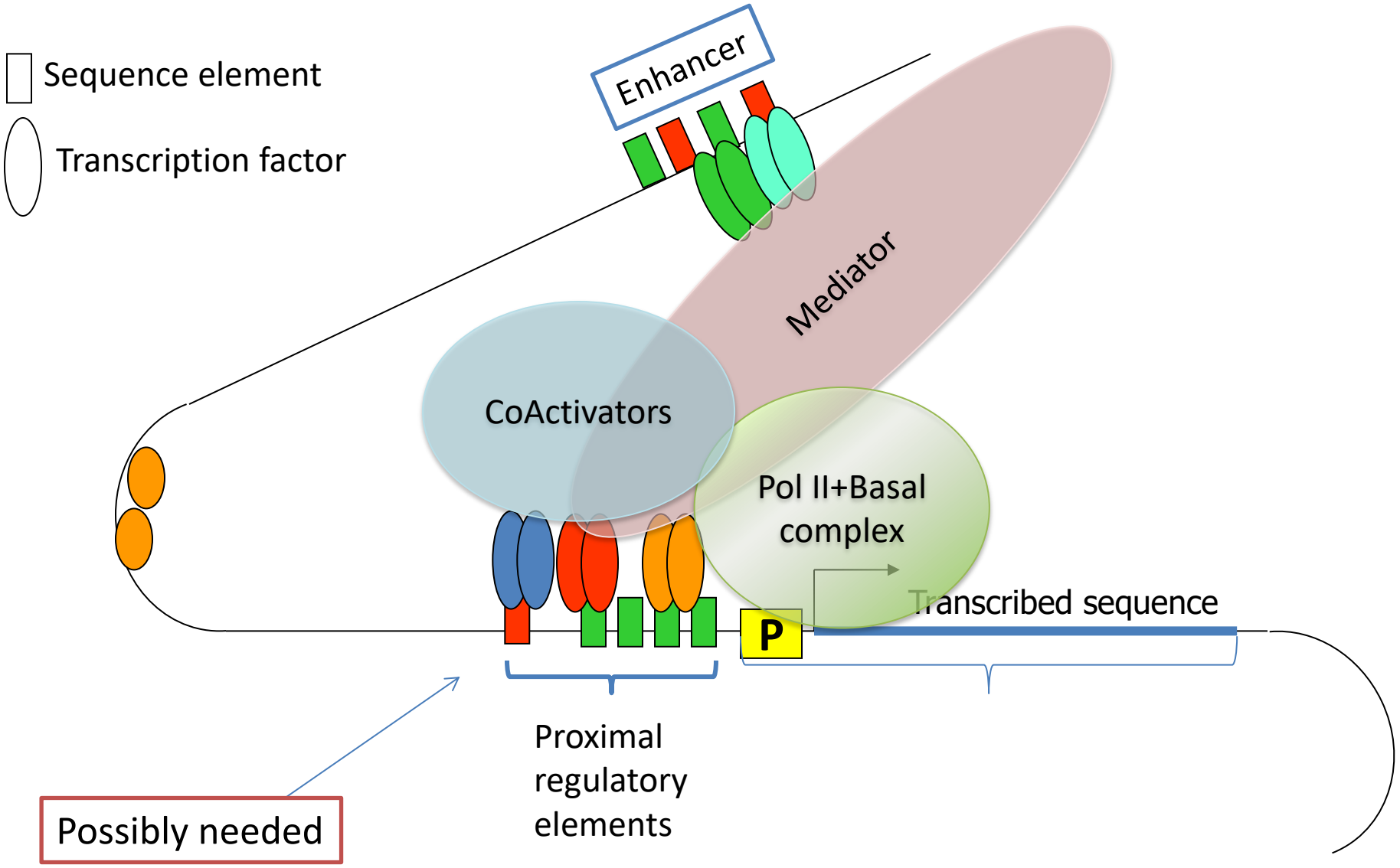


Fig. 2. Conformations of the mammalian mediator complexes. (A) EM composites of the ARC-L and CRSP complexes, which illustrate the size and structural differences between the two. (B) EM composites showing the distinctly different structural conformations adopted by CRSP when isolated via affinity interactions with either the VP16 or SREBP activator. EM composites were generously provided by Dylan Taatjes and Bob Tjian (Naar et al., 2002; Taatjes et al., 2002).

Take-home message:

Mediator is a large, multi-subunit protein complex. showing both common and facutative subunits.

The composition, shape and MW is dictated by the context where Mediator operates, i.e. TFs bound to Enhancers and Promoters involved in any specific interaction.


Sequence element
Transcription factor
Enhancer
Mediator
CoActivators
Pol II+Basal complex
Transcribed sequence
P
Proximal regulatory elements
Possibly needed

Coactivators and Corepressors:

Large protein complexes exhibiting several functions:

- Interaction with Transcription Factors (sequence-specific TF)
- Histone PTM writers/erasers (in particular, HAT histone acetyl transferase)
- Chromatin remodeling factors
    - (ATP-dependent chromatin remodelers are a class of proteins that «remodel» nucleosomes over DNA, usually in ATP-dependent fashion)

CoA and **CoR** may be recruited:

a) directly by Transcription Factors

b) through interaction with PIC or Mediator subunits

Prototype Coactivator: CBP

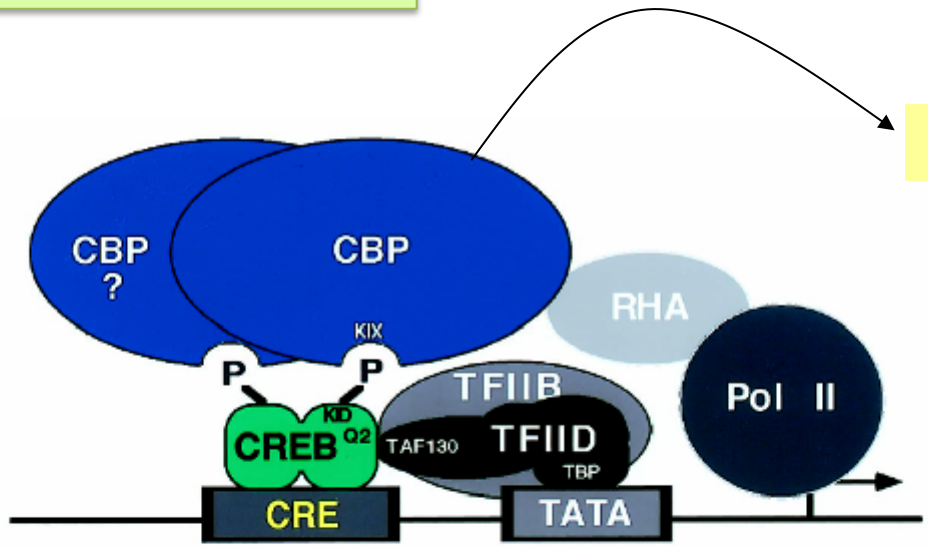


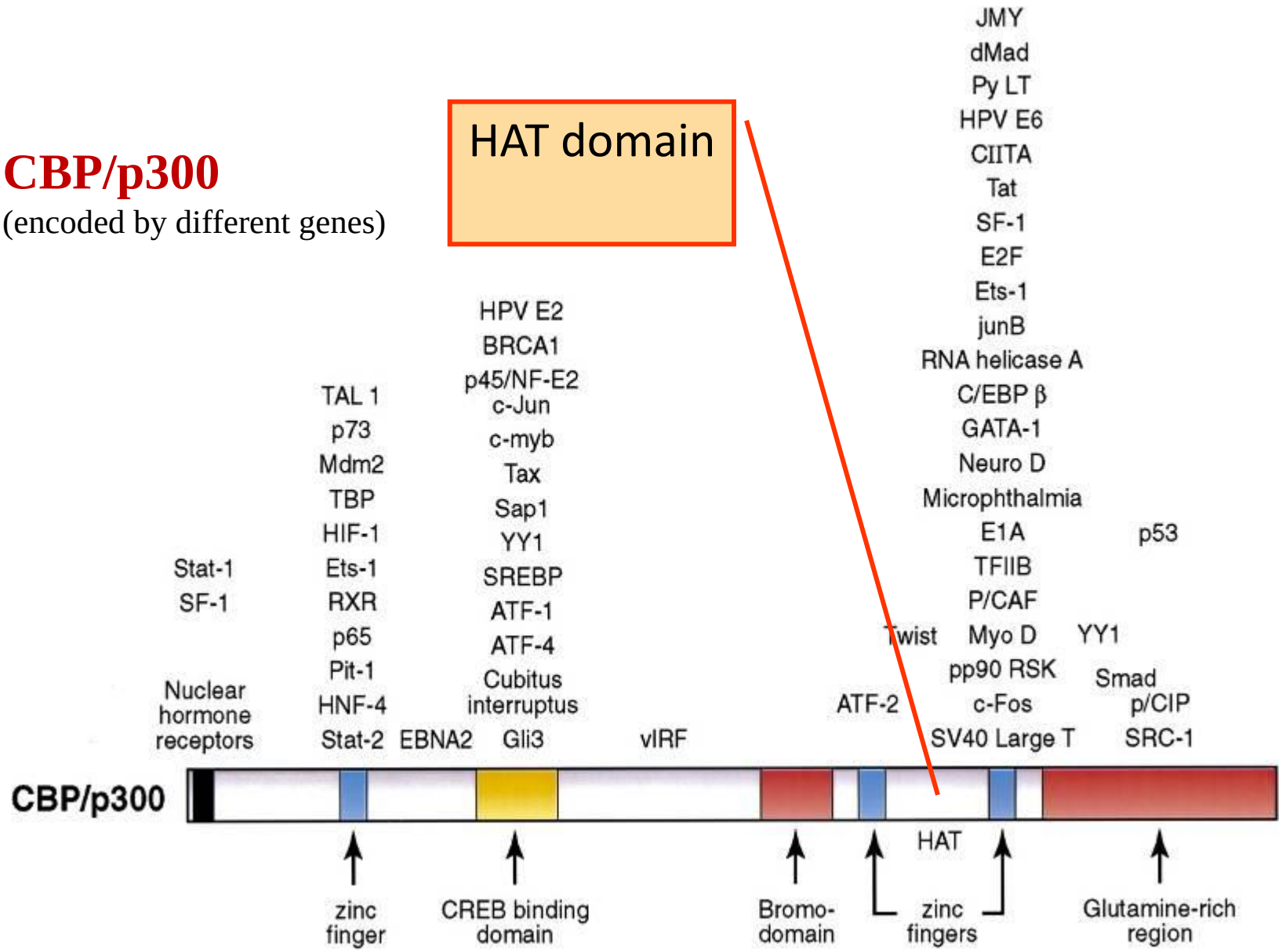
Figure 4 Multiple domains of CREB contribute to transcriptional activation. Different domains of CREB bind distinct coactivators and basal transcription factors to activate transcription. Shown is a CREB dimer bound to its cognate CaRE/CRE element on the promoter of a CREB target gene. Downstream of the CaRE/CRE is the TATA box, which binds the multiprotein TFIID basal transcription factor (via the TBP protein). Another factor within TFIID, TAF130, binds to the Q2 domain of CREB. The Q2 domain of CREB has also been shown to interact with TFIIB, which is a part of the basal transcription machinery as well. A distinct domain of CREB, the KID, contributes to signal-induced transcriptional activation. When phosphorylated at Ser133, the KID of CREB can bind to the KIX domain of the CBP. It is presently unclear whether CBP associates with Ser133–phosphorylated CREB as a dimer. CBP associates indirectly with Pol II via the RNA helicase A (RHA) protein. Therefore, recruitment of CBP to Ser133–phosphorylated CREB results in recruitment and stabilization of Pol II on the promoter of CREB target genes, whereas the Q2 domain interacts with other elements of the basal transcription machinery that are required for transcription, such as TFIID and TFIIB.

cAMP response pathway, CREB

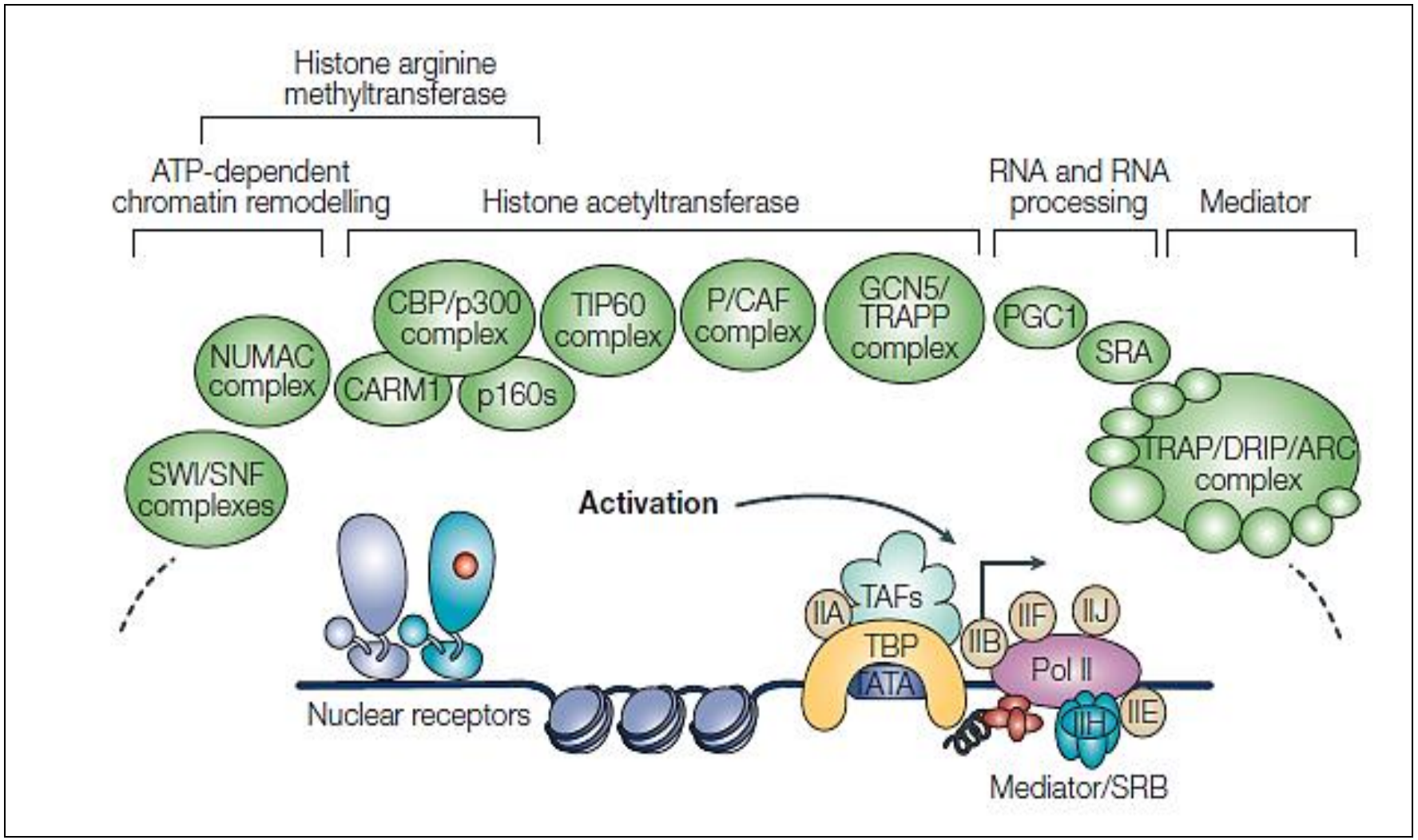
CBP = CREB binding protein (265KDa) and **p300** are in fact **general coactivators**

CBP/p300

(encoded by different genes)



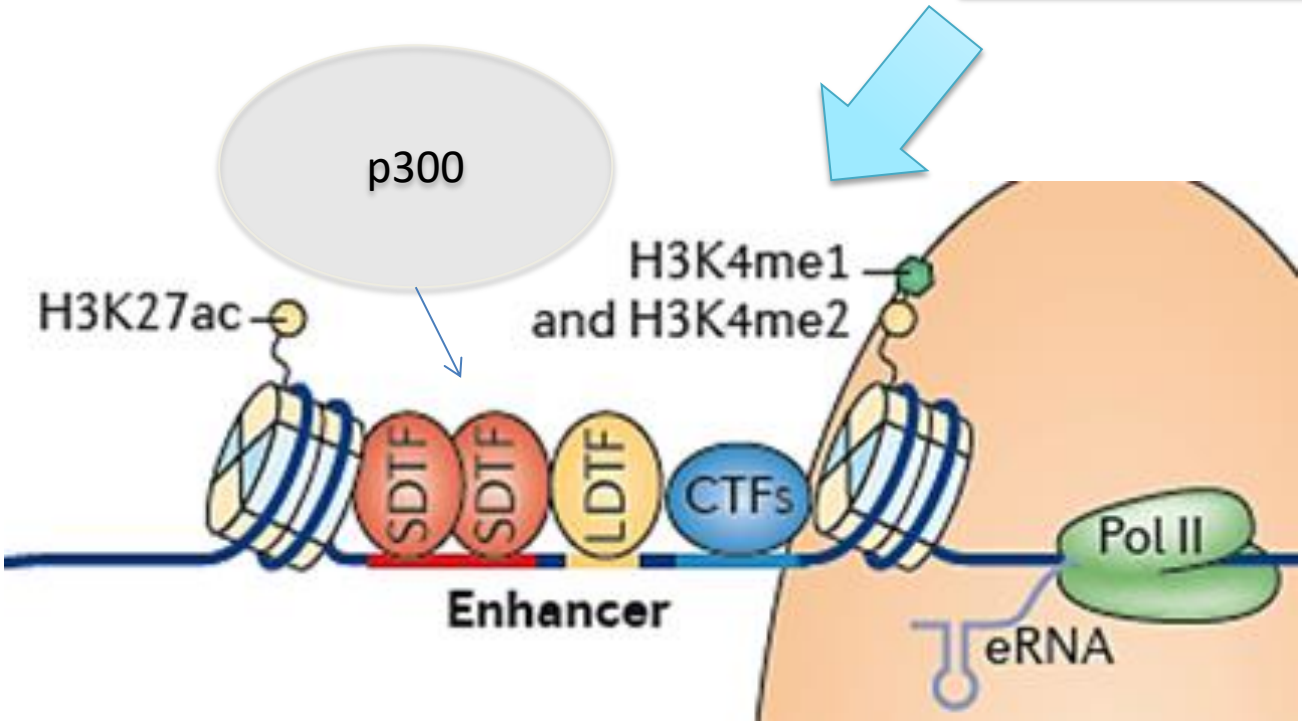
Co-activators participating in transcriptional activation by Nuclear Receptors



From: Perissi & Rosenfed, 2005, Nature Rev Mol Cell Biol, 6: 542-554

- **do TFs induce any change in chromatin ?**

see your Research paper 3



Enhancer activation.

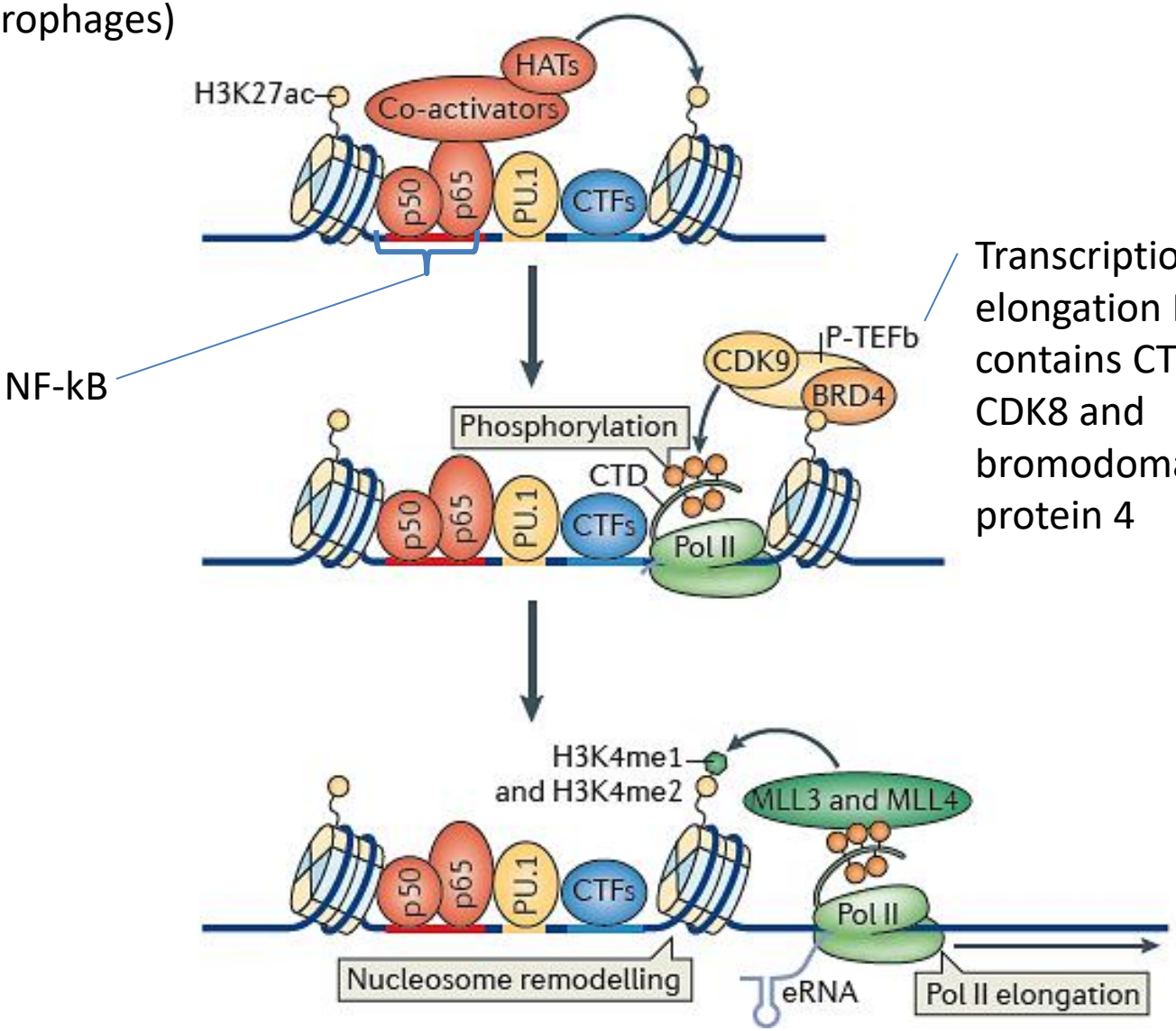
Markers of enhancer activation: (discussed in Heinz 2015)

TF binding
H3K4me1/me2 (see Henriques et al.)
H3K27ac
eRNA

co-regulators (co-activator and co-repressor) including the HAT enzymes p300/CBP (binds directly to several TFs)

Among others, the Mediator, BRG1, MLL, BRD4 are also found at active enhancers

Activation of eRNA transcription
(example in macrophages)



Transcription elongation Factor b contains CTD kinase CDK8 and bromodomain protein 4

Heinz et al., 2015

Enhancer transcription

Production of unstable short trancripts in both directions from the Enhancer (called eRNA) is considered today as an essential mark of enhancer activity.

nature Vol 465|13 May 2010|doi:10.1038/nature09033

ARTICLES

Widespread transcription at neuronal activity-regulated enhancers

Tae-Kyung Kim[1]*†, Martin Hemberg[2]*, Jesse M. Gray[1]*, Allen M. Costa[1], Daniel M. Bear[1], Jing Wu[3], David A. Harmin[1,4], Mike Laptewicz[1], Kellie Barbara-Haley[5], Scott Kuersten[6], Eirene Markenscoff-Papadimitriou[1]†, Dietmar Kuhl[7], Haruhiko Bito[8], Paul F. Worley[3], Gabriel Kreiman[2] & Michael E. Greenberg[1]

We used genome-wide sequencing methods to study stimulus-dependent enhancer function in mouse cortical neurons. We identified ~12,000 neuronal activity-regulated enhancers that are bound by the general transcriptional co-activator CBP in an activity-dependent manner. A function of CBP at enhancers may be to recruit RNA polymerase II (RNAPII), as we also observed activity-regulated RNAPII binding to thousands of enhancers. Notably, RNAPII at enhancers transcribes bi-directionally a novel class of enhancer RNAs (eRNAs) within enhancer domains defined by the presence of histone H3 monomethylated at lysine 4. The level of eRNA expression at neuronal enhancers positively correlates with the level of messenger RNA synthesis at nearby genes, suggesting that eRNA synthesis occurs specifically at enhancers that are actively engaged in promoting mRNA synthesis. These findings reveal that a widespread mechanism of enhancer activation involves RNAPII binding and eRNA synthesis.

In the nervous system, hundreds of genes are induced in response to sensory experience-dependent neuronal activation.

Exposure of **primary neuronal cultures** to an elevated level of potassium chloride (KCl) leads to membrane depolarization and an influx of calcium through L-type voltage-sensitive calcium channels.

The resulting increase in intracellular calcium level then triggers several calcium dependent signalling pathways that ultimately lead to changes in gene expression.

We used this in vitro neuronal culture system to characterize neuronal activity-regulated enhancers

- **Enhancers identified using ChIP-Seq with CBP antibodies**
- **RNA Pol II colocalizes with several enhancers (ChIP-Seq)**

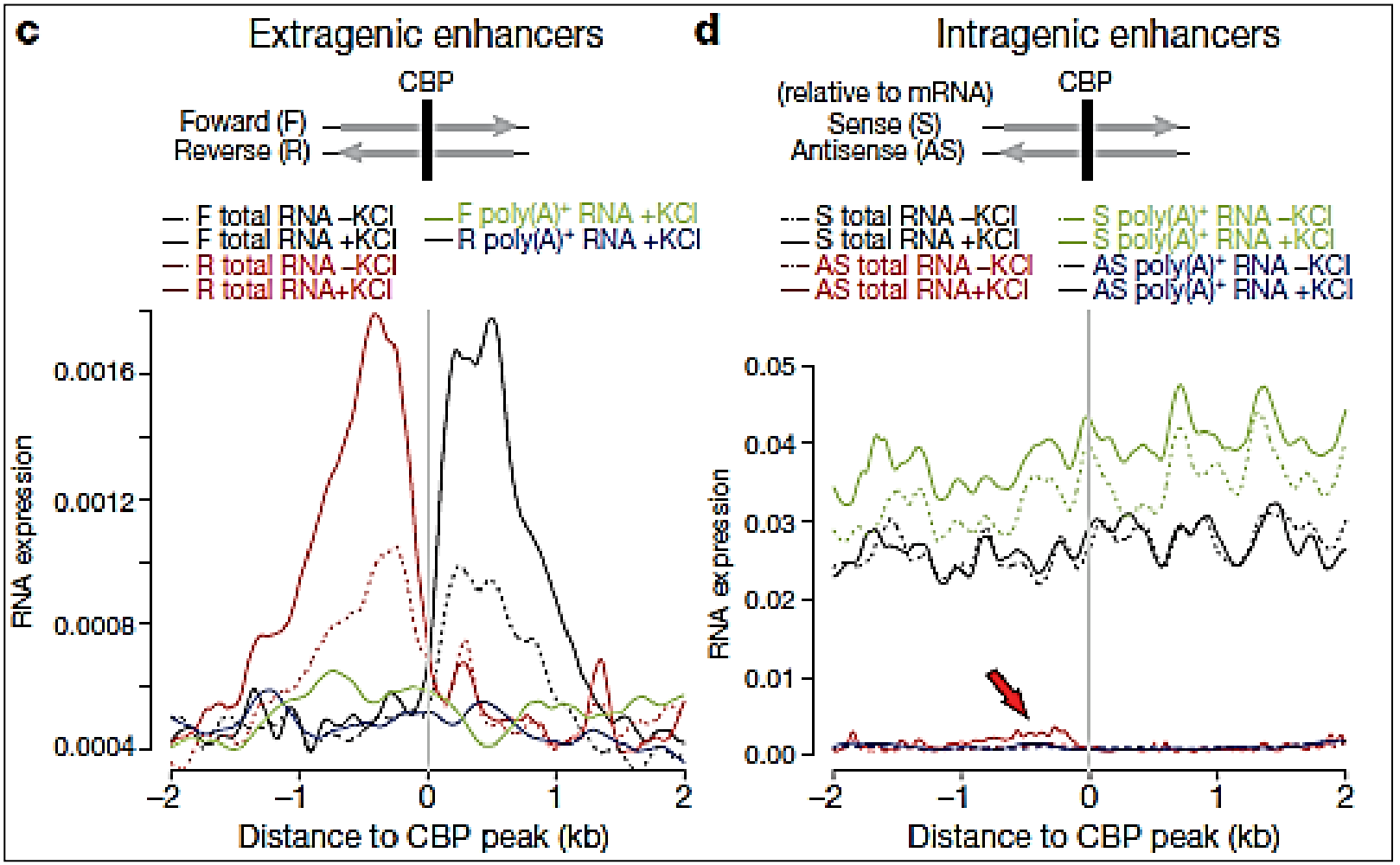


Figure 4 | Enhancers bind RNA polymerase II (RNAPII) and produce eRNAs.
In c, F and R denote forward (1) and reverse (2) genomic strands. In d, enhancers are aligned oriented relative to the gene in which they reside to allow for sense and antisense RNA-Seq reads to be shown separately. Although sense eRNAs cannot be detected due to overlapping mRNA transcription, the red arrow indicates a local increase in antisense RNA expression attributable to eRNAs. Note different scales on the y axis in c and d.

eRNA are induced after stimulation

eRNA are **quantitatively correlated** with enhancer-regulated mRNA

eRNA transcription confirmed by subsequent studies including ENCODE

(described in one of the 2012 ENCODE articles by Djebali et al., Nature 2012)

eRNA unstable

Difficult to map using common RNA-Seq technologies

«Nascent» RNA methods required (such as GRO-seq).

eRNA are unstable, long noncoding transcripts in both directions around enhancers

eRNA are mainly poly(A-)

eRNA transcription is increased in active enhancers, i.e. when the enhancer contacts a promoter and activates transcription

There is indication that knockdown of eRNA can influence enhancer function ?

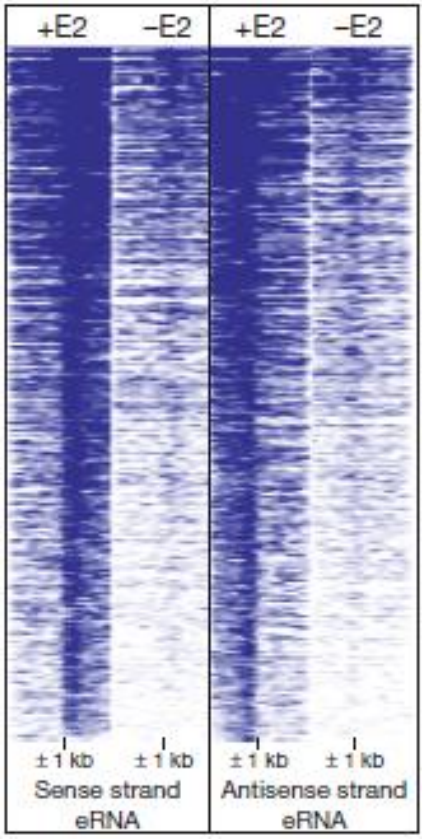
LETTER

doi:10.1038/nature12210

Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation

Wenbo Li[1]*, Dimple Notani[1]*, Qi Ma[1,2], Bogdan Tanasa[1,3], Esperanza Nunez[1], Aaron Yun Chen[1], Daria Merkurjev[1,2], Jie Zhang[1], Kenneth Ohgi[1], Xiaoyuan Song[1], Soohwan Oh[1,4], Hong-Sook Kim[1], Christopher K. Glass[5] & Michael G. Rosenfeld[1]

The functional importance of gene enhancers in regulated gene expression is well established[1–3]. In addition to widespread transcription of long non-coding RNAs (lncRNAs) in mammalian cells[4–6], bidirectional ncRNAs are transcribed on enhancers, and are thus referred to as enhancer RNAs (eRNAs)[7–9]. However, it has remained unclear whether these eRNAs are functional or merely a reflection of enhancer activation. Here we report that in human breast cancer cells 17β-oestradiol (E2)-bound oestrogen receptor α (ER-α) causes a global increase in eRNA transcription on enhancers adjacent to E2-upregulated coding genes. These induced eRNAs, as functional transcripts, seem to exert important roles for the observed ligand-dependent induction of target coding genes, increasing the strength of specific enhancer–promoter looping initiated by ER-α binding. Cohesin, present on many ER-α-regulated enhancers even before ligand treatment, apparently contributes to E2-dependent gene activation, at least in part by stabilizing E2/ER-α/eRNA-induced enhancer–promoter looping. Our data indicate that eRNAs are likely to have important functions in many regulated programs of gene transcription.

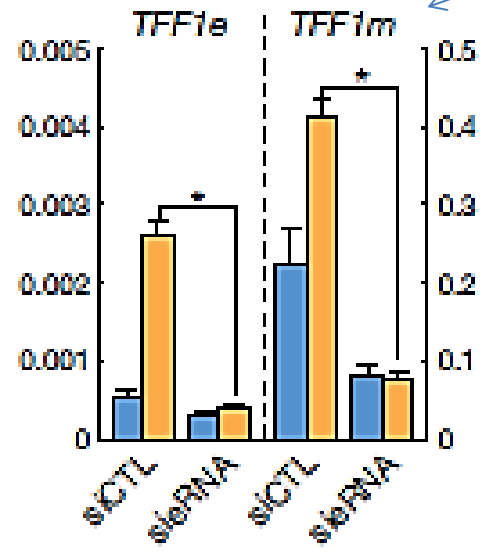


Do eRNAs have any functional role ?

One approach is to knock them down and see if transcription of the connected genes is affected

eRNA at enhancer

mRNA



eRNA knock-out using siRNA/LNA abrogates the enhancer effect on transcription

LNA= Locked Nucleic Acid

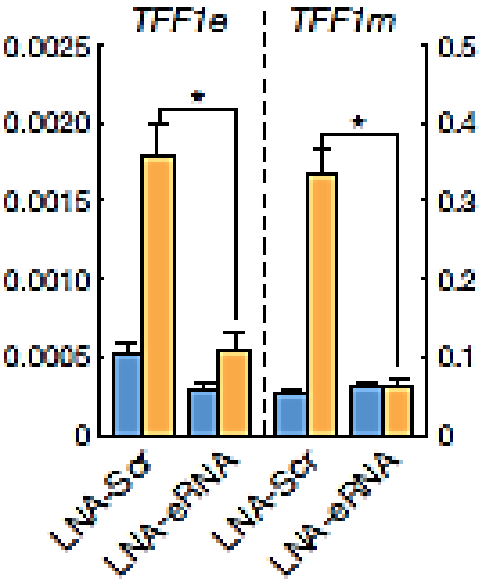


Figure 2 | Importance of eRNA for target gene activation. a, b, siRNA/LNA knockdown of eRNAs. Efficacy and effects on coding gene transcription were assessed by qPCR for the TFF1, FOXC1 and CA12 eRNAs and corresponding coding transcription units. Lower case ‘e’ and ‘m’ after gene names denote eRNA and gene mRNA, respectively. CTL, control; Scr, scramble. (Li et al., 2013)

RNA in the loop ?

Model of ncRNA-a function as described by Lai et al. (2013).
An ncRNA-a interacts with the multisubunit Mediator complex to facilitate the formation of a long-range DNA loop, bringing the enhancer-like ncRNAa locus into physical proximity with its target locus. This then leads to robust expression of the target gene.

ncRNA-a = noncoding RNA activator

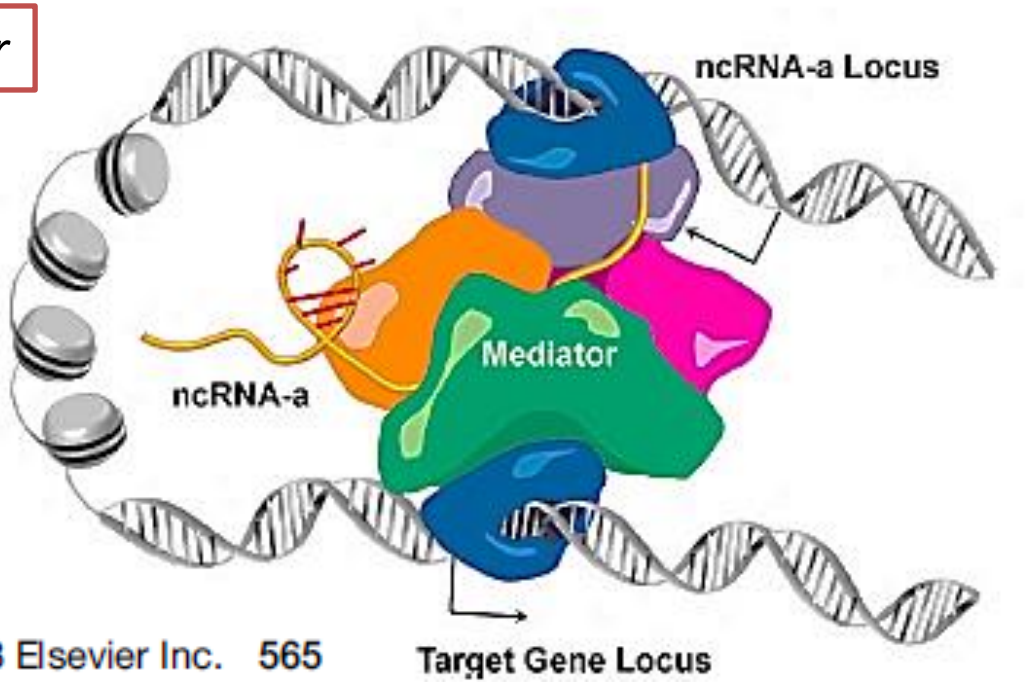


Figure 1. Schematics of lncRNA-Directed cis-Regulatory Mechanisms

Developmental Cell 24, March 25, 2013 ©2013 Elsevier Inc. 565

28 FEBRUARY 2013 | VOL 494 | NATURE | 497

LETTER

doi:10.1038/nature11884

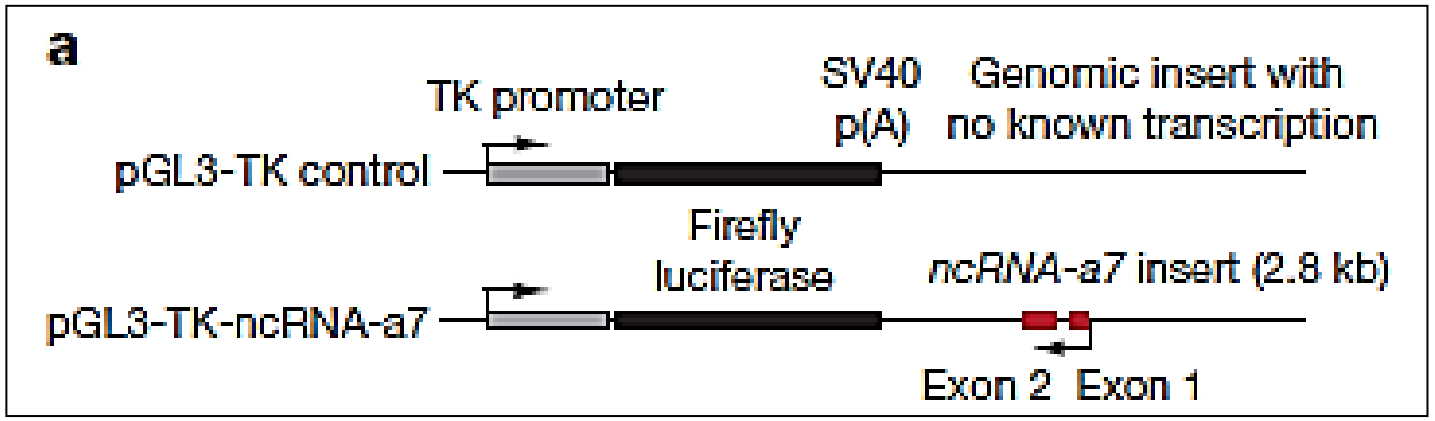
Activating RNAs associate with Mediator to enhance chromatin architecture and transcription

Fan Lai[1], Ulf A. Orom[2], Matteo Cesaroni[1], Malte Beringer[3], Dylan J. Taatjes[4], Gerd A. Blobel[5] & Ramin Shiekhattar[1]

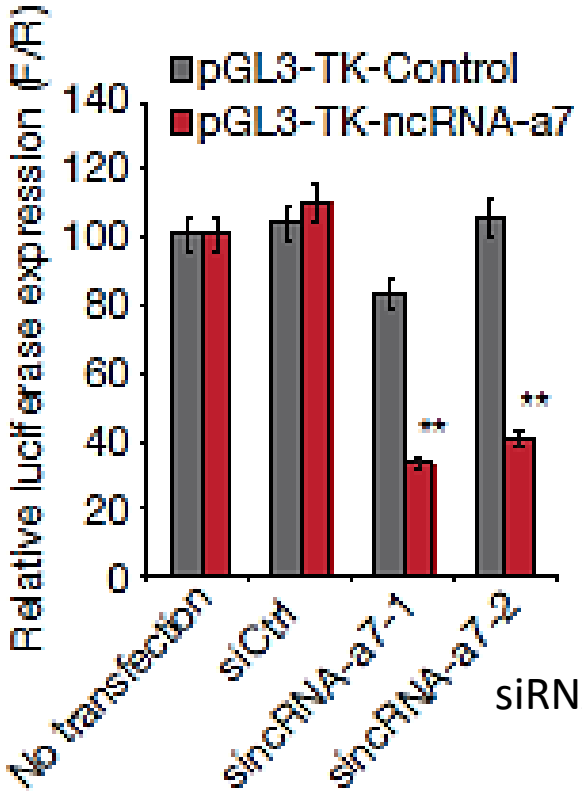
In previous work they found lncRNAs with enhancer-like properties:
A class of lncRNAs, termed **ncRNA-activating** (ncRNA-a), that function to activate their neighbouring genes using a cis-mediated mechanism.

They systhematically siRNA noncoding RNAs and identified neighbouring down-regulated genes

1st question: is activation ncRNA dependent ?



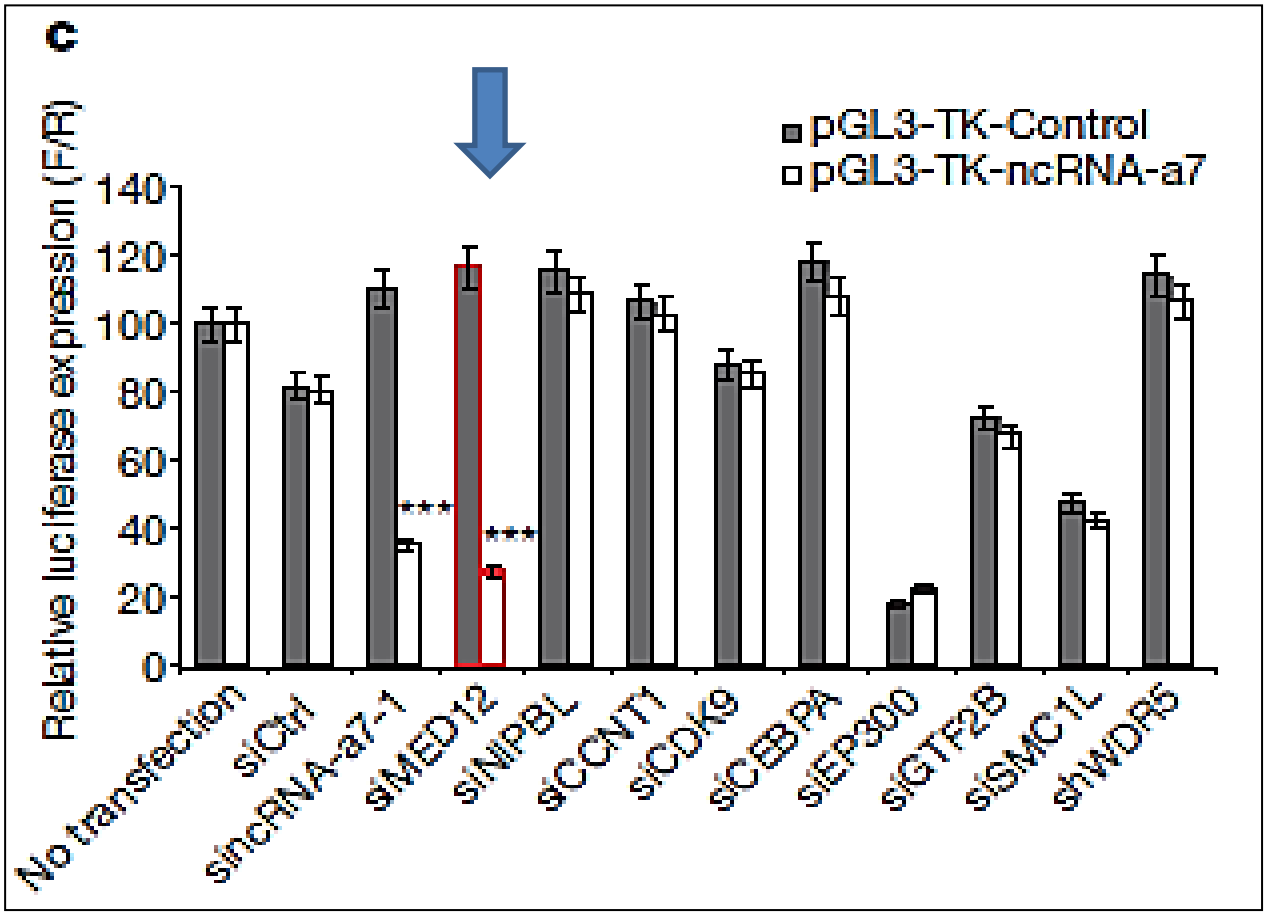
HEK293 cells
(Human embryonal kidney)



siRNA down-regulation

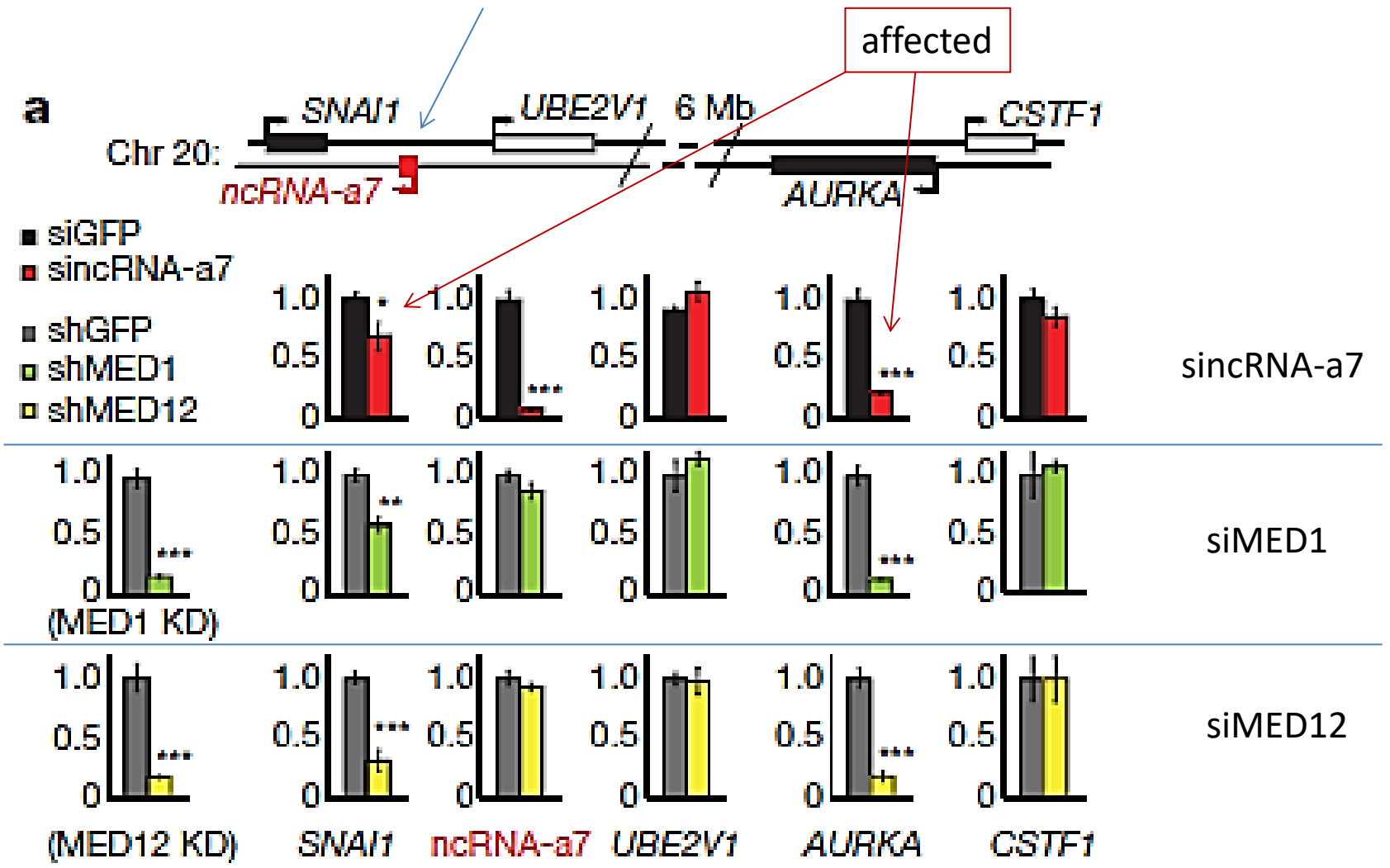
2nd question: which component of the transcriptional machinery is involved ?

Screening protein components for function in gene activity



MED12 is the only protein, among those tested, that affects RNA-a function

3° question: is this effect reproducible on the endogenous loci ?

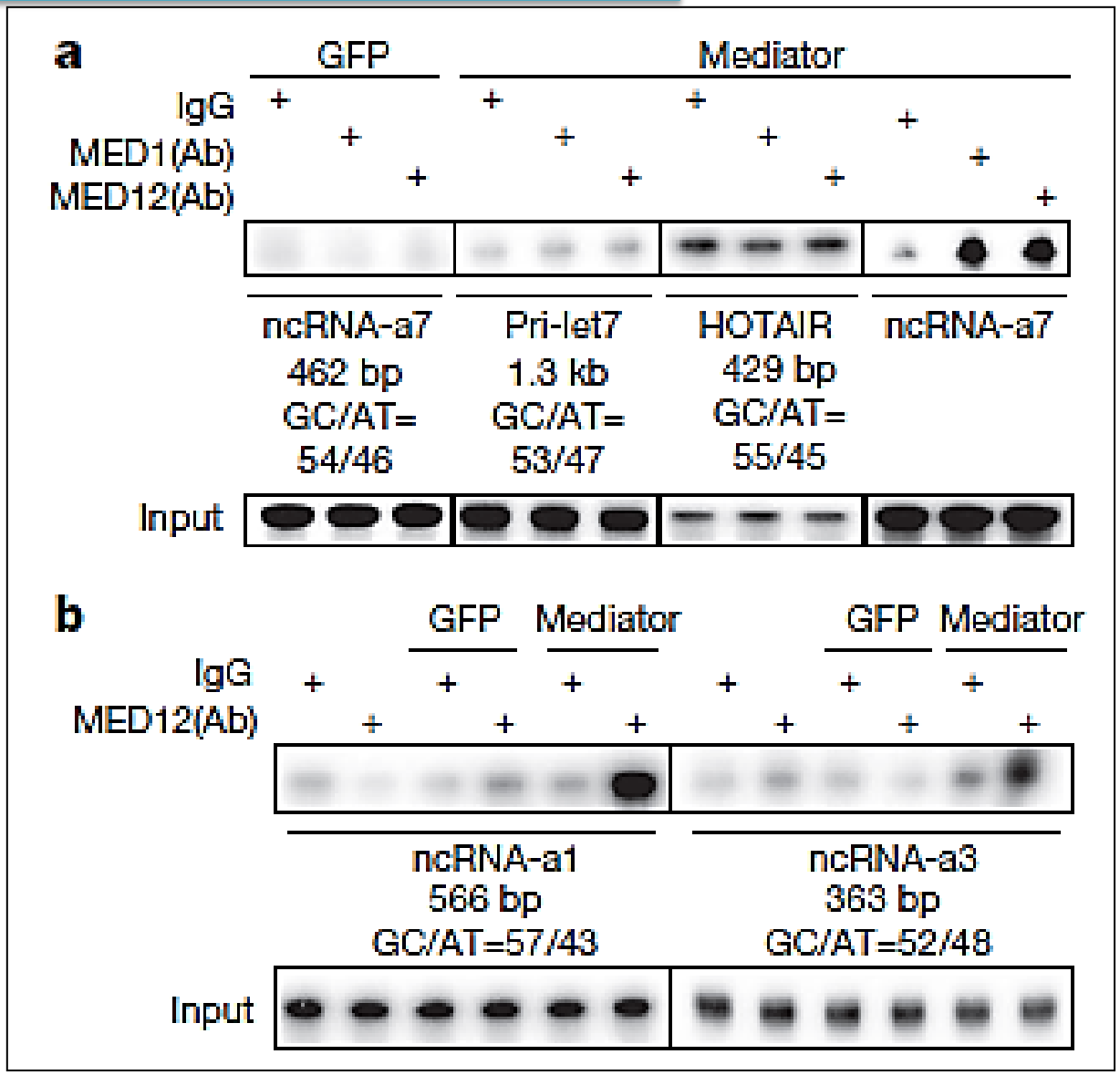


Authors demonstrated ncRNA-a/MED binding

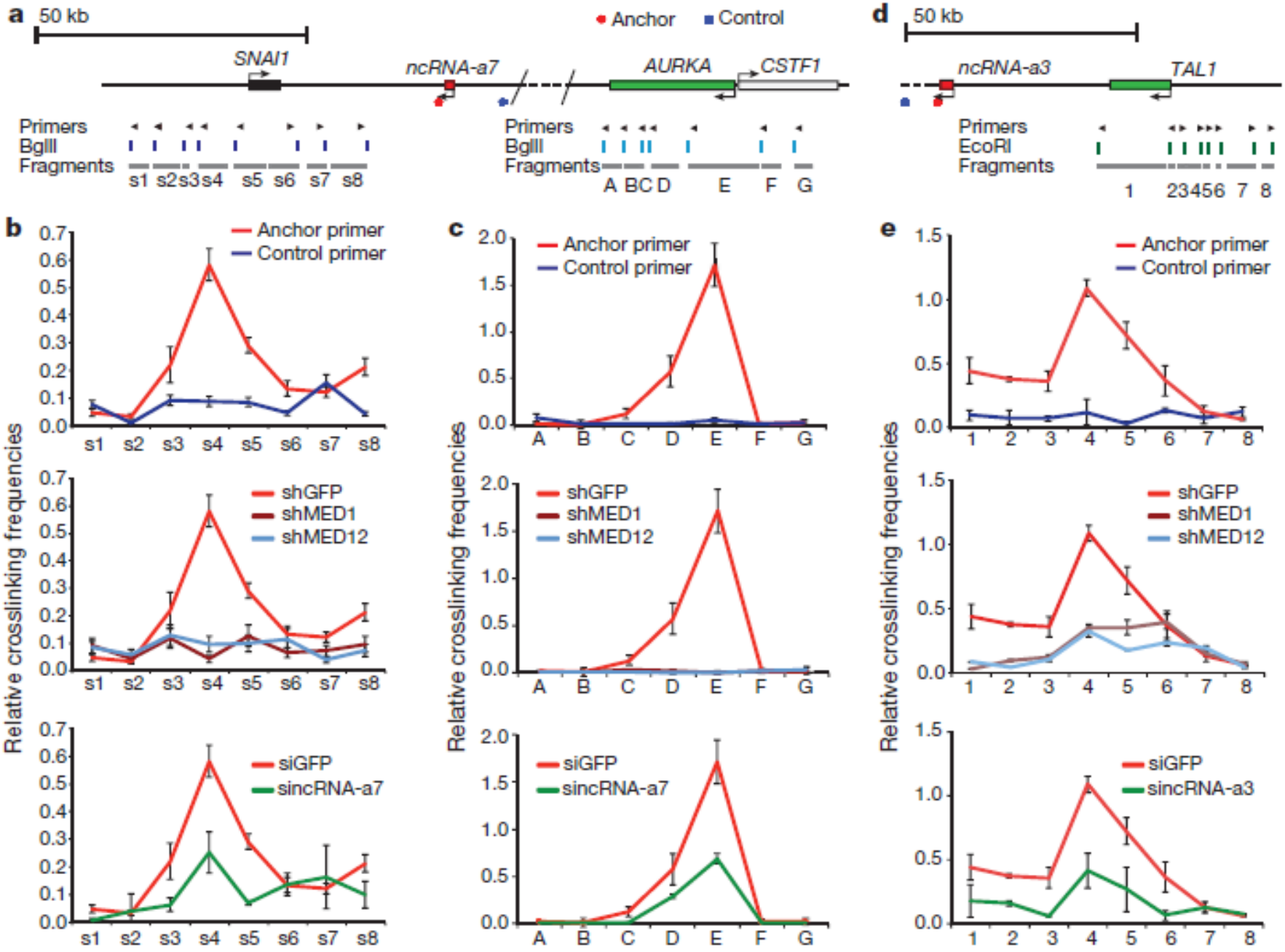
RIP (RNA immunoprecipitation) performed using IgG or MED1-Ab or MED12-Ab, using in vitro transcribed ncRNA-a7 and controls.

Mediator purified using FLAG-tagged Med12

Controllo: FLAG-GFP



Looping analysis by **3C**



Conclusions

A new action of ncRNA was discovered

ncRNA-a binds to Mediator

These ncRNAs are involved in looping interactions

Next Lesson we will discuss aspects of cell-specific enhancer establishment and activation

Read you Textbook by Heinz et al. 20015

Watch Dr Chris Glass lesson video !