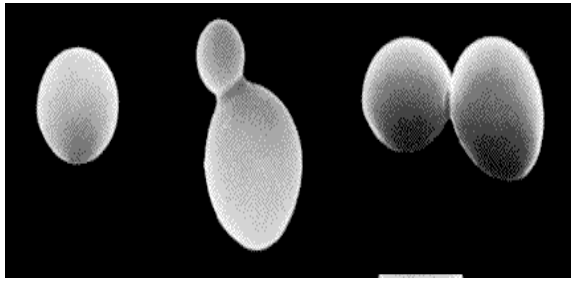
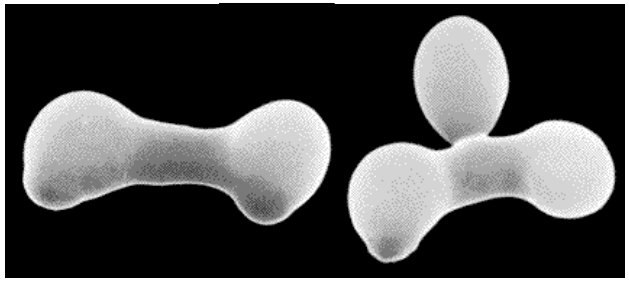
Regulación de la expresión génica





Francisco Navarro

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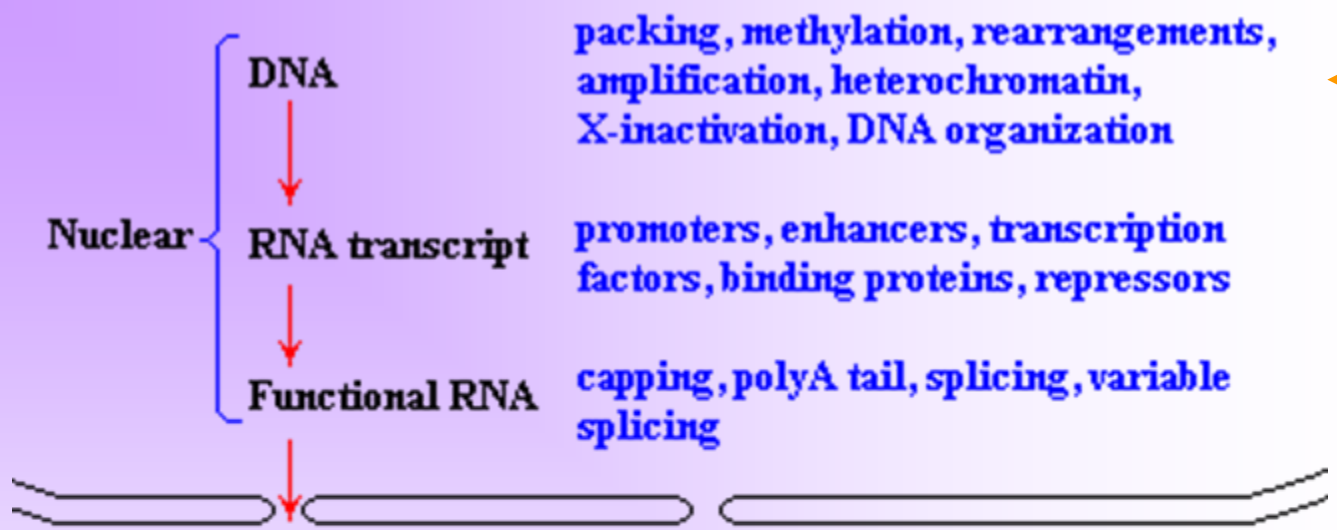
Gene expression

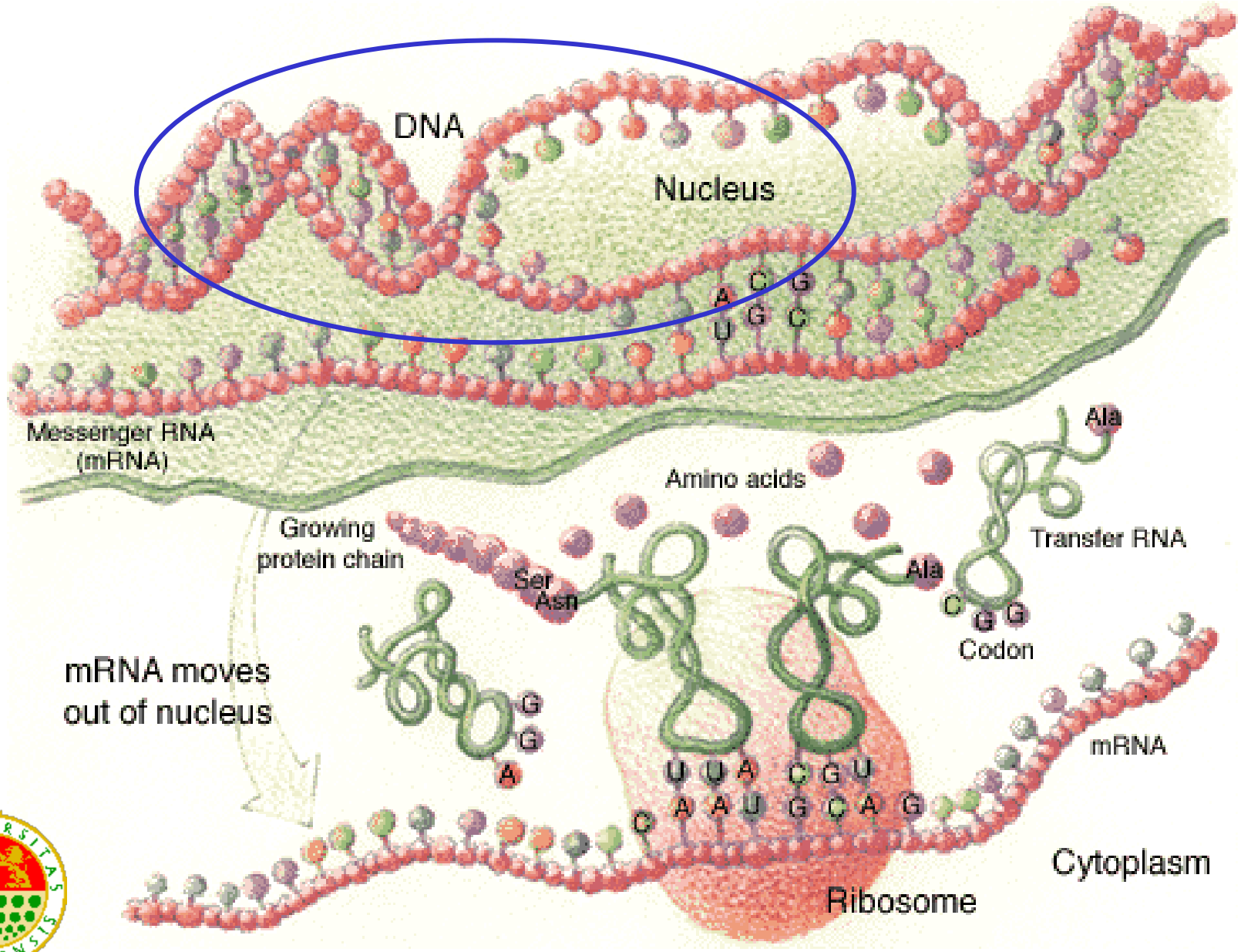


Cytosol

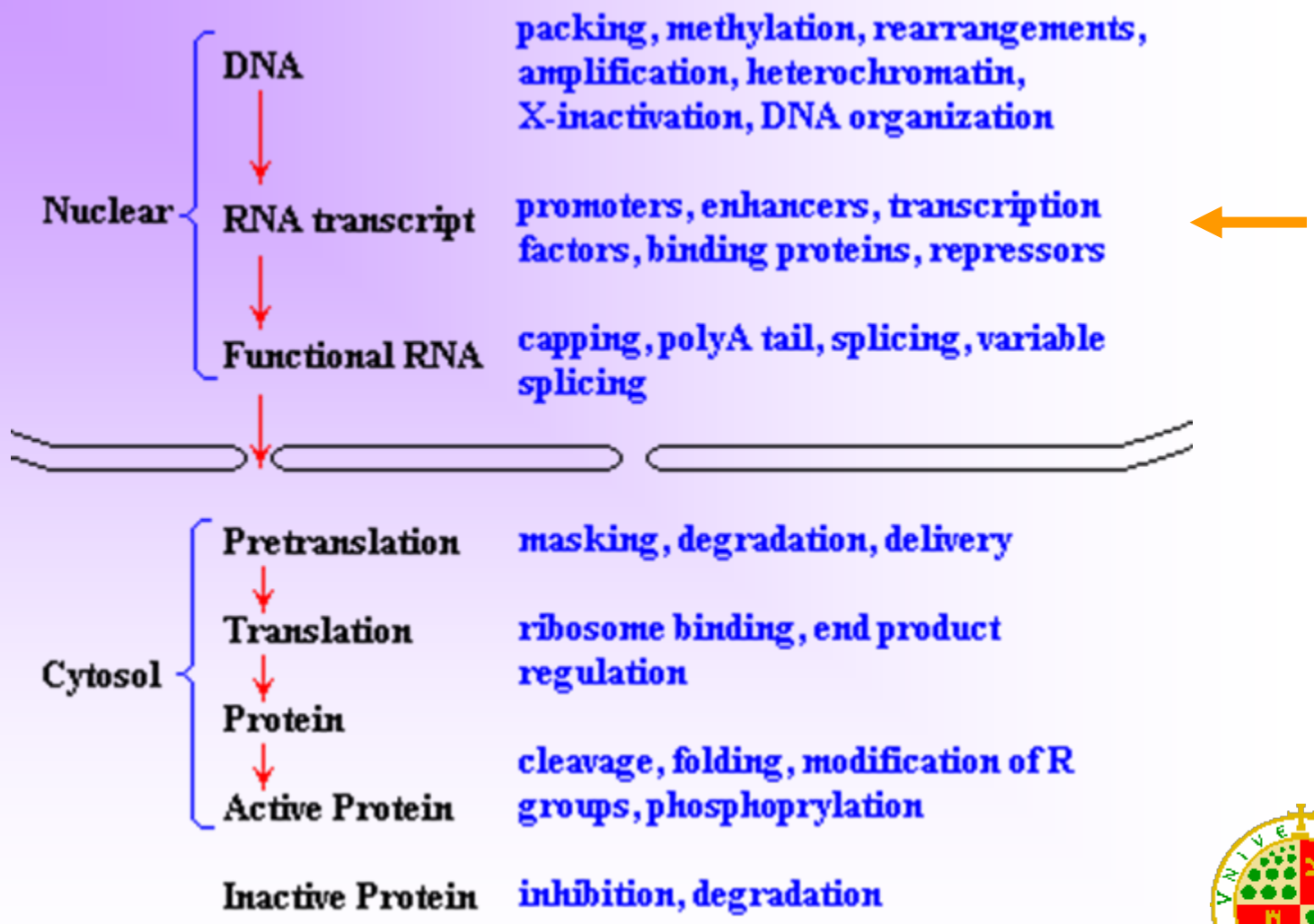
- Pretranslation — masking, degradation, delivery
- Translation — ribosome binding, end product regulation
- Protein
- Active Protein — cleavage, folding, modification of R groups, phosphoprylation

Inactive Protein — inhibition, degradation

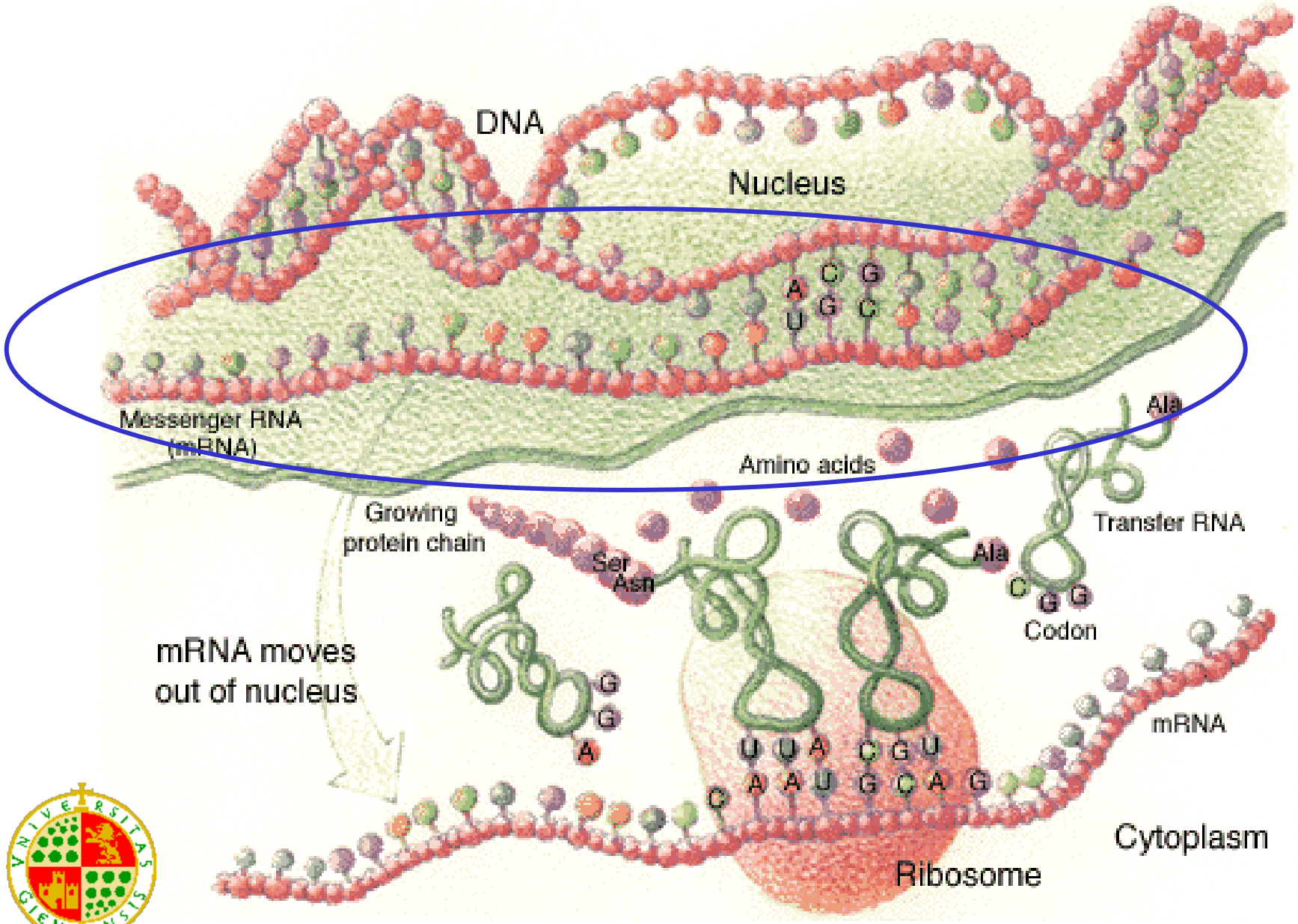
From genes to proteins



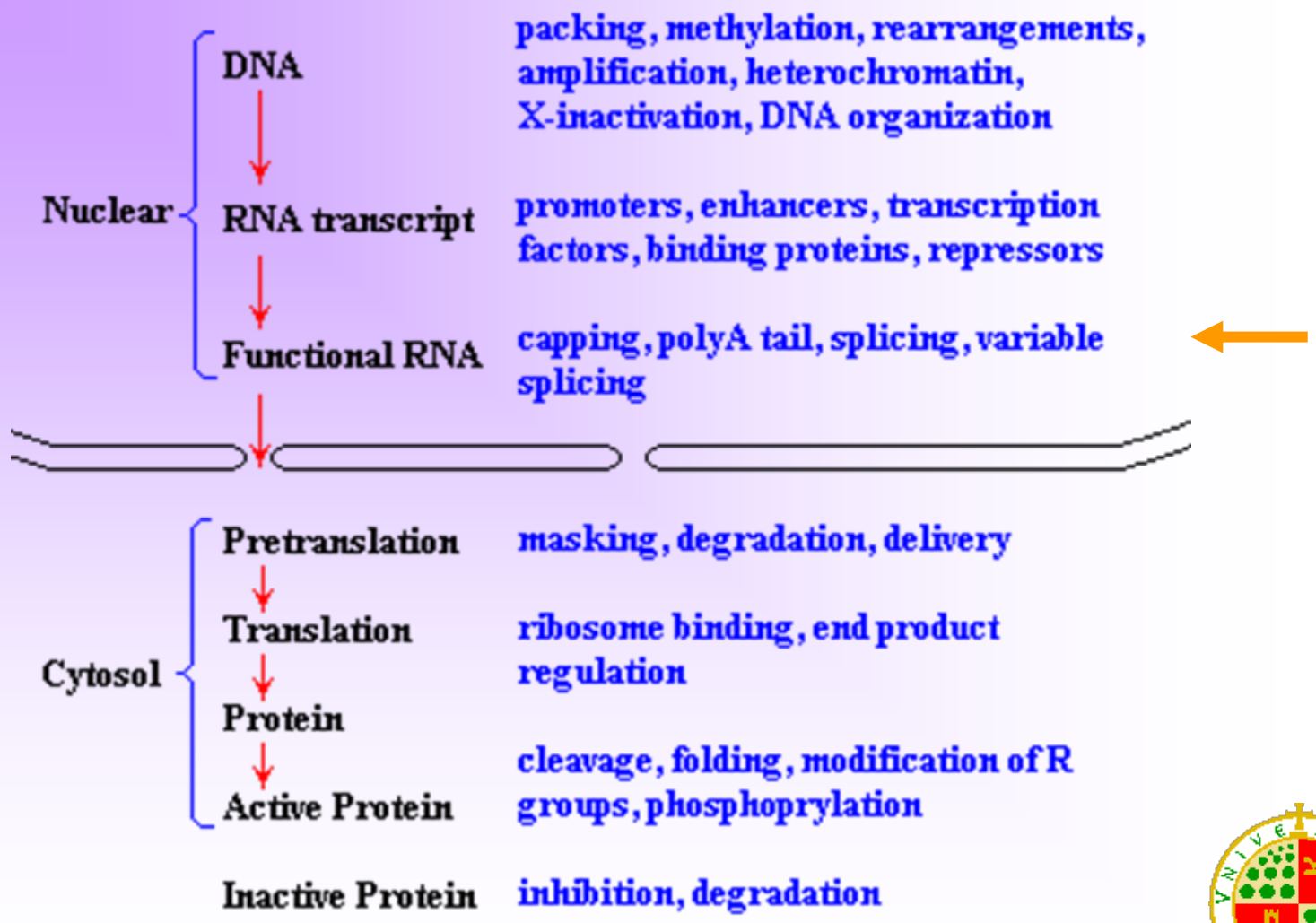
Gene expression



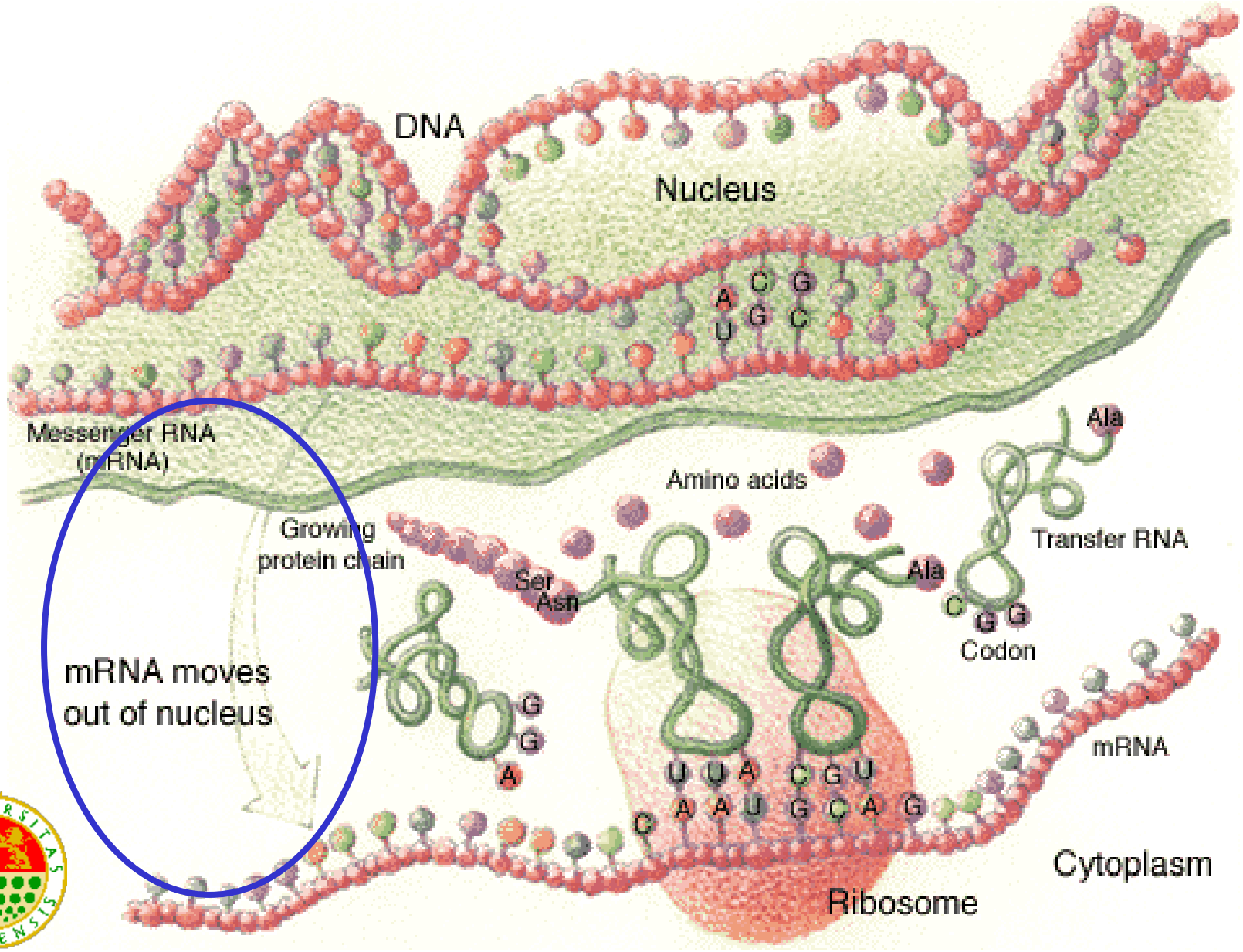
From genes to proteins



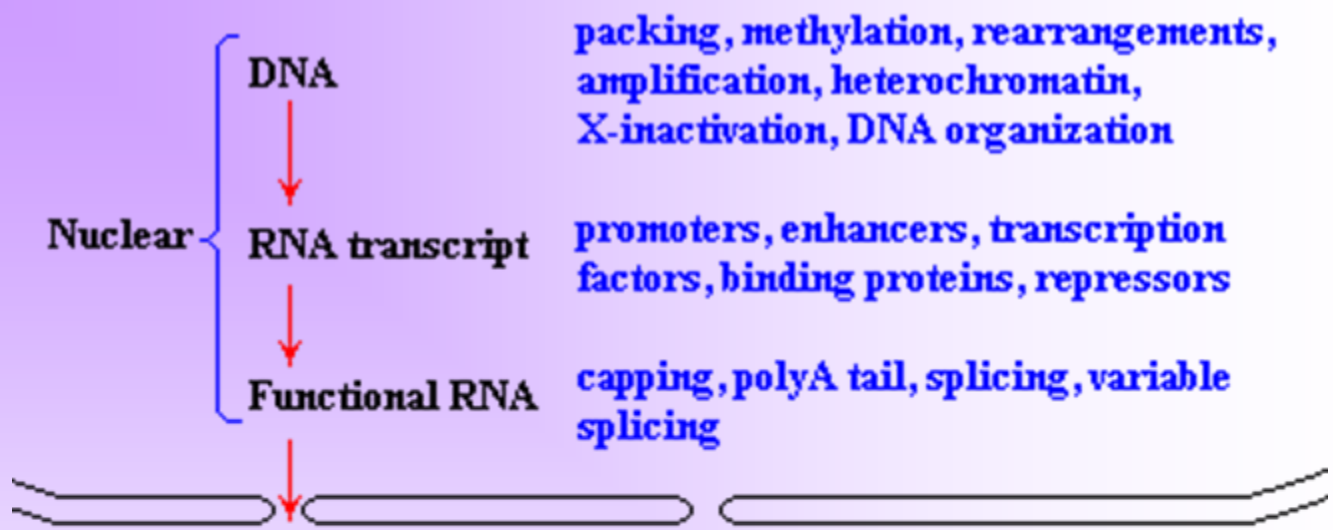
Gene expression



From genes to proteins



Gene expression



Cytosol

- Pretranslation — masking, degradation, delivery
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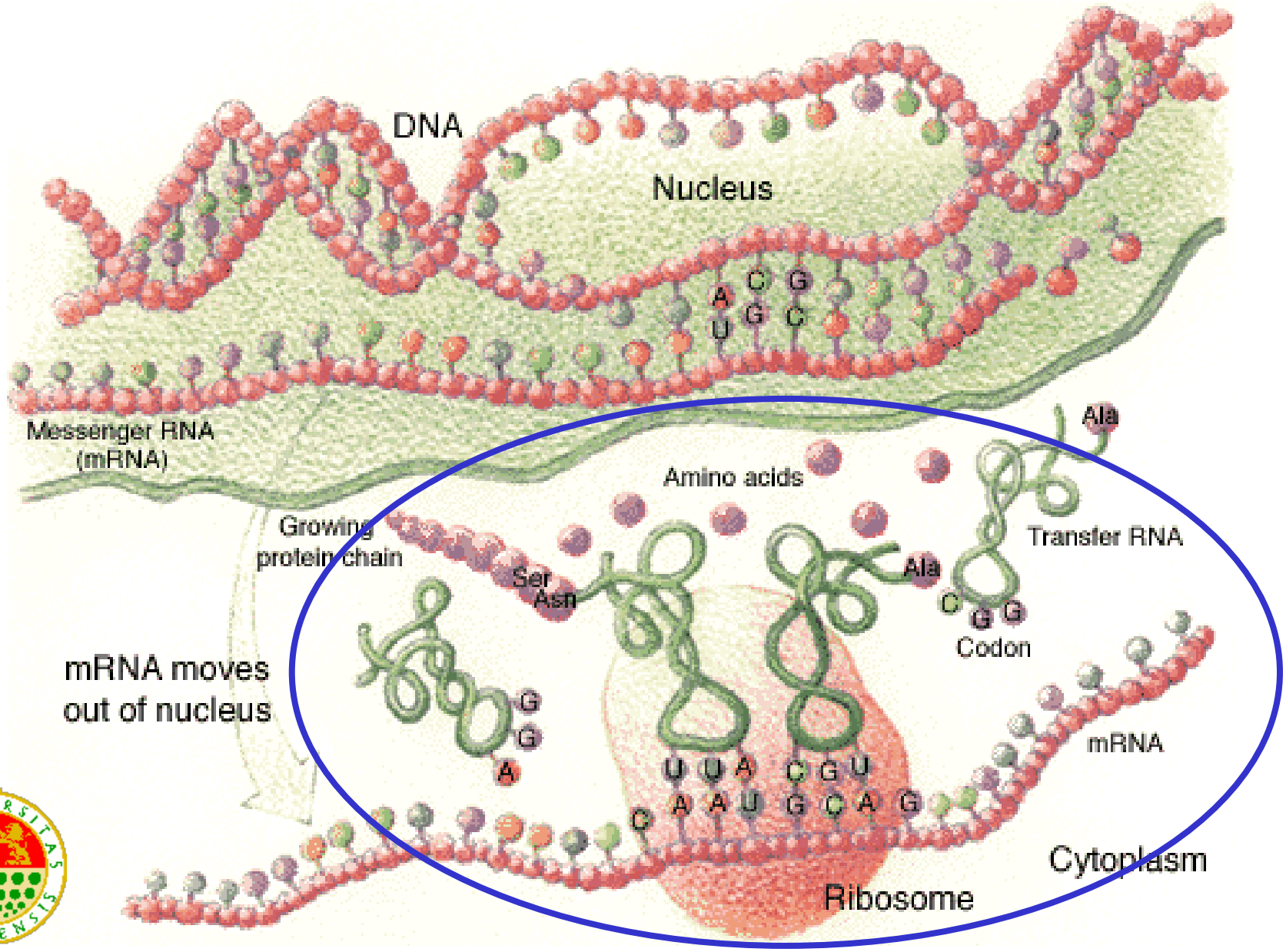
From genes to proteins



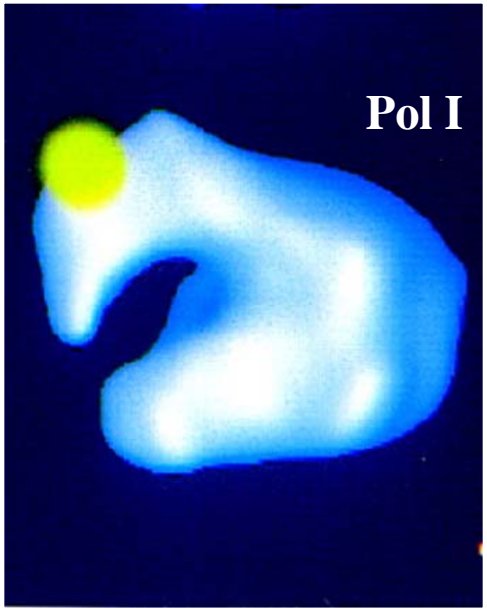
Transcription in eukaryots

Pol I	rRNA 35 S >>>> 25S/28S, 18S, 5.8S
Pol II	mRNA, small non coding RNAs
Pol III	rRNA 5 S, tRNA and small non coding RNAs

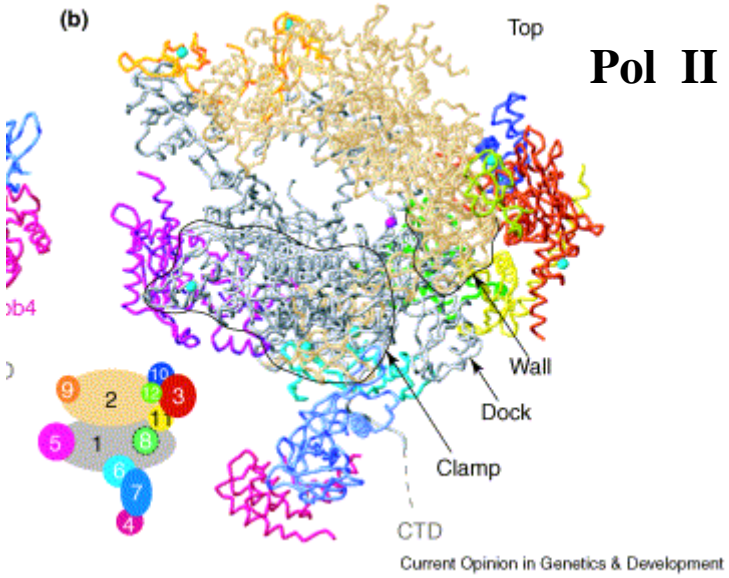
RNA accumulation

rRNA	75%
tRNA	15%
mRNA	5%
SnoRNA	5%

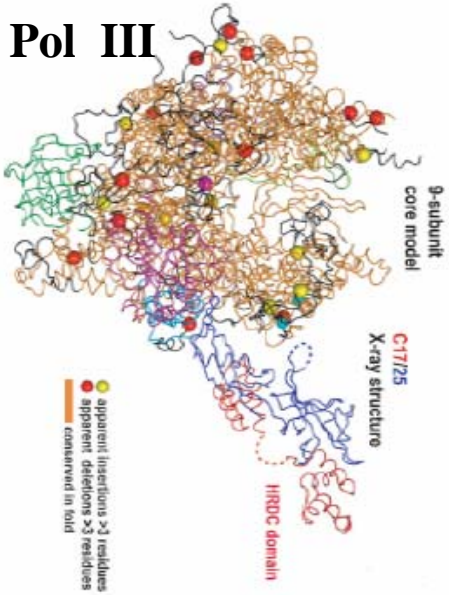
RNA polymerases



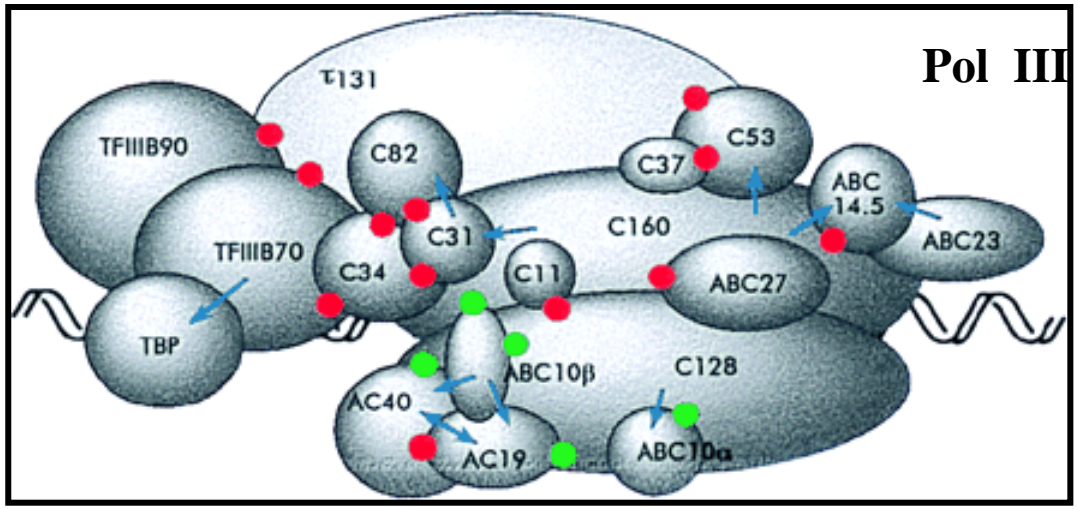
Schultz *et al*., 1993



Cramer *et al*., 2000

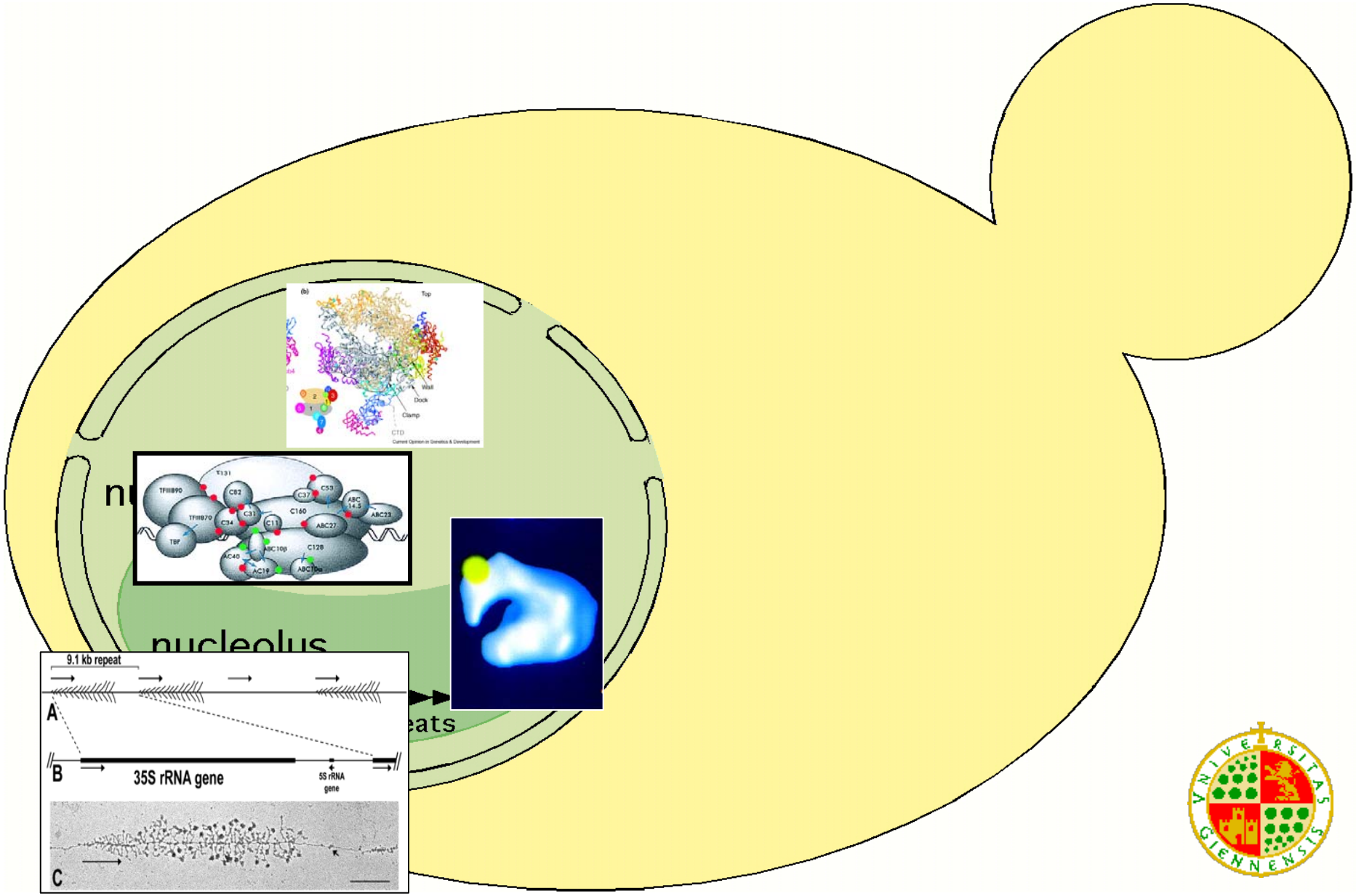


Cramer *et al*., 2006

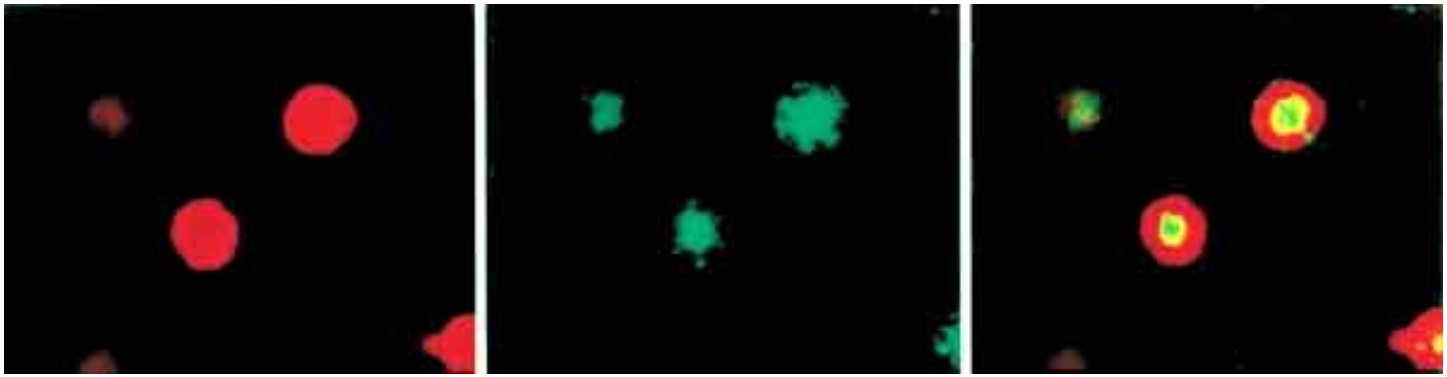


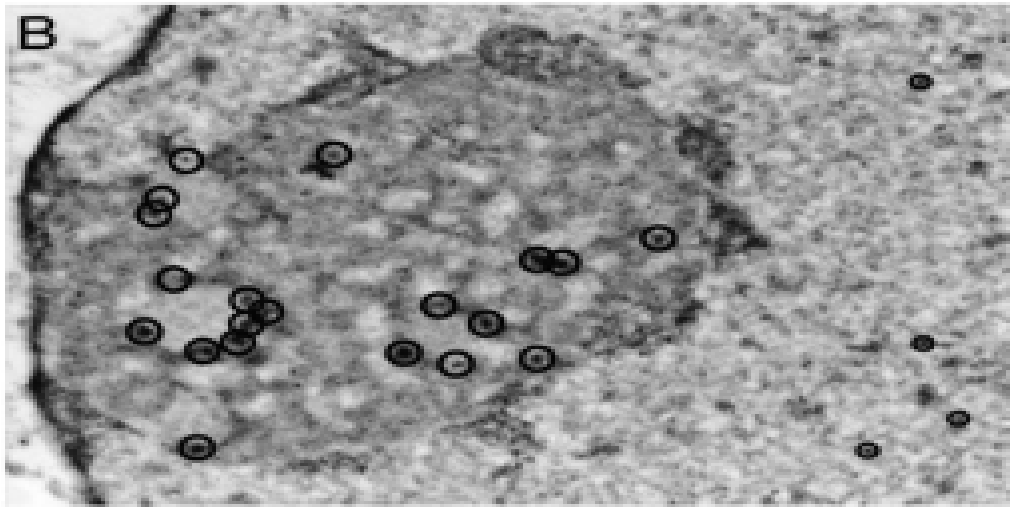
Flores *et al*., 1999

RNA polymerases localisation

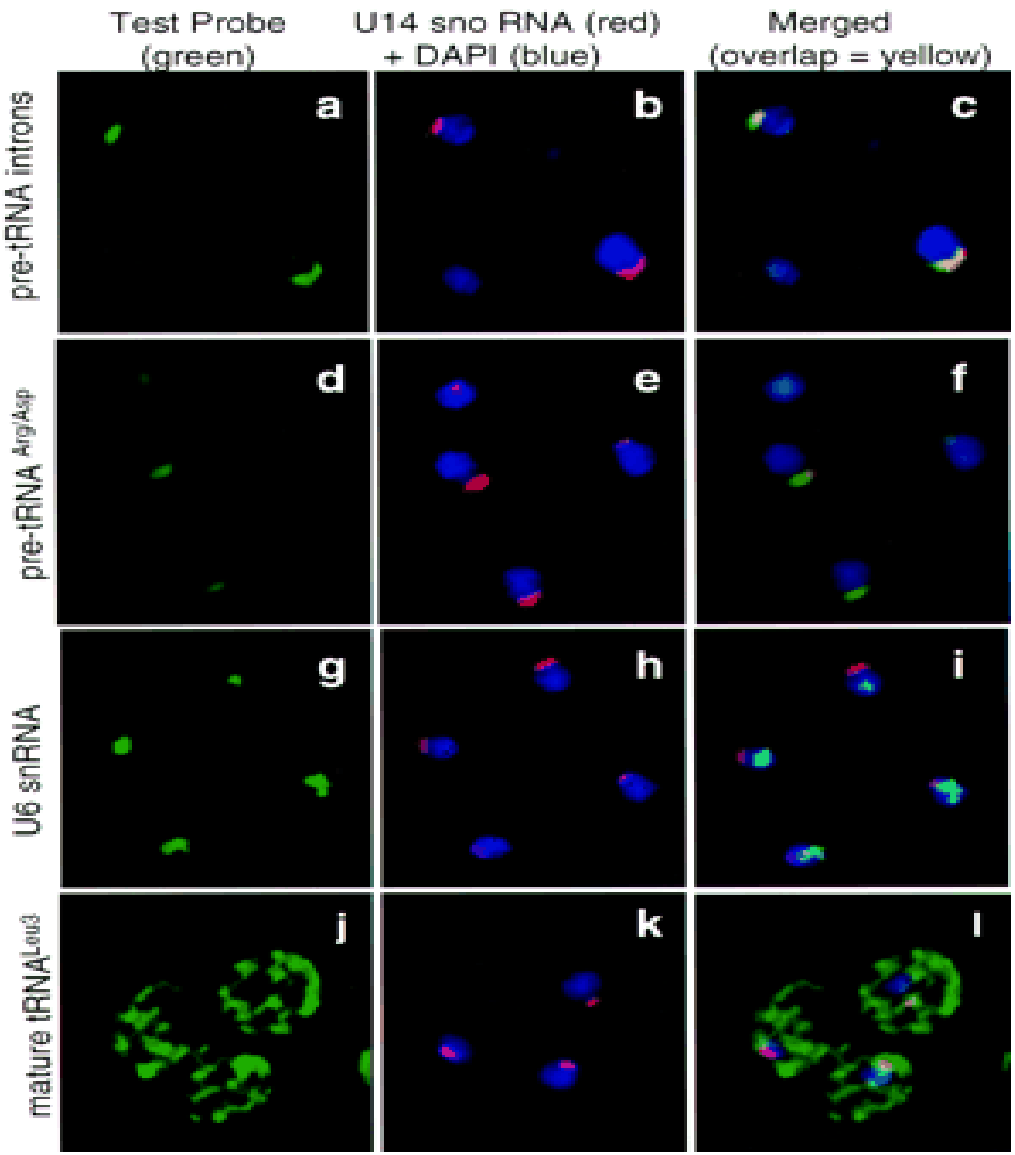


Nucleolar Localisation of rRNAs

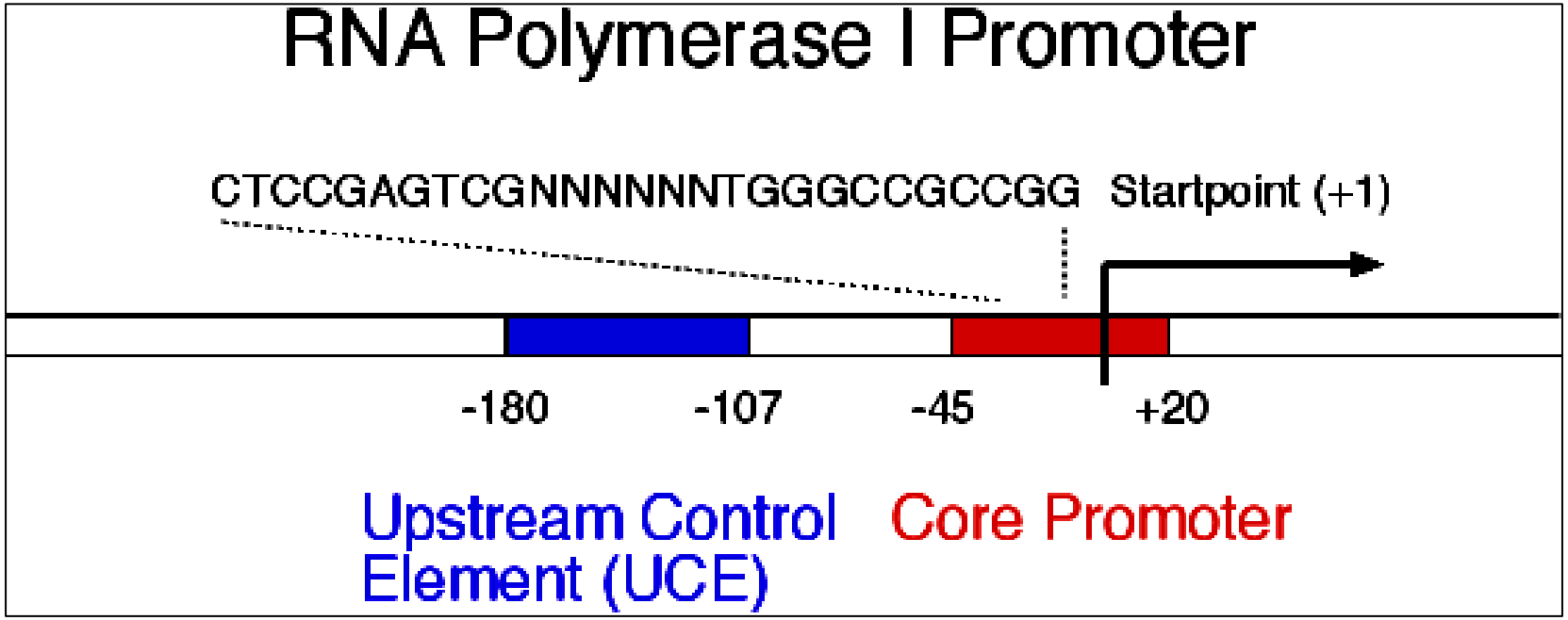




Nucleolar Localisation of tRNAs



Pol I promoter



Pol I Trancription factors

Core-binding factors

TIF-IB (mouse, *A.castellanii*)
SL1 (human, rat)
Rib1 (*Xenopus*)
CF (yeast)

Termination factors

TTF-I (mouse, human)
Reb1p (*S.cerevisiae*)

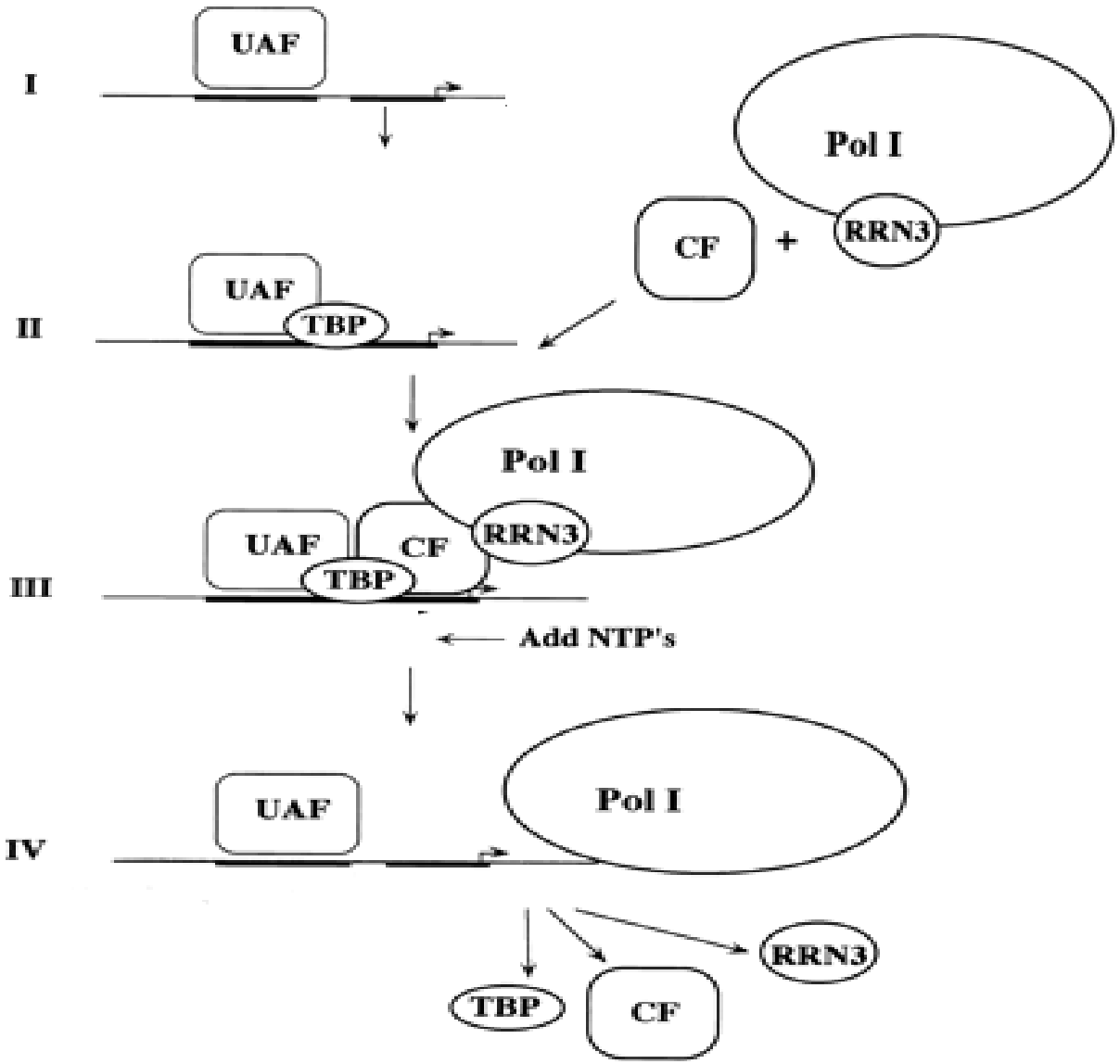
UPE-binding factors

UBF (human, mouse, rat, *Xenopus*)
UAF (*S.cerevisiae*)

Enhancer-binding factors

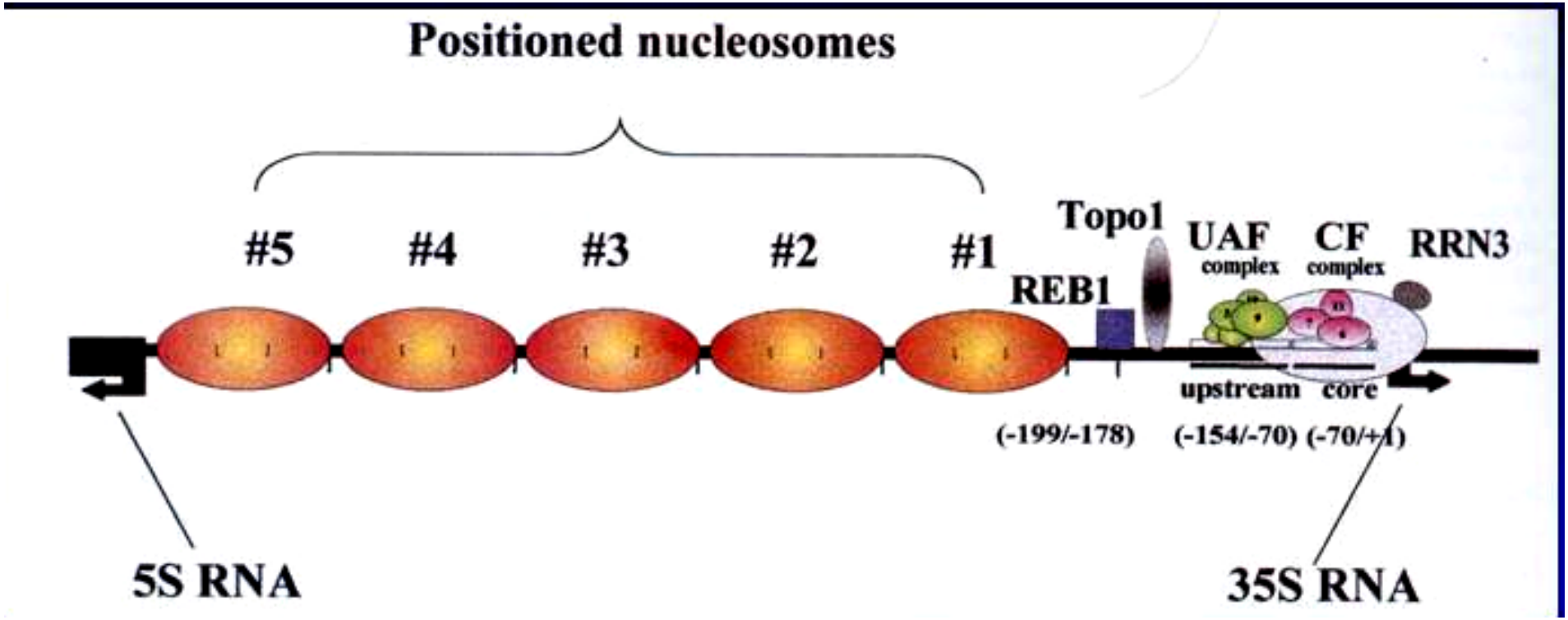
UBF (human, mouse, rat, *Xenopus*)
Reb1p (*S.cerevisiae*)
EBF (*A.castellanii*)
E_1BF (rat)

Model of the RNA Pol I transcription

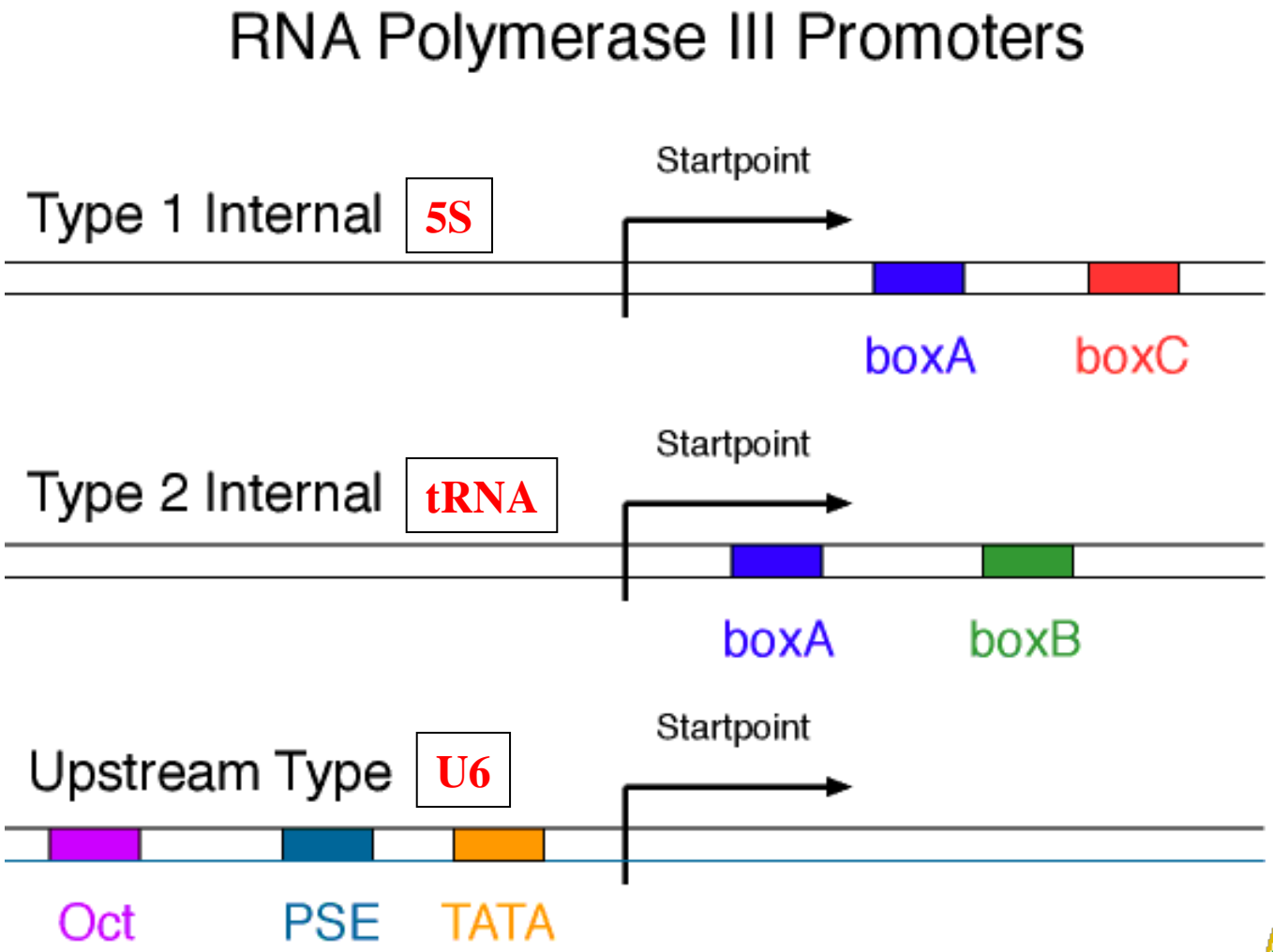


Aprikian *et al*., 2001

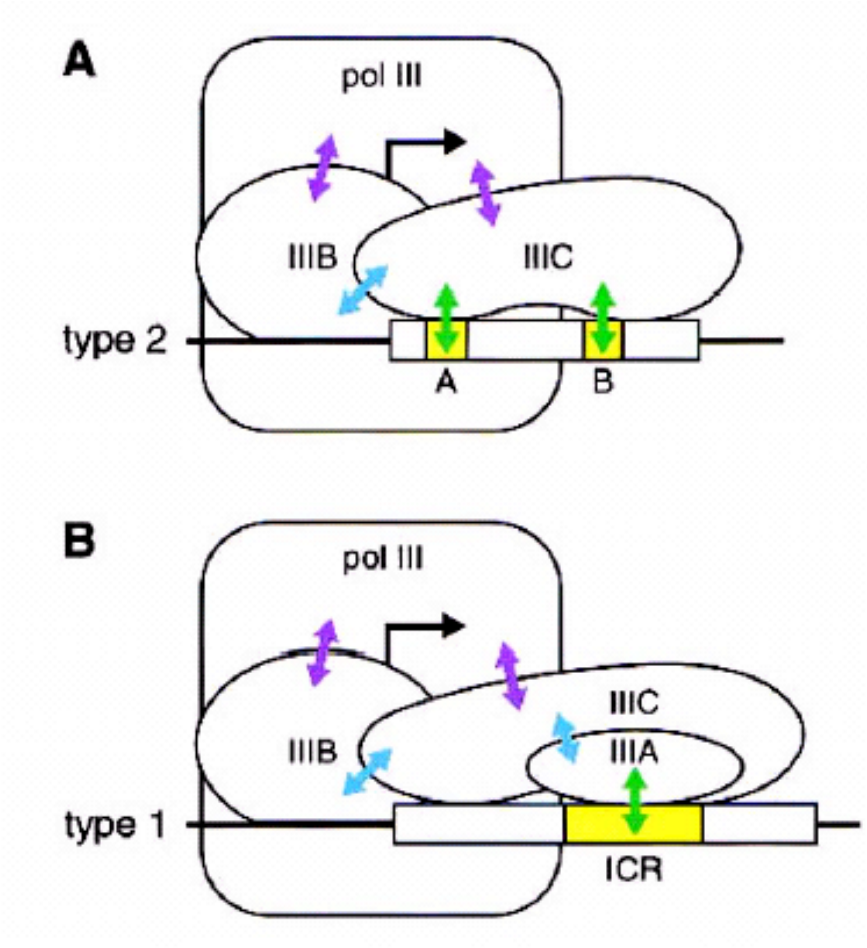
Model of the RNA Pol I transcription

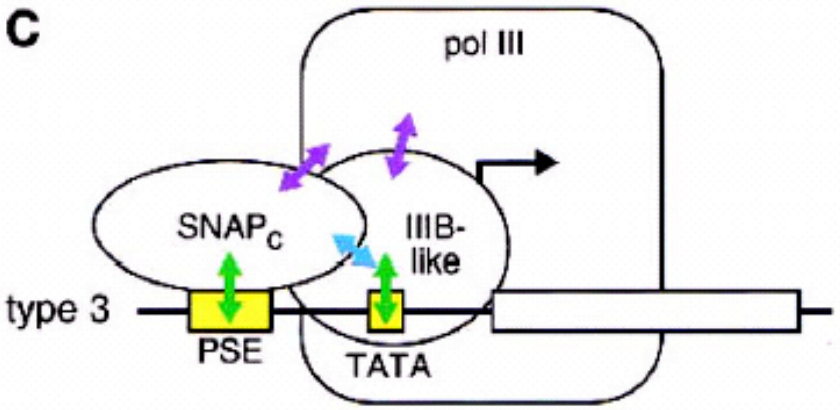


Pol III promoters



Model of the RNA Pol III transcription

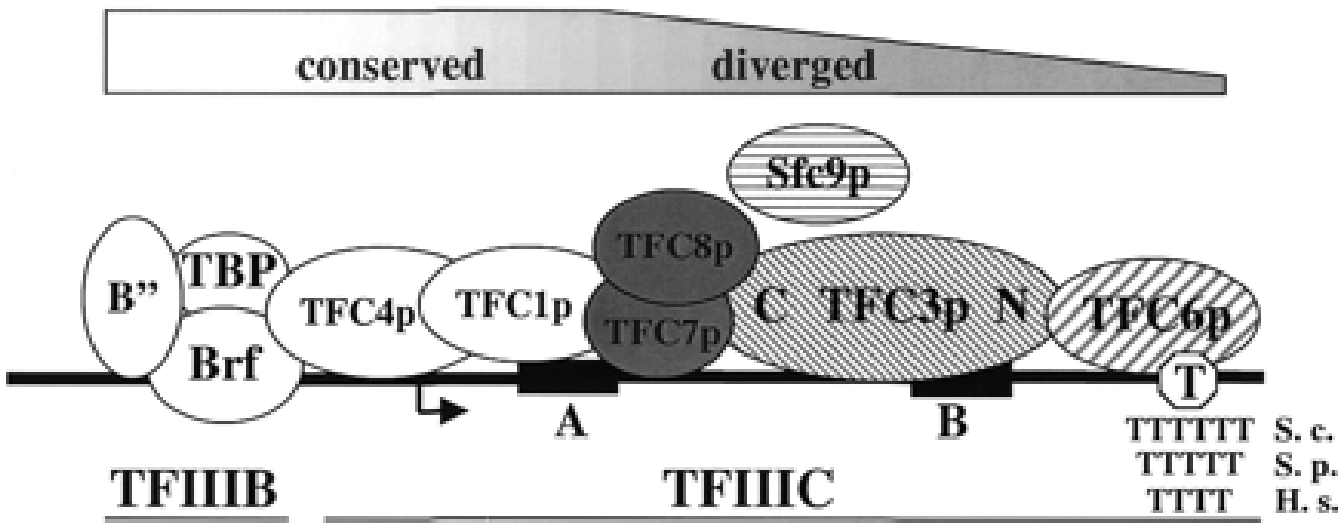




All three promoter types converge on recruitment of TFIIIB and RNA polymerase III

Schramm & Hernandez (2002) *Genes Dev.* **16**:2593

Model of the RNA Pol III transcription



Orthologs present in *S. cerevisiae*, *S. pombe* and humans (each independently finds homolog of two others)

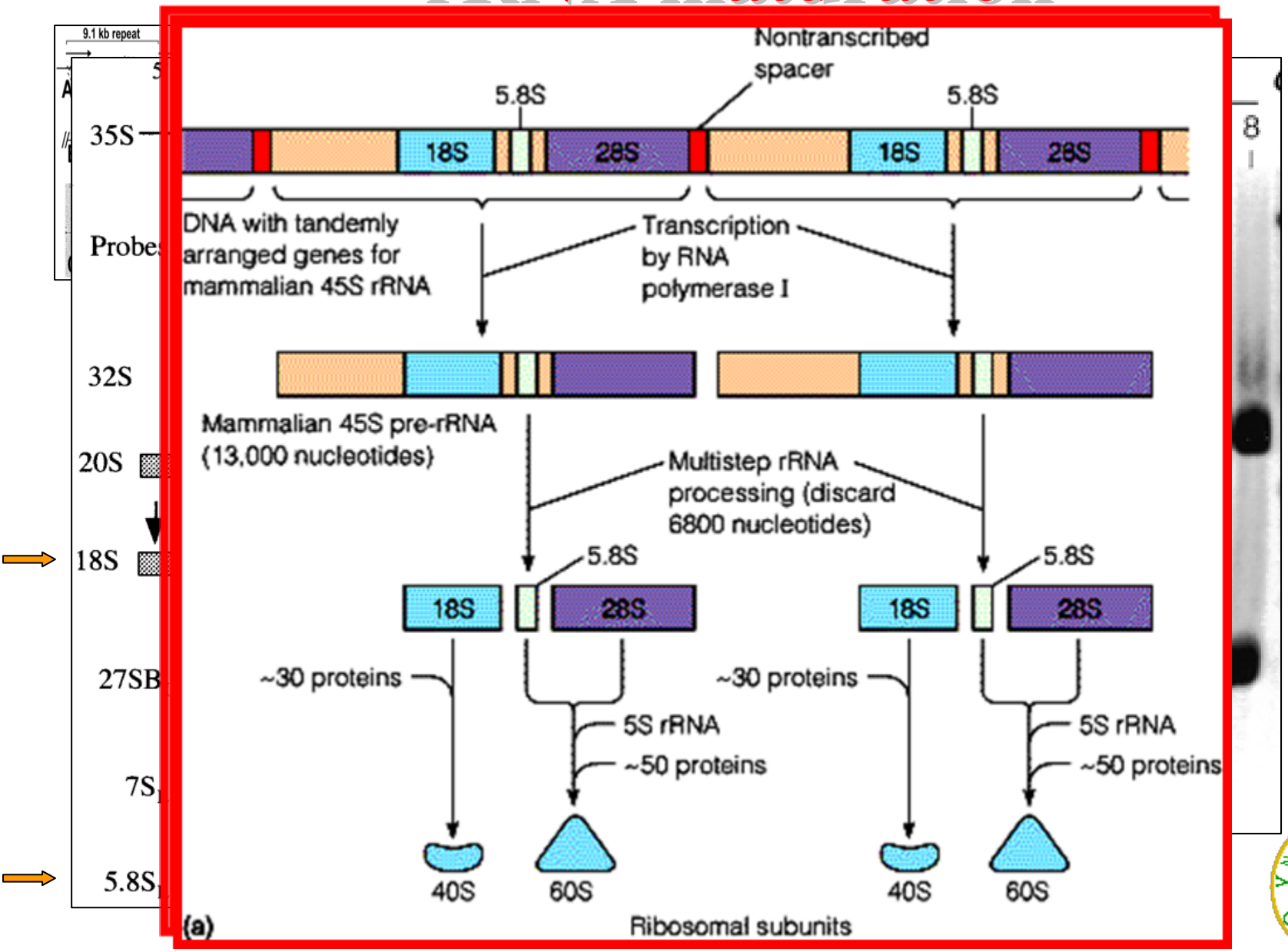
Ortholog present in *S. cerevisiae* and *S. pombe* (no apparent sequence relatedness to hTFIIIC220)

Present only in *S. cerevisiae*

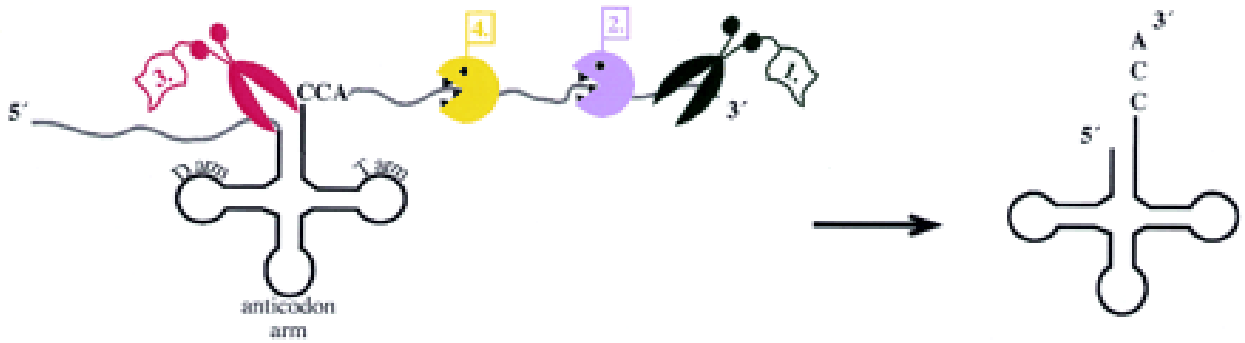
Homologs detectable in *S. pombe* and humans but not *S. cerevisiae*

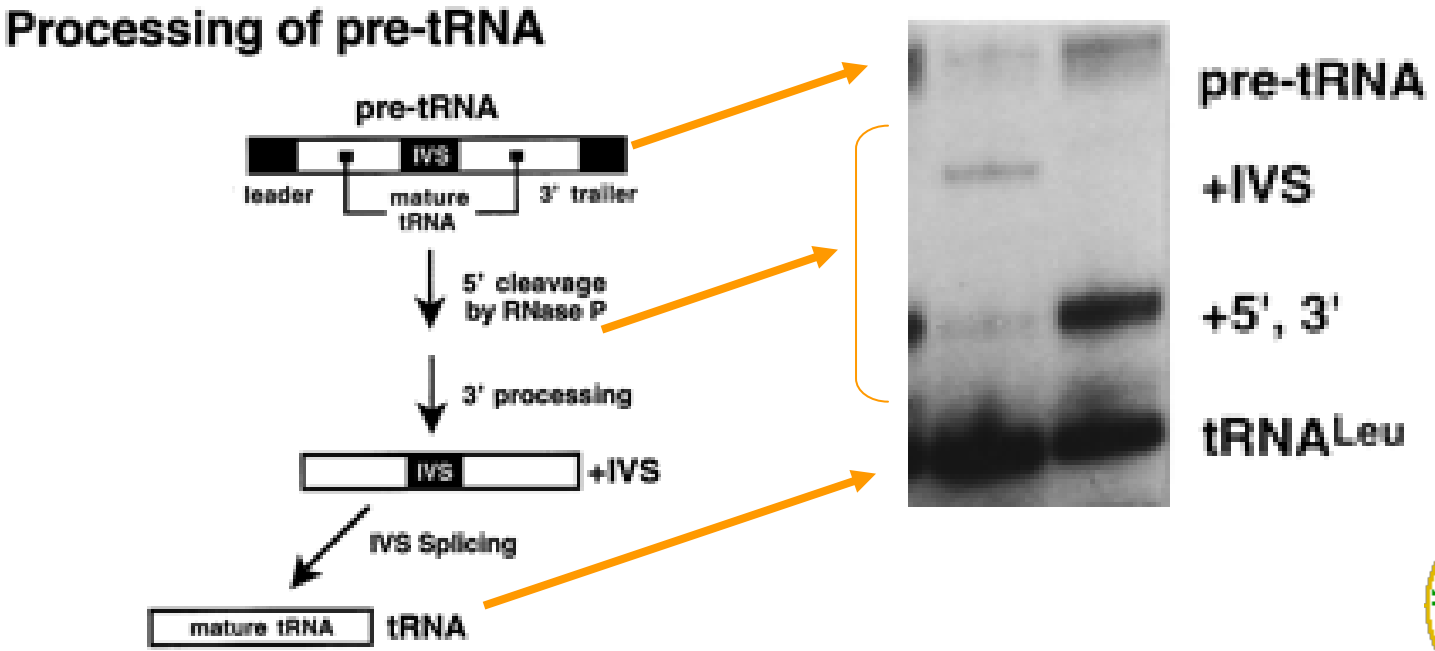
Homology between *S. cerevisiae* and human can only be descerned by comparison to *S. pombe* Sfc6p

rRNA maturation

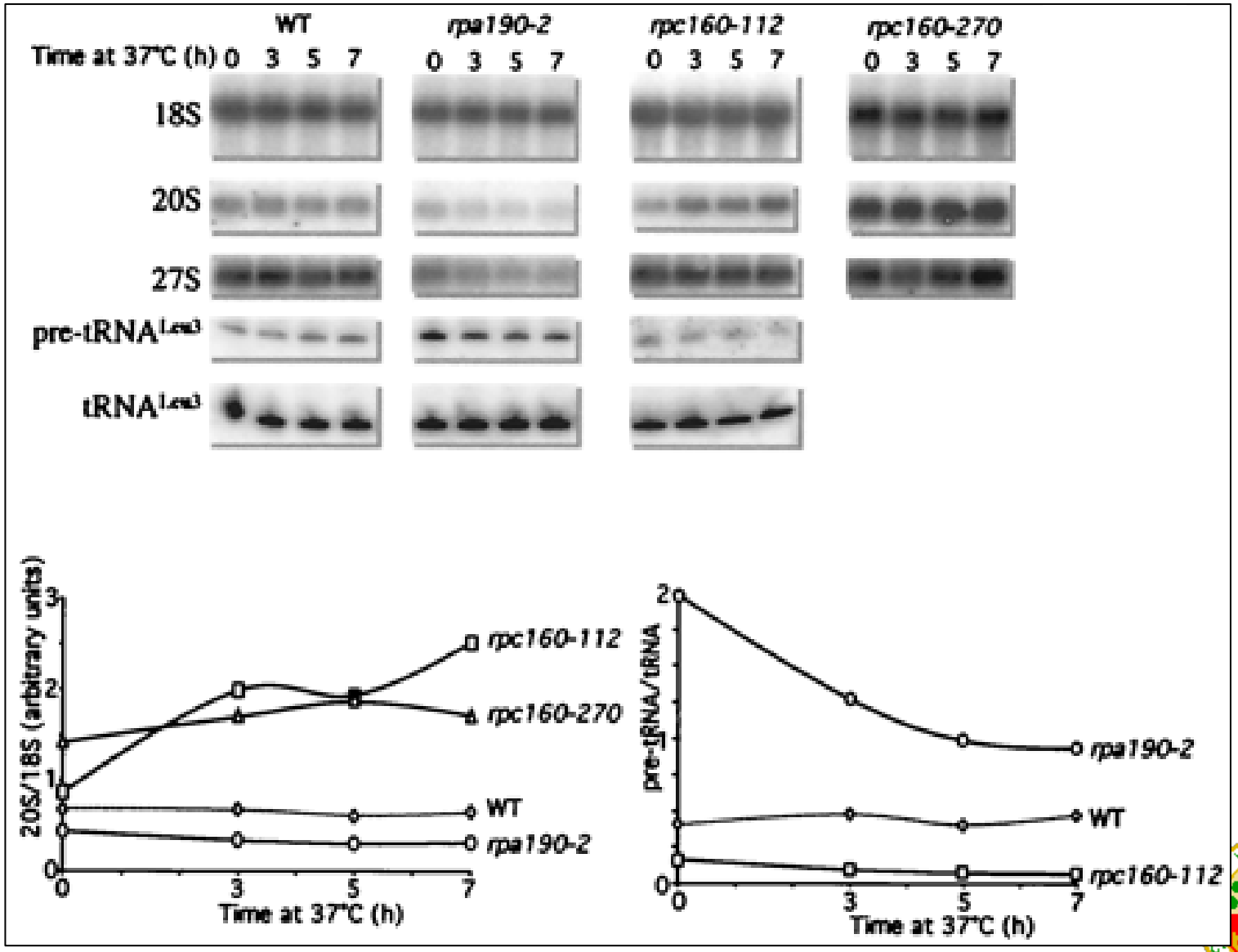


tRNA Maturation

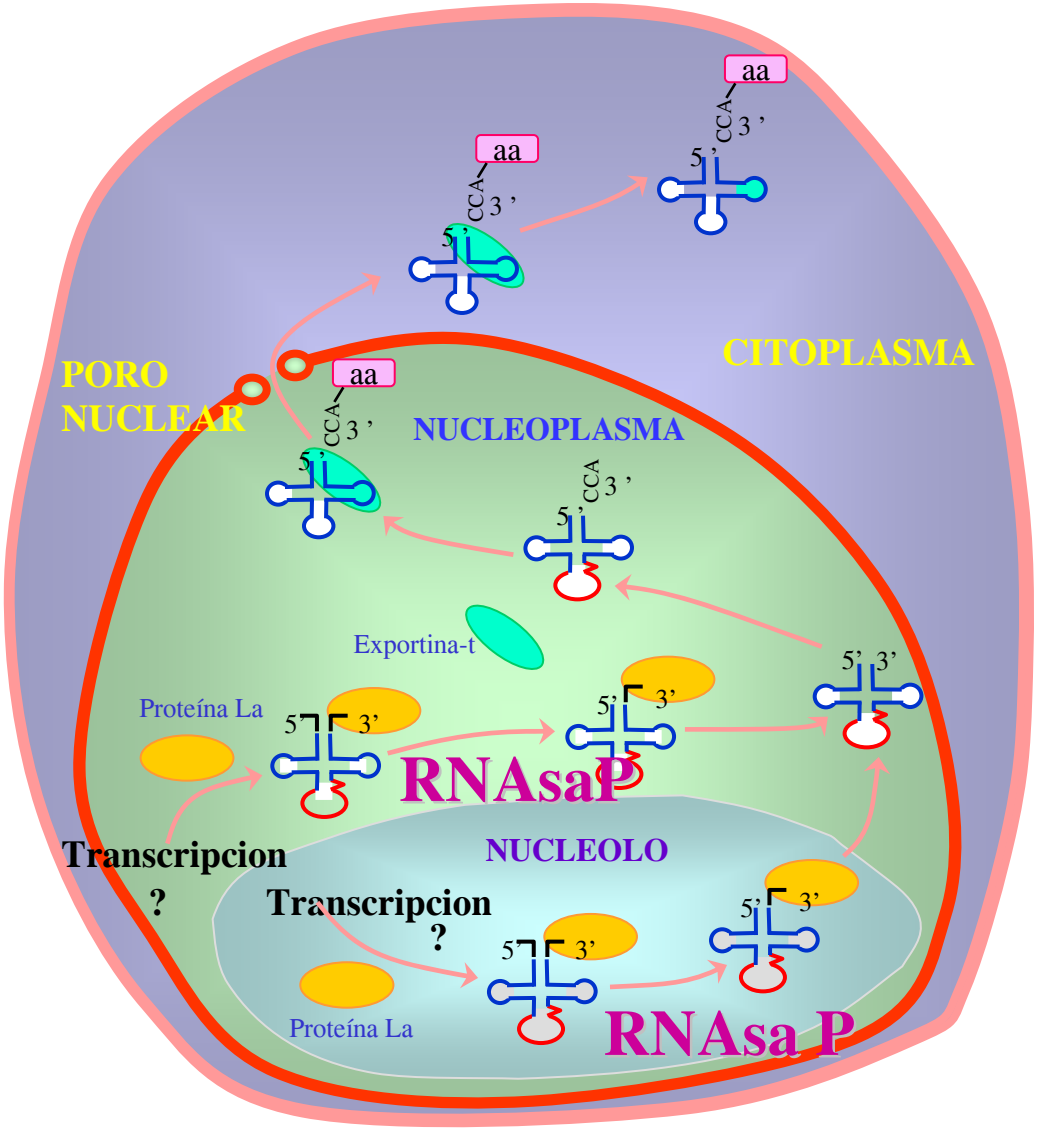




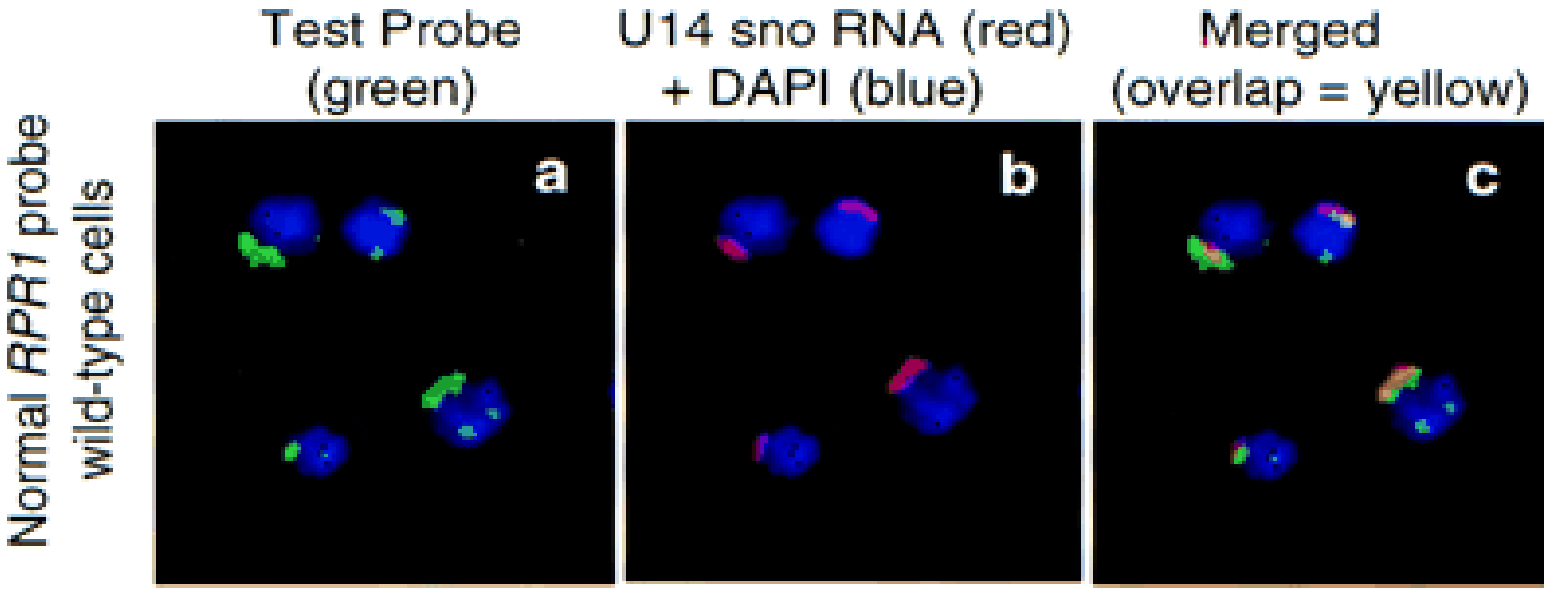
RNA Pol I/ Pol III coregulation



tRNA maturation > RNAaseP

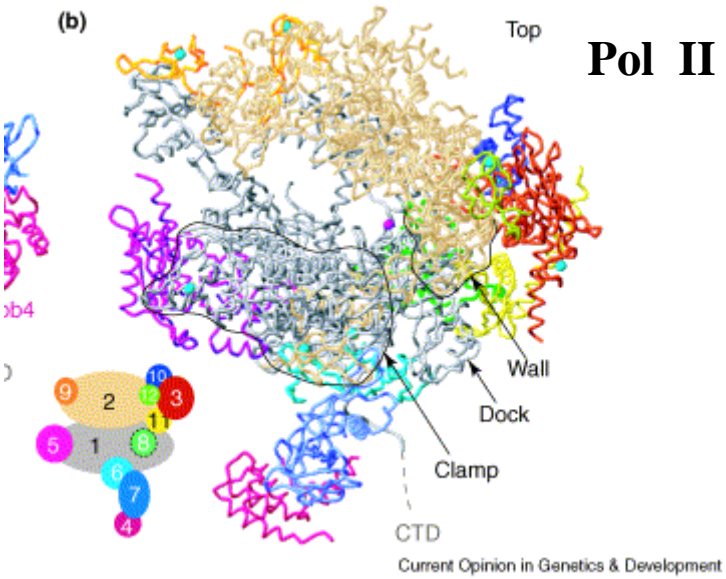


Nucleolar localisation of RNAaseP



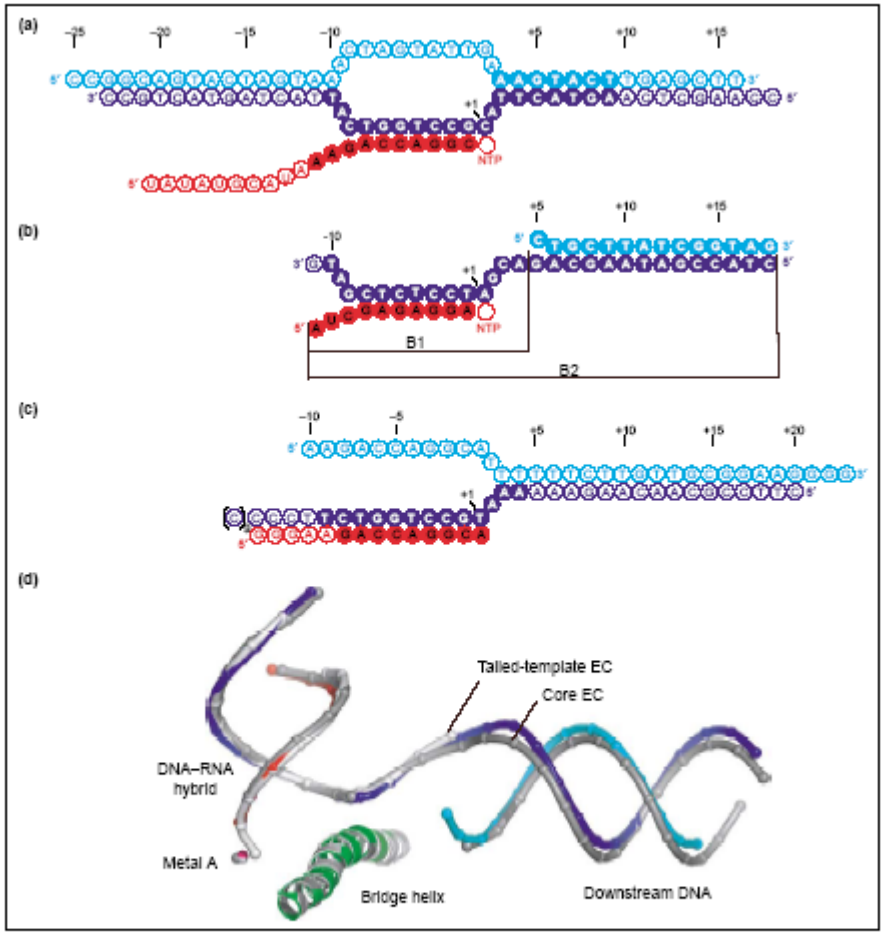
Bertrand *et al*., 2000

RNA polymerase II



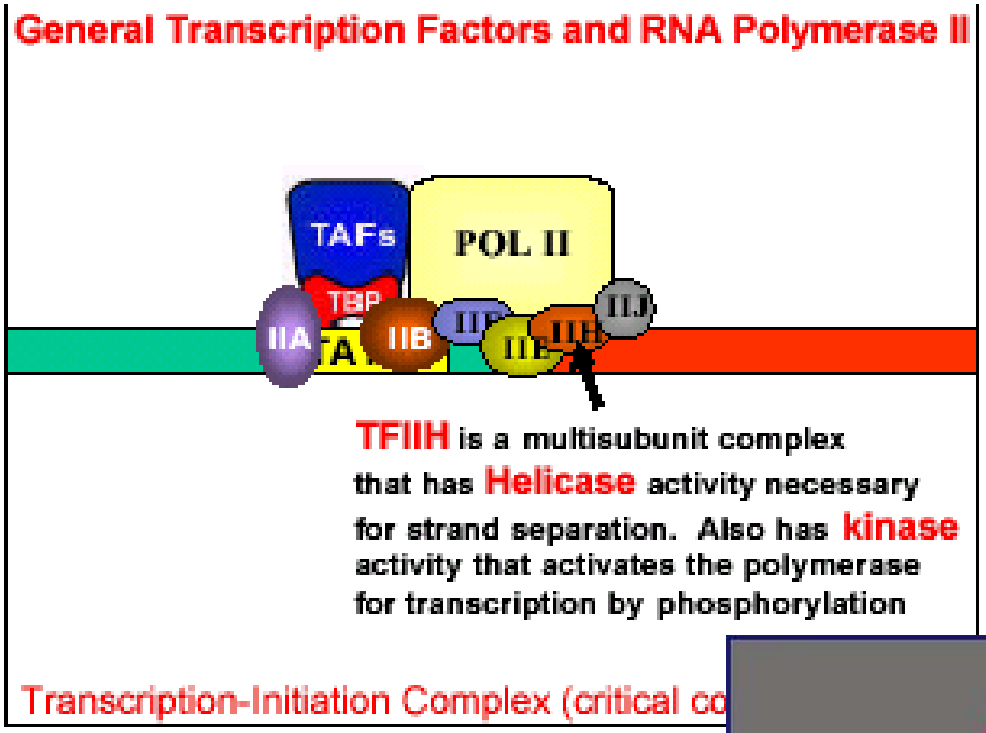
Cramer *et al*., 2000

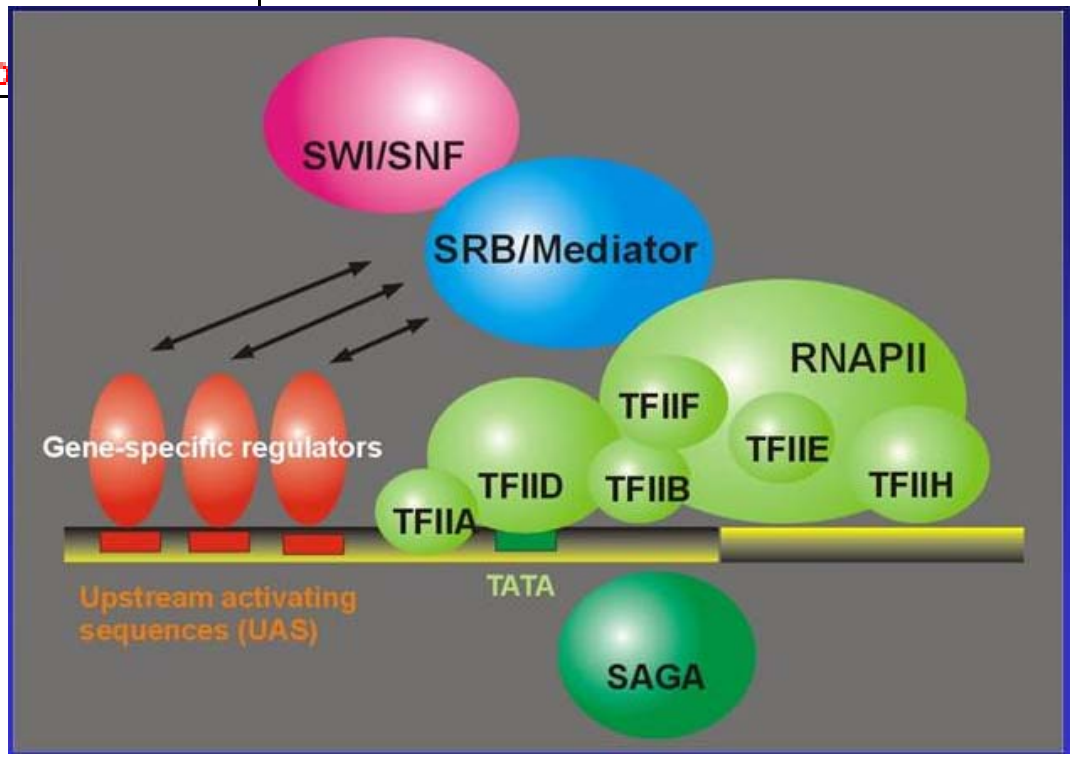
Figure 1



Nucleic acids in Pol II elongation complexes. (a–c) Schematic diagrams of nucleic acids used in structural studies of Pol II ECs: (a) complete Pol II EC (complete EC) [18••]; (b) core Pol II EC (core EC) (B1 [16•], B2 [17••]); (c) core Pol II tailed-template EC [9]. Template DNA, non-template DNA and RNA strands are in blue, cyan and red, respectively. All nucleotides present in the crystals are shown, with structurally resolved nucleotides highlighted as filled circles. The register is shown above the sequences, with downstream positions indicated by positive numbers. Figures prepared with Pymol (http://www.pymol.org). (d) Superposition of the nucleic acid backbones in the structures of Pol II ECs. The nucleic acids in the complete EC are colored as in (a–c), and the nucleic acids in the core EC and tailed-template EC are in dark and light grey, respectively. The active site metal ion A is depicted as a pink sphere.

Model of the RNA Pol II transcription

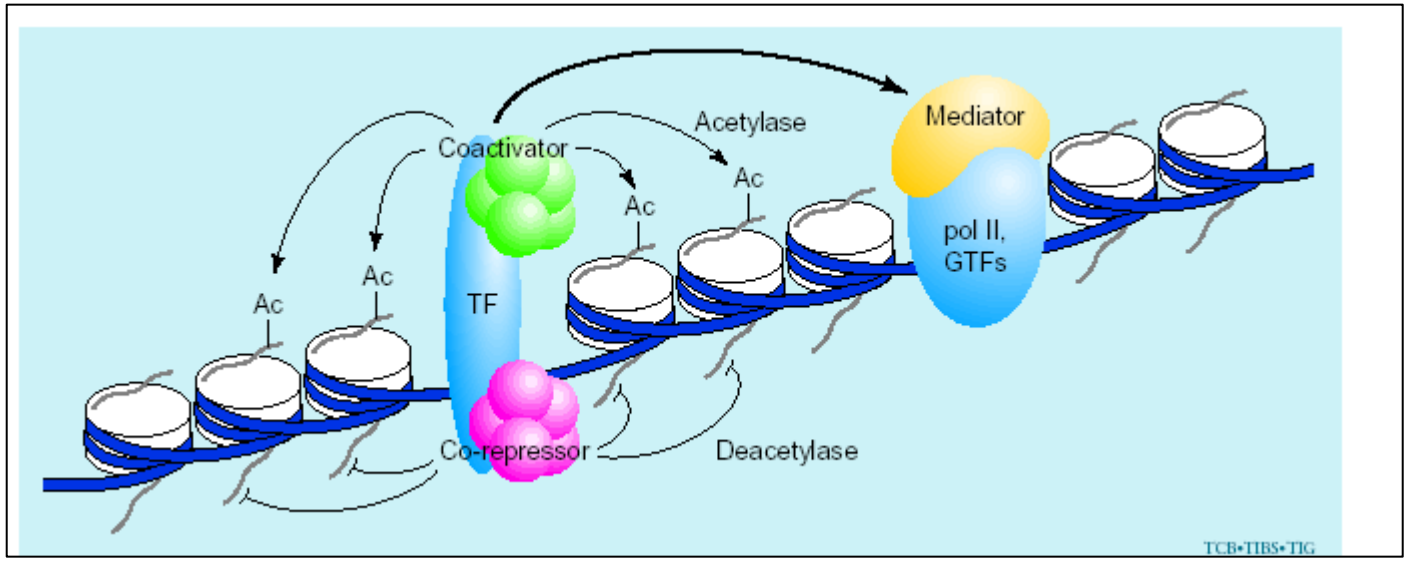




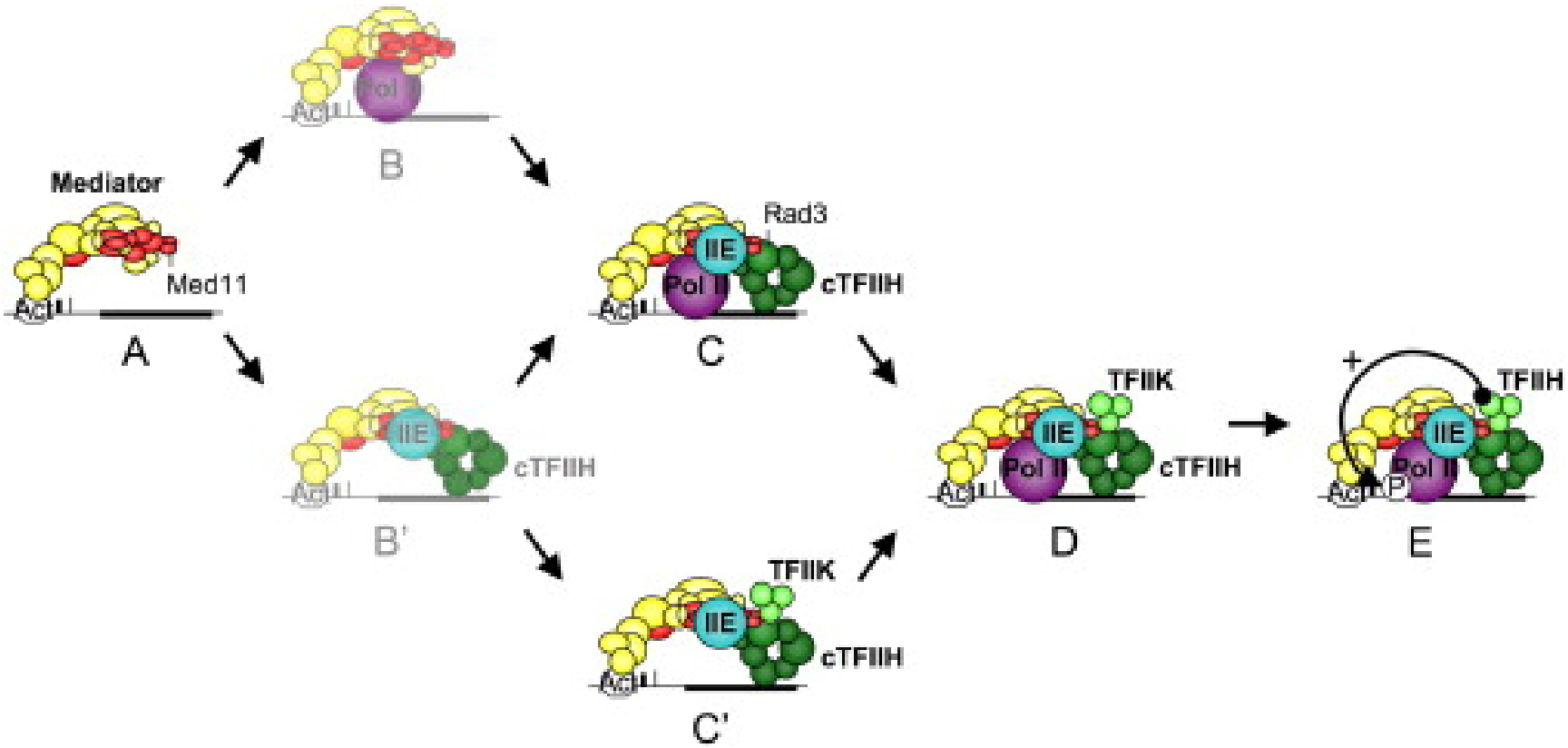
Model of the RNA Pol II transcription

TABLE 1. RNA polymerase II transcription machinery[a]

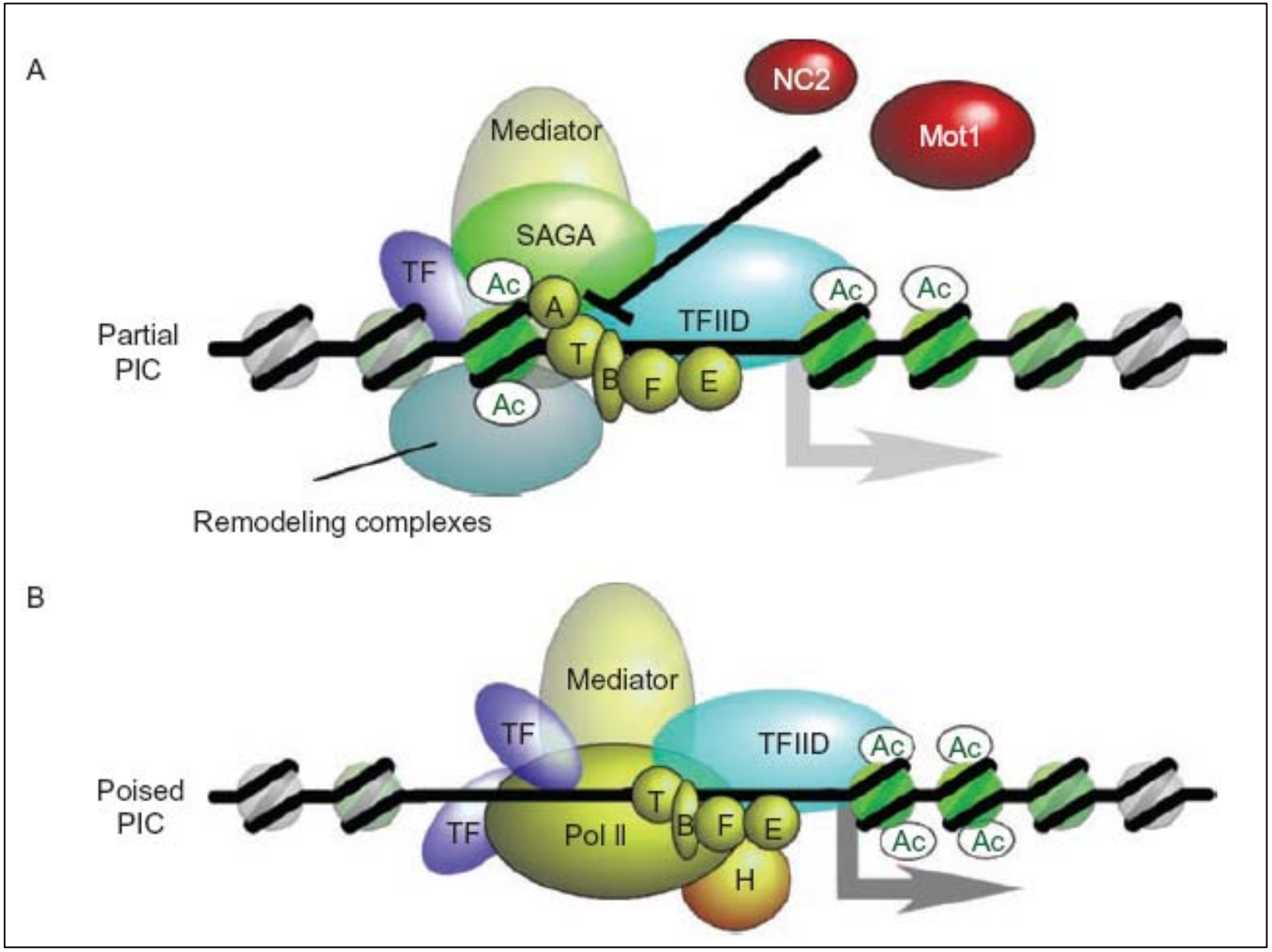
	Number of subunits	Catalytic subunit(s)
Histone acetyltransferase[b,c]		
CBP/p300 (*H.s.*)	1	
SAGA (*S.c.*)	≥14	Gcn5p
PCAF (*H.s.*)	~20	PCAF
Elongator (*S.c.*)	3	Elp3p
Histone deacetylase[b,c]		
mSin3 (*H.s.*)	~10	HDAC1, HDAC2
NURD (*H.s.*)	~18	HDAC1, HDAC2 (also CHD3, CHD4 ATPases)
Chromatin-remodelling complex[b,c]		
SWI/SNF (*S.c.*)	11	Swi2p/Snf2p (ATPase)
NURF (*D.m.*)	4	ISWI (ATPase)
RNA polymerase II	12	
General transcription factor[c]		
TFIIB	1	
TFIID	9	
TFIIE	2	
TFIIF	2	
TFIIH (*S.c./H.s.*)	9	Ssl2p/Xpb (helicase), Rad3p/Xpd (helicase), Kin28p (kinase)
Mediator	20	



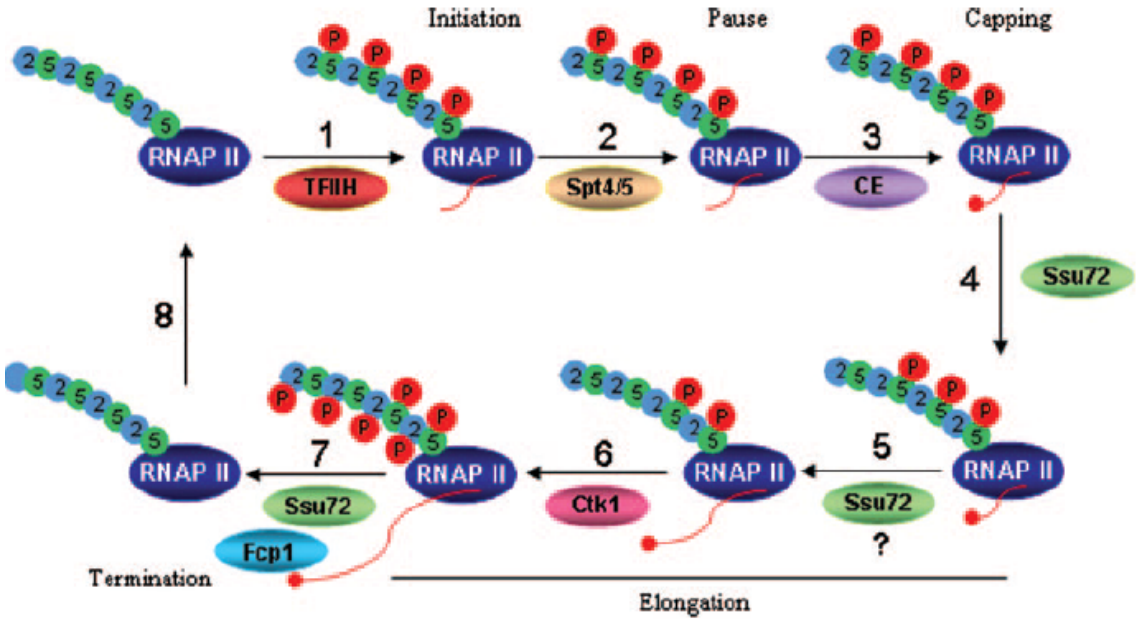
PIC formation

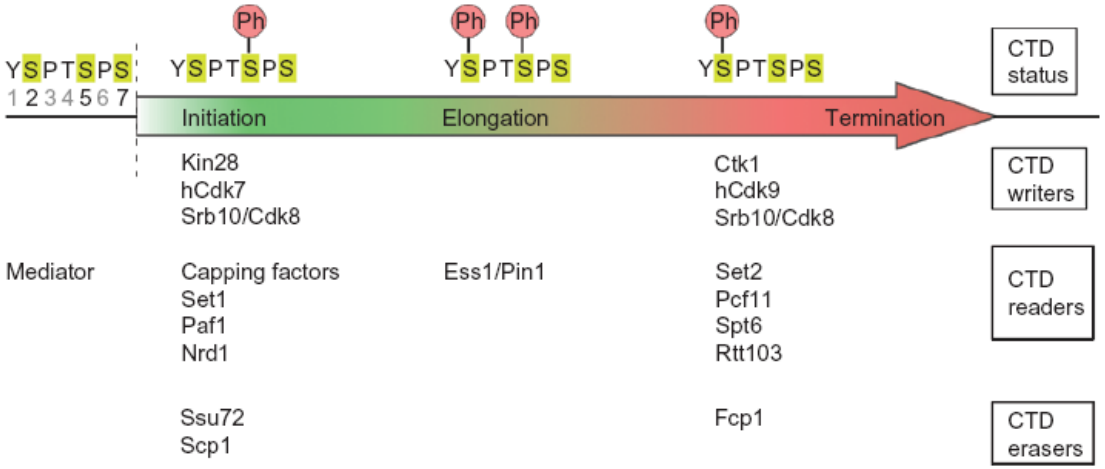


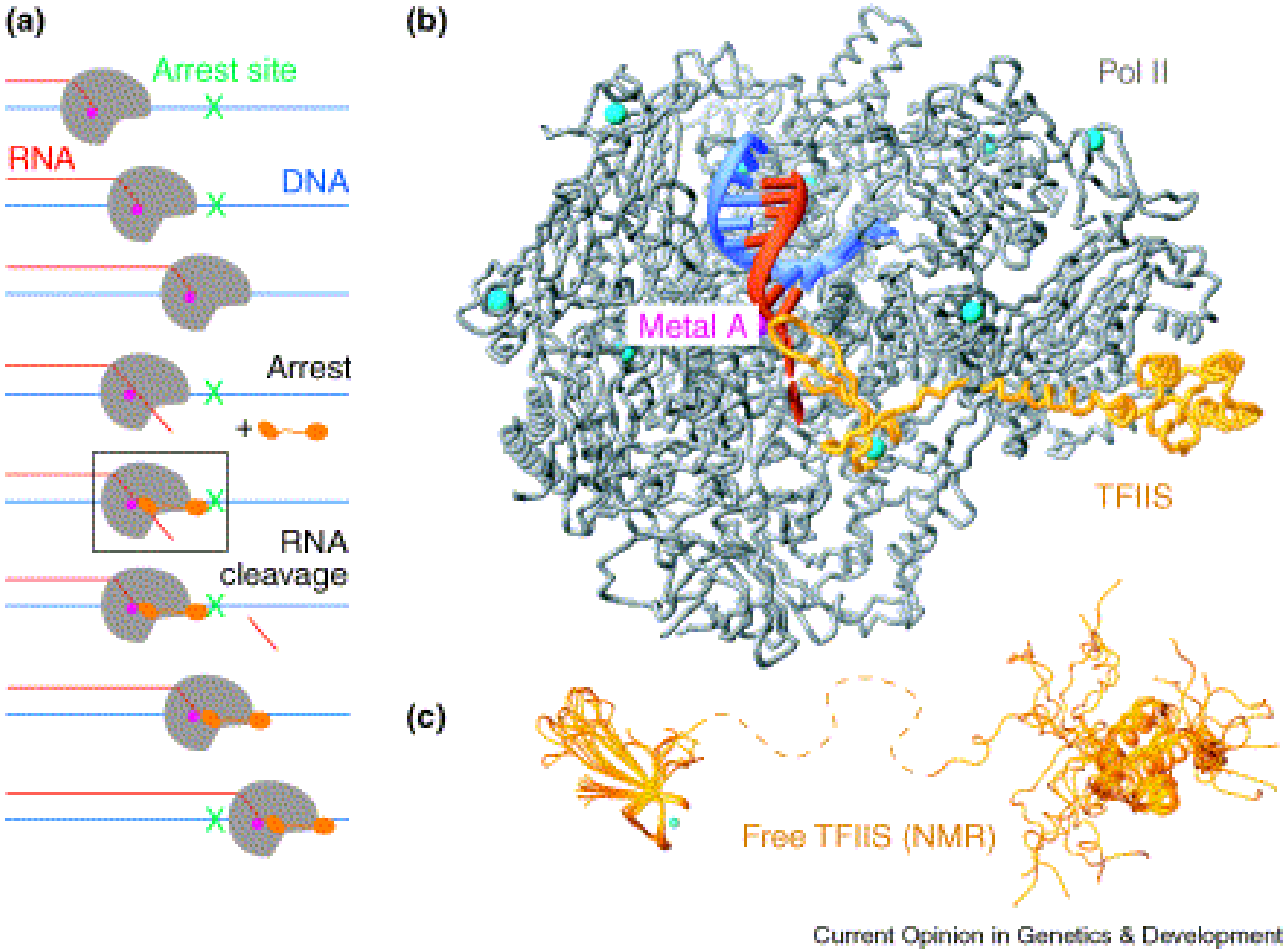
PIC formation



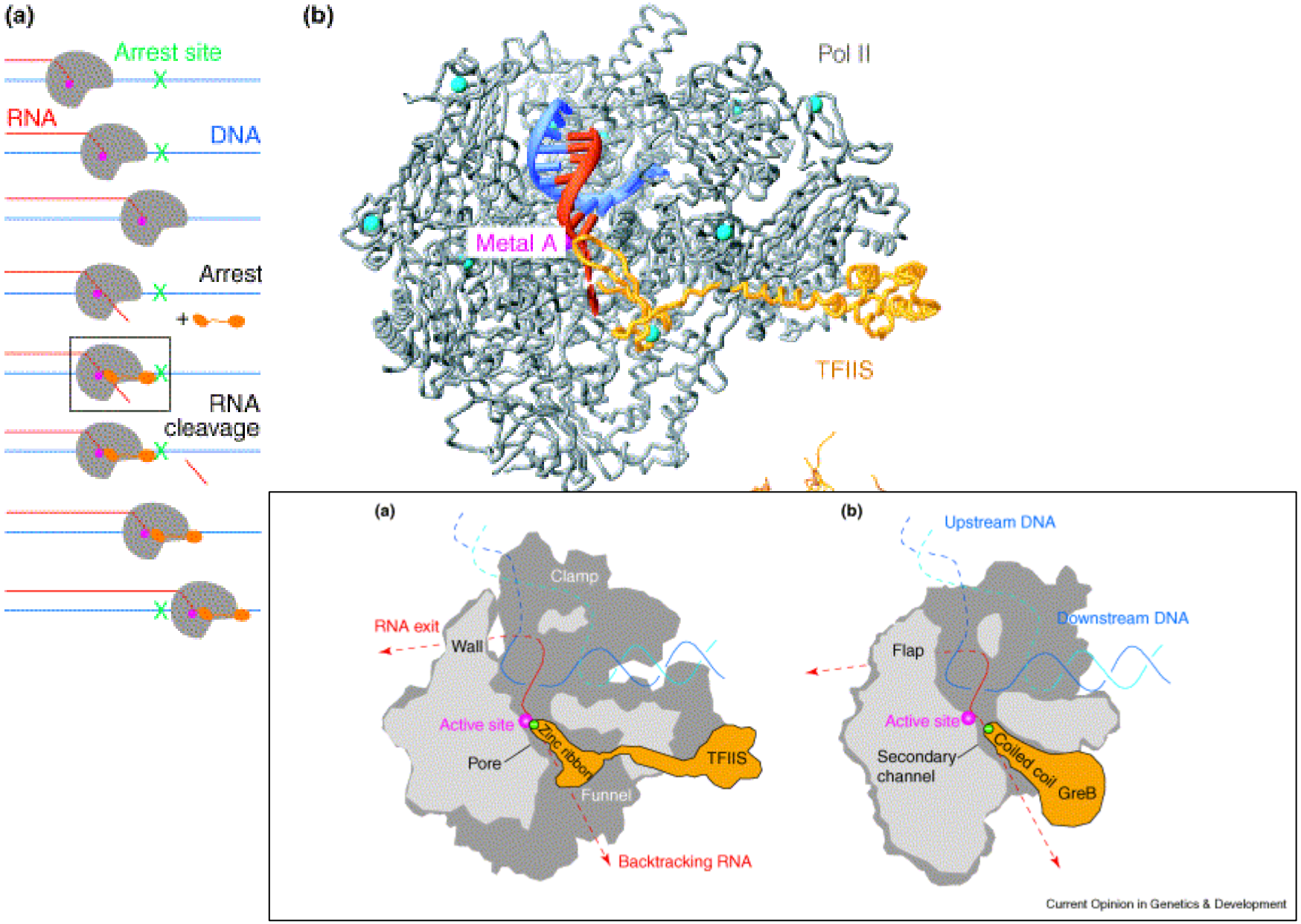
Pol II transcription steps



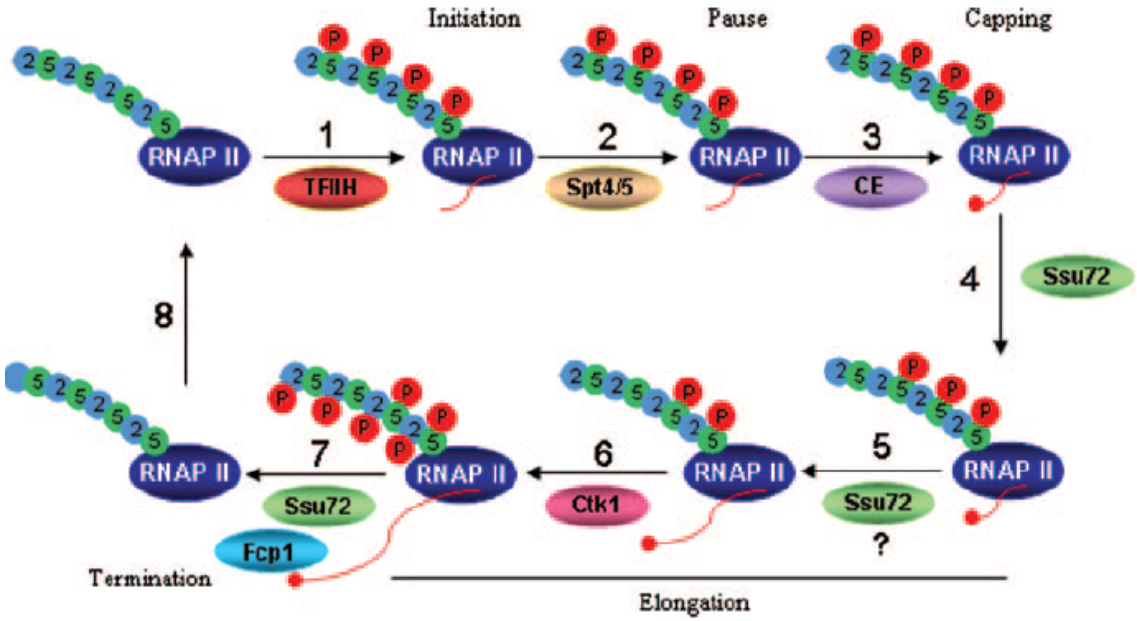


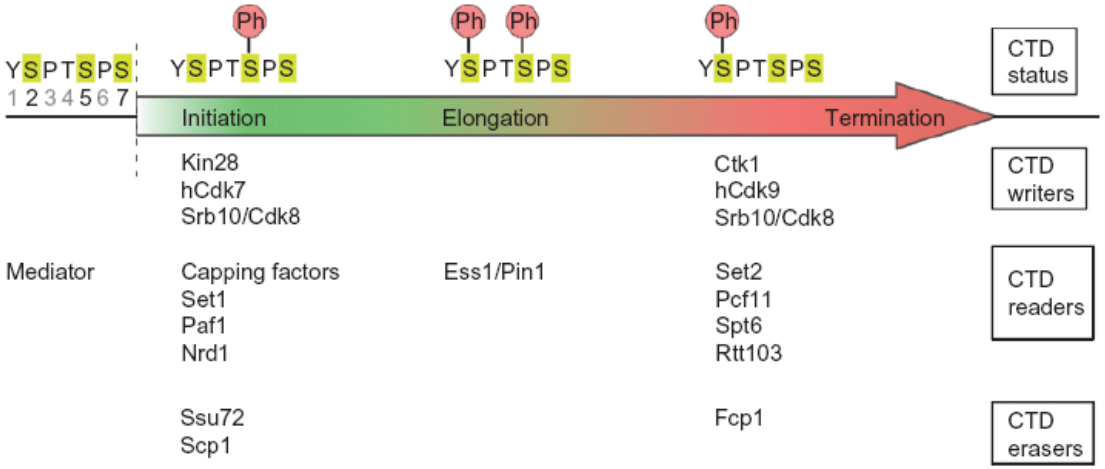


Current Opinion in Genetics & Development


(a)
Arrest site
RNA
DNA
Arrest
+
RNA
cleavage
(b)
Pol II
Metal A
TFIIS
(a)
Clamp
RNA exit
Wall
Active site
Zinc ribbon
TFIIS
Pore
Funnel
Backtracking RNA
(b)
Upstream DNA
Downstream DNA
Flap
Active site
Secondary
channel
Coiled coil
GreB
Current Opinion in Genetics & Development

Pol II transcription steps





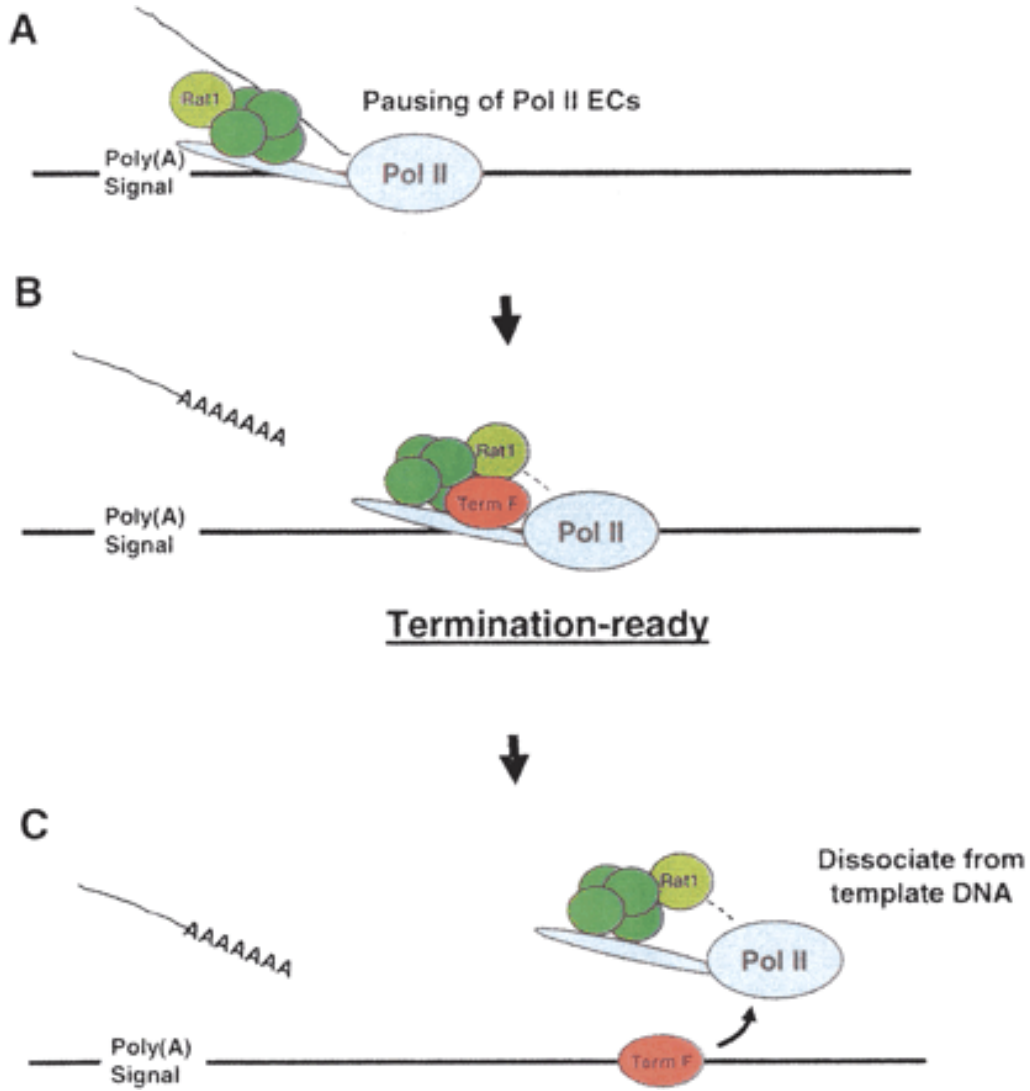


Figure 2. Concerted torpedo model for termination by RNA Pol II. (*A*) Immediately after passing through the poly(A) site, Pol II ECs pause transcription induced by one of several mechanisms (see text); for example, through a conformational change involving the 3'-end processing factors (green). (*B*) Endonucleolytic cleavage at the polyadenylation site and subsequent degradation of 3'-cleaved RNA by Rat1/Xrn2. Simultaneously, Rat1/Xrn2 recruits termination factors (Term F) and delivers them to the Pol II active site. (*C*) The resulting "termination-ready" Pol II is targeted by Term F. RNA-binding proteins, and especially helicase-like ATPases such as TTF2 (see text), are candidates for Term F.

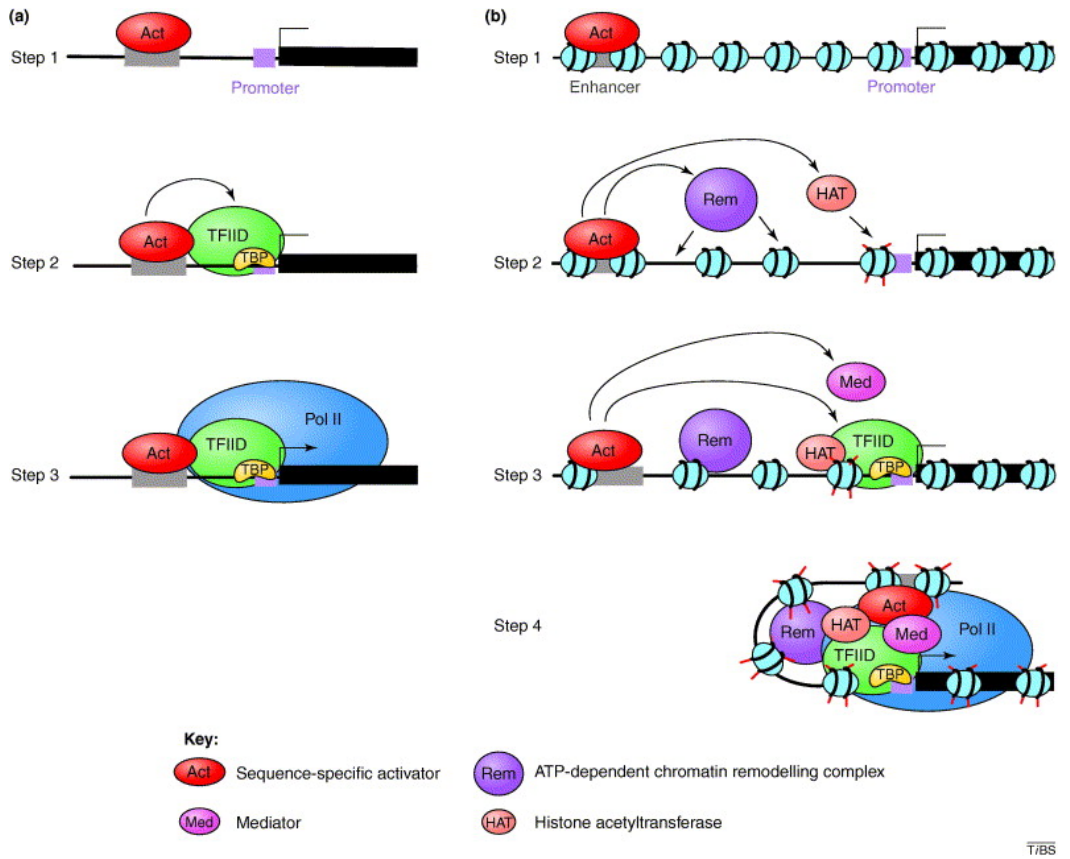
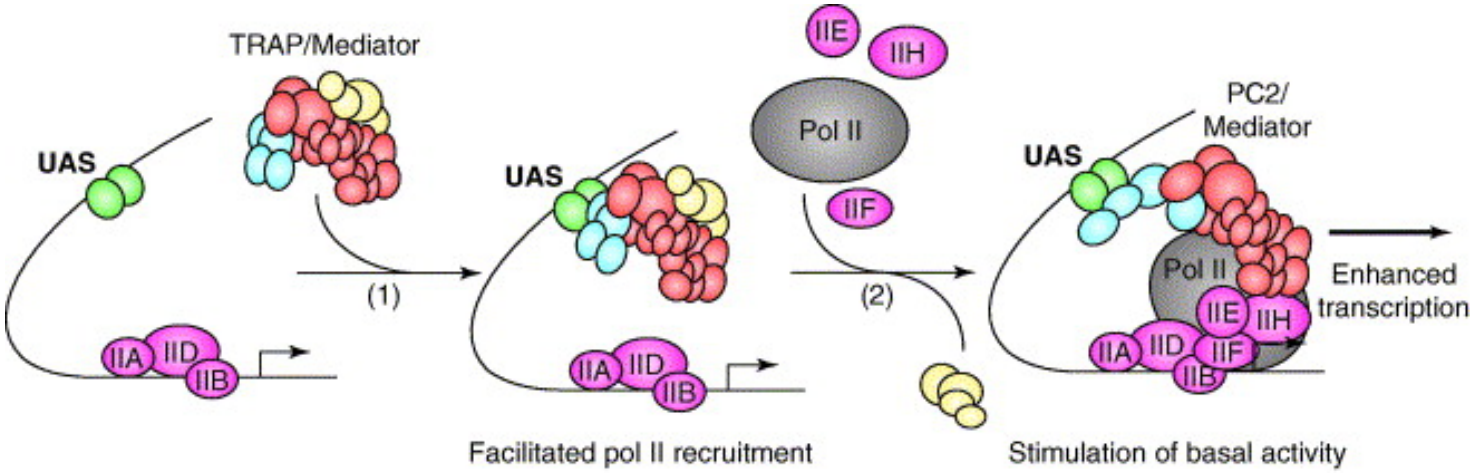


Figure 1. Classical models for the activation of gene transcription. **(a) Transcriptional activation from a naked DNA template. The model is based on data from** *in vitro* reconstituted transcription reactions [57] containing a simple reporter gene (black box) that is controlled by two *cis*-acting elements, the core promoter (purple box) and a proximal promoter element (grey box). Activation is initiated by binding of a sequence-specific activator (Act) to the enhancer (step 1), which recruits the basal transcription machinery to the core promoter (step 2). Binding of TFIID is followed by ordered interactions between the other basal factors (TFIIA, TFIIB, TFIIE, TFIIF and TFIIH, not shown for simplicity) and Pol II to yield an active PIC (step 3); transcriptional activation is indicated by a black arrowhead from the gene. **(b) Model describing the activation process of a eukaryotic gene in chromatin. The gene (black box) has a core promoter element (purple box) and is regulated by a distal enhancer (grey box). The enhancer-bound activator (step 1) recruits ATP-dependent chromatin-remodelling complexes (Rem) and histone-modifying factors [e.g. histone acetyltransferases (HATs)] to the core promoter (step 2). This model also implies that, after rendering the gene accessible by chromatin remodelling mechanisms, Mediator complexes (Med) establish the link between the enhancer-bound activator and the basal transcription machinery at the promoter (step 3). The PIC remains inactive until tracking (not shown) or a direct contact (looping) occurs between the distal enhancer and the promoter. Establishment of enhancer–promoter contact results in Pol II recruitment and the initiation of transcription from the gene (step 4).**

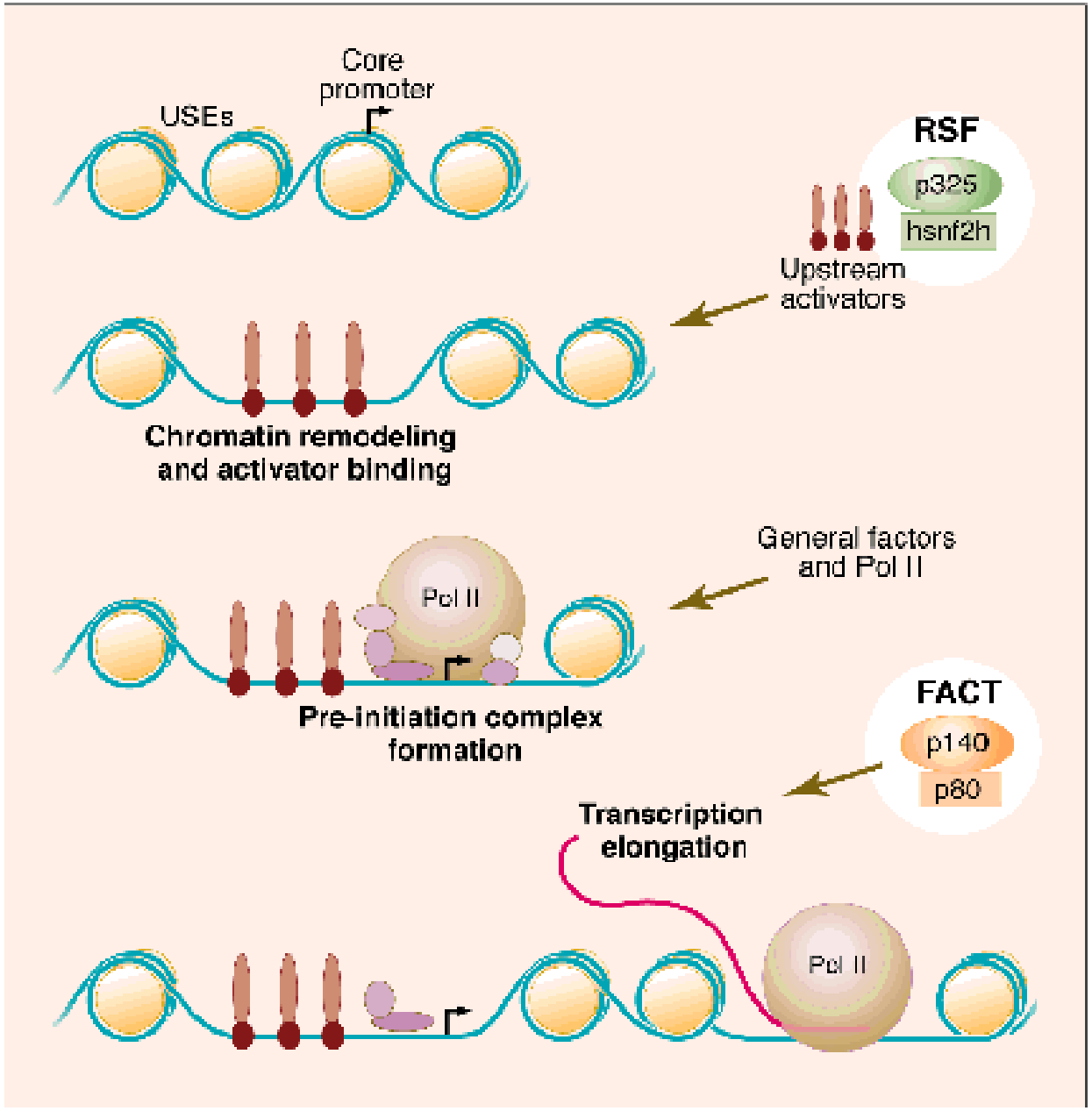
(a)

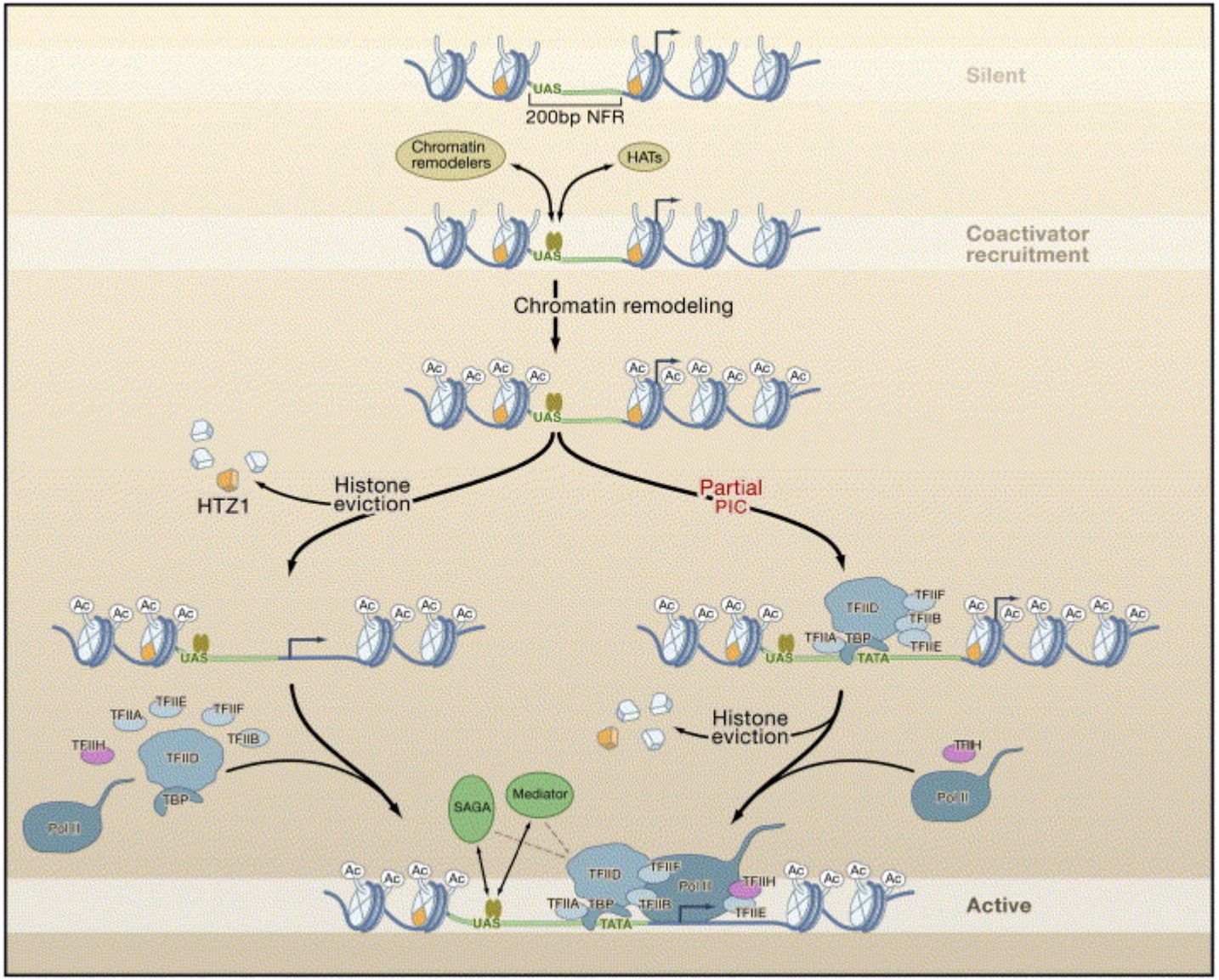
MED11/HSPC296
MED17/TRAP80
MED20/ TRFP
MED23/ Surf5
MED12/ TRAP230
MED8/ ARC32
MED19/LCMR1
MED18/p28b
MED6
MED13/TRAP240
MED30/ TRAP25
MED21/SRB7
MED4/p36
SRB10/CDK8
MED7
SRB11/CycC
MED31/ SOH1
MED10/NUT2
MED1/TRAP220
MED27/ TRAP37
MED26/CRSP70
MED25/ ARC92/ACID1/ TRAP97
MED15/ TIG 1/ PC QAP
MED23/SUR2
MED14/TRAP170
MED24/TRAP100
MED16/TRAP95



TiBS

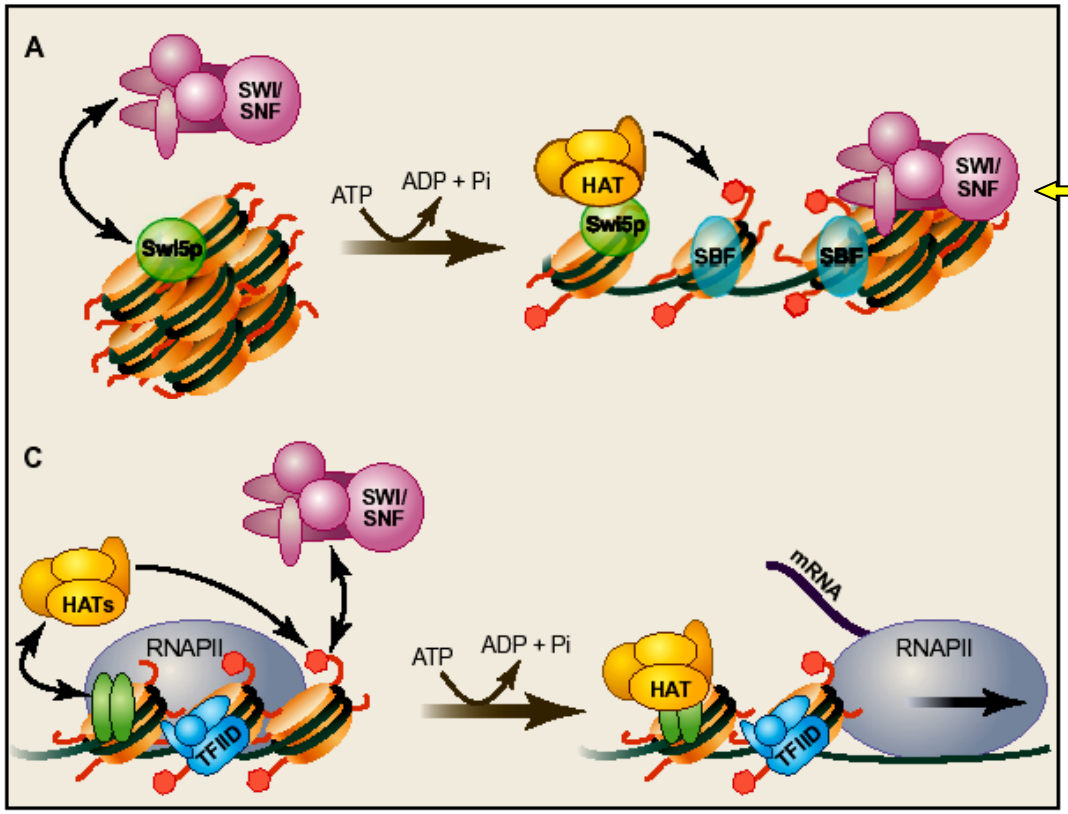
Model of the RNA Pol II transcription



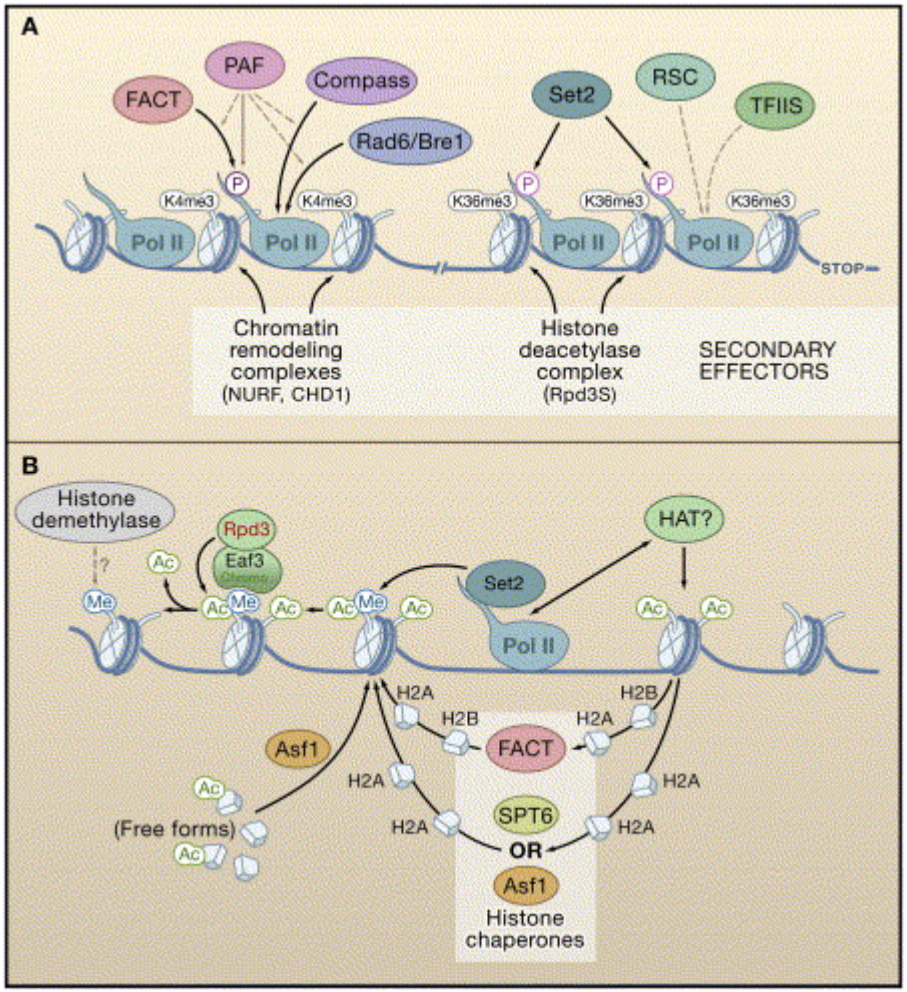


Models of Chromatin Regulation during Transcription Initiation

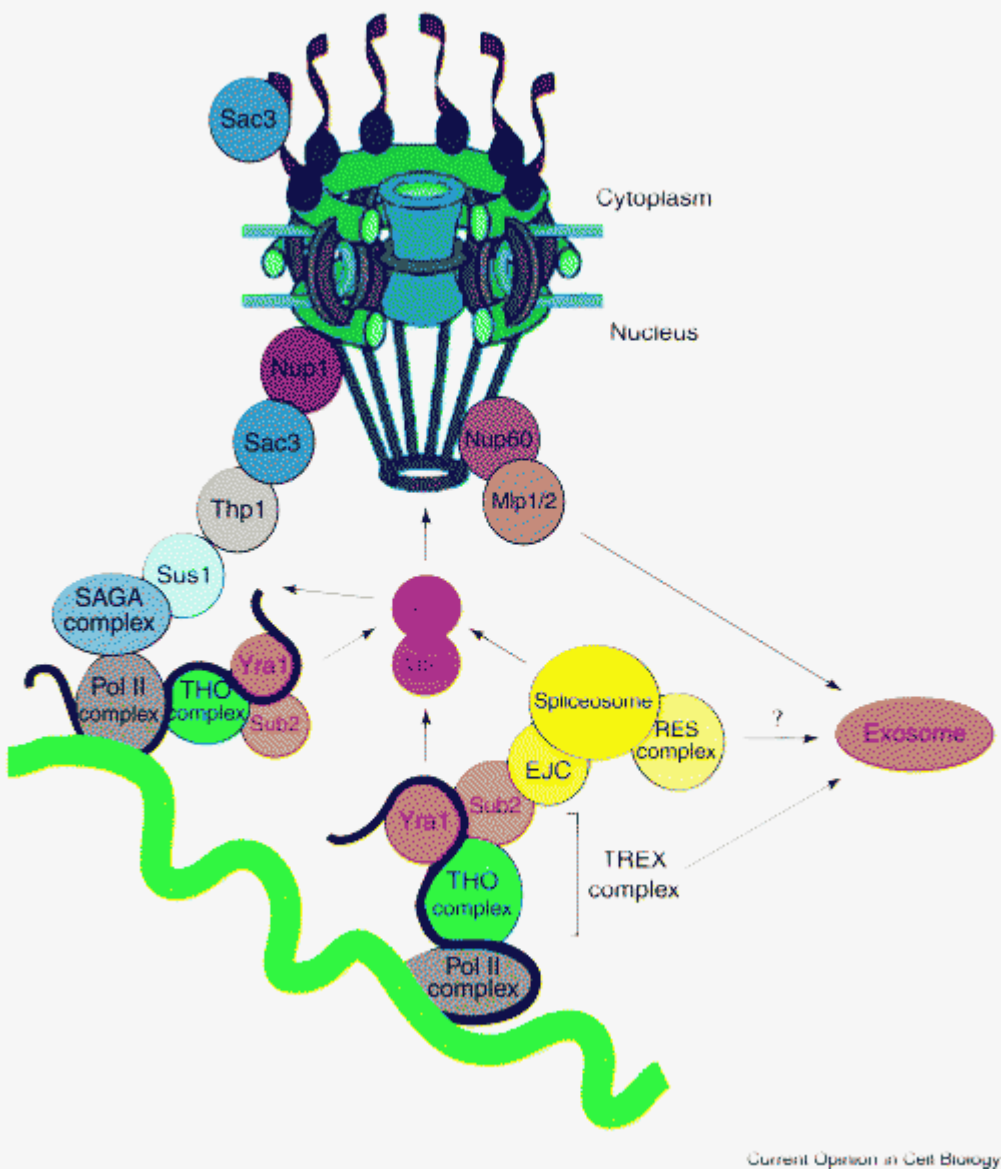
Model of the RNA Pol II transcription



UAF



Regulation of Nucleosome Dynamics during Transcription Elongation


Sac3
Cytoplasm
Nucleus
Nup1
Sac3
Nup60
Mlp1/2
Thp1
Sus1
SAGA complex
Pol II complex
THO complex
Yra1
Sub2
Spliceosome
RES complex
?
Exosome
EJC
Sub2
Yra1
THO complex
TREX complex
Pol II complex
Current Opinion in Cell Biology

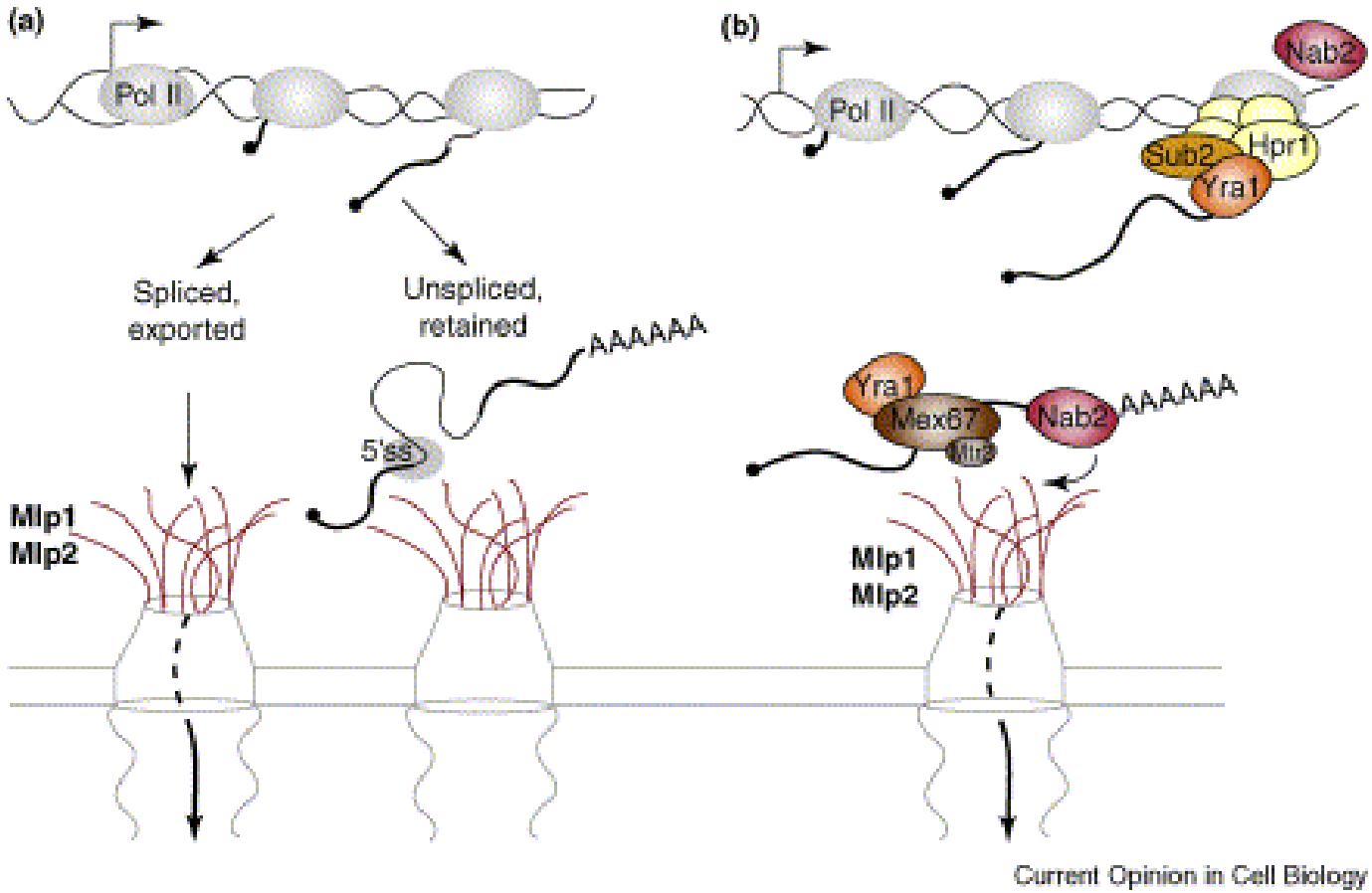
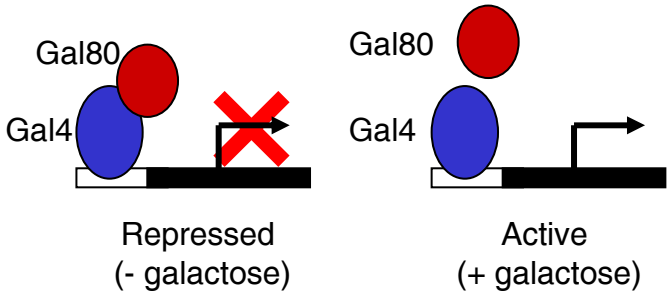
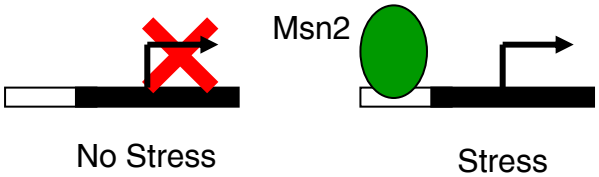


Figure 3. Mlp proteins form a selective filter at the entrance of the nuclear pore complex. **(a) The perinuclear Mlp1p protein contributes to mRNP surveillance by retaining unspliced transcripts within the nucleus, possibly via recognition of a component associated with the 5′ splice site** [57.••]. **(b) Nab2p, a shuttling mRNA binding protein involved in polyA tail length regulation, directly interacts with Mlp proteins** [58.•]. Nab2p may be important for the docking of mRNPs to the Mlp barrier, perhaps by signaling proper 3′ end formation. Consistent with their inessential nature, Mlp proteins may play a general role in mRNP surveillance by preferentially interacting with properly packaged mRNP complexes, preventing mRNPs that lack essential signals from reaching the central channel of the NPC.

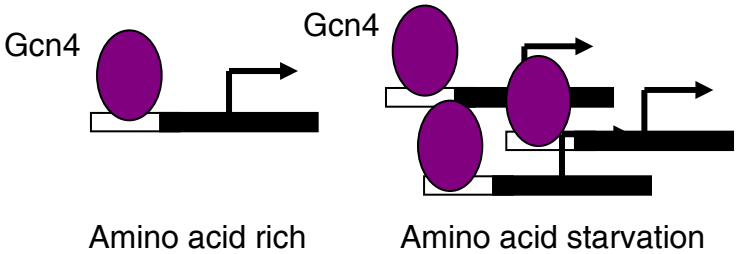
Examples of Gene Regulatory Mechanisms



Regulator is bound under both conditions but is modified upon activation



Regulator binds only in activating conditions due to nuclear exclusion



Regulator binds expanded set of targets in activating conditions

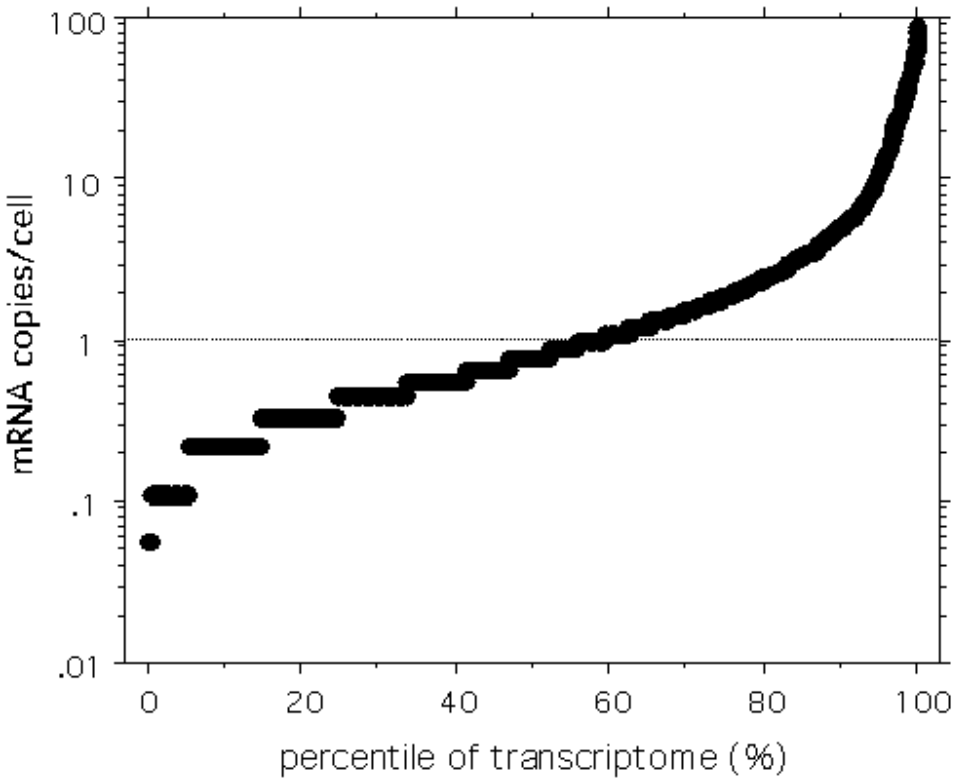
Several RNA Poll II holoenzymes implicated in transcriptional regulation?

Transcriptome Statistics

5460 transcripts

15000 polyA-RNA's per cell

80% of the transcriptome is expressed at 0.1 - 2 mRNA copies/cell



Transcriptome analysis

Microarrays

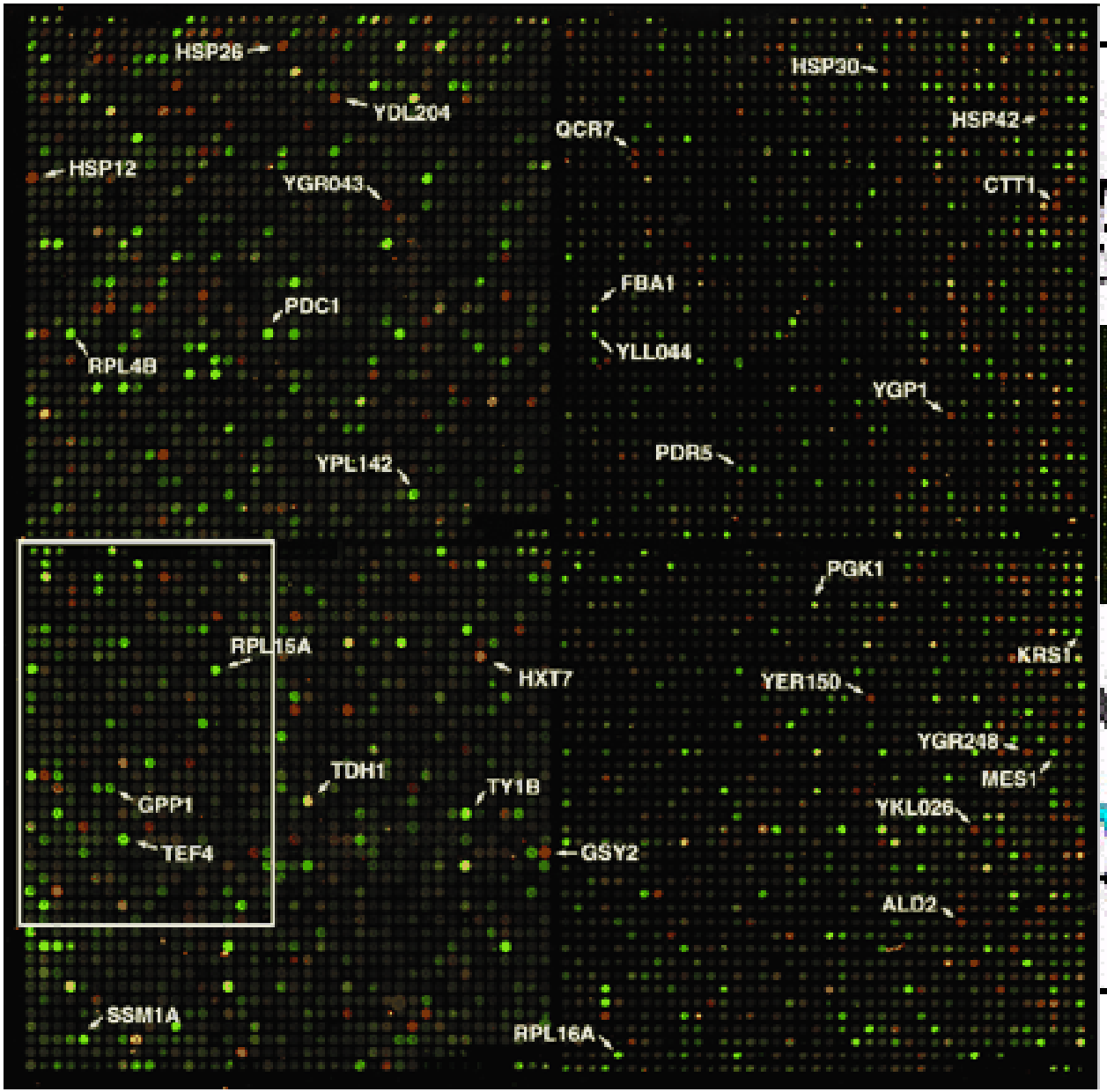
- • Allows researchers to analyze the expression of thousands of genes simultaneously.
- • Using genomic databases one can analyze the complete transcriptional program of an organism under specific conditions.

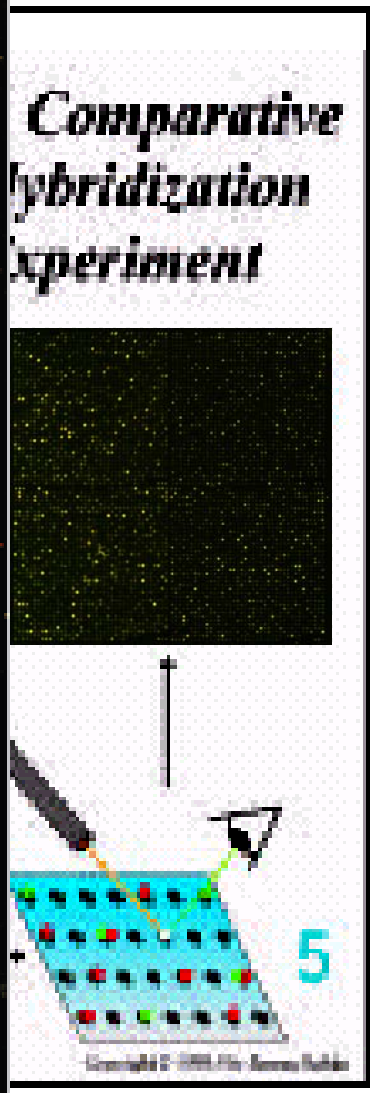
There are two major applications of DNA microarray technology

1) identification of sequence(gene or gene mutation).

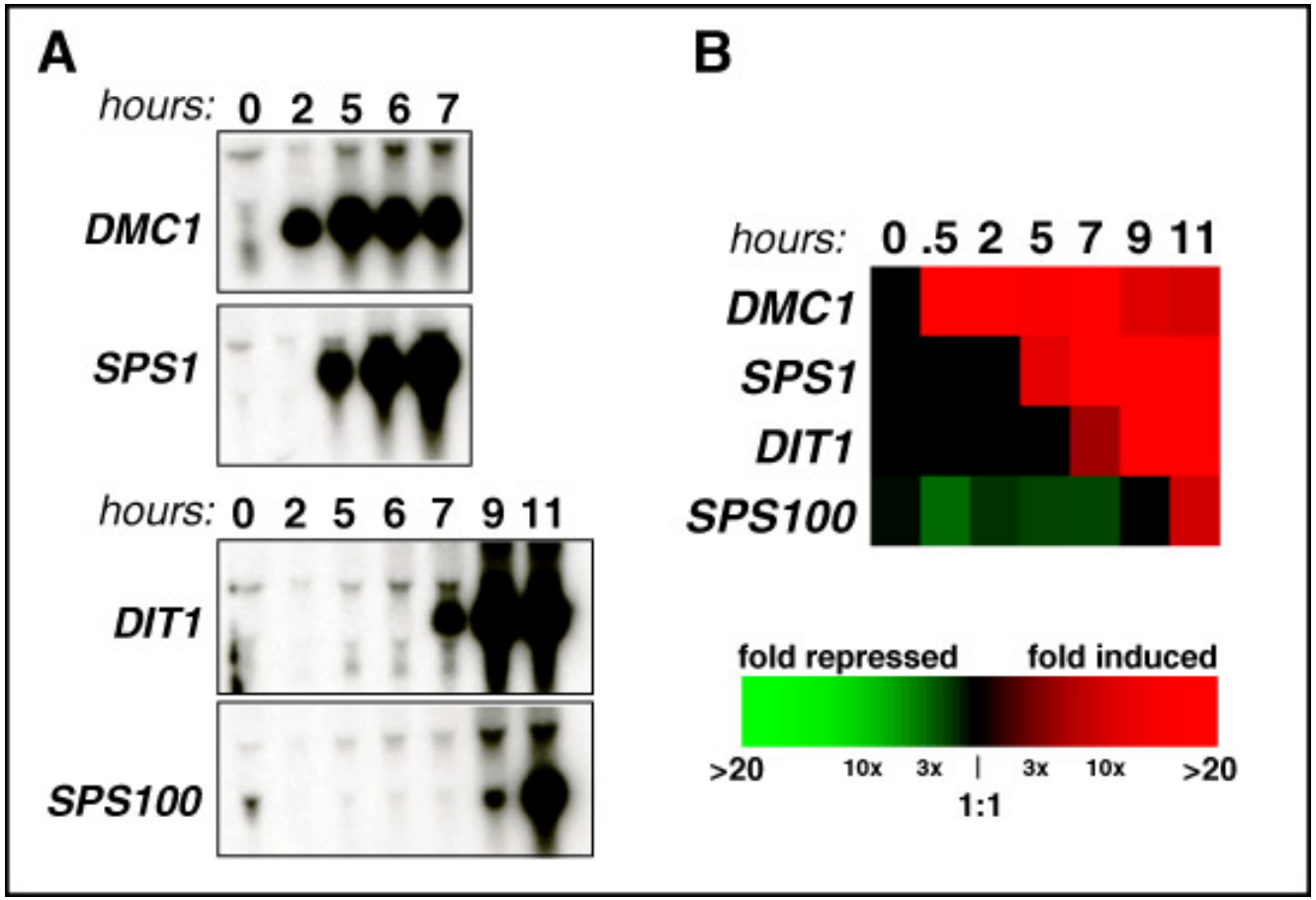
. 2) determination of expression level of genes.

Transcriptome analysis

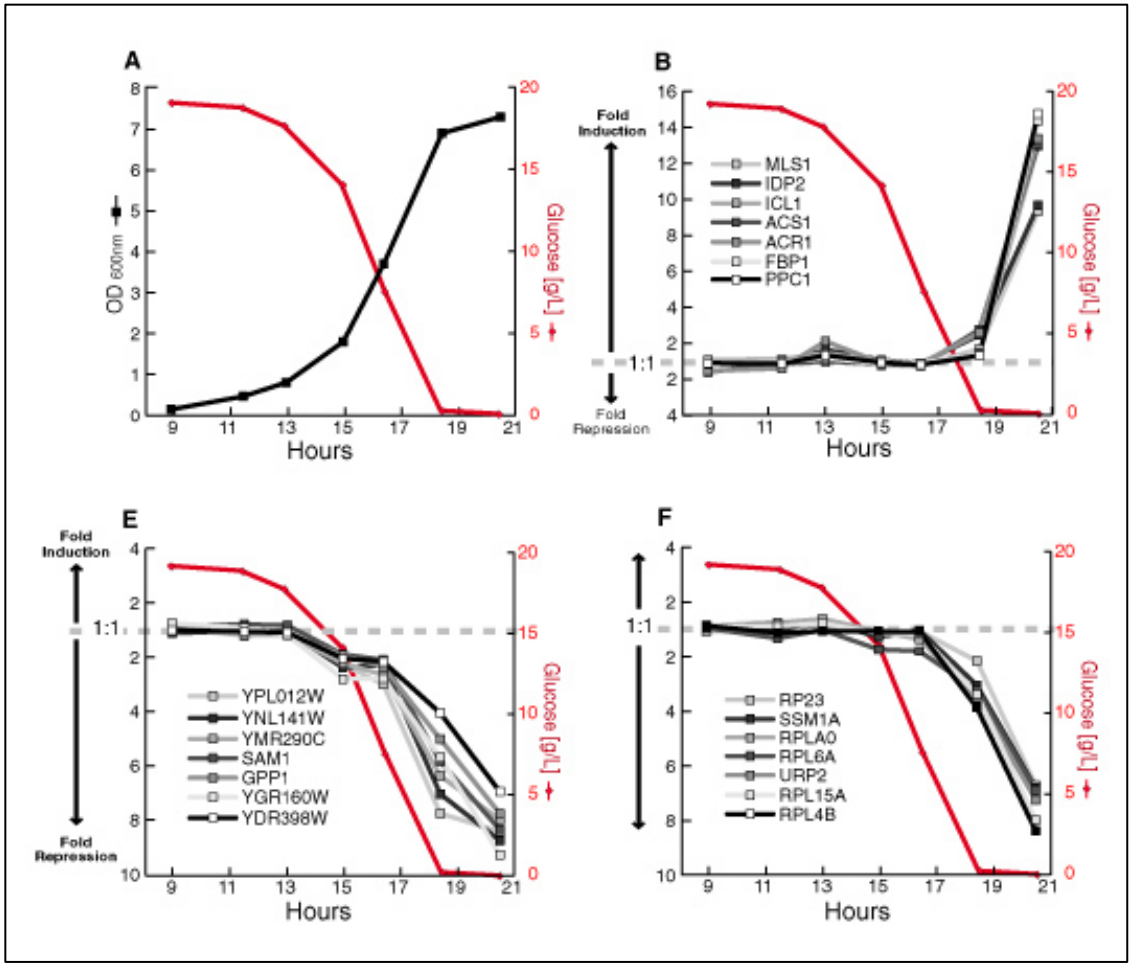


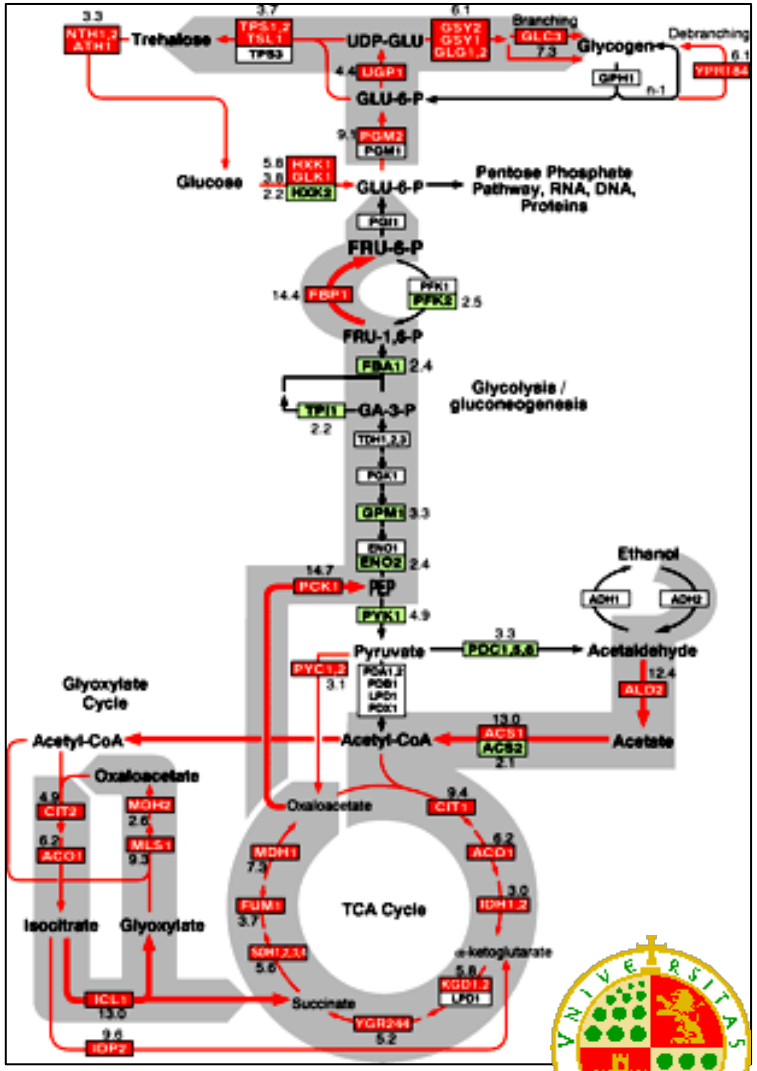


Sporulation analysis

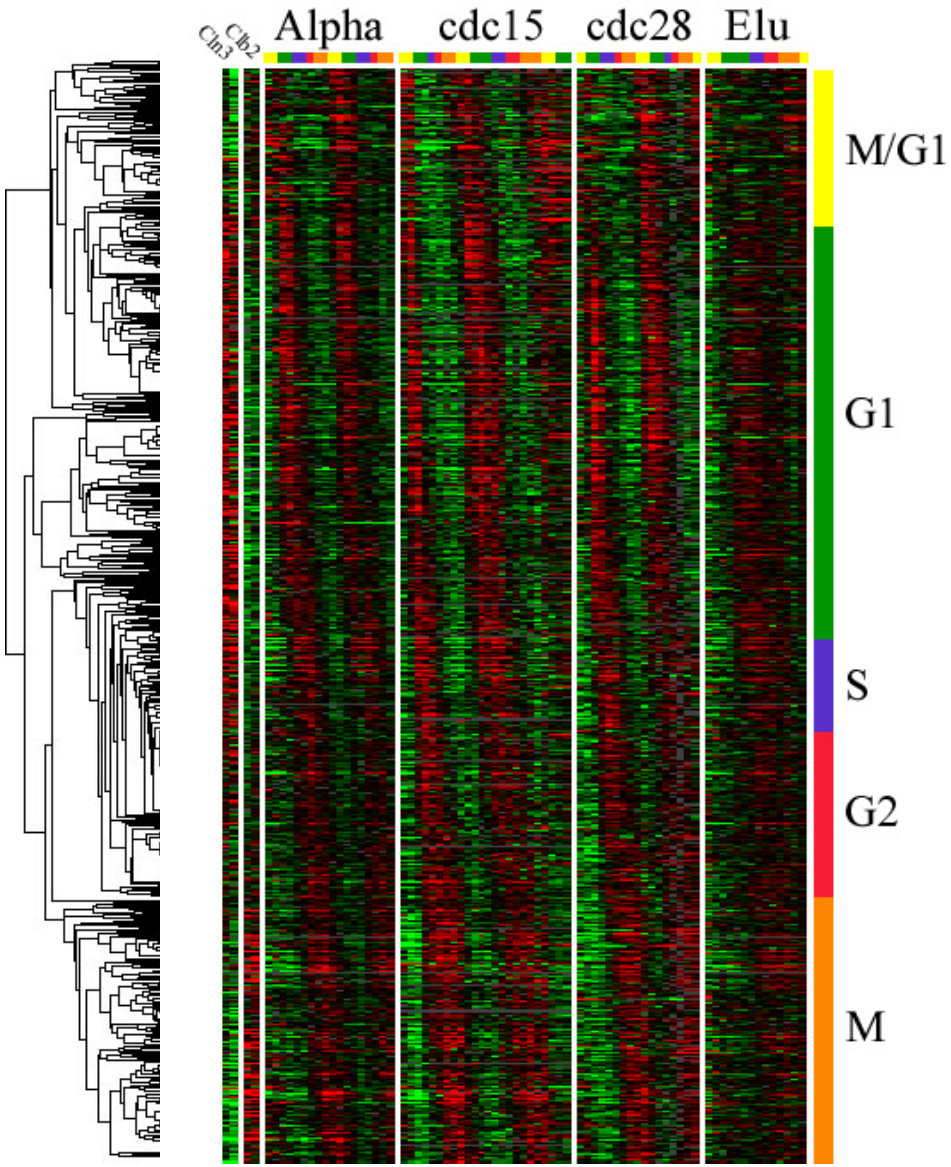


Transcriptome analysis

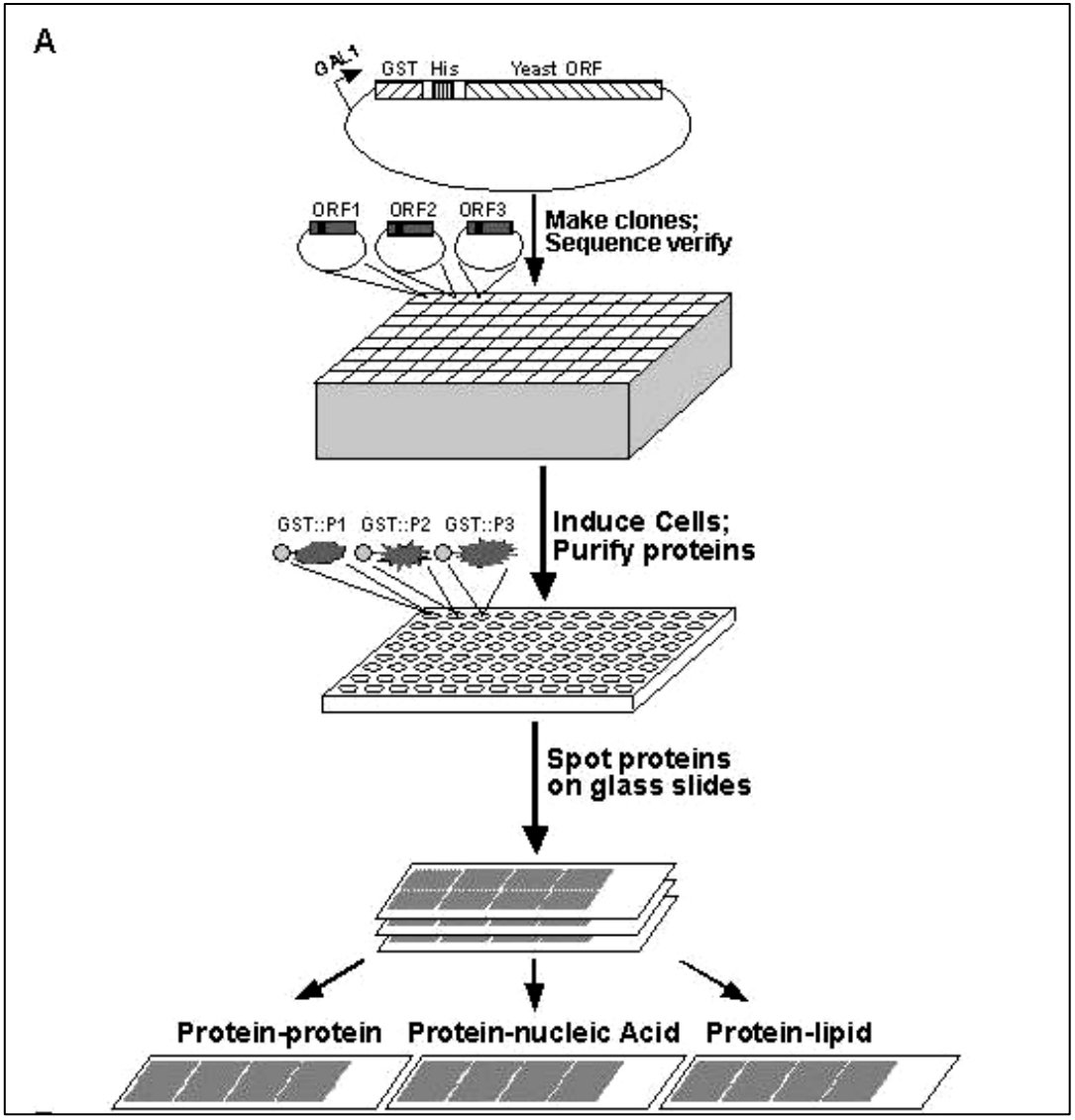


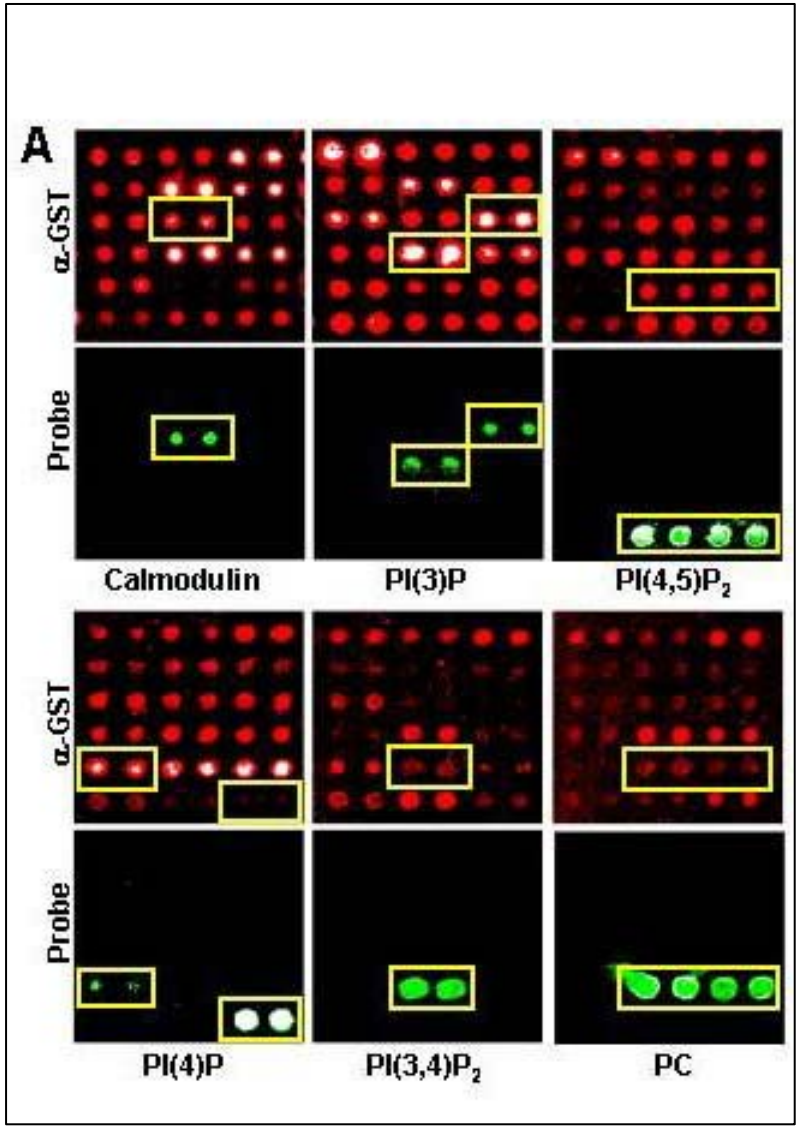


Cell Cycle analysis

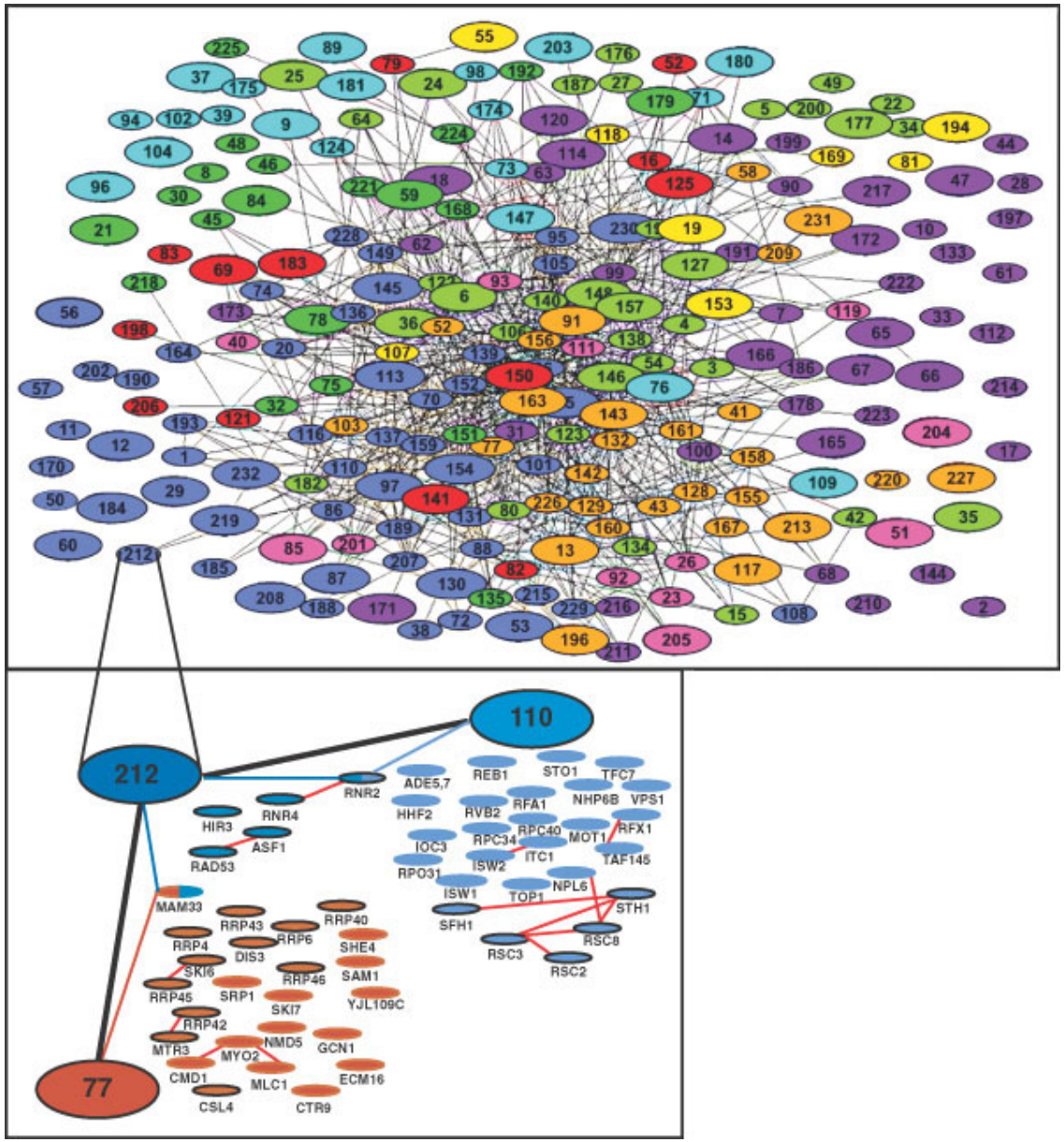


The procedure of yeast proteome analysis using protein chip technology





Protein complex network



Proteome analysis