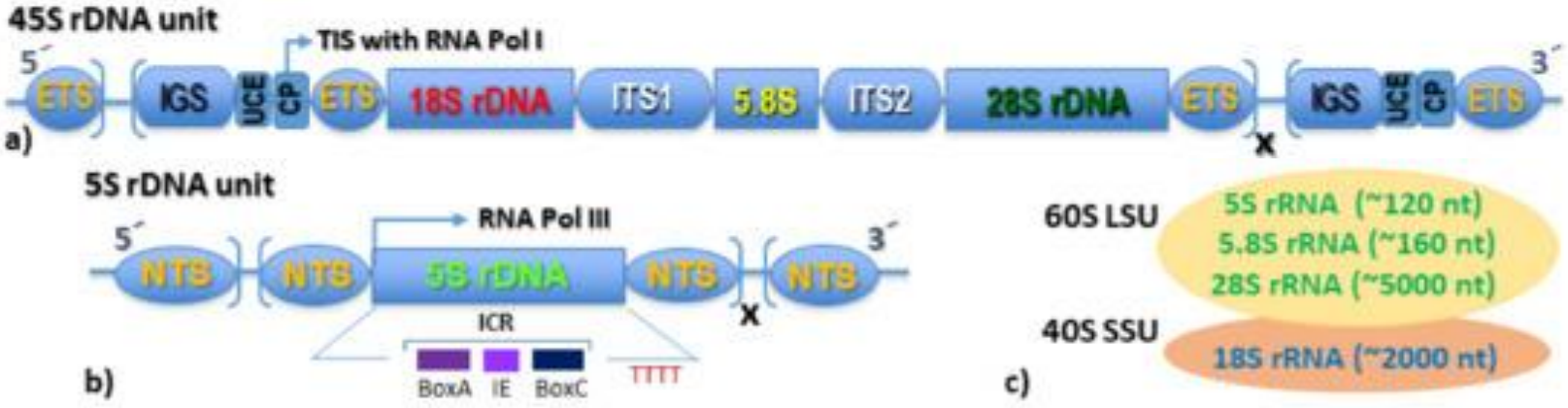
Genome Editing and Engineering

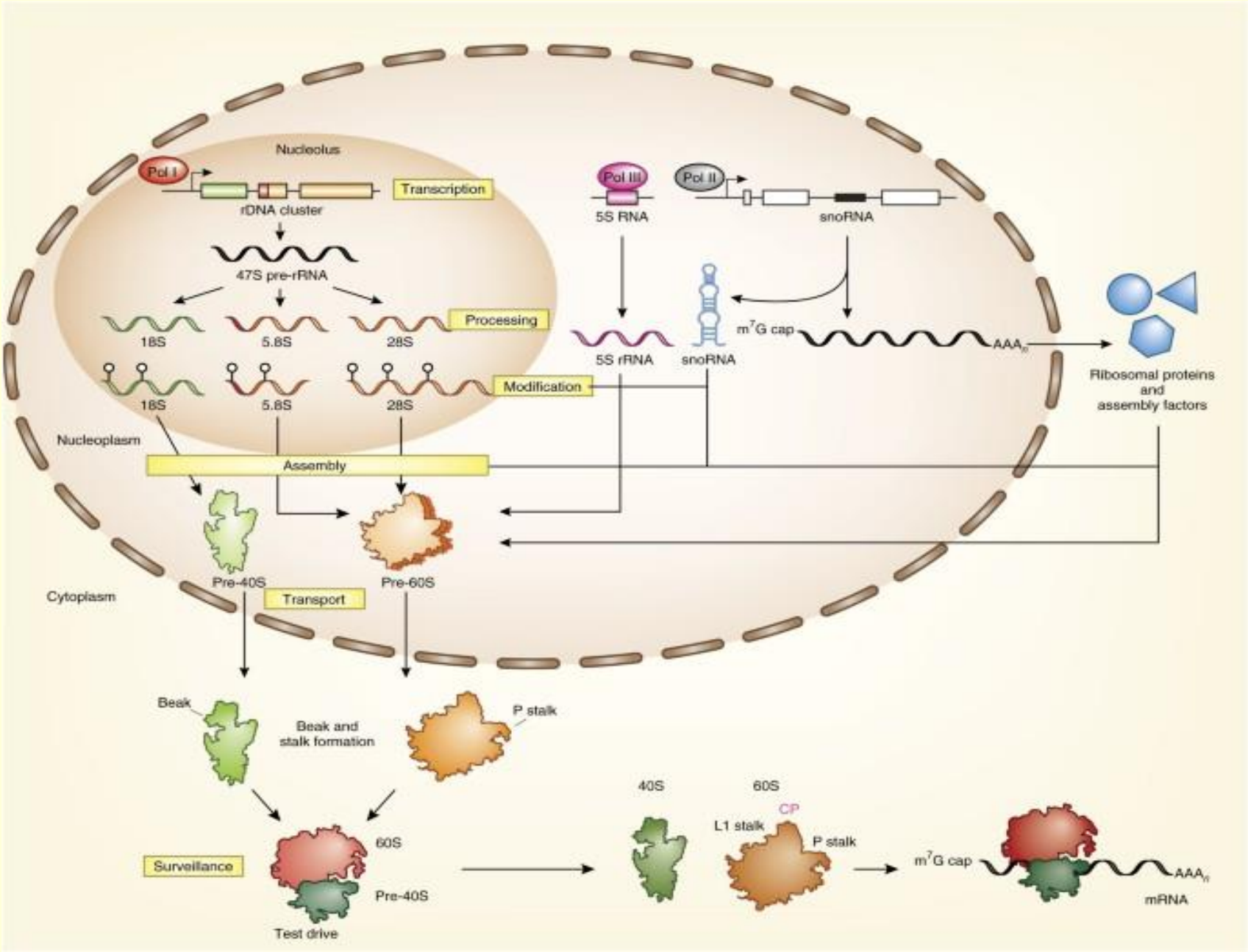
Course No: BT-637

LECTURE-9

Dr. Kusum K. Singh
Department of Biosciences and Bioengineering
Indian Institute of Technology Guwahati



Symonova 2019

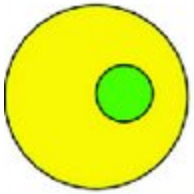

Nucleolus
Pol I
Transcription
rDNA cluster
47S pre-rRNA
18S
5.8S
28S
Processing
18S
5.8S
28S
Modification
Nucleoplasm
Assembly
Pre-40S
Pre-60S
Cytoplasm
Transport
Pol III
5S RNA
Pol II
snoRNA
5S rRNA
snoRNA
m7G cap
AAAn
Ribosomal proteins and assembly factors
Beak
Beak and stalk formation
P stalk
Surveillance
60S
Pre-40S
Test drive
40S
60S
CP
L1 stalk
P stalk
m7G cap
AAAn
mRNA

Introduction

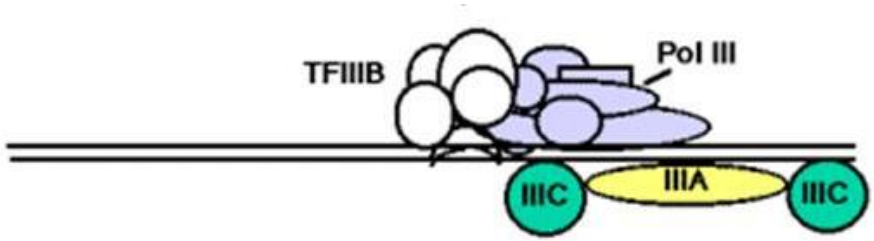
- Xenopus laevis

Transcription Factor IIIA

Somatic
(body) cell



Egg cell



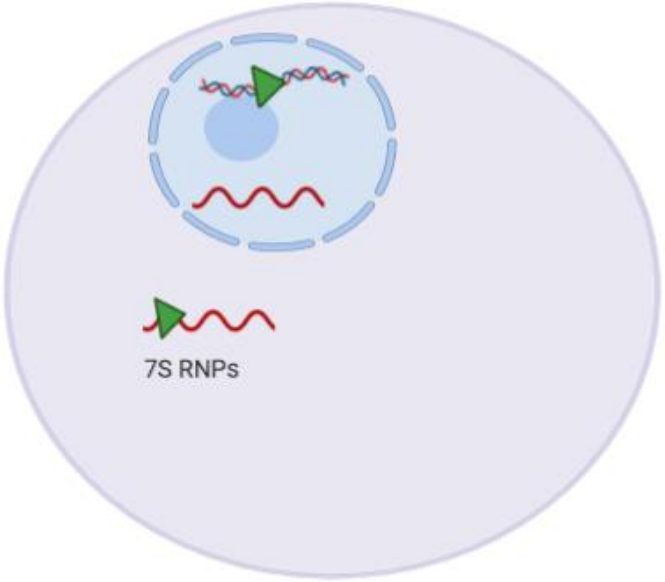
- Gene expression & Transcription mechanism

Isolation of a 7S particle from *Xenopus laevis* oocytes A 5S RNA–protein complex

(oogenesis/ribonucleoprotein particles/ribosome unfolding)

Proc. Natl. Acad. Sci. USA
Vol. 76, No. 1, pp. 241–245, January 1979
Biochemistry

BRIGITTE PICARD AND MAURICE WEGNEZ*



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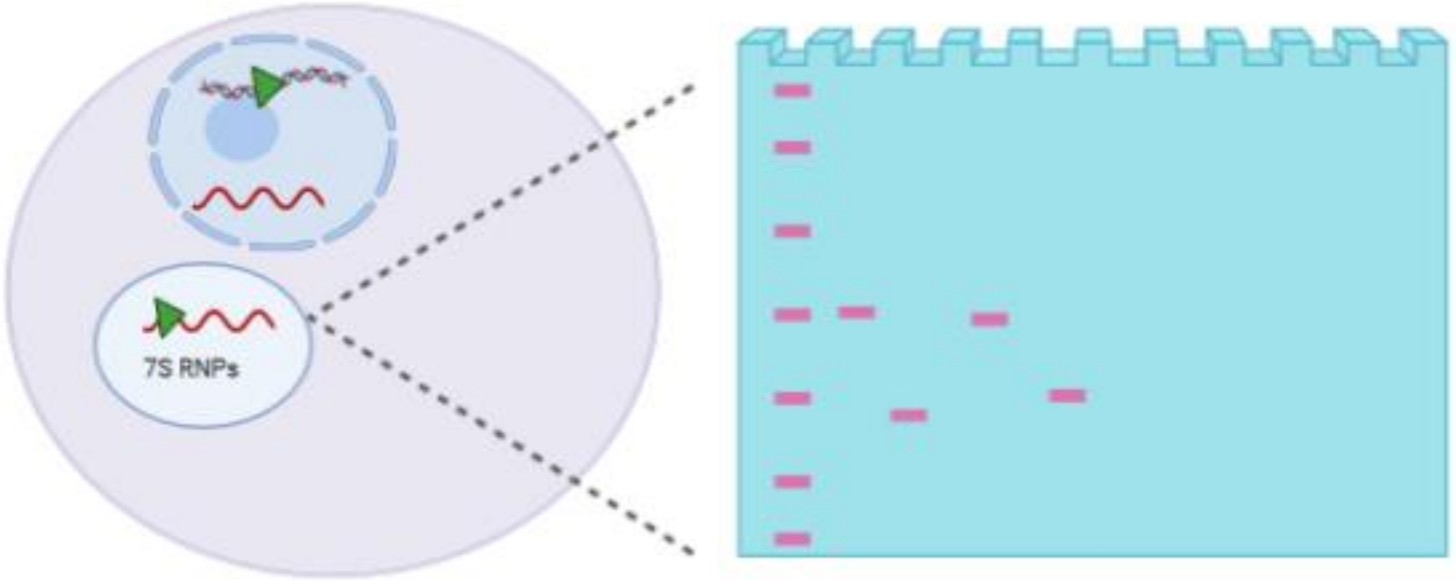

Table 1. Physical properties of the 7S particles from *X. laevis* ovaries

Property	5S RNA	Protein	7S particle
Molecular weight	40,000	45,000	85,000
Sedimentation coefficient s_0	—	—	7.53 ± 0.15
Buoyant density in 25 mM KCl, g cm^{-3}	1.169 ± 0.004	1.275 ± 0.006	1.222 ± 0.005
Buoyant density in 25 mM KCl/5 mM $MgCl_2$, g cm^{-3}	1.184 ± 0.004	1.278 ± 0.006	1.259 ± 0.006

A specific transcription factor that can bind either the 5S RNA gene or 5S RNA

(*Xenopus* oocytes/protection from DNase/ribonucleoprotein particles/developmental control of transcription)

HUGH R. B. PELHAM AND DONALD D. BROWN

Proc. Natl. Acad. Sci. USA
Vol. 77, No. 7, pp. 4170–4174, July 1980
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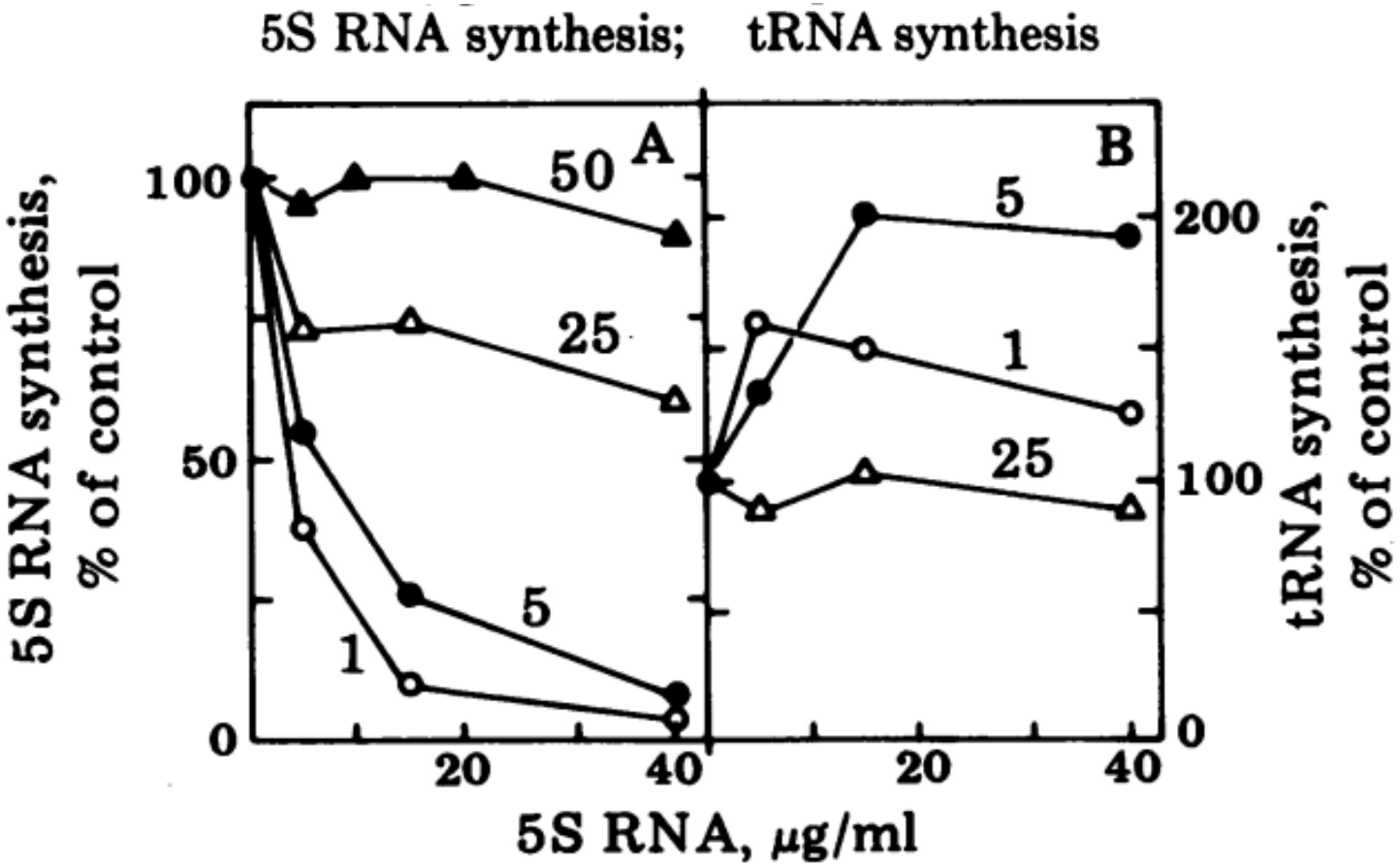

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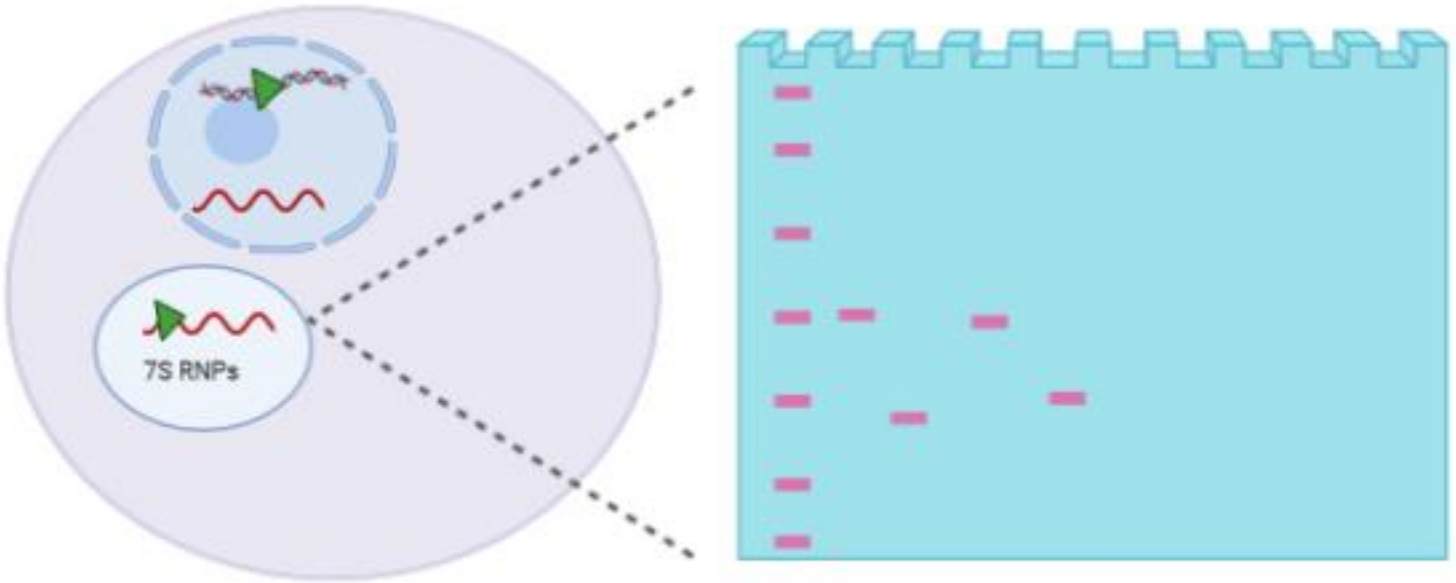
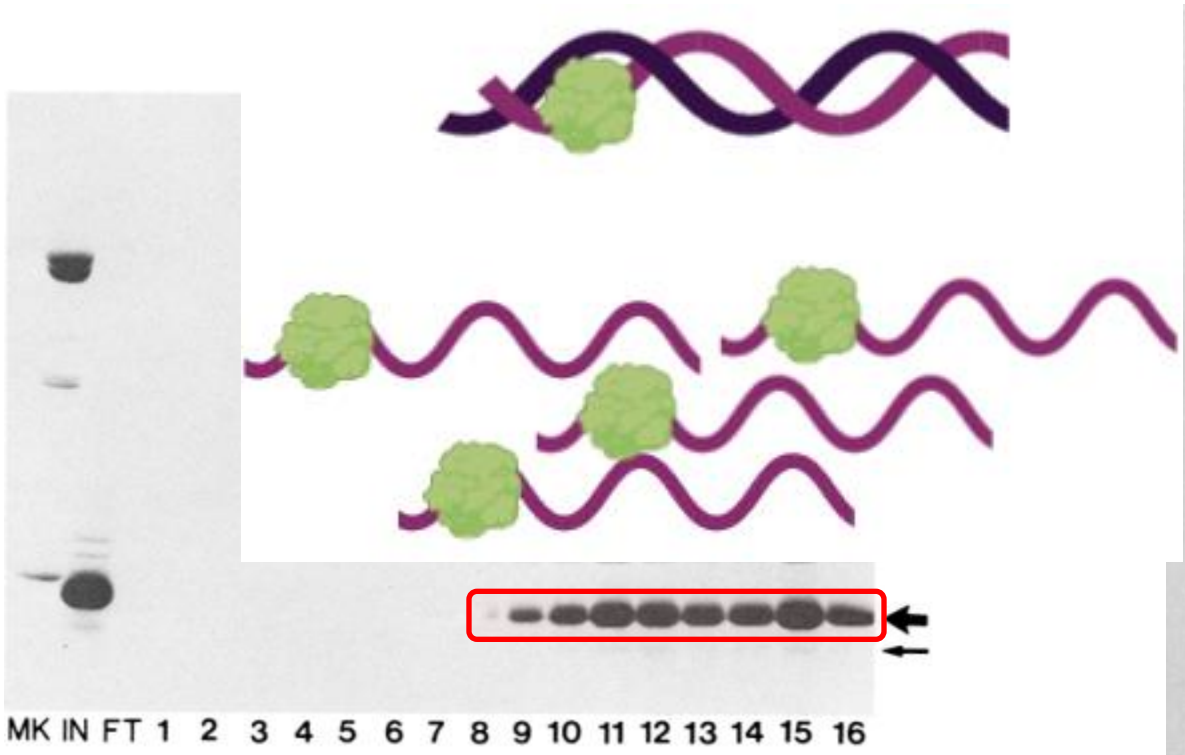
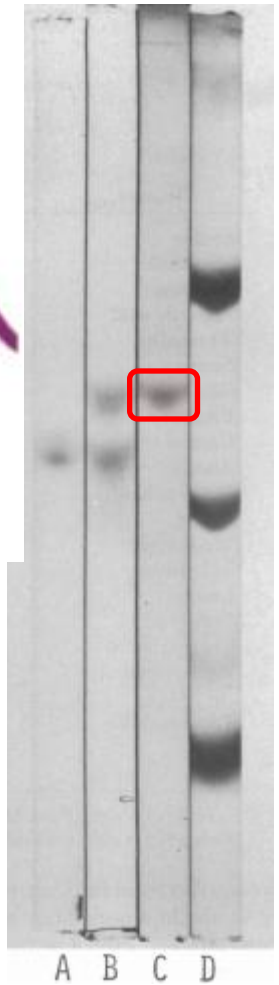


Comparison of factor A & 7S RNP



Engelke et al., 1980



Picard & Wegnez., 1979

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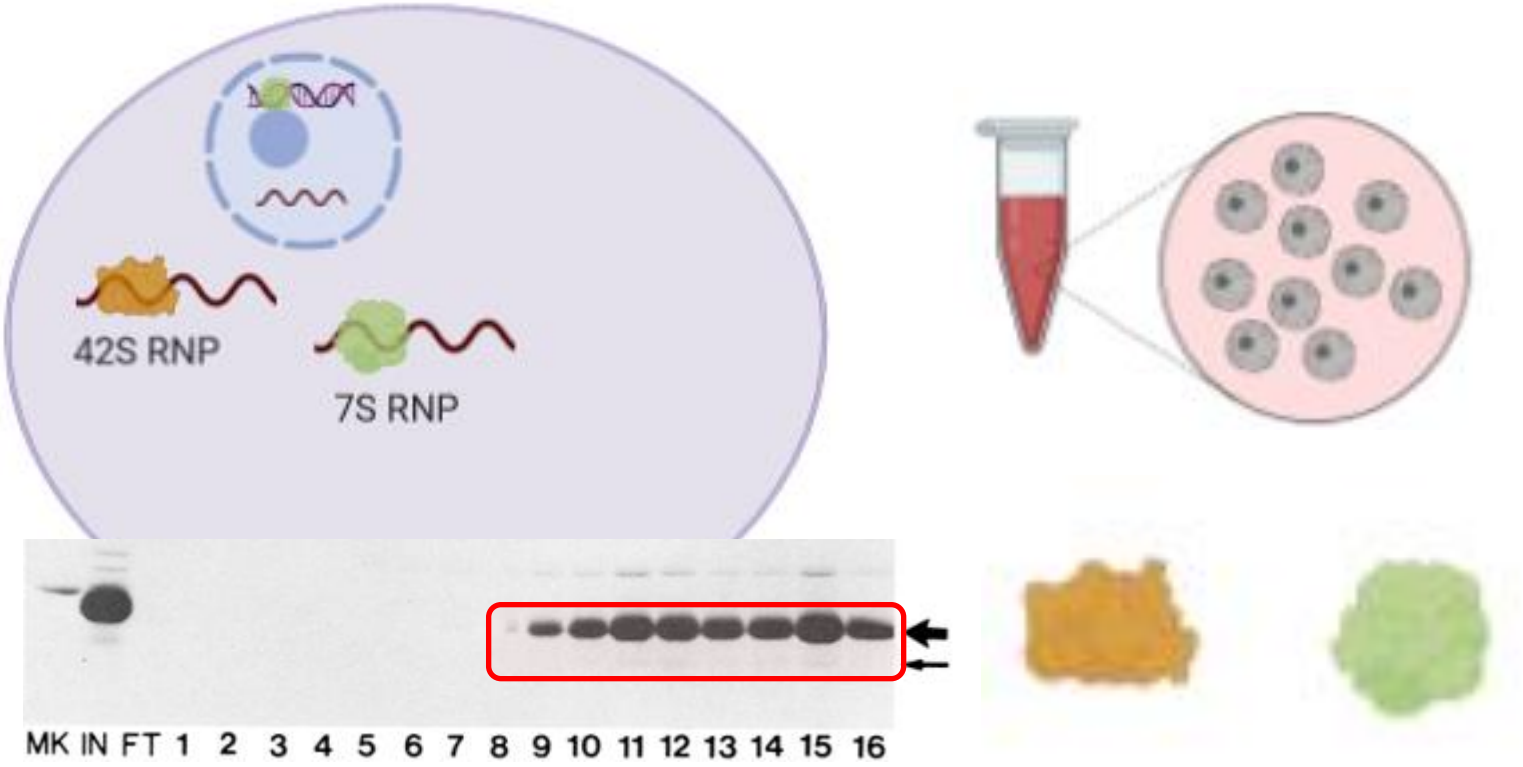

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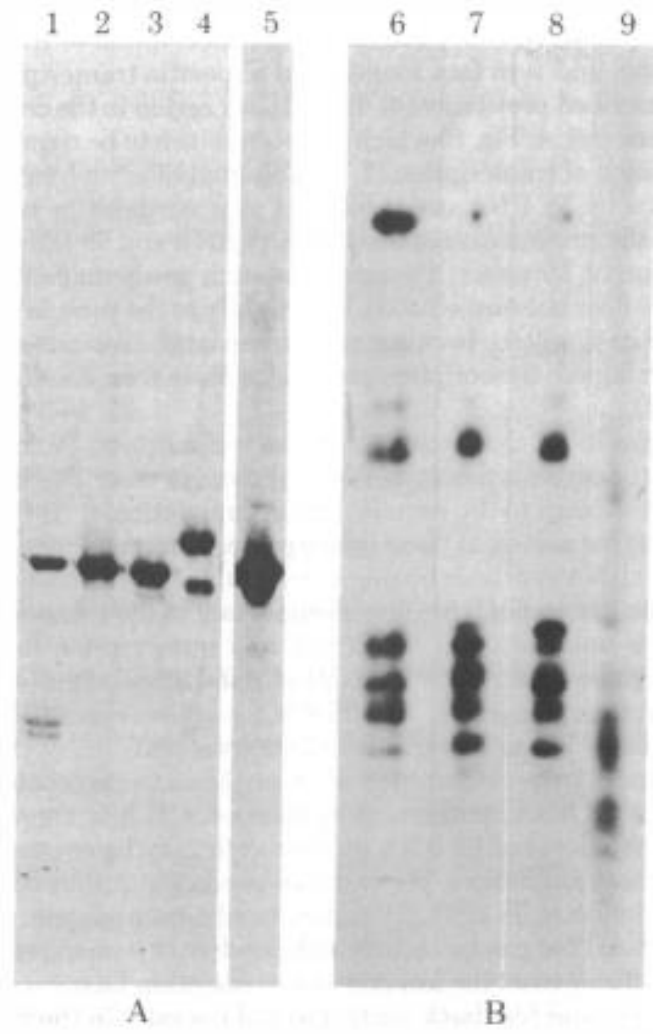

Purified protein from 7S matched with Transcription factor but not with 42S purified protein of similar size.

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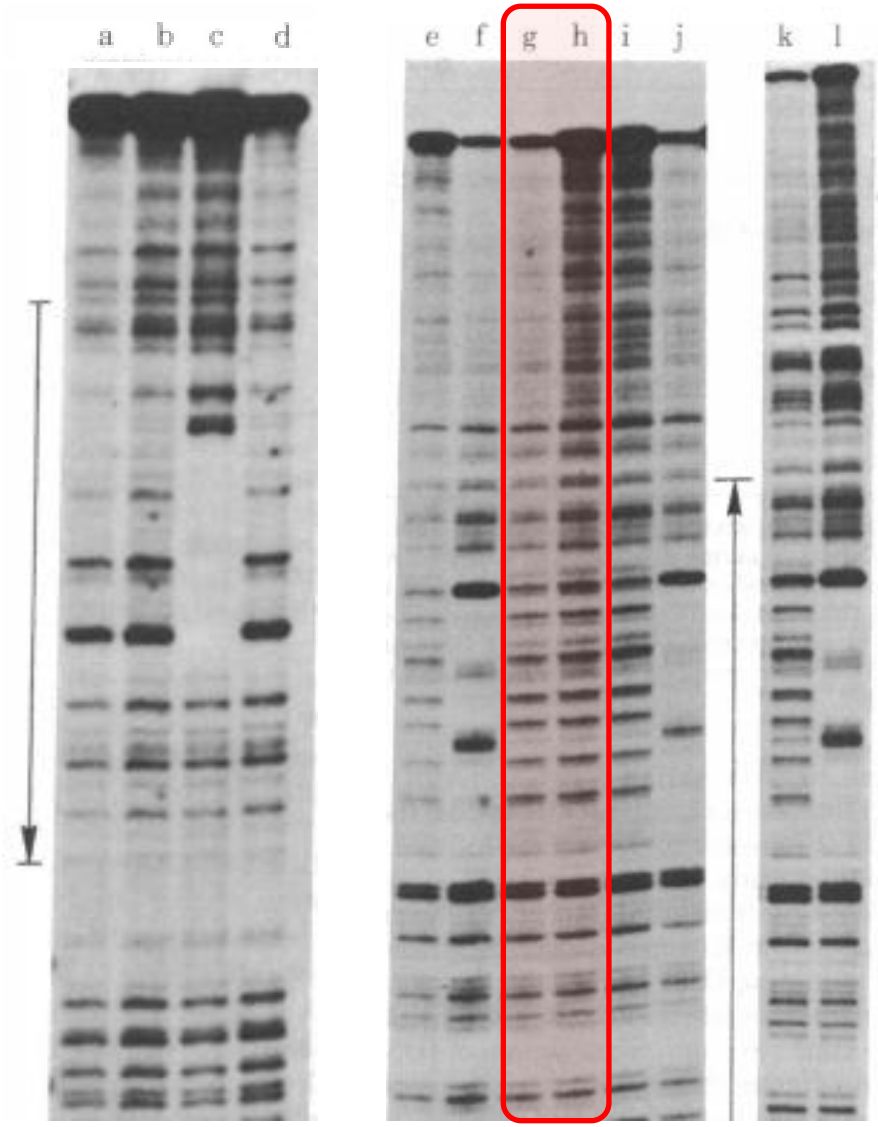


Protein from 7S = Transcription factor

Binds in the center of the gene

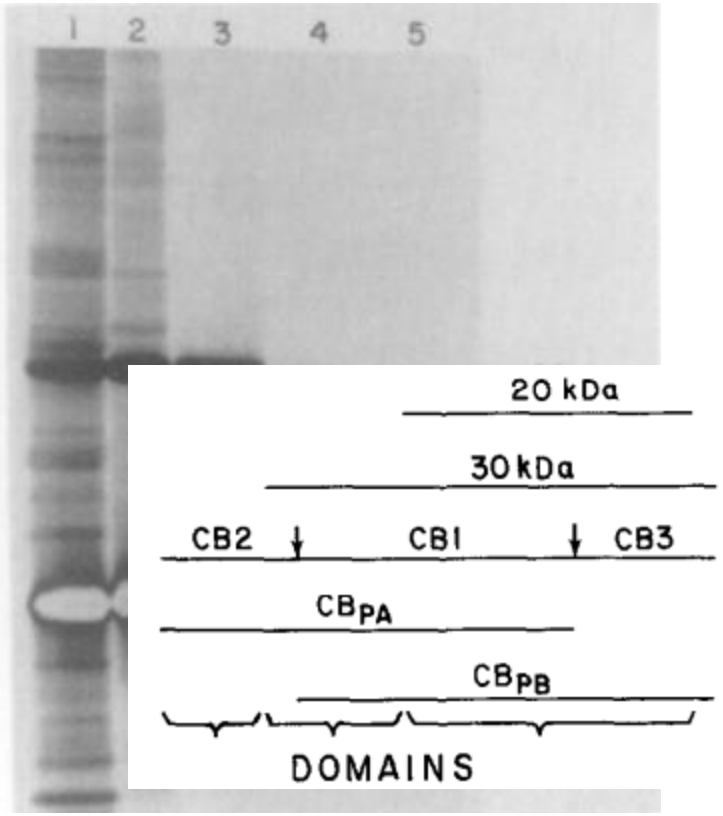
Required for the initiation of transcription

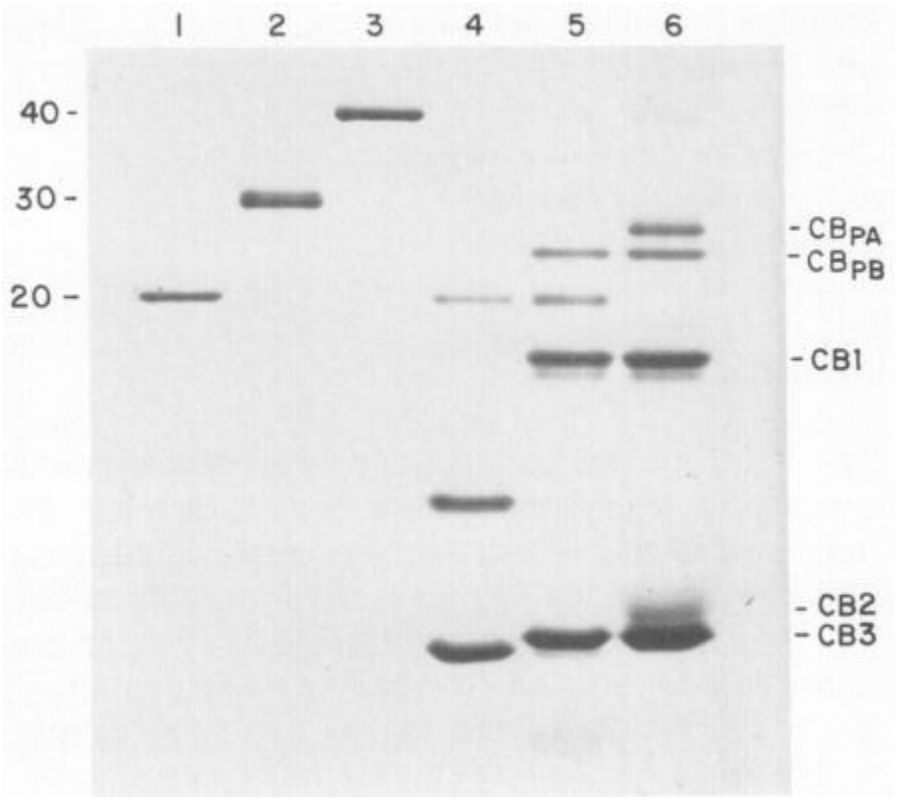
Dual binding ∴ inhibits its own synthesis

Domains of the Positive Transcription Factor Specific for the Xenopus 5S RNA Gene

Douglas R. Smith,* Ian J. Jackson,† and Donald D. Brown

Cell, Vol. 37, 645–652, June 1984,





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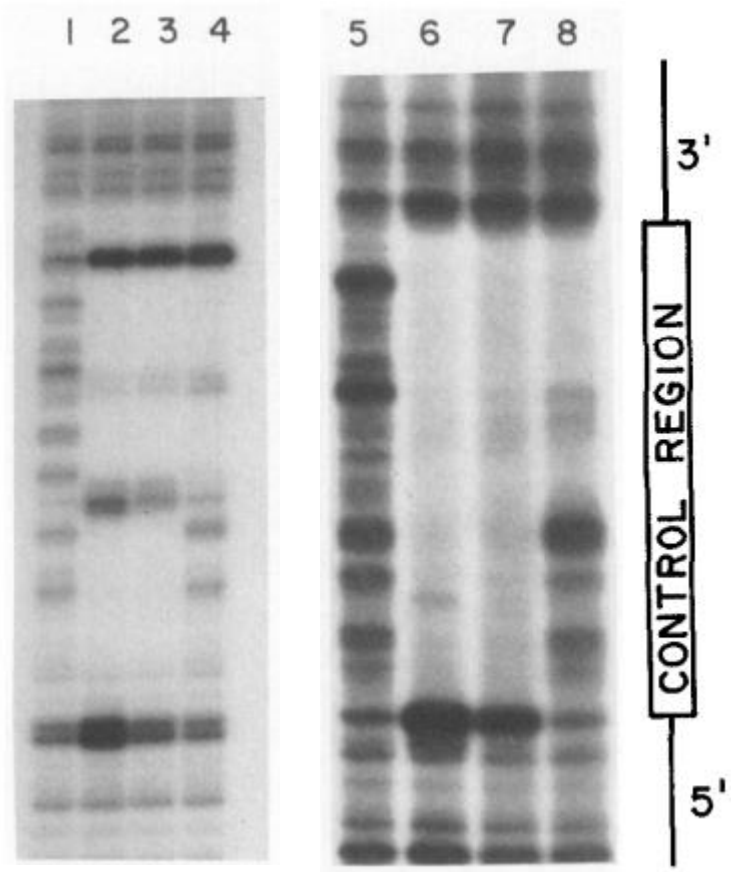

Noncoding strand

Coding strand

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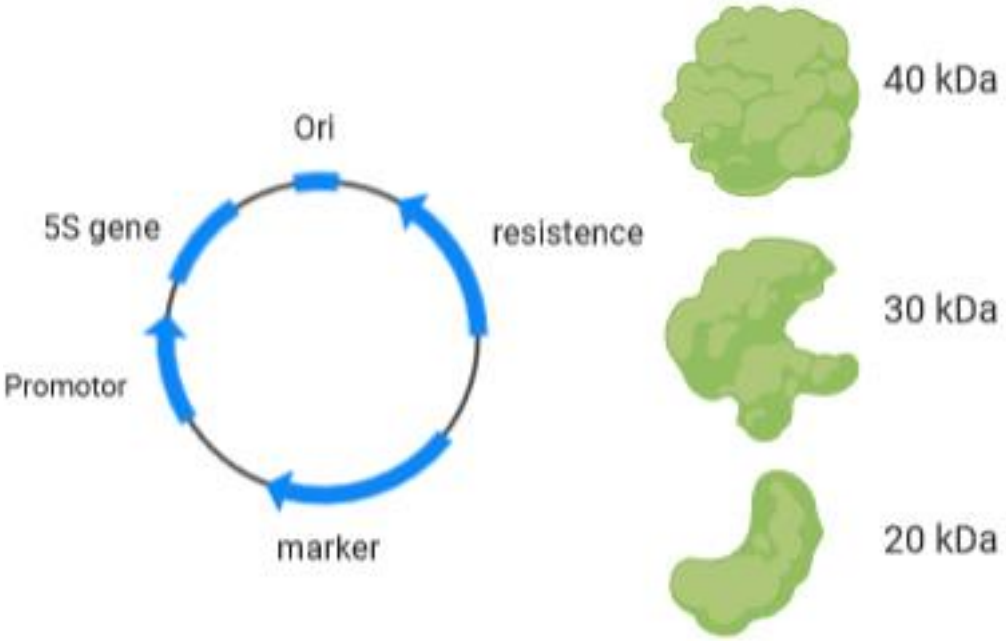

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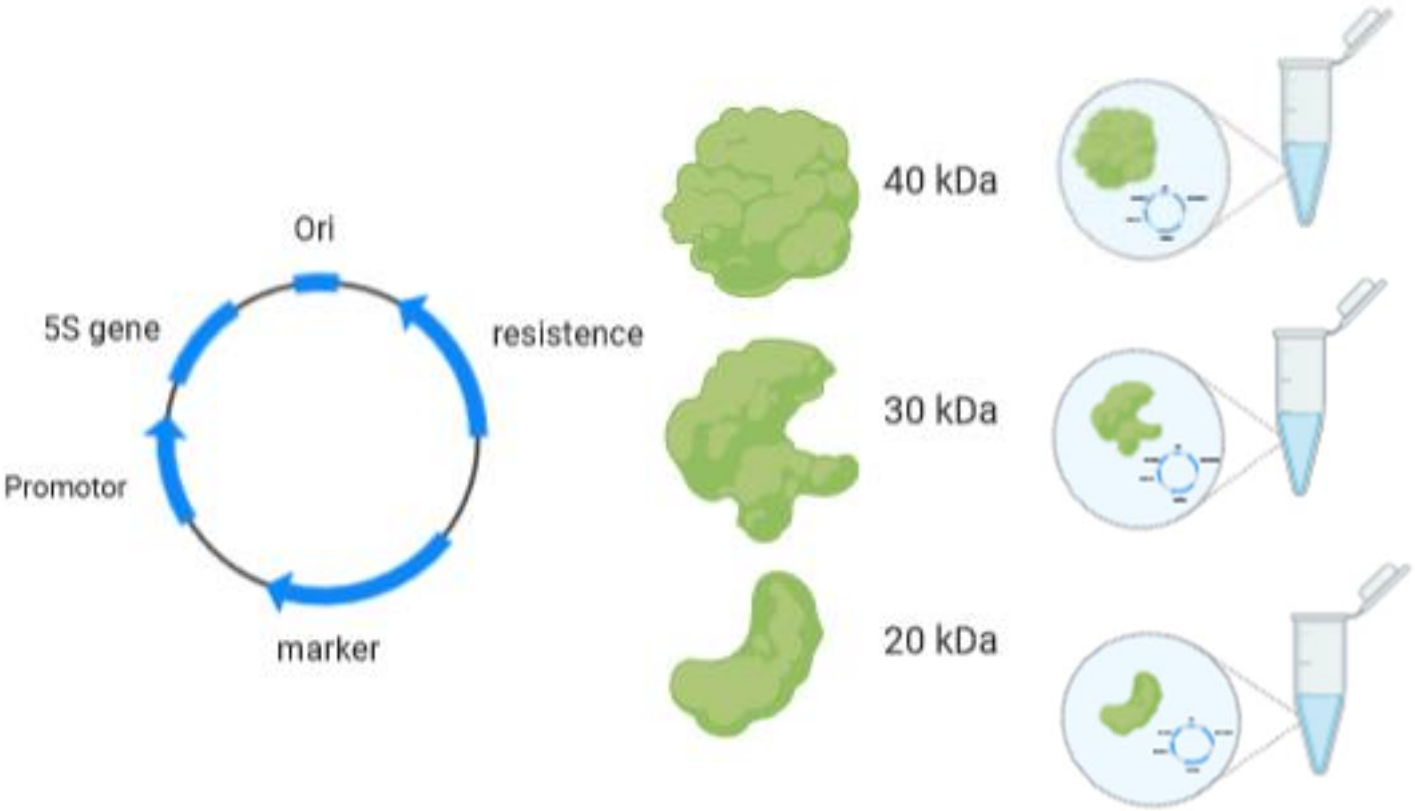

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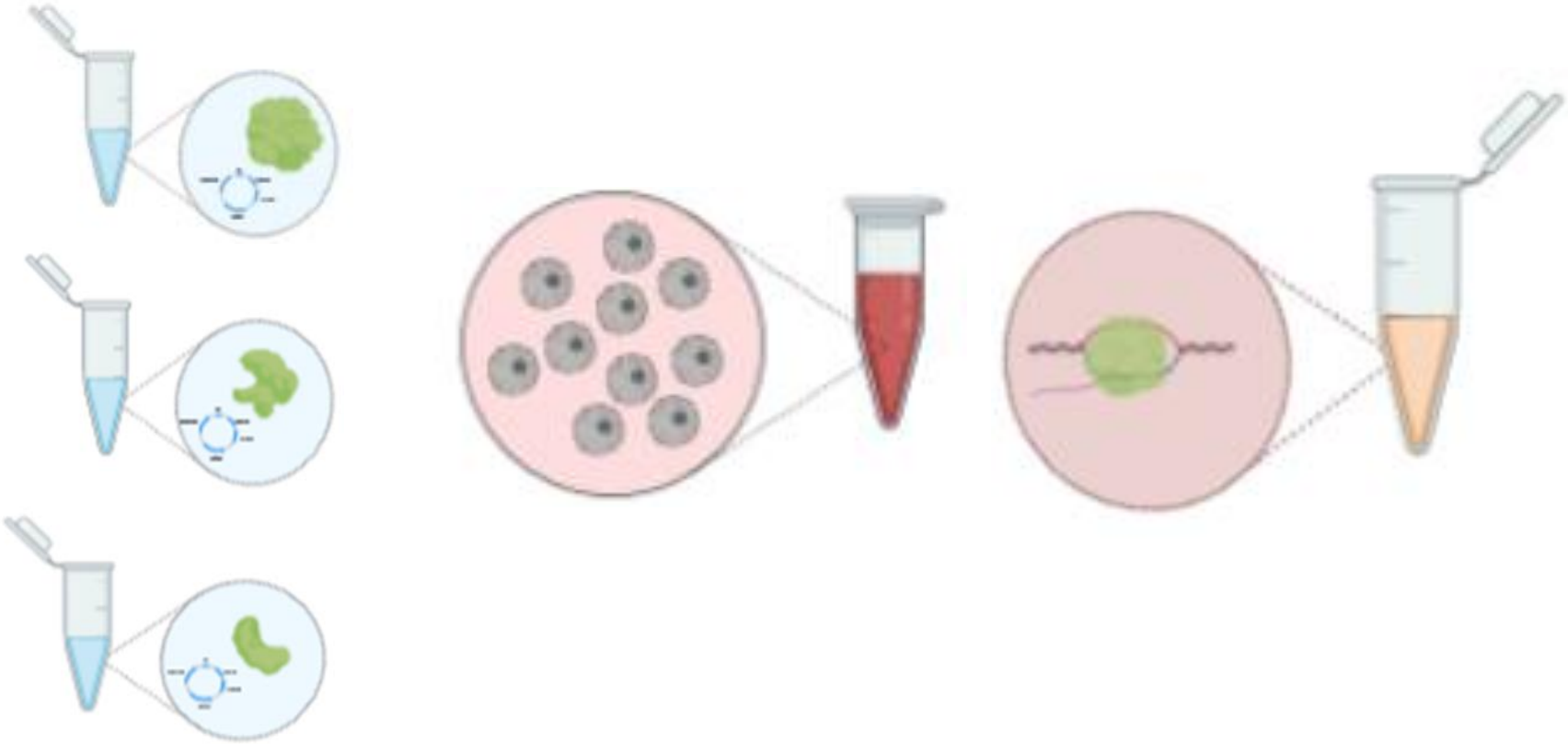

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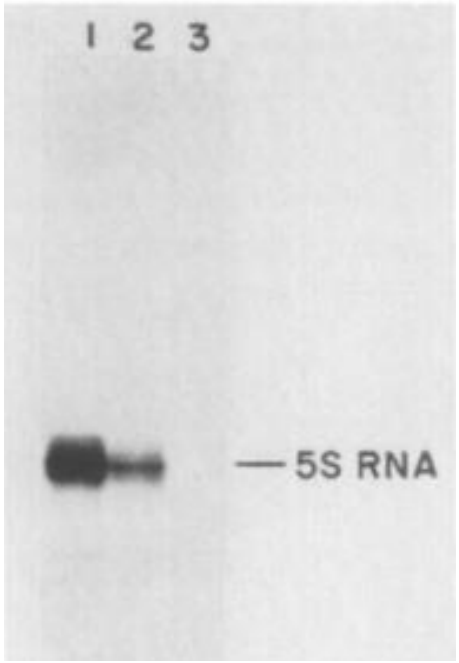

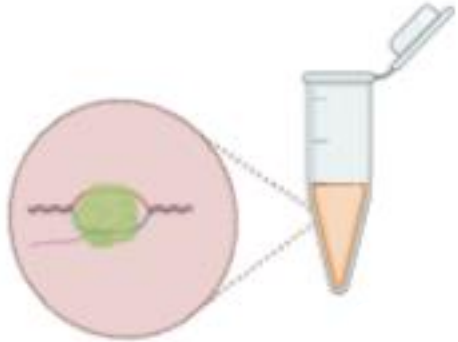

30 kDa has only 20% transcription activity

20 kDa is totally inactive

∴ 10 kDa missing from 30 kDa is required

for 5S transcription

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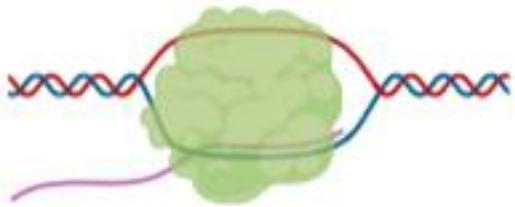

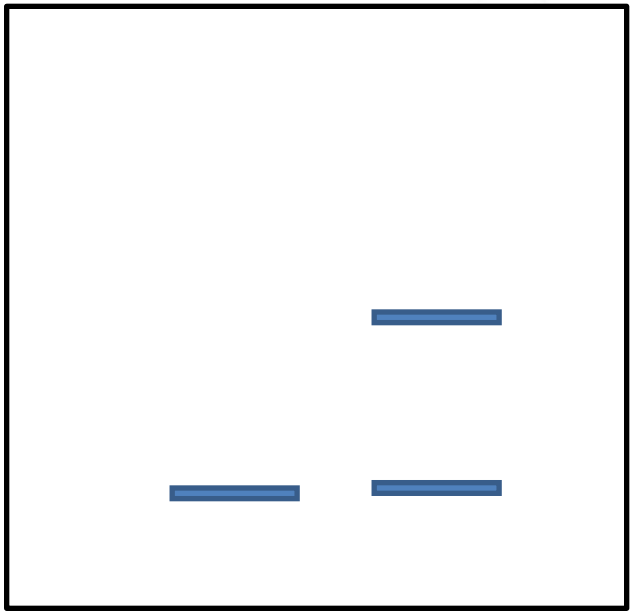

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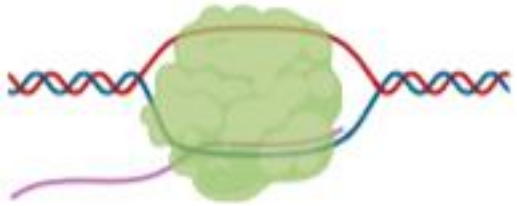

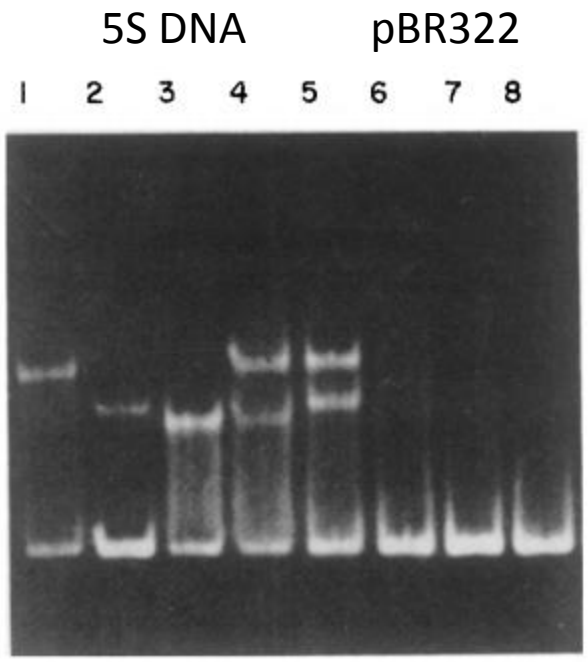

- **Only mixture of original sized proteins**
- **No complexes of intermediate mobility**

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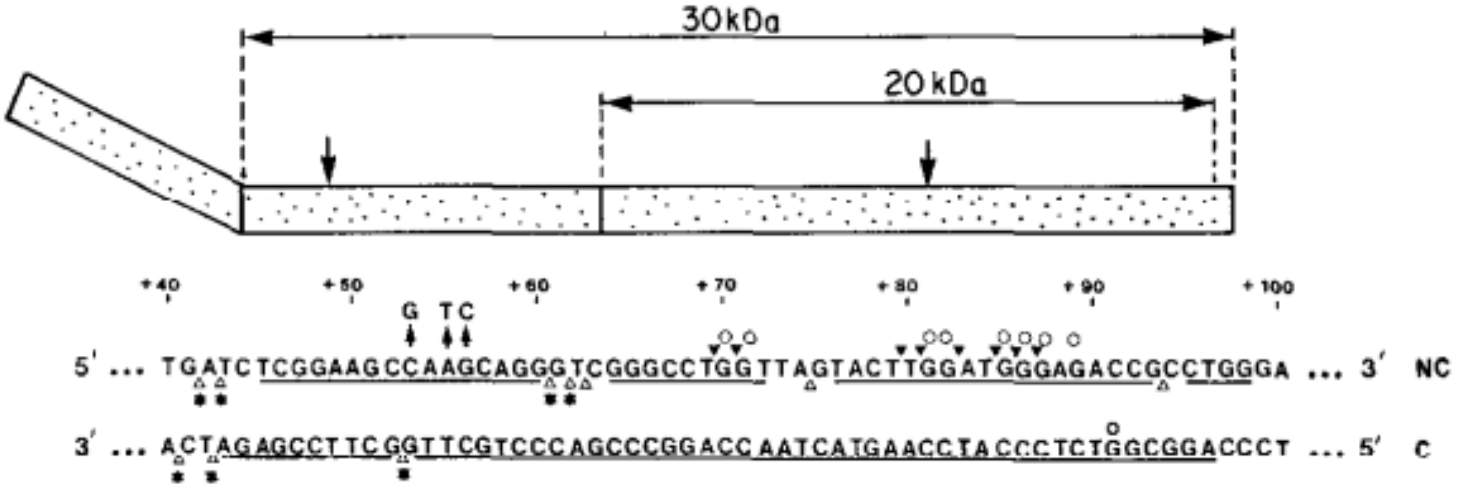

Figure 9. Projection of the 40 kd Transcription Factor onto the Internal Control Region of the 5S RNA Gene

Conclusions of Lecture-9

- TF IIIA binds 5S RNA (7S) and 5S DNA
- TF contain three structural domains: 20 kDa at one end and two smaller 10 kDa domains at the other end.
- 20 kDa domain contains the sequence specific DNA binding site = that recognizes key nucleotides on the 3' side of the control region.
- 20kDa by itself is not sufficient to activate the gene transcription, an adjacent 10 kDa domain in conjunction with 20 kDa extends binding to the 5' end of the control region.
- The third 10 kDa does not bind directly to DNA but is responsible for most of the transcription enhancing activity of intact protein.

Thank You!