

# **Eukaryotic Gene Expression: Basics & Benefits**

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# **Lecture 1**

## **Introduction to Gene Regulation in Eukaryotes: Eukaryotic RNA polymerases and basal transcription factors**

All cells in an organism contain the same set of genes.

However, only a fraction of these genes are transcribed in an individual cell at any given time.

It is the pattern of gene expression that determines the structure, physiological function and health of all cells, tissues, and organisms.

In many instances, aberrant gene expression can lead to disease.

In eukaryotes, the temporal and spatial expression of specific genes is vital to key life processes such as development, differentiation and homeostasis.

Gene expression is regulated primarily at the level of transcription and an understanding of the molecular basis for this regulation continues to be a major challenge in the 21<sup>st</sup> century.

The process of synthesis of RNA from DNA is known as TRANSCRIPTION and the key enzyme involved in RNA synthesis is the RNA POLYMERASE.

In eukaryotes, there are three distinct RNA polymerases of which transcription of genes by RNA polymerase II results in the synthesis of messenger RNAs (mRNAs) in the nucleus. Translation of these mRNAs by the protein synthesizing machinery in the cytoplasm results in the synthesis of proteins.

Synthesis of proteins in cells is a highly regulated process.

A specific protein is synthesized in the cell only when it is necessary.

When a particular protein is to be synthesized, the specific gene encoding the cognate mRNA has to be transcribed.

THE MECHANISM BY WHICH TRANSCRIPTION OF GENES IS ACTIVATED OR INHIBITED IS REFERRED TO AS **GENE REGULATION**

Gene regulation has been extensively studied in prokaryotes.

Bacteria respond to external conditions by regulating levels and/or activity of key enzymes. For example, if a bacterium is grown in medium containing lactose rather than glucose, it must equip itself to:

- 1)import lactose,
- 2)cleave lactose to glucose and galactose and
- 3)convert the galactose to glucose.

Bacteria therefore have to produce the enzymes required for lactose metabolism when exposed to lactose.

While allosteric regulation of enzymes by small molecules was rather well studied at that time (1960s), the method of controlling the enzyme production by small molecules was not well understood.

A series of investigations including those by Jacob and Monod (1961) revealed that in bacteria, genes involved in specific metabolic and biosynthetic pathways are grouped together as OPERONS and their expression is coordinately regulated by small molecules (JACOB & MONAD 1961).

In a bacterial operon, the binding of RNA polymerase to its cognate promoter is regulated by an operator/repressor mechanism

### **There are two types of operons in bacteria**

#### **INDUCIBLE AND REPRESSIBLE OPERONS**

In an inducible operon, the ability of RNA polymerase to bind to the promoter and transcribe the structural genes located downstream of the promoter is controlled by an operator

The function of the operator is controlled by a repressor protein which binds to the operator and prevents RNA polymerase from binding to the promoter.

The repressor protein is kept in an active state by an inducer molecule which is usually the substrate for the enzymes encoded by the structural genes of the operon.

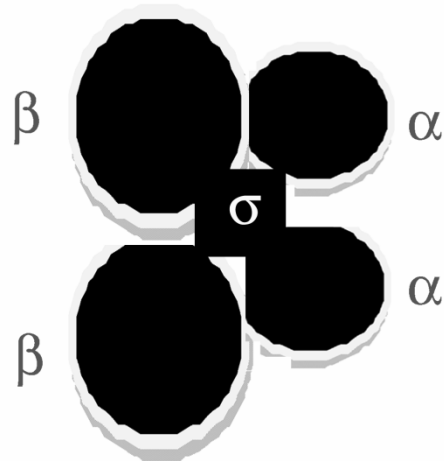
The repressible operon is similar to an inducible operon except that the repressor in this case is activated by a chemical substance called co-repressor, which is usually the end product.

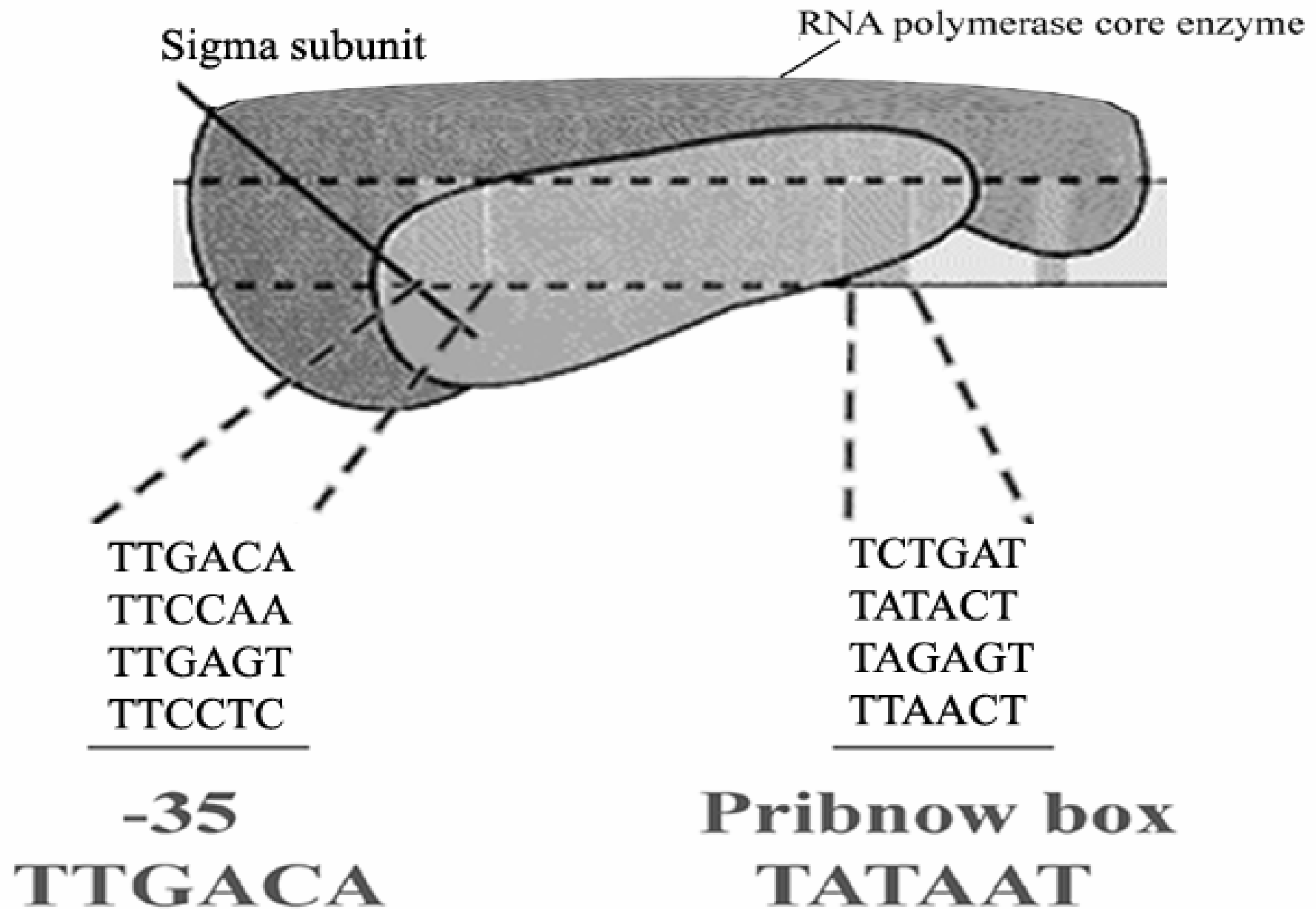
## How about regulation of *E. coli* genes and PROMOTERS which are not organized into operons?

Not all genes in bacteria are organized into operons

There are a number of genes which are not organized into operons and such genes contain their own promoters. Since all such genes are not expressed at the same level, we need to understand how differential regulation is brought about in their case.

This is achieved primarily by variations in the smallest subunit of bacterial RNA polymerase known as the sigma factor.







# Regulation of gene expression by Sigma subunits in E. coli

Sigma subunit	Genes controlled by sigma subunit
<b>RpoD</b>	<b>Growth-related genes (1000)</b>
<b>RpoN</b>	<b>Nitrogen-regulated/stress response genes (15)</b>
<b>RpoS</b>	<b>Stationary phase/stress response genes (100)</b>
<b>RpoH</b>	<b>Heat shock/stress response genes (40)</b>
<b>RpoF</b>	<b>Flagella-chemotaxis genes (40)</b>
<b>RpoE</b>	<b>Extreme heat shock/extracytoplasmic genes (5)</b>

**In prokaryotes, gene regulation allowed them to respond to their environment efficiently and economically.**

**A single RNA polymerase with different sigma subunits can bring about differential gene regulation together with activators and repressors.**

**In case of eukaryotes, in addition to environmental response, gene regulation became essential for the control of a number of cellular processes such as cell differentiation during development, immune responses, tissue-specific functions etc.**

**To meet this complexity, as a first step, the number of RNA polymerases were increased from 1 to 3 in eukaryotes.**

# **Eukaryotic RNA polymerasees**

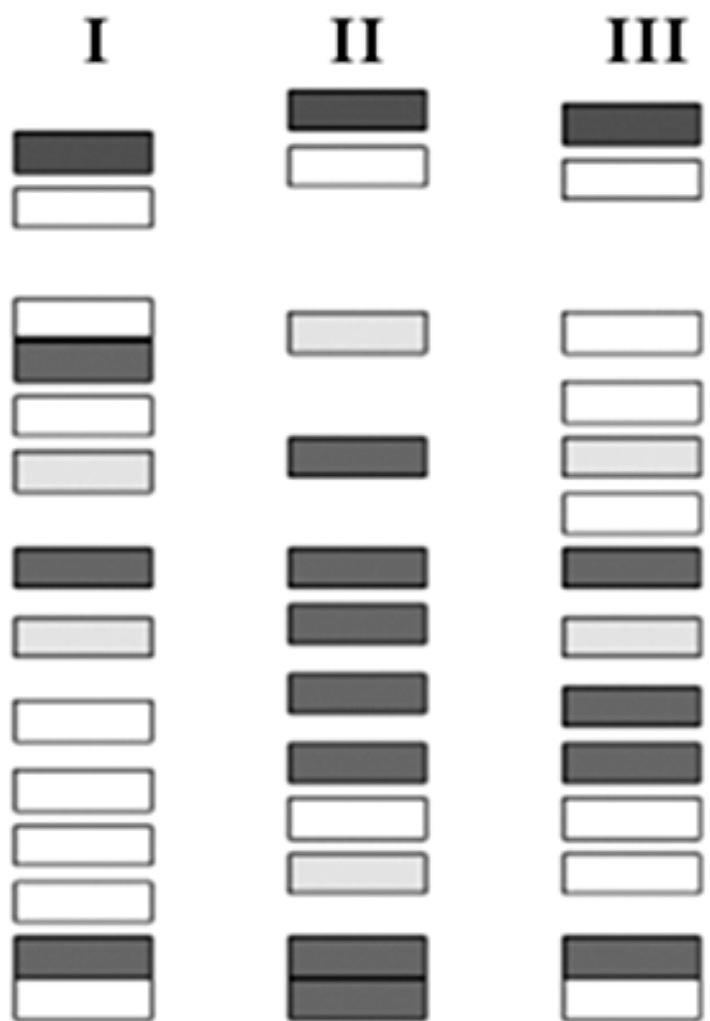
Bacteria

Eukaryotes

RNA polymerases

■  $\beta'$   
□  $\beta$

■  $\alpha$   
■  $\alpha$



# Purification of Eukaryotic RNA polymerases (1940-1970)

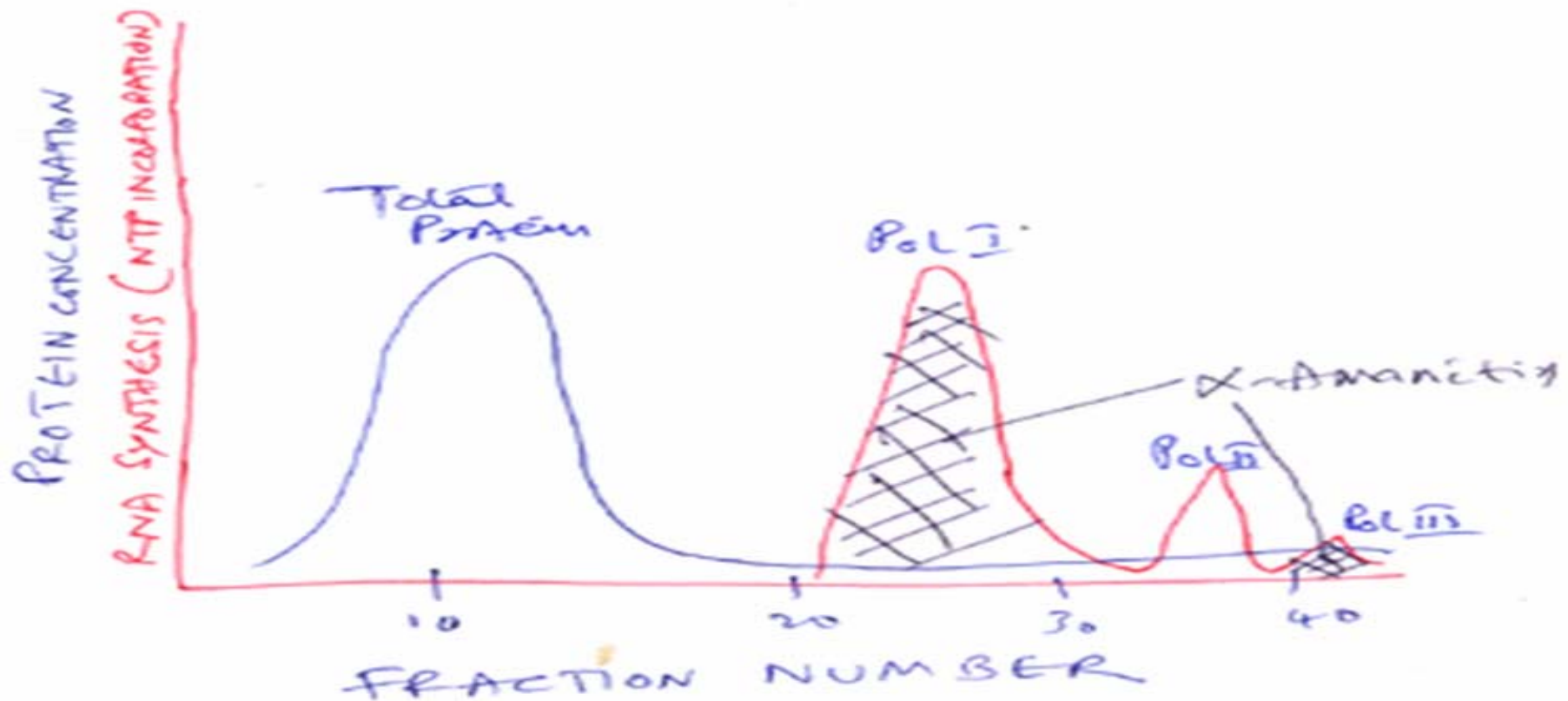
Mammalian RNA polymerase was tightly bound to chromatin as a result of its engagement in active transcription, it became essential to develop new methods to solubilize the RNA polymerase and remove the interfering DNA and histones.

- Nuclear disruption and histone dissociation from DNA at high salt concentration
- DNA breakage and dissociation of RNA polymerase by sonication
- Selective precipitation of DNA-histone complexes by rapid dilution to a low salt concentration.

The resulting soluble enzyme preparation then was subjected to conventional protein purification.

Chromatography on a DEAE-Sephadex column yielded three peaks of activity, which were designated as RNA polymerases I, II and III.

- Template-dependent incorporation of all four ribonucleoside triphosphates
- Distinct salt and metal ion responses and
- Distinct chromatographic behavior.



*Nature* 224, 234-237 (1969).

# Three types of eukaryotic RNA polymerases

$\alpha$ -amanitin isolated from *Amanita Phalloides*. It binds tightly to RNA Pol II and blocks transcriptional elongation.

Purified RNA polymerases I, II and III could be distinguished on the basis of differential sensitivities to the mushroom toxin  $\alpha$ -amanitin.

By monitoring the  $\alpha$ -amanitin sensitivities of specific transcription events by endogenous RNA polymerases in isolated nuclei, it was demonstrated that the rRNA is synthesized by Pol I, adenovirus pre-mRNA is synthesized by Pol II and the synthesis of cellular 5S and tRNA were synthesized by Pol III

Type	Location	RNA synthesized	$\alpha$ -amanitin
I	Nucleolus	Pre-rRNA for 18, 5.8 and 28S rRNAs	Insensitive
II	Nucleoplasm	Pre-mRNA, snRNAs	Sensitive to 1 $\mu$ g/ml
III	Nucleoplasm	Pre-tRNAs, 5S rRNA, some snRNA	Sensitive to 10 $\mu$ g/ml

## Eukaryotic RNA polymerase II

<b>Subunits</b>	<b>Size (kDa)</b>
<b>Rpb1</b>	<b>190</b>
<b>Rpb2</b>	<b>140</b>
<b>Rpb3</b>	<b>35</b>
<b>Rpb4</b>	<b>25</b>
<b>Rpb5</b>	<b>25</b>
<b>Rpb6</b>	<b>18</b>
<b>Rpb7</b>	<b>19</b>
<b>Rpb8</b>	<b>17</b>
<b>Rpb9</b>	<b>14</b>
<b>Rpb10</b>	<b>8.3</b>
<b>Rpb 11</b>	<b>14</b>
<b>Rpb12</b>	<b>7.7</b>



- In yeast, all three polymerases have five core subunits which have that homology with the  $\beta$ ,  $\beta'$ ,  $\alpha$ ,  $\omega$  subunits in *E. coli* RNA polymerase.
- RNA polymerases I and III contain the same two non-identical  $\alpha$ -like subunits. Polymerase II has two copies of a different  $\alpha$ -like subunit.
- Four subunits are common for all the three RNA polymerases. Each RNA polymerase contains 3-7 unique smaller subunits.
- The largest subunit of RNA polymerase II contains an essential C-terminal domain (CTD).
- The yeast and human CTDs have 27 and 52 copies of YSPTSPS.
- Phosphorylation of CTD has a key role in transcription and RNA processing.

**Thus, early studies of the mammalian RNA polymerases provided the first indication that the eukaryotic transcription machinery is far more complex than that existing in prokaryotes.**

# Two Approaches



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graph TD; A[Two Approaches] --> B[Determination of DNA sequence requirements for initiation, using cell-free transcription and transfection assays]; A --> C[Isolation and characterization of the unknown protein factors that are involved in accurate transcription initiation by RNA polymerases]; C --> D[Fractionation of HeLa extracts];
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**Determination of DNA sequence requirements for initiation, using cell-free transcription and transfection assays**

**Isolation and characterization of the unknown protein factors that are involved in accurate transcription initiation by RNA polymerases**

**Fractionation of HeLa extracts**

# Basic promoter elements of a gene transcribed by RNA Pol II

	A	17	22	13	7	97	7	85	63	88	50	32	15	
	C	55	37	51	2	2	0	0	0	0	12	37	47	
Frequency of bases (%)	T	16	27	9	81	2	91	10	37	10	31	12	14	
	G	14	12	22	10	0	0	4	0	2	3	16	18	
	5'	_____												Transcription start site
					T	A	T	A	T	A	_____			
									A					
					-34 to -26						15-25 BASES			

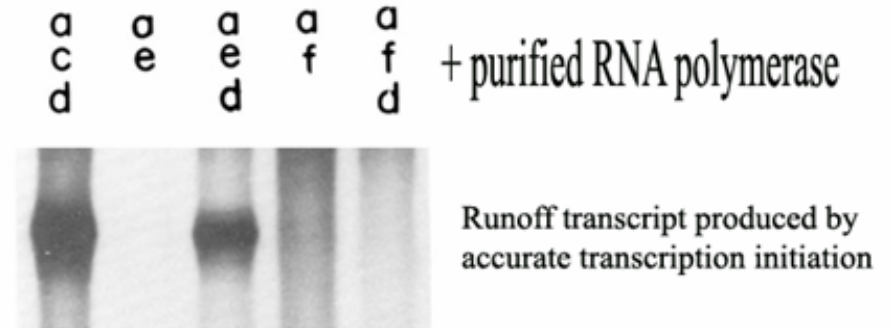
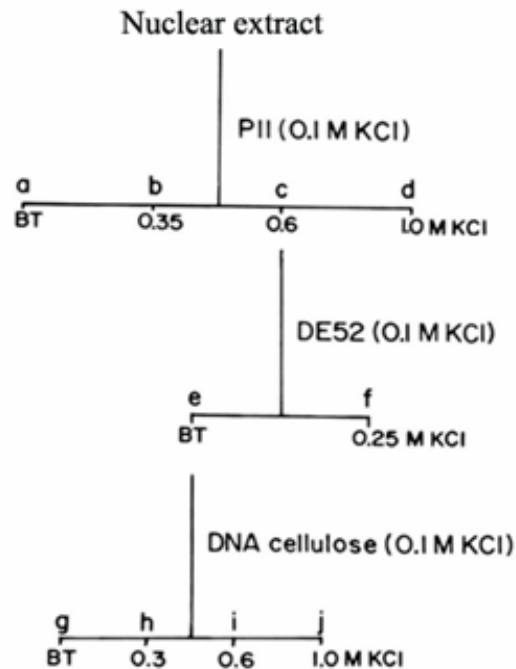
**In 1977, Carl Parker showed that purified Pol III, but not Pol I, Pol II or *E. coli* RNA polymerase, could accurately transcribe 5S RNA genes in purified chromatin from immature oocytes but not in total cellular or cloned 5S DNA templates**

**This was the first demonstration of accurate transcription by a eukaryotic RNA polymerase in a reconstituted cell-free system.**

**It suggested the presence of essential (chromatin-bound) RNA polymerase-specific accessory factors**

**Parker & Roeder (1978) further demonstrated accurate transcription of cloned 5S RNA, tRNA and adenovirus VA RNA genes by purified Pol III in conjunction with soluble subcellular fractions from *Xenopus* oocytes and human HeLa cells**

# Fractionation of nuclear extracts and identification of factors essential for accurate transcription initiation



In 1980, Jacki Segall, Takashi Matsui and Tony Weil identified two factors, TFIIC and TFIIB, that are generally required for transcription of class III genes and several factors, including TFIID, that were required for transcription of class II genes

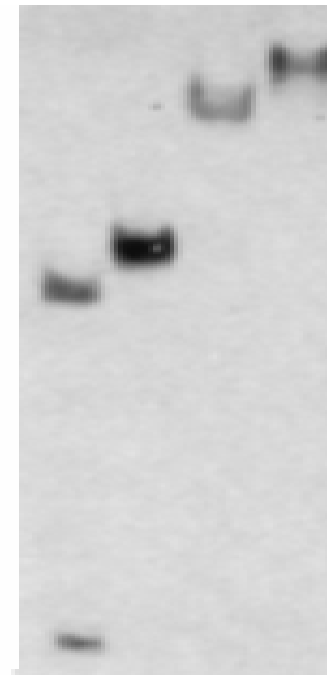
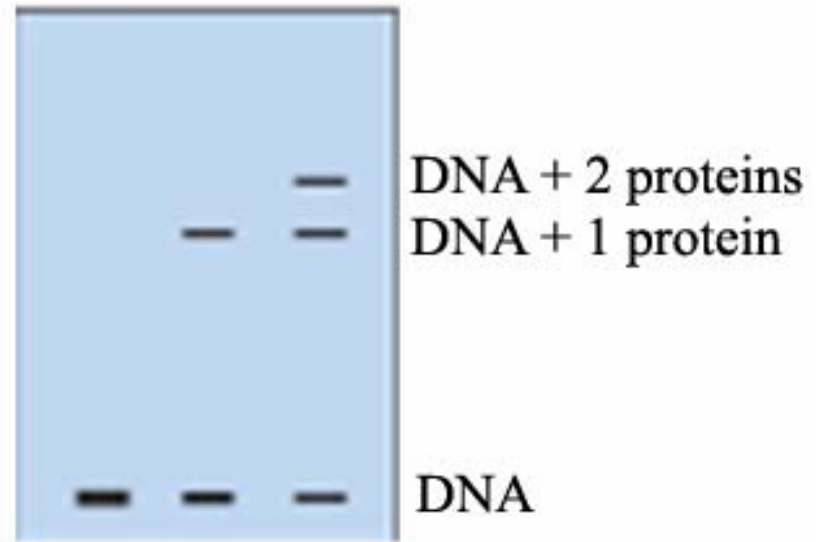
**Five intermediate complexes in transcription initiation by RNA polymerase II.**

**Buratowski S, Hahn S, Guarente L, Sharp PA.**

**Cell. 1989 Feb 24; 56(4): 549-61.**

A native gel electrophoresis DNA binding assay was used to identify five sets of complexes generated by sequential binding of TFIID, TFIIA, TFIIB, RNA polymerase II, and TFIIE.

A model for the interactions of components of transcription initiation by RNA polymerase II was proposed.



**Between 1980 and 1990, studies from various laboratories established that:**

**Accurate transcription initiation by RNA pol III requires two factors (TFIIIC and TFIIIB) which contain at least nine distinct polypeptides**

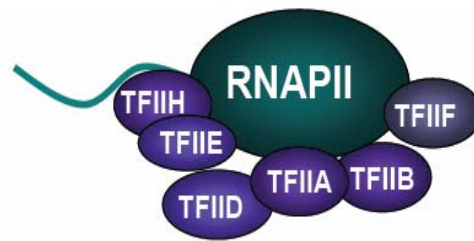
**Six factors required for accurate transcription initiation by RNA pol II (TFIID, TFIIA, TFIIIB, TFIIIE, TFIIIF and TFIIH) with a total of about 32 distinct polypeptides were identified.**

**Several Pol I factors were identified.**

**These factors required for accurate transcription initiation by Pol I, Pol II and Pol III factors are structurally and functionally distinct.**

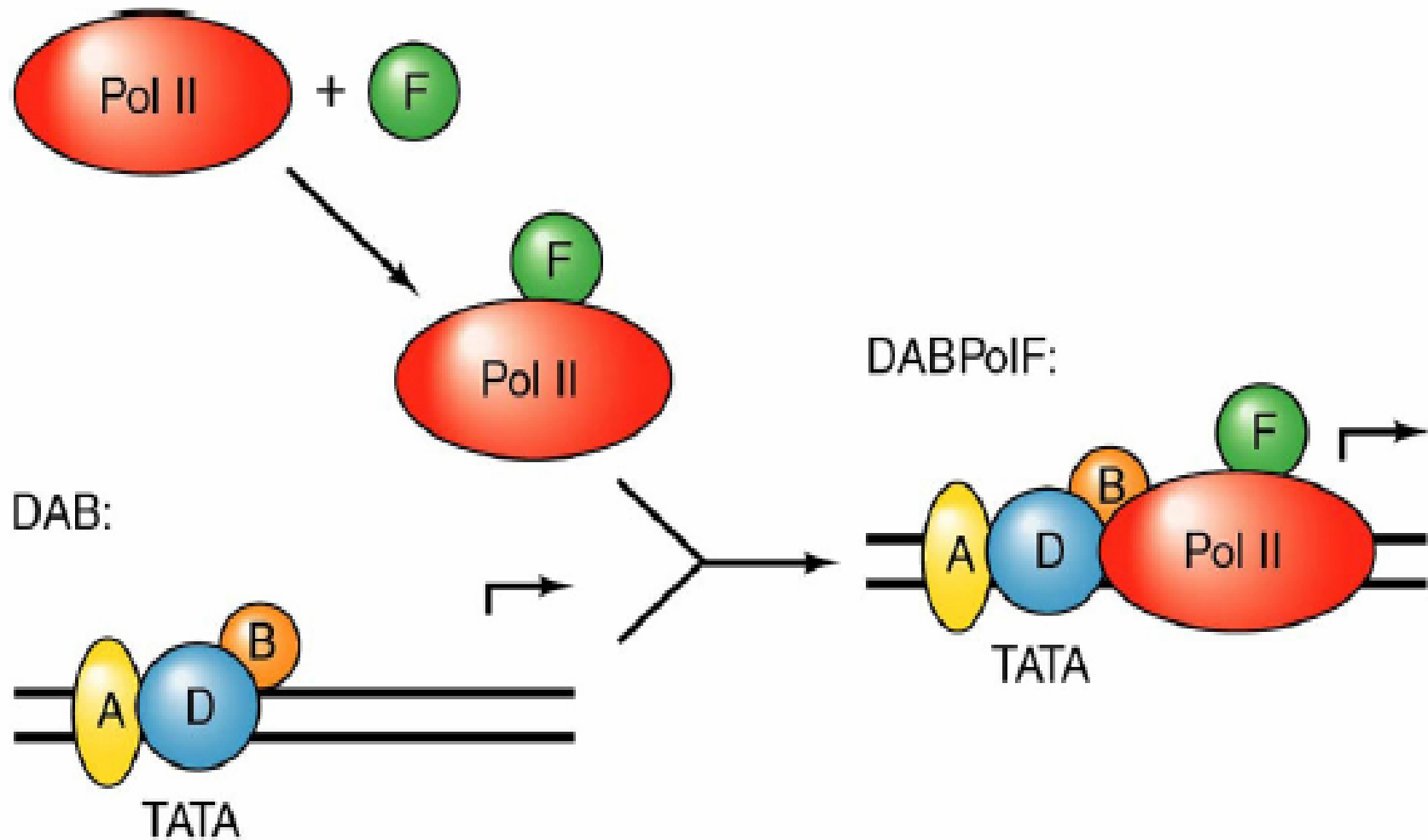
**The general /basal transcription initiation factors are highly conserved from yeast to humans.**



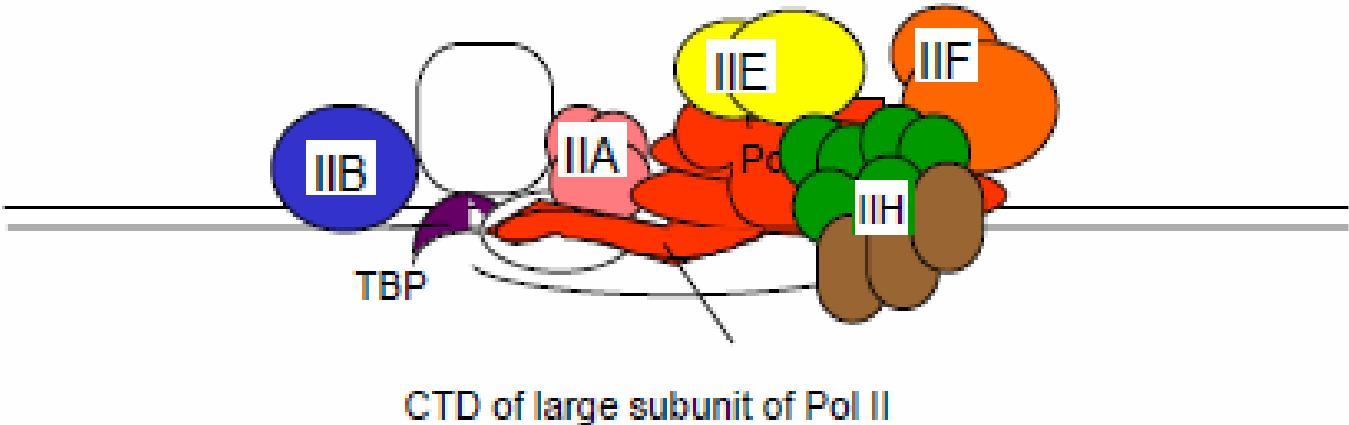
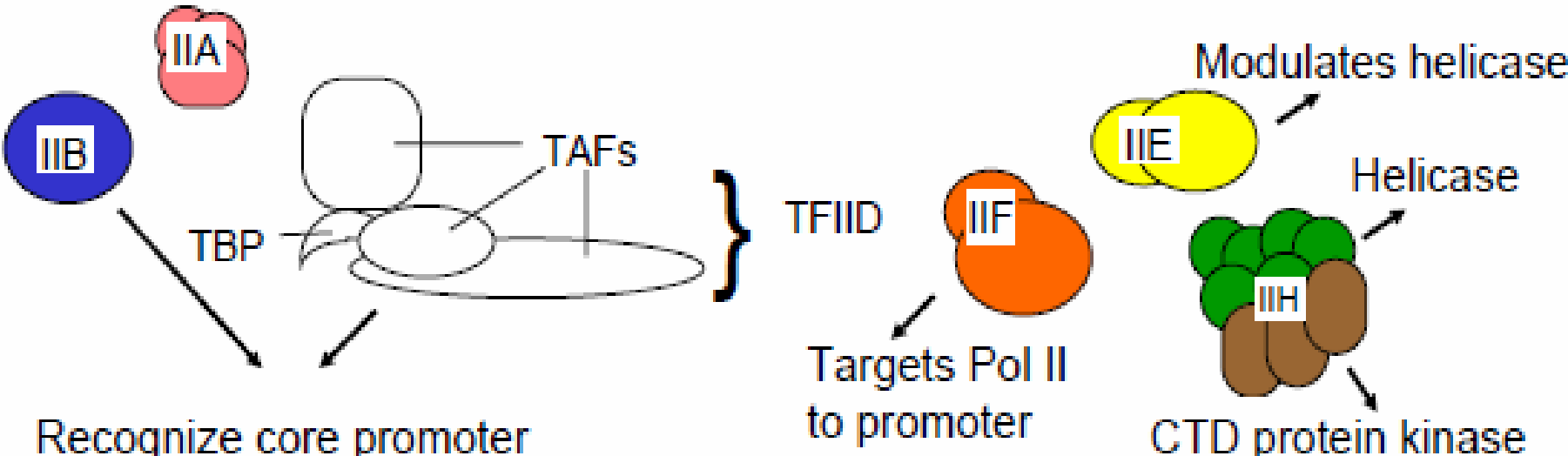


Factor	Number of Subunits	Mw. (kD)	Function
TFIID - TBP	1	38	Recognize core promoter; Recruit TFIIB
TFIID - TAFs	12	15 - 250	Assist transcription activation; Assist promoter recognition
TFIIA	3	12, 19, 35	Stablize TFIID and promoter binding
TFIIB	1	35	Recruit RNA Pol II and TFIIF
TFIIF	2	30, 74	Assist RNA Pol II to reach promoter
TFIIE	2	34, 57	Recruit TFIIH; Modulate TFIIH helicase, ATPase and kinase activities

# Ordered assembly of the Pre-Initiation Complex (PIC)



# Summary



## Original Research Articles

Roeder, R.G. & Rutter, W.J. Multiple forms of DNA-dependent RNA polymerase in eukaryotic organisms. *Nature* 224, 234-237 (1969).

Multiple ribonucleic acid polymerases and ribonucleic acid synthesis during sea urchin development. Roeder RG, Rutter WJ. *Biochemistry*. (1970) 2543-53

Lindell TJ, Weinberg F, Morris PW, Roeder RG & Rutter WJ. Specific inhibition of nuclear RNA polymerase II by alpha-amanitin. *Science*. 1970 Oct 23; 170(956): 447-9

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Parker, C.S. & Roeder, R.G. Selective and accurate transcription of the *Xenopus laevis* 5S RNA genes in isolated chromatin by purified RNA polymerase III. *Proc. Natl. Acad. Sci. USA* 74, 44-48 (1977).

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Weil, P.A., Segall, J., Harris, B., Ng, S.-Y. & Roeder, R.G. Faithful transcription of eukaryotic genes by RNA polymerase III in systems reconstituted with purified DNA templates. *J. Biol. Chem.* 254, 6163-6173 (1979).

Segall, J., Matsui, T. & Roeder, R.G. Multiple factors are required for the accurate transcription of purified genes by RNA polymerase III. *J. Biol. Chem.* **255**, 11986-11991 (1980).

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