

Eukaryotic Gene Expression: Basics & Benefits

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Lecture 4

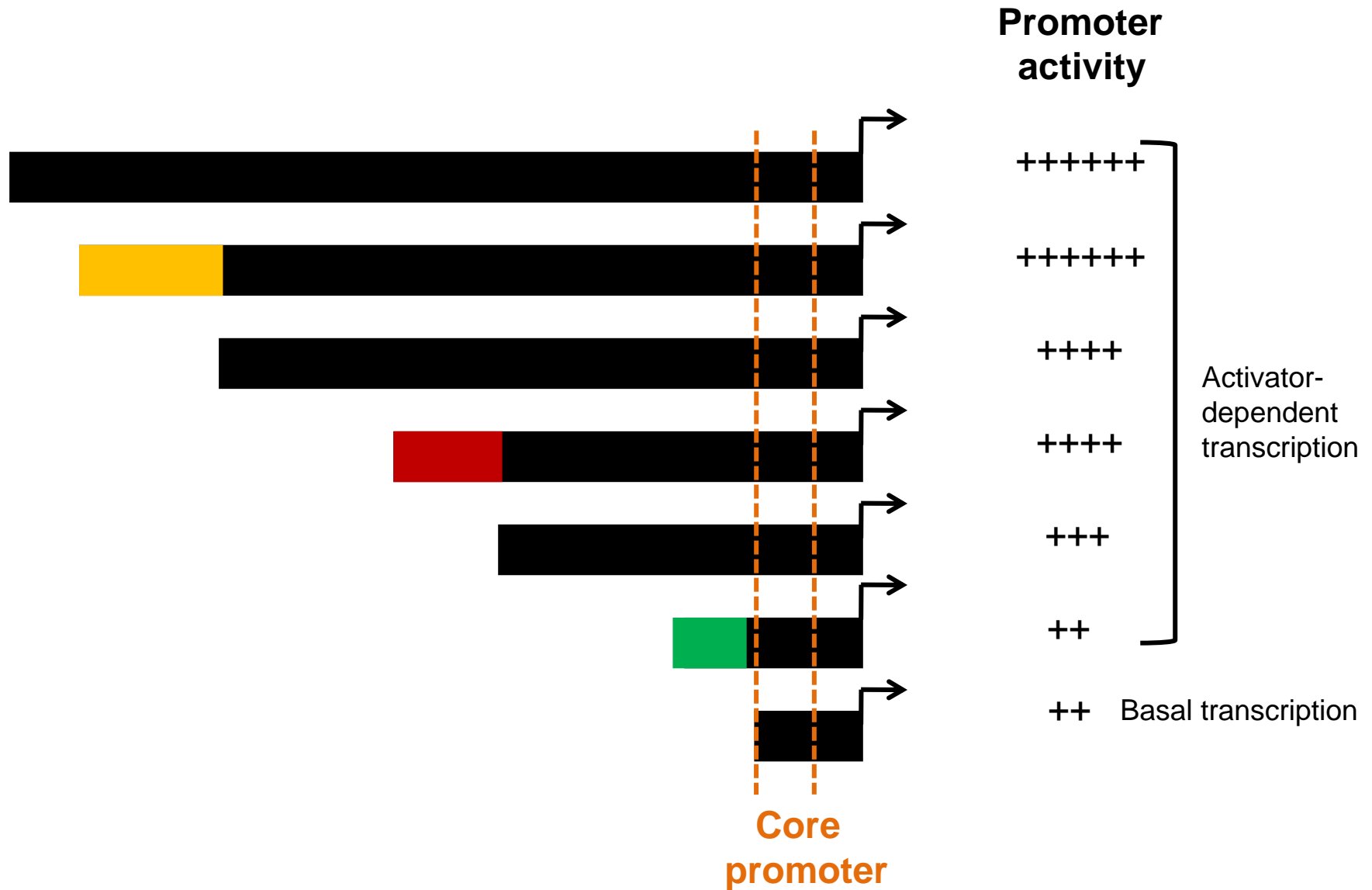
**Gene Regulation in Eukaryotes:
Proximal & Distal Promoter Elements,
Enhancers and Silencers,
Gene-specific Regulators**

So far.....

- **Eukaryotic RNA polymerases (lecture 1)**
- **Core promoter sequences and their variants (lecture 2)**
- **General transcription factors and their variants (lecture 3)**

In this lecture.....

- **Identification of proximal & distal promoter elements & enhancers**
- **Identification and characterization of transcriptional activators**
- **Benefits arising out of study of promoters and transcription factors**



How does one assay promoter activity?
 How does one identify upstream regulatory elements?

PROMOTER ACTIVITY ASSAYS

- Assay of reporter gene activity in cell lines



Examples of reporter genes: Green fluorescent protein (GFP)
 β -galactosidase (β -gal)
 Luciferase (luc)
 Chloramphenicol acetyl transferase (CAT)

or

any other protein that can be visualized or measured in some way

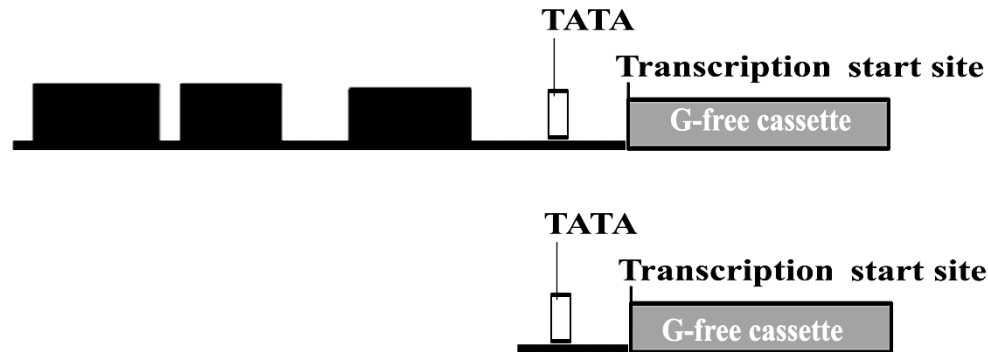


Transfect into cell lines
 Harvest cells
 Lyse cells
 Assay reporter activity

HeLa	3T3
293	PA317
K562	COS
L Cells	
293T	

PROMOTER ACTIVITY ASSAYS

- Cell-free transcription studies



What is a G-free cassette?

It is a guanosine-free (G-free) region of DNA cloned immediately downstream from a promoter. Transcription in the presence of RNase T1 and 3' O-Me-GTP eliminates non-specific transcripts, and produces the G-free transcripts initiated at the promoter.

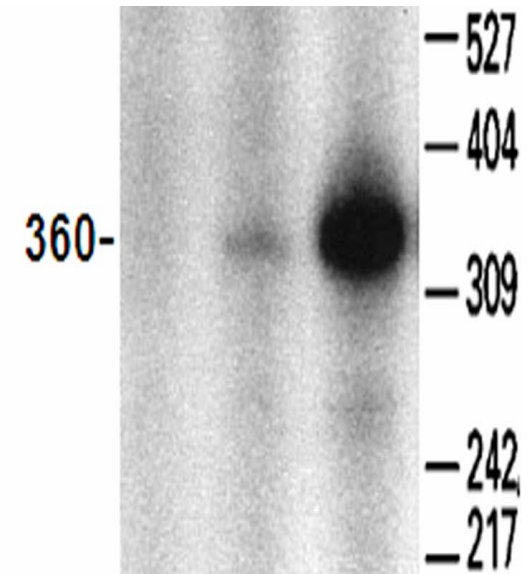
Sawadogo and Roeder, Proc. Natl. Acad. Sci. USA 82 (1985) 4394-4398

In vitro transcription assay

Crude nuclear extracts (He La, Drosophila embryo etc.)

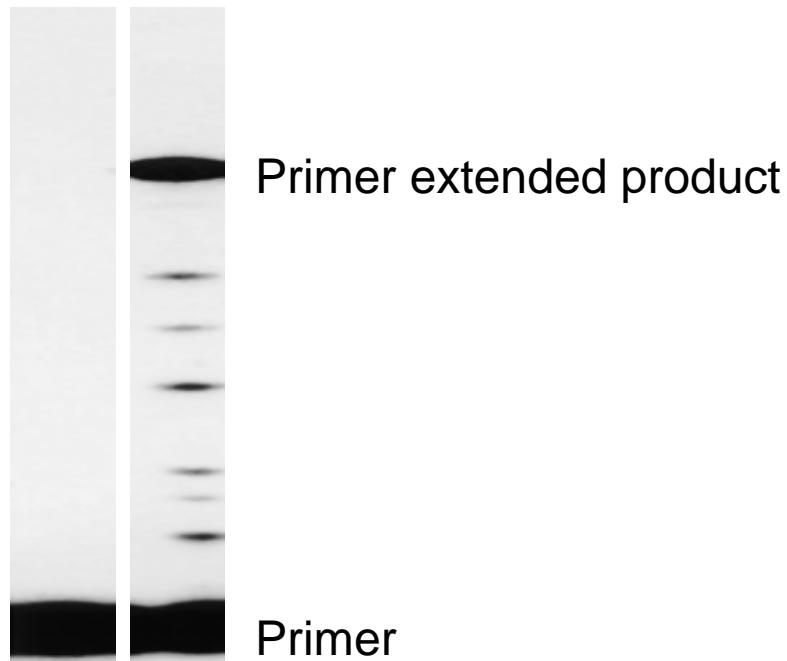
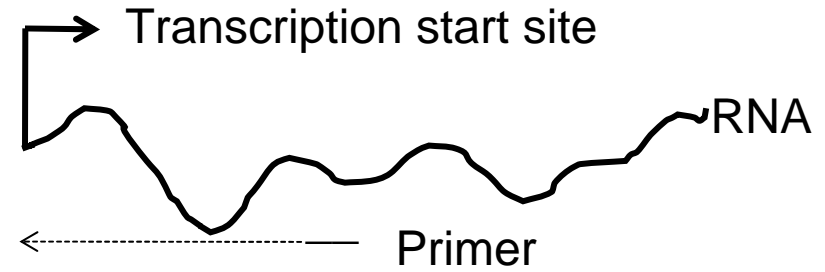
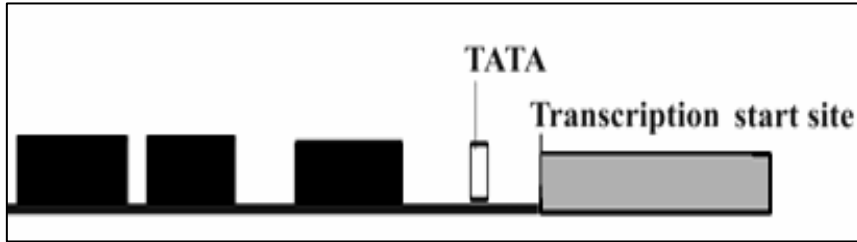
Purified RNA Pol II + purified general transcription factors

Purified RNA Pol II + recombinant general transcription factors



PROMOTER ACTIVITY ASSAYS

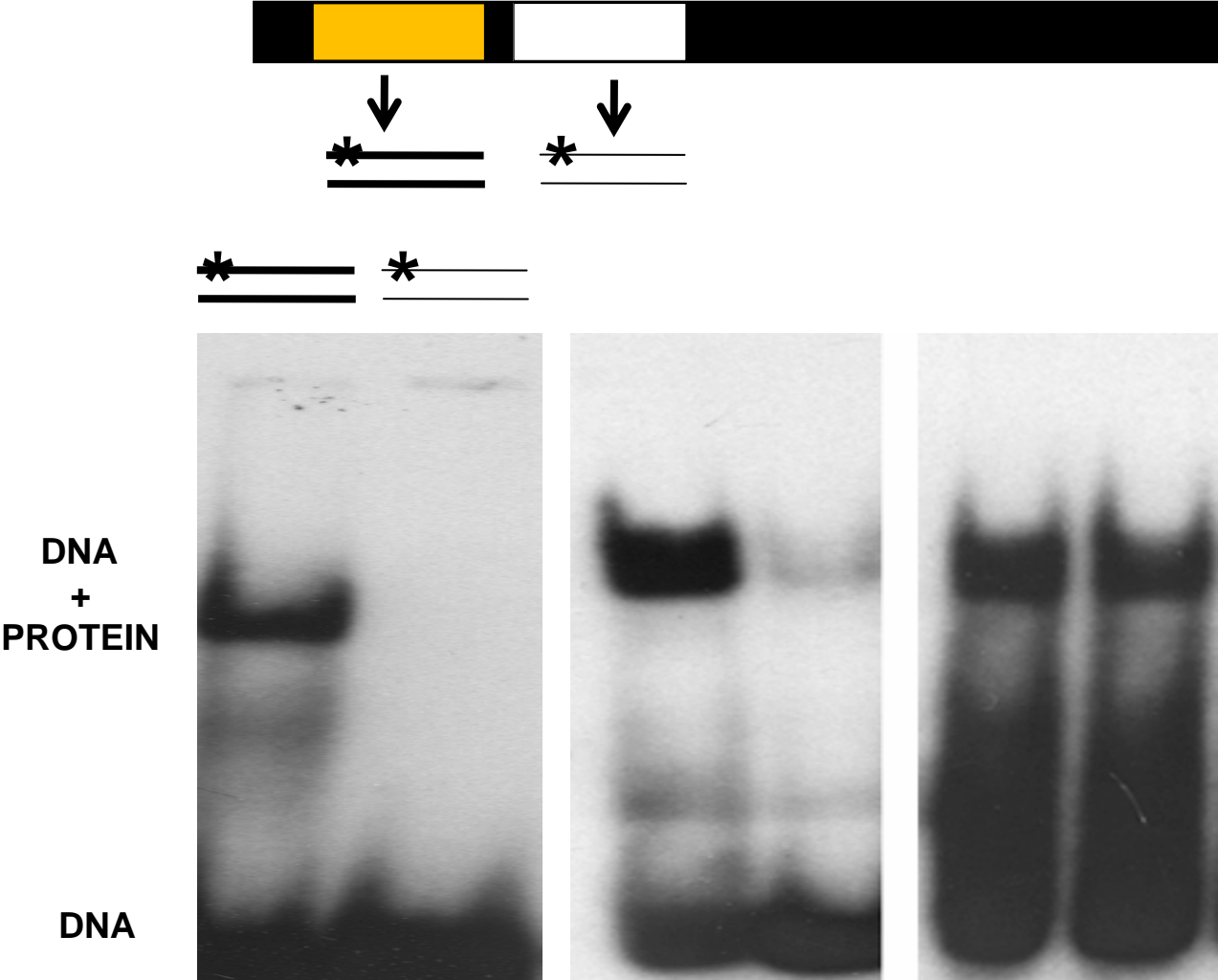
- Cell-free transcription studies



Primer Extension

IDENTIFICATION OF TRANSCRIPTION FACTOR BINDING SITES IN PROMOTERS

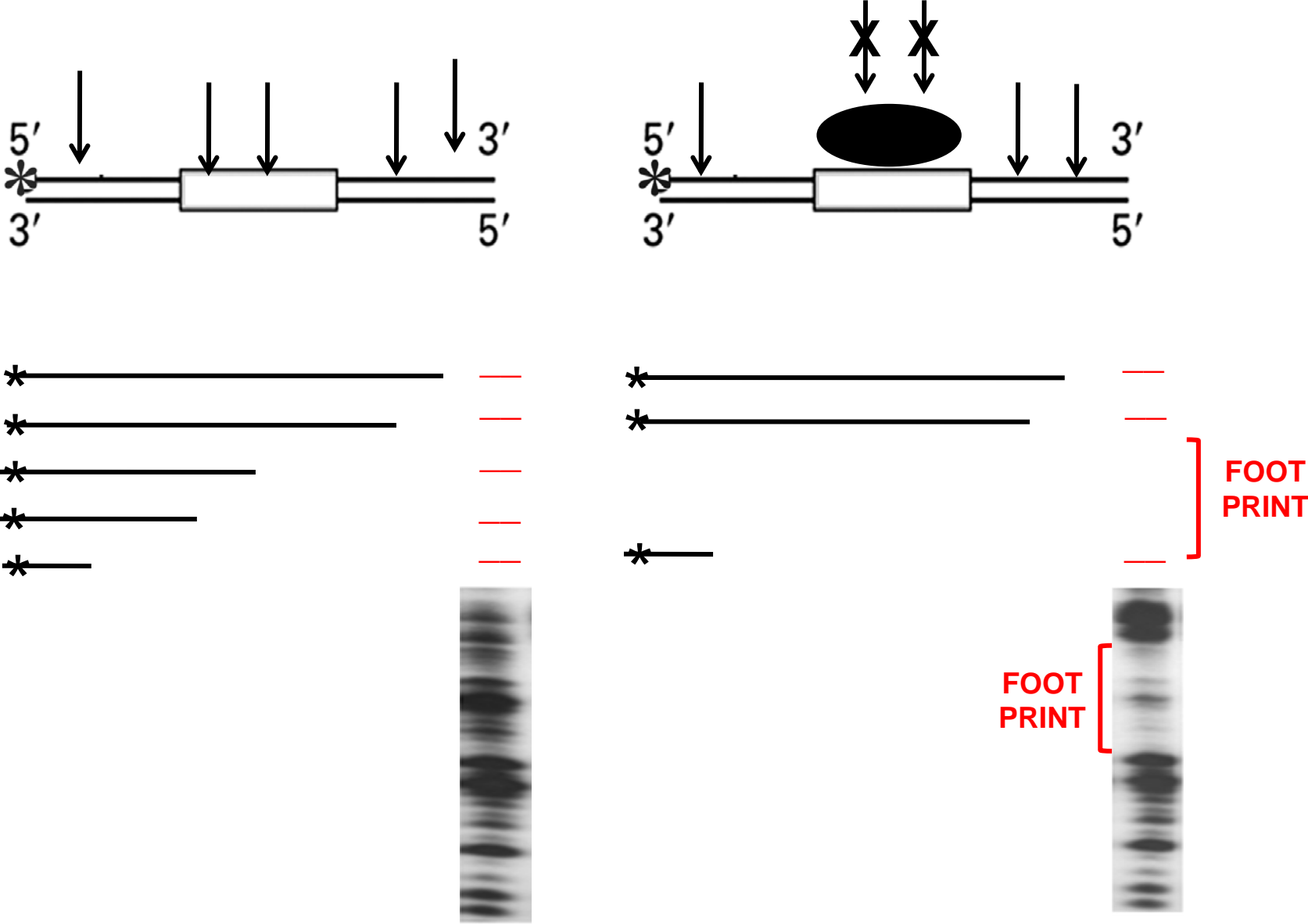
- Electrophoretic mobility shift assay (EMSA)



Specific vs nonspecific binding

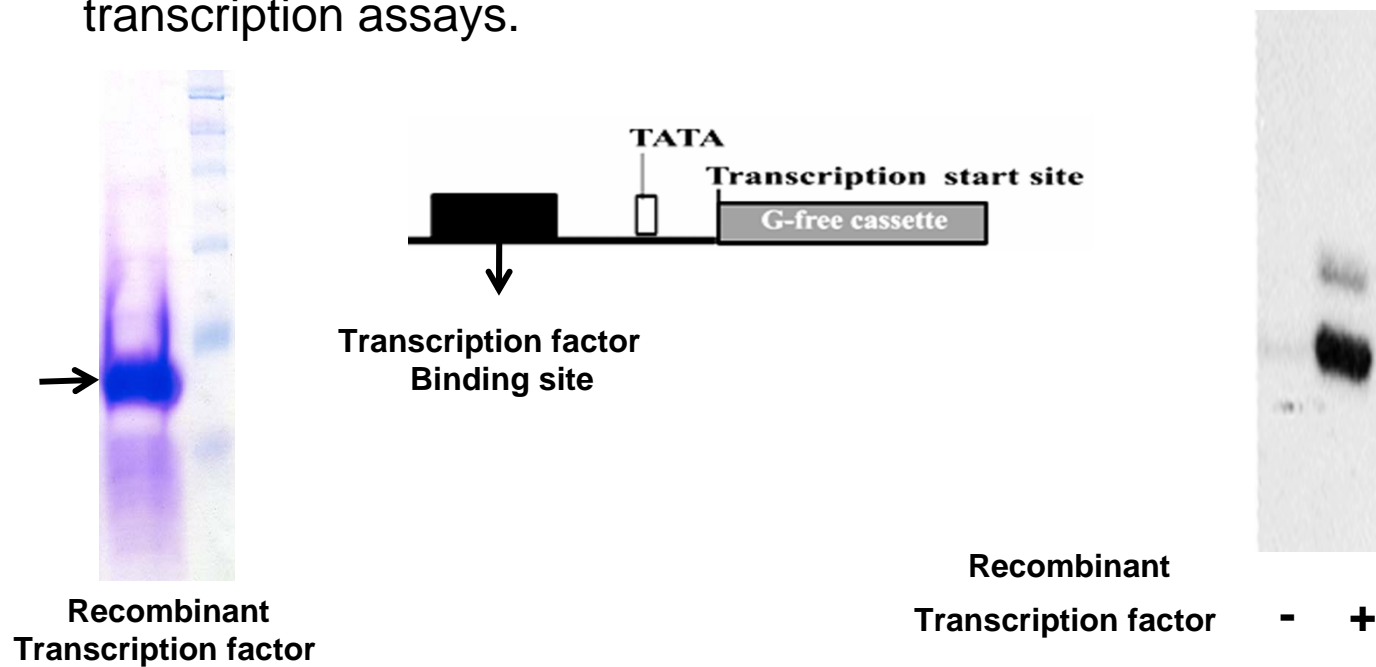
IDENTIFICATION OF TRANSCRIPTION FACTOR BINDING SITES IN PROMOTERS

- DNase I Foot printing

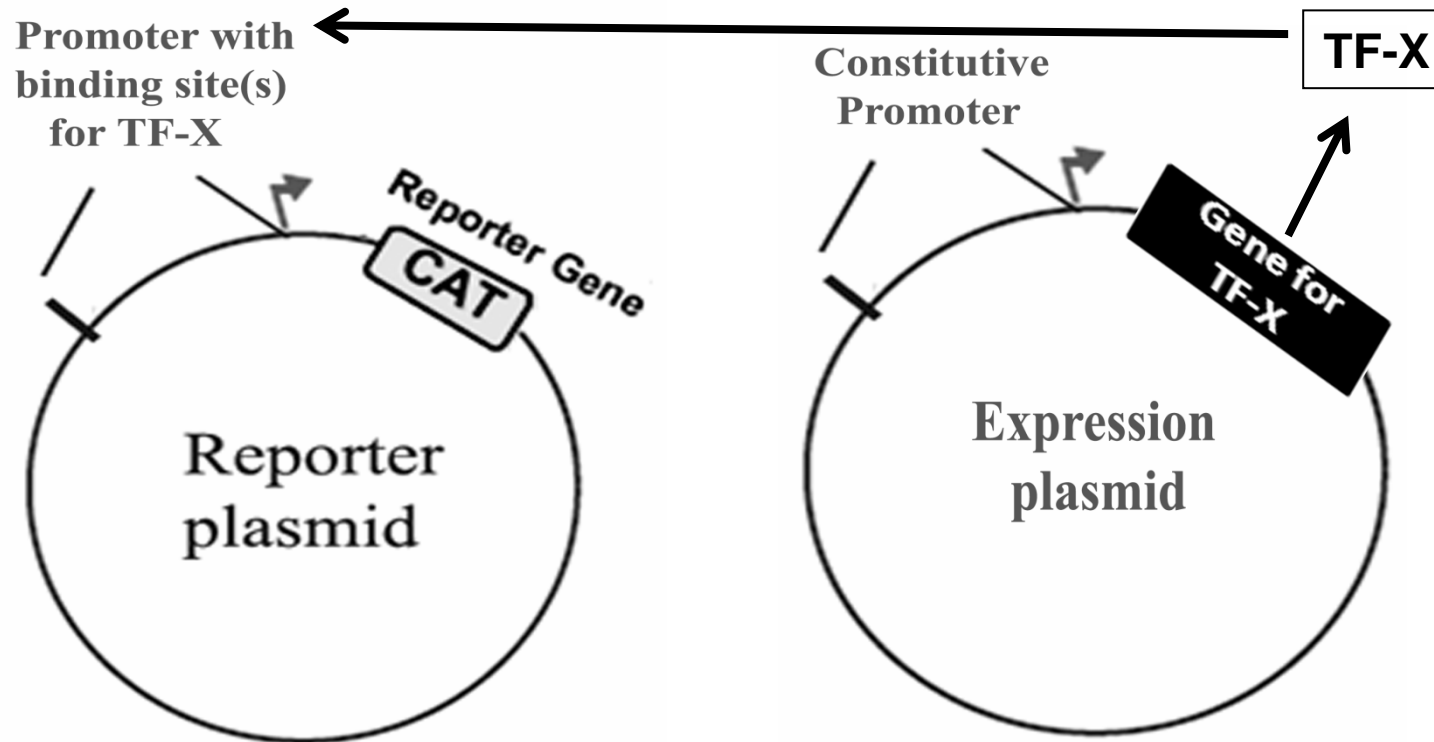


Using assays such as EMSA, DNase I footprinting, transfection of reporter plasmids and cell-free transcription studies coupled to biochemical purification of DNA binding proteins/transcription factors, a large number of promoter elements and transcription factors binding to them were identified.

Using recombinant DNA technology, genes encoding these transcription factors were cloned into *E. coli* expression vectors and the transactivation functions of the recombinant transcription factors were studied in cell-free transcription assays.



Cis-Trans co-transfection assay



Genes encoding mammalian transcription factors were cloned into mammalian expression vectors and these were transfected into mammalian cells and their transactivation functions were studied by cis-trans co-transfection assays.

Genes encoding the transcription factors were cloned into bacterial expression vectors, the recombinant transcription factors were purified from bacterial cell extracts and their DNA binding properties were studied by EMSA, DNase I foot printing etc.

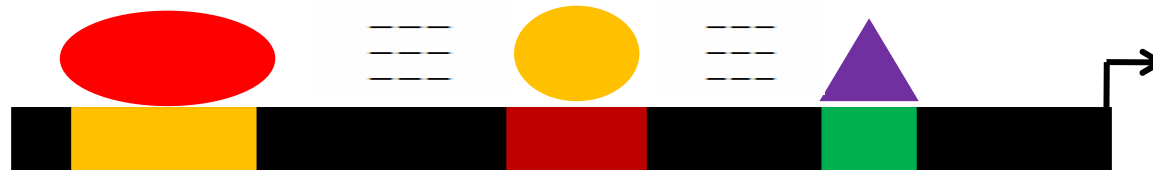
- identification of upstream activation sequences, enhancer elements etc., in a number of eukaryotic promoters
- Identification and characterization of a number of eukaryotic transcription factors binding such sequences.

STRONG PROMOTERS

WEAK PROMOTERS

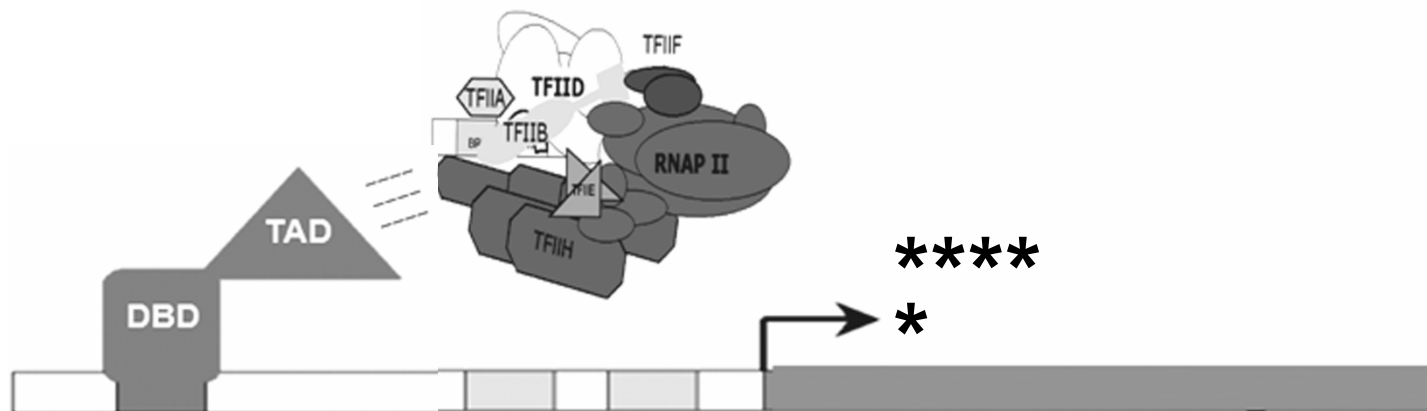
CONSTITUTIVE PROMOTERS

INDUCIBLE PROMOTERS



Structural and functional studies revealed that many of the transcription factors are modular proteins composed of distinct and separable functional domains:

- DNA binding domain (DBD) interacts with specific DNA sequences
- Transcription Activation domain (TAD) interacts with other proteins (general transcription machinery, coactivators etc.), to stimulate transcription from the core promoter



The function of DBD and TAD can be modulated by post-translational modifications or by small molecules

Database of eukaryotic transcription factors and their binding sites

<http://jaspar.cgb.ki.se/>

JASPAR is an open-access database of annotated, high-quality, matrix-based transcription factor binding site profiles for multicellular eukaryotes.

Nucleic Acids Research, 2004, Vol. 32, Database issue **D91-D94**
DOI: 10.1093/nar/gkh012

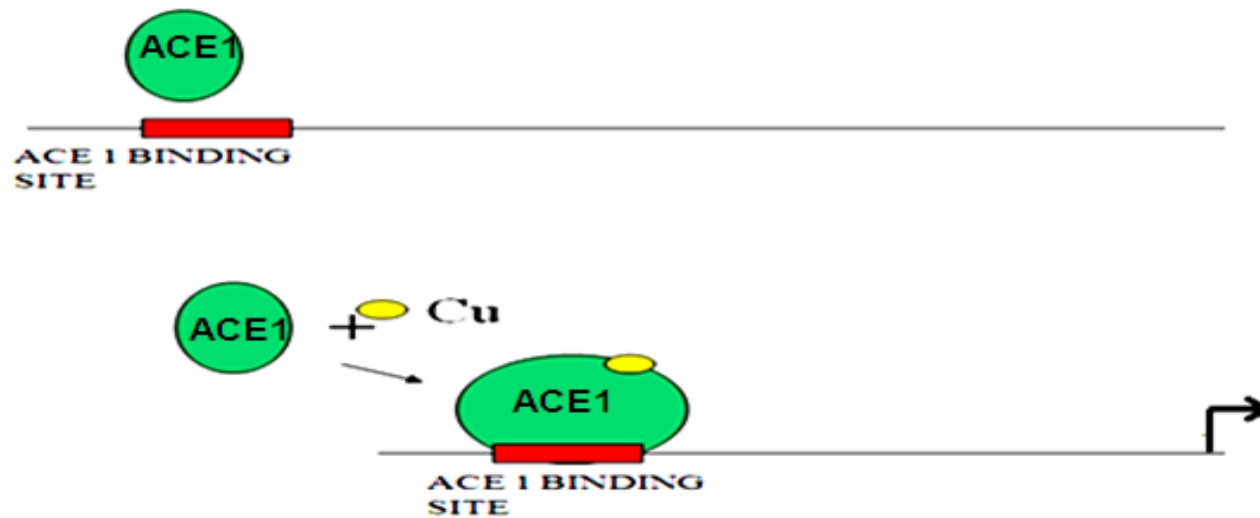
JASPAR: an open-access database for eukaryotic transcription factor binding profiles

Albin Sandelin, Wynand Alkema, Pär Engström, Wyeth W. Wasserman¹ and Boris Lenhard*

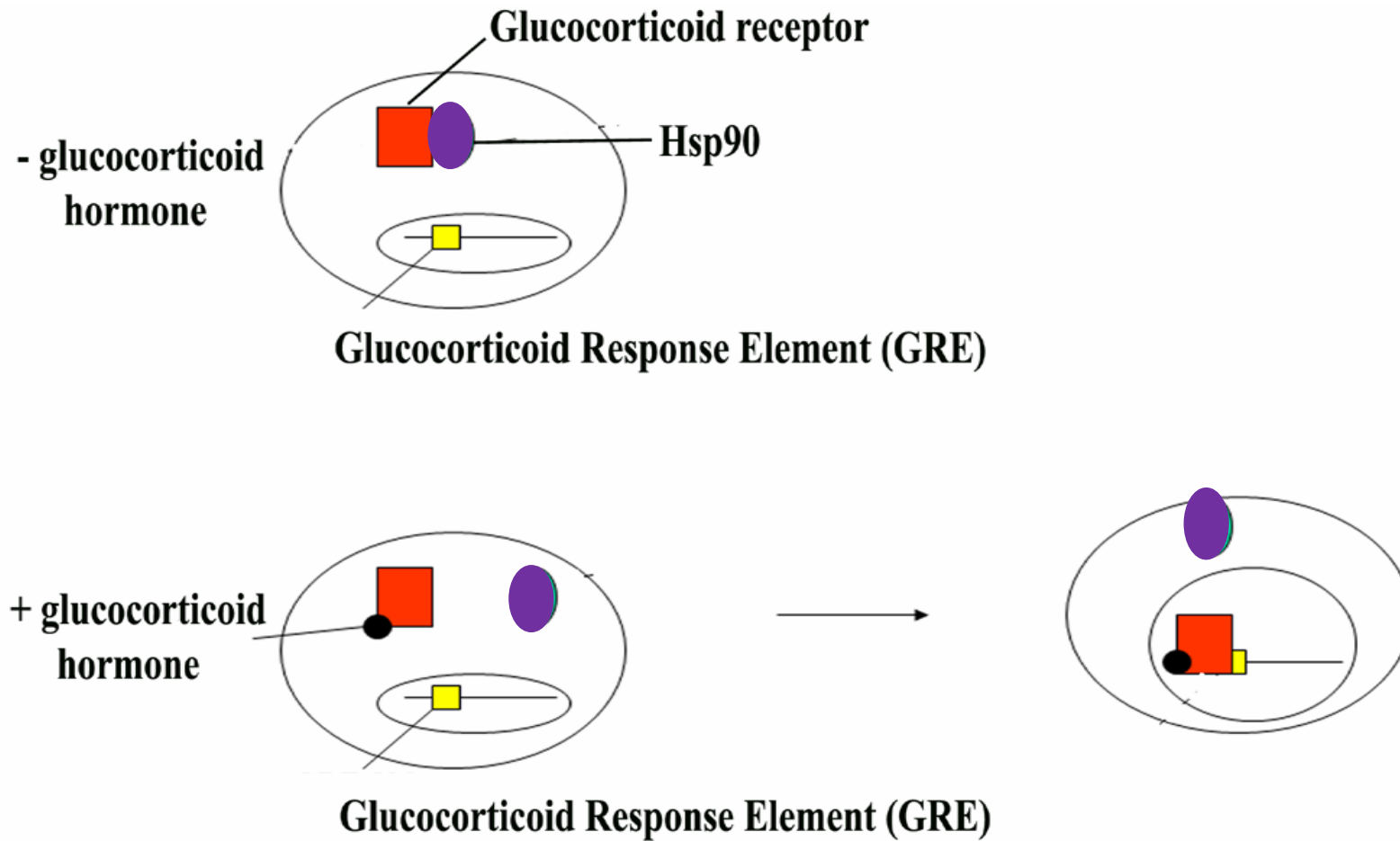
Transcriptional activation of genes in response to heat shock:



Transcriptional activation of metallothionin gene:



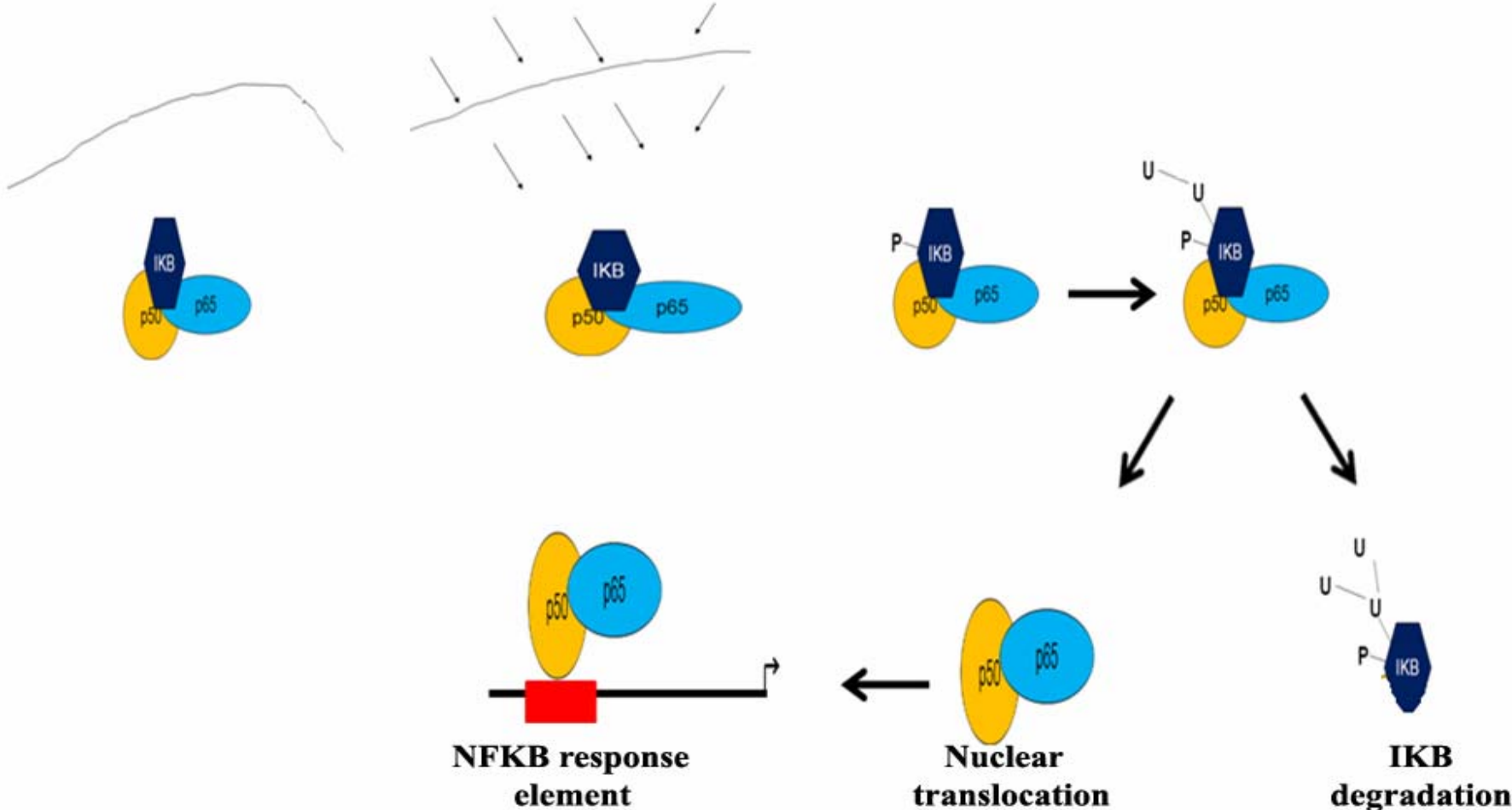
**Transcriptional activation by steroid hormones
(glucocorticoids, estrogen, progesterone etc).**



NF κ B

- Involved in inflammatory response and belongs to Rel family of transcription activators
- Inactive form is present in cytoplasm
- Activation involves translocation from cytoplasm to nucleus

Transcriptional regulation by NFkB



Examples of transcription factor families

Family

Transcription factors

Homeodomain:

Hox

Hoxa-1, Hoxb-2, etc.

POU

Pit-1, Unc-86, Oct-2

LIM

Lim-1, Forkhead

Pax

Pax1, 2, 3, etc.

Basic helix-loop-helix (bHLH)

MyoD, achaete, daughterless

Basic leucine zipper (bZip)

C/EBP, AP1, c-JUN, c-FOS, CREB etc.

Zinc finger:

Nuclear hormone receptors

Glucocorticoid receptor,
Estrogen receptor,
Progesterone receptor,
Retinoic acid receptors,
Thyroid hormone receptor etc.

Transcriptional activation in mammals depends on ~2000 site-specific transcription factors which interact with the general transcription machinery (~40-50 proteins) either directly or through intermediary proteins known as coactivators or corepressors (~200-300).

As development and differentiation occurs, selection among these >2000 transcription factors for the regulation of cell-specific gene expression involves:

- a cascade of regulation of expression of transcription factor genes,
- signals from the extracellular milieu that activate posttranscriptionally, pre-synthesized transcription factors already present in the embryo.

During embryonic development, promoters of certain genes contain Transcription-factor binding sites that are often organized into clusters called **cis-regulatory modules (CRMs)**, which span a few hundred nucleotides and can contain dozens of binding sites for 3–10 transcription factors.

Eukaryotic Gene Expression: Basics & **Benefits**

Understanding promoters and transcription factors has helped us to develop expression systems to produce recombinant proteins

Plant Promoter

Gene of Interest

Insulin

Growth hormone

Hepatitis B antigen

Factor VIII

http://www.accessexcellence.org/RC/AB/IWT/The_Biopharmaceuticals.php

USFDA has approved >200 peptide and protein pharmaceuticals

Many transcription factors and promoter elements are targets for developing novel drugs to fight a number of diseases.