Eukaryotic Gene Expression: Basics & Benefits

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Lecture 3

Gene Regulation in Eukaryotes: Diversity in general transcription factors

Recap……

DIFFERENTIAL GENE REGULATION BY CORE PROMOTER VARIANTS

THERE ARE MANY DIFFERENT CORE PROMOTER ELEMENTS IN EUKARYOTES

THERE IS NOTHING LIKE UNIVERSAL CORE PROMOTER ELEMENTS IN EUKARYOTES

CORE PROMOTERS ARE ESSENTIAL NOT ONLY FOR THE ASSEMBLY OF RNA POLYMERASE MACHINERY AND TRANSCRIPTION INITIATION BUT ALSO FOR THE MODULATION OF THE FUNCTION OF OTHER UPSTREAM CIS-ACTING ELEMENTS (ENHANCERS) AND TRANSCRIPTION FACTORS (WHICH BIND TO ENHANCER SEQUENCES).

DIVERSITY IN GENERAL TRANSCRIPTION FACTORS INTERACTING WITH THE CORE PROMOTER ELEMENTS

Cis-acting DNA sequences that regulate RNA polymerase II transcription include:

Core promoter elements

Proximal promoter (encompassing −250 to +250 nt from transcription start site)

Enhancers

Silencers

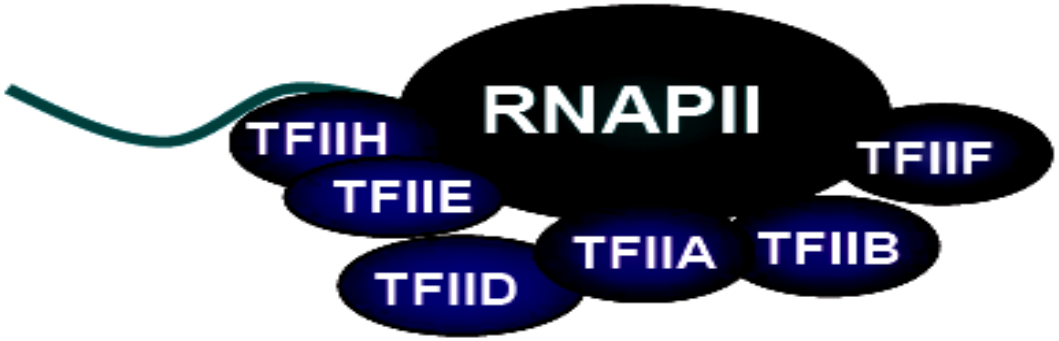
Boundary/insulator elements

Transcriptional activation in eukaryotes involves atleast three classes of trans-acting factors:

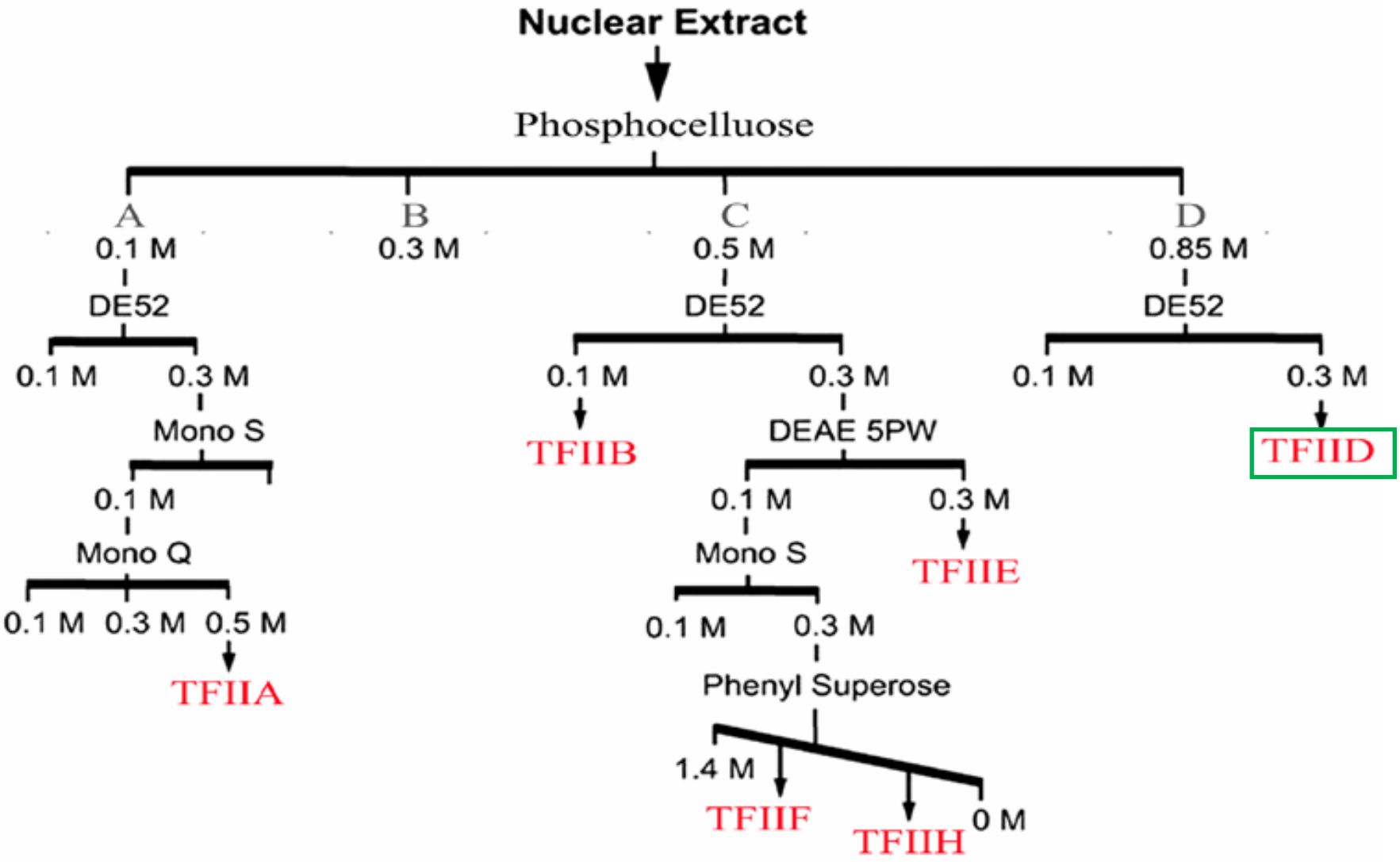
General / basal transcription factors that interact with core promoter elements

Transcriptional activators which bind to proximal / distal promoter sequences

Coactivators which act as a bridge between transcriptional activators and general transcription factors



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



PURIFICATION OF GENERAL TRANSCRIPTION FACTORS

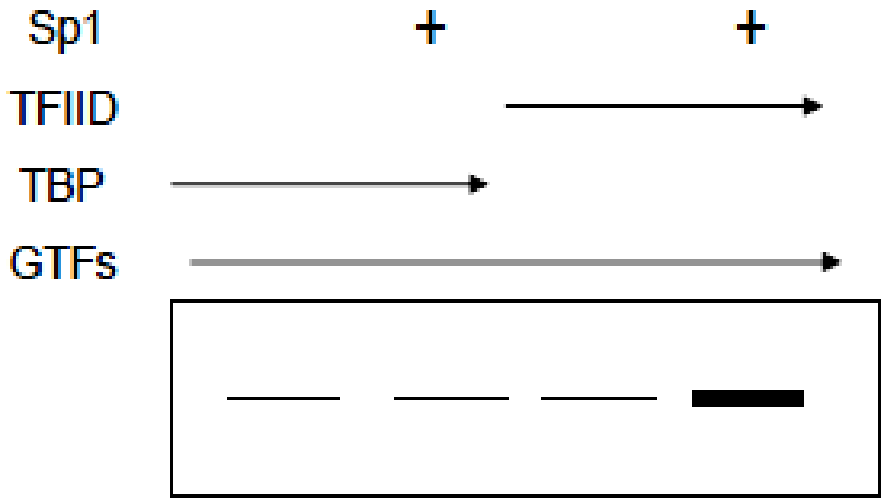
TFIID

•TFIID is a multiprotein complex and the protein responsible for recognizing the TATA box is called ‘TATA-Binding Protein’ (‘TBP’)

• The other proteins (10-14) of the TFIID complex are referred to as TBP Associated Factors (TAFs)

•TBP can specifically bind to TATA-boxes on its own

•*In vitro, TBP is sufficient for “basal”transcription, but not for “activated” transcription*



Studies in the late 1980s revealed that in addition to TBP, certain TAFs can also bind to core promoter elements suggesting that in addition to the diversity of core promoter elements, diversity amongst proteins that bind to core promoter elements, may also contribute to differential gene regulation.

Further, components of TFIID, including TBP and TAFs, have gene paralogs (*i.e., related proteins encoded* by different genes belonging to the same family) which are often expressed in a tissue- and development-specific manner.

These TBP-related factors (TRFs) and TAF variants can play a role in differential regulation of gene expression

The excitement began with the observation that certain components of TFIID are cell type-specific suggesting that the so-called basal apparatus comprising of general transcription factors and RNA polymerase II that forms the heart of the pre-initiation complex may exist in different cell-type-specific forms.

For example, an ovarian-specific TAF4b was identified in humans and several other tissue-specific TAF subunits were identified in flies, mice, and humans.

Further, several proteins referred to as TBP related factors (TRFs) were discovered.

All these studies indicated that just as different sigma factors contribute to differential gene regulation in prokaryotes, diversity in general transcription factors may bring about cell-, tissue- and developmental-specific regulation of gene expression in eukaryotes.

Identification of tissue-specific TAFs

The first evidence for the presence of tissue-specific TAFs came from the identification of a TAF4 variant (TAF4b) by Dikstein et al, in 1996 in cultured B cells.

TAF4b was later found to be present in mouse testis and ovary as well. Interestingly, TAF4b knockout females are infertile due to deficient folliculogenesis, and knockout males exhibit age-dependent infertility as a result of defect in spermatogenic maintenance (Falender et al., 2005).

TAF4b was shown to be required for the transcription of a subset of ovarian granulosa cell-specific genes and genes required for spermatogonial stem cell maintenance.

TAF4b was identified as the key mediator of extracellular signals that specify the developmental and proliferative program of granulosa cells.

Identification of TAF variants

The importance of tissue-specific TAFs in gene regulation was further strengthened by the identification of five testis-restricted (tTAF) subunits in *Drosophila* by Lin et al., in the year 1996:

No hitter (paralog of TAF4),
Cannonball (paralog of TAF5),
Meiosis I arrest (paralog of TAF6),
Spermatocyte arrest (paralog of TAF8), and
Ryan express (paralog of TAF12)

In the year 2001, Hiller et al., demonstrated that disruption of each of these tTAFs results in alteration of expression of testis-specific genes, and together these tTAFs form a stable complex, which is required for meiotic cell-cycle progression and normal spermatid differentiation.

In mice, a variant of TAF7 called TAF7l was shown to be expressed in testis in late spermatocytes and haploid spermatids.

TAF7l knockout mice exhibit altered patterns of spermatocyte gene expression and defects in spermiogenesis resulting in reduced fertility (Cheng et al., 2007).

Several such TAF variants have been identified in recent times indicating that diversity in TAFs can contribute to differential gene expression in eukaryotes.

The presence of tissue-specific TAF variants enables TFIID to work in conjunction with germ cellspecific transcription factors, cofactors, or other components of the general transcription machinery and contribute to differential gene regulation

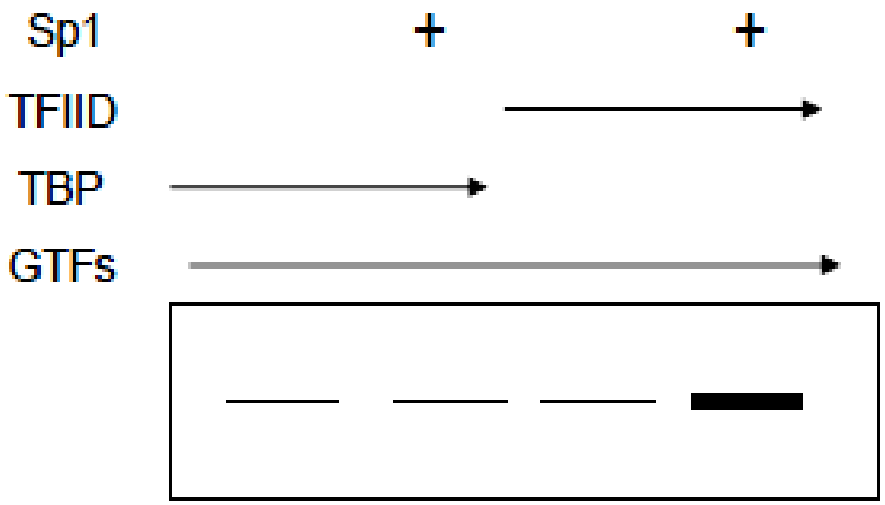
Enzyme activities of TAFs

Many of the TAFs possess multiple enzymatic activities to post translationally modify histones and transcription factors, thus allowing TFIID to serve as a core promoter-binding factor in the context of chromatin and as a coactivator mediating activator response.

For example, Human, *Drosophila and yeast TAF1, a* TFIID-specific subunit, functions as a histone acetyltransferase (HAT) in acetylating histones H3 and H4 *in vitro*

Drosophila TAF1 has also been shown to act as a kinase in phosphorylating histone H2B

The ability of some of the TAFs to modify histones explained how only TFIID (TBP + TAFs) but not TBP alone can enhance activator-dependent transcription from chromatin templates



Only TFIID, rather than TBP, can work in conjunction with a TFIID-deficient pol II holoenzyme complex to facilitate activator-dependent transcription from an *in vitro* reconstituted chromatin template, suggesting a unique involvement of TAFs in chromatin transcription

In addition to modifying histones, certain TAFs such as TAF1, either can also covalently modify other general transcription factors and cofactors:

For example, TAF1 can acetylate TFIIE*β, phosphorylate RAP74,* histone H2B *,* PC4 *and the β subunit of TFIIA and* ubiquite TAF5

Identification of TBP related factors (TRFs)

All multicellular organisms contain at least two genes coding for TBP family members that share sequence homology at their C-terminal 180 amino acid core DNA-binding domains.

The first gene codes for TBP, considered a universal TATA-binding transcription factor present in all eukaryotes

The second gene encodes TBP-related factor 2 (TRF2), a.k.a. TBP-related protein (TRP), TBP-like factor (TLF) or TBP-like protein (TLP).

TRF2 shares □60% sequence homology and 41% identity within the C-terminal core with that of TBP, it recognizes a sequence element distinct from the TATA box

Identification of TBP related factors (TRFs)

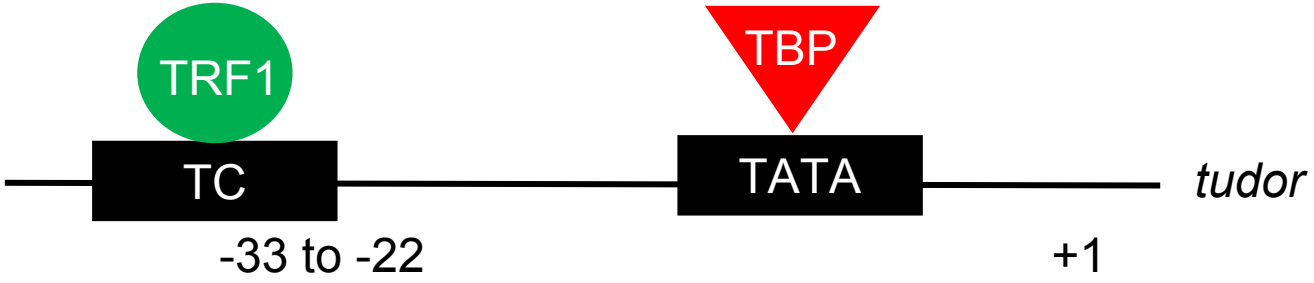
In addition to TRF2, three species-specific TRFs (TRF1, TRF3, and TRF4) have also been identified.

TRF1

TRF1 was the first TRF to be identified in Drosophila where it was shown to be involved in regulation of a specific subset of genes in nervous system and gonads. (Crowley et al., 1993; Hansen et al., 1997).

TRF1 binds TFIIA, TFIIB, his its own TAFs (nTAFs) and promotes formation of the pre-initiation complex in neural tissue.

In Drosophila, a gene known as *tudor* has two promoters, one with a TATA box that binds TBP, and the other a TC box that binds TRF-1.



A TC-rich sequence, located between −22 and −33 relative to the transcription start site of this upstream TRF1-responsive *Tudor* promoter preferentially nucleates TRF1-mediated PIC assembly and transcription.

Promoter selectivity by TRF1 is likely enhanced by some transcriptional activators or by its association with neuron-specific TRF1-associated factors (nTAFs) to form a multiprotein complex, which is distinct from TFIID

TRF2

TRF2 binds TFIIA and TFIIB and displays high conservation of the TBP DNA-binding domain,

TRF2 does not bind TATA box-containing DNA and fails to complement TFIID in basal or activated in vitro transcription assays

TRF2 associates with the DRE-binding factor (DREF) and selectively regulates the transcription of DRE-containing promoters

Genome-wide localization studies in *Drosophila* revealed that TRF2 occupies over a thousand promoters, most of which are not bound by TBP and are enriched for DREs but lack a canonical TATA box

In mouse ,TRF2 is highly enriched in the testis

TRF2 knockout mice display spermiogenesis defects and altered patterns of testis-specific gene expression

TRF3 /TBP2

TRF3 is the most recently identified member of the TBP related factor family (2003) and is expressed in several mammalian tissues.

Studies of TRF3 function have focused on oogenesis and early embryonic development.

Murine TRF3 expression increases rapidly during oocyte growth from primordial follicle formation until preovulation at the same time that TBP expression is effectively eliminated.

Recent studies indicate a key role for TRF3 in skeletal muscle differentiation (M.D. Deato and R. Tjian 2007).

TRF3 knockout mouse: homozygous females display complete sterility due to oocyte arrest at the primordial follicle stage and eventual ovarian failure (Gazdag et al., 2009)

Thus, variants of TFIID subunits play key roles in a range of biological processes.

Drosophila TRF1:	Transcription of targets of Pol III
Mammalian TAF4 homolog (TAF4b)	folliculogenesis in the ovary,
Human TAF9L	Apoptosis
Drosophila testis TAFs	Spermatogenesis

It is now becoming increasingly clear that TFIID variants have a key role in of cell type–specific development and differentiation.

Alternate forms of core transcription machinery components are used during metazoan development of eukaryotes to switch between transcription programs as cells differentiate.

For example, incorporation of TAF4b in place of one of the two subunits of the generally expressed TAF4 allows TFIID to stimulate binding of certain DNA binding transcription factors to specific target genes, turning on a cell type–specific transcription program (Liu et al. 2008).

Strikingly, differentiation of skeletal muscle involves destruction of TFIID and its replacement by a novel complex of TBP (TRF3) and TAF (TAF3) homologs required for expression of differentiation genes (Deato and Tjian 2007).

TFIIA variants

A cell type-specific TFIIA*αβ-like factor (ALF) has been identified* identified in human testis (Upadhyaya *et al., 1999; Ozer et al.,* 2000) and is shown to work in conjunction with TFIIA*γ* to stabilize TBP binding to the promoter (Upadhyaya *et al., 2002).*

ALF is also found in immature oocytes of the frog *Xenopus laevis,* in which ALF replaces TFIIA during oogenesis (Han *et al., 2003).*

ORIGINAL RESEARCH ARTICLES

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ORIGINAL RESEARCH ARTICLES

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