Lecture 7

Read p330-347

I. Transposon and transposon tagging

Ac/Ds type
Retrotransposon
Transposon mutagenesis
Gene cloning using transposon as a probe

II. Gene structure and regulation

A. Cis-regulatory elements
B. Trans-acting regulators
DNA-binding type
non-DNA binding type

Similar transpoable elements:

Snapdragon	Maize
Tam1	Ac/Ds
Tam3	*Spm/dSpm*

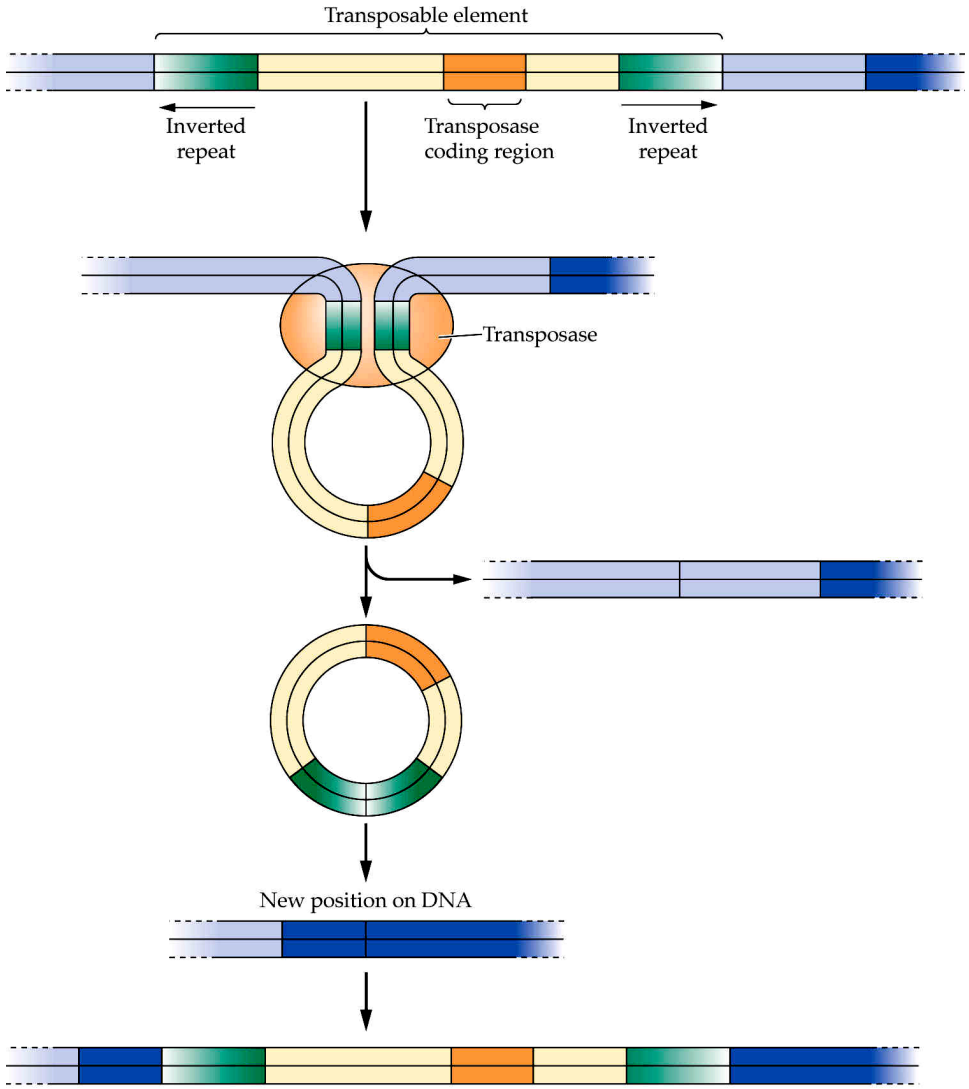


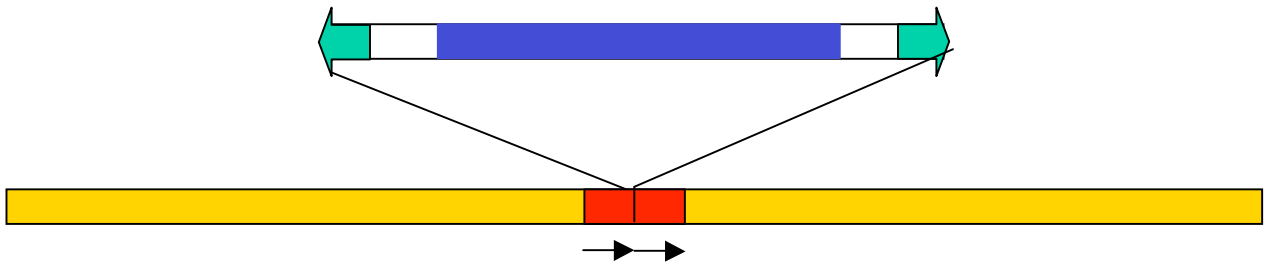
Fig. 7.33

Ac: activator, autonomous, 4.6 kb long, enocdes a 3.5 kb transcript of transposase

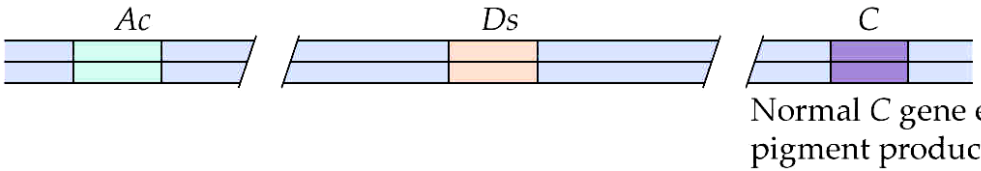
Ds: dissociation, non autonomous

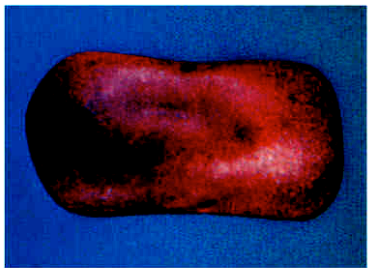
-both *Ac* and *Ds* have 11 bp inverted repeats at the ends, which function in the transposase recognition

-an 8 bp direct repeat generated from the host genome--footprint

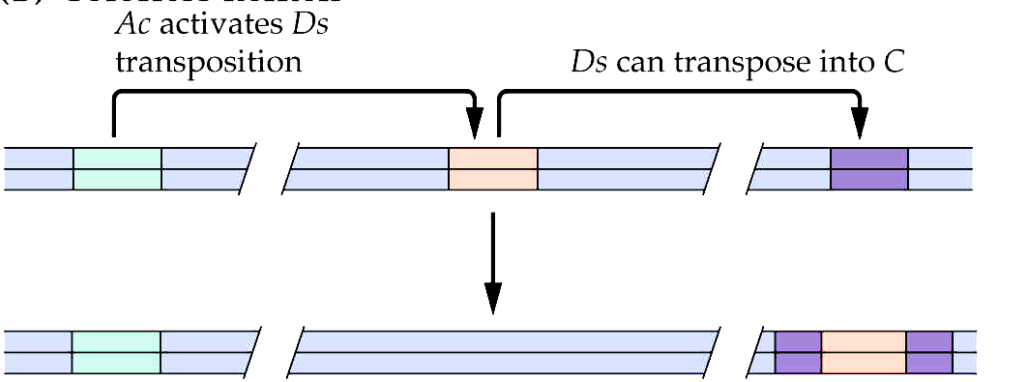


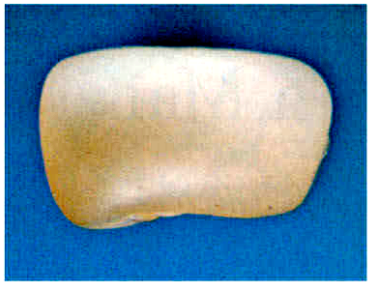
(A) Purple kernels



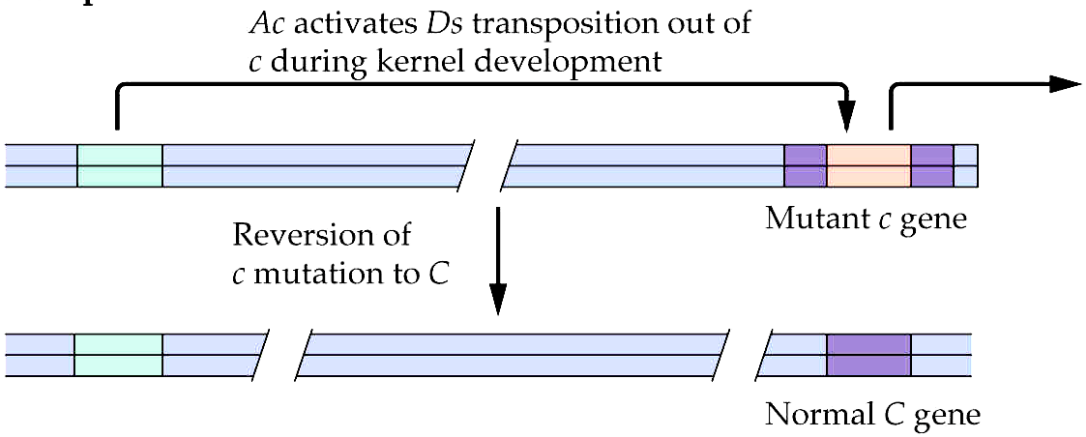


(B) Colorless kernels





(C) Spotted kernels



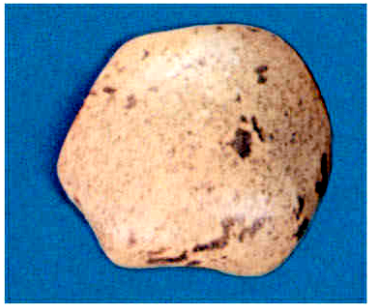


Fig. 7.36

Retrotransposons-viral in origin

Yeast	*Ty*
Drosophila	*copia*
Maize	*Mu*

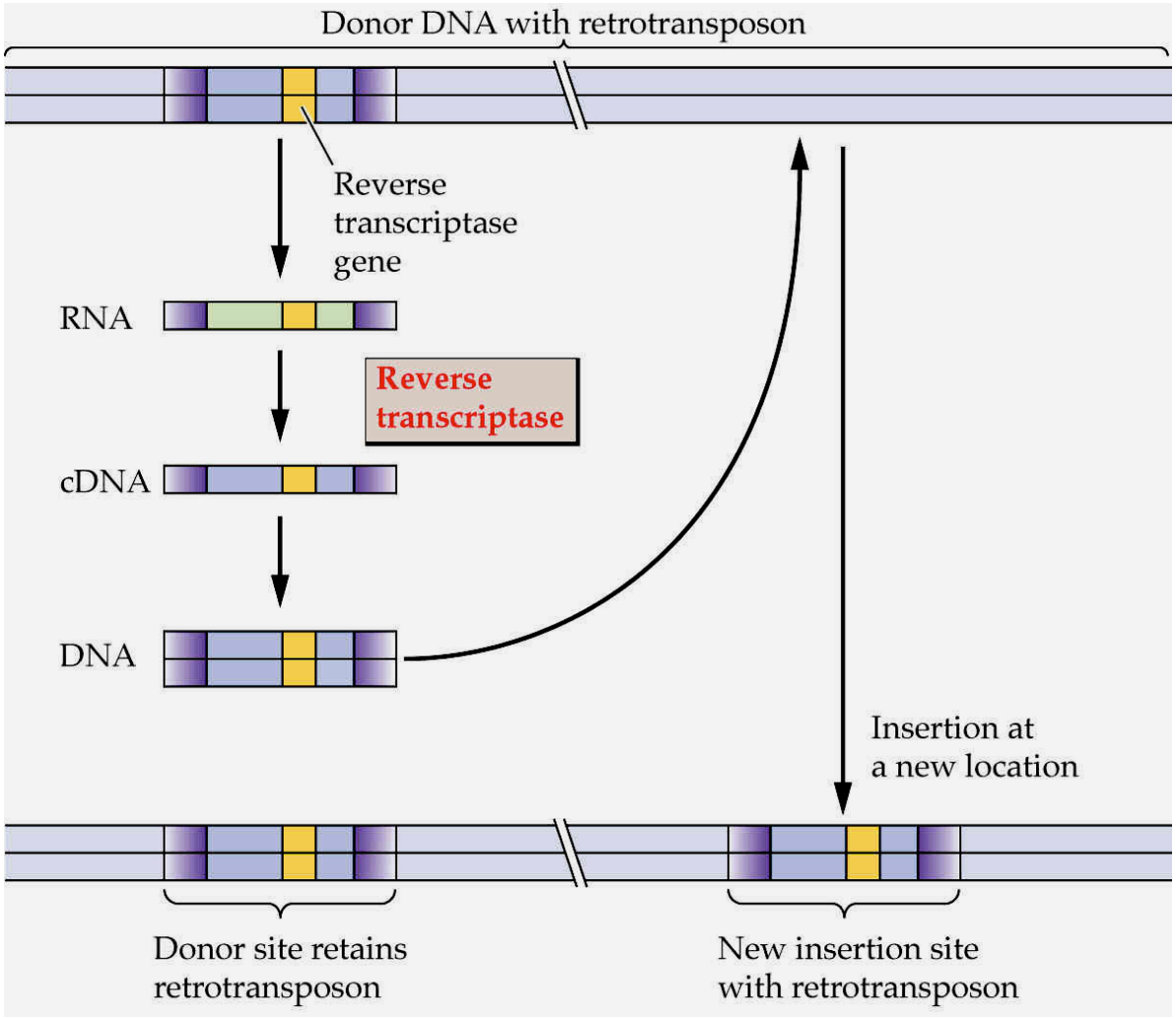


Fig. 7.34

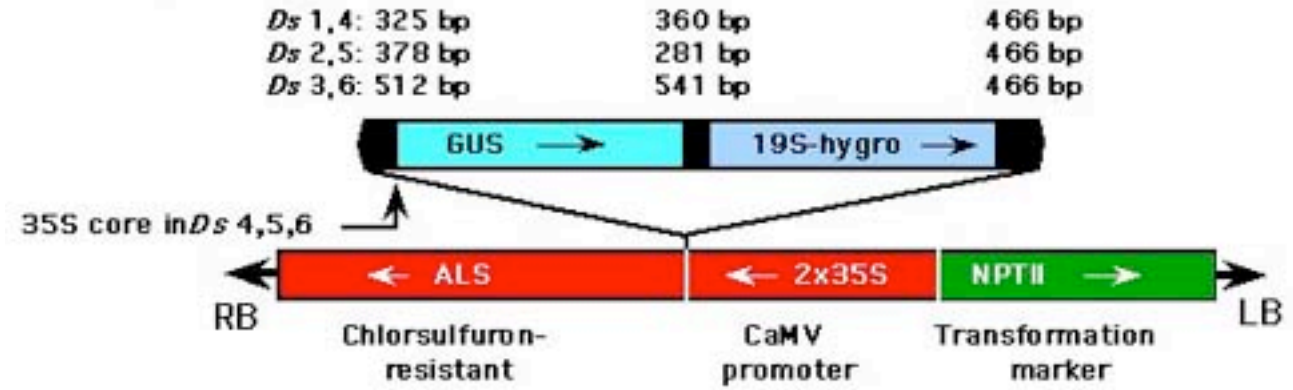
Transposon tagging using *Ac/Ds in Arabidopsis*

-*Ac/Ds* transposons were isolated in Nina Fedoroff's lab
-they are shown to be able to transpose in many plants
both monocot and dicot: tobacco, tomato, Arabidopsis, flax
-they are powerful tools for tagging and cloning genes

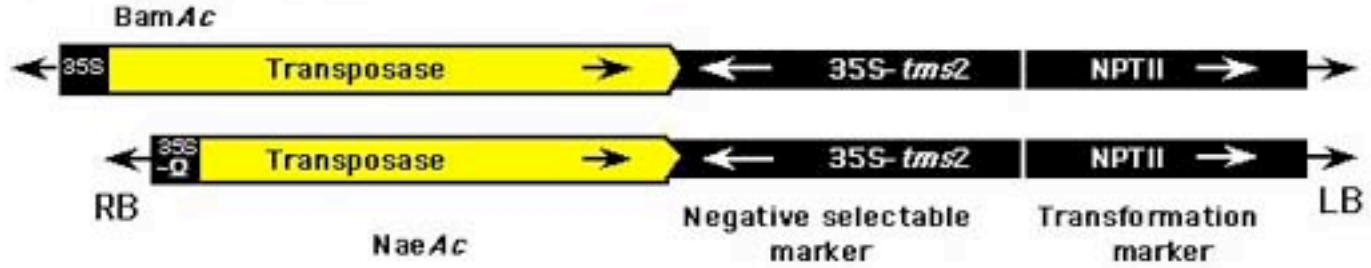
Nina Fedoroff lab (http://www.lsc.psu.edu/ptl/transtag.html)

Figure 1: Structure of *Ac*- and *Ds*-GUS T-DNAs

a. Transposon T-DNAs

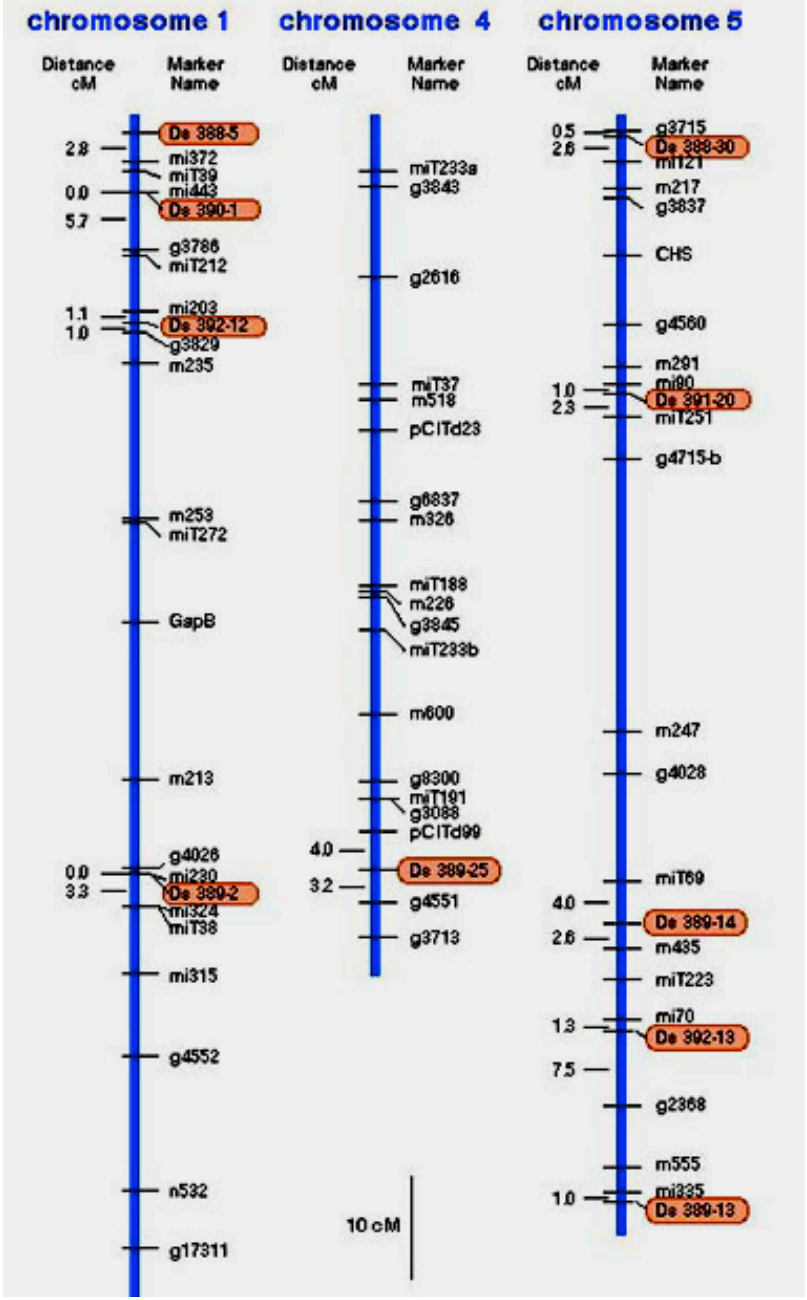


b. Transposase T-DNAs



Structure of the T-DNA segments containing the transposon (a.) and the transposase gene (b.), as well as the selectable markers associated with each. The constructs used here have been described in Fedoroff and Smith (Plant J., 3: 273-289 [1993]). The amount of the *Ac* element in each *Ds* construct is represented by the filled portions at the beginning, middle and end of the transposon and the exact number of base pairs is indicated above the diagram. *Ds*1 and 4 contain only the first of the *Ac* element's 4 transcription start sites, while the others contain all of them with (*Ds*3 and 6) or without (*Ds*2 and 4) part of the element's untranslated leader. *Ds* constructs 4-6 contain the 35S core sequence upstream from the GUS gene, while *Ds* constructs 1-3 do not. Abbreviations: RB: T-DNA right border; LB: T-DNA left border; GUS: β-glucuronidase; 35S and 19S: the promoters of the CaMV 35S and 19S transcripts; the Nae*Ac* contains the Ω sequence of the tobacco mosaic ; ALS: chlorsulfuron-resistant acetolactate synthase gene (Haughn et al., Mol.Gen.Genet. 211: 266-271 [1988]); hygro: the bacterial *aph*4 gene, confers hygromycin resistance; NPTII: bacterial neomycin phosphotransferase II gene; *tms*2: the agrobacterial *tms*2 gene, confers sensitivity to auxin amides; Bam- and Nae-*Ac*: deleted and promoter-substituted *Ac* elements.

Figure 12: chromosomal locations of *Ds*-GUS-TDNAs



II. Gene structure and regulation

A. Cis-regulatory elements

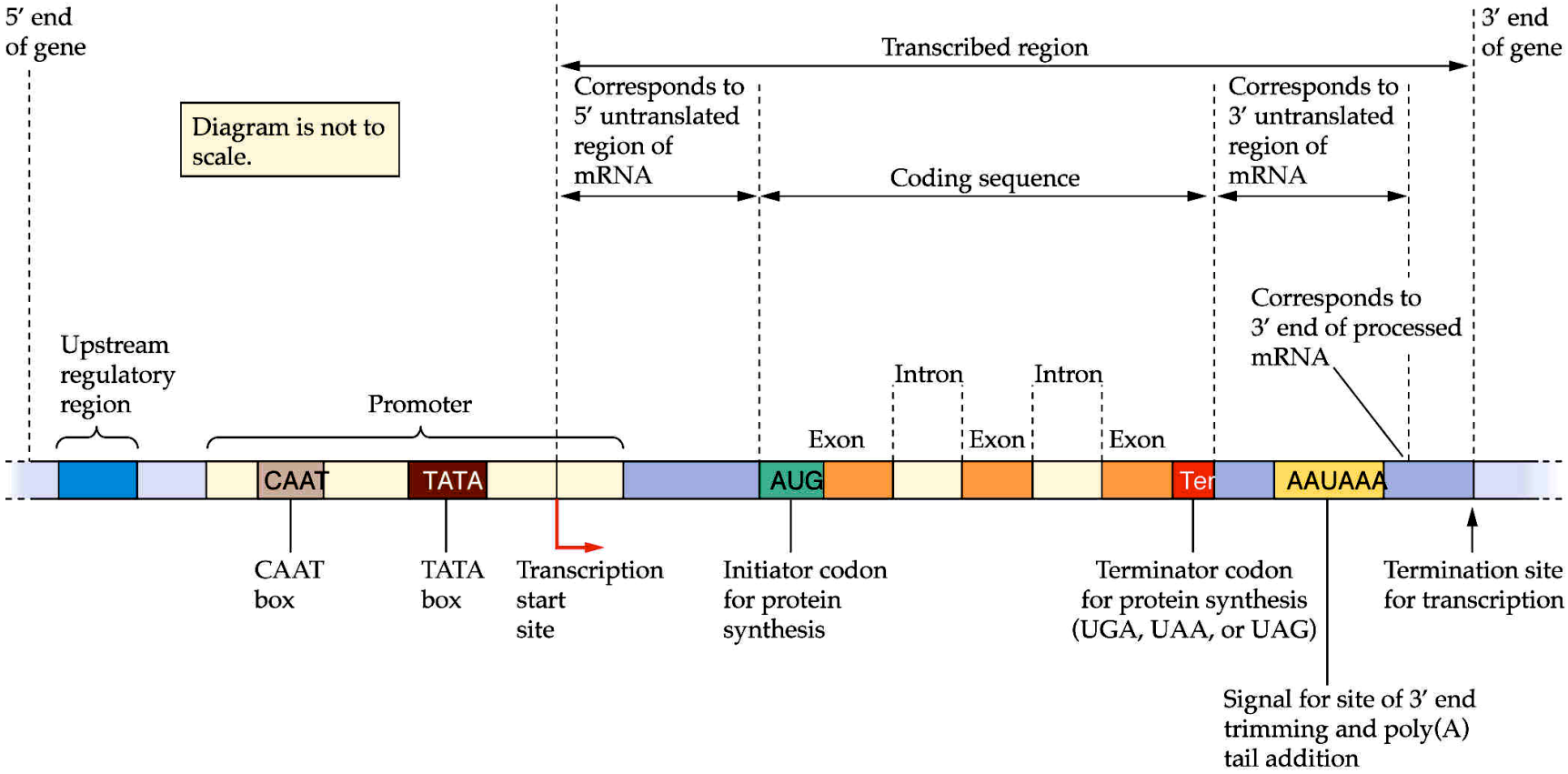


Fig. 7.41

Certain genes are only expressed under certain conditions

CDeT6-19-GUS expression

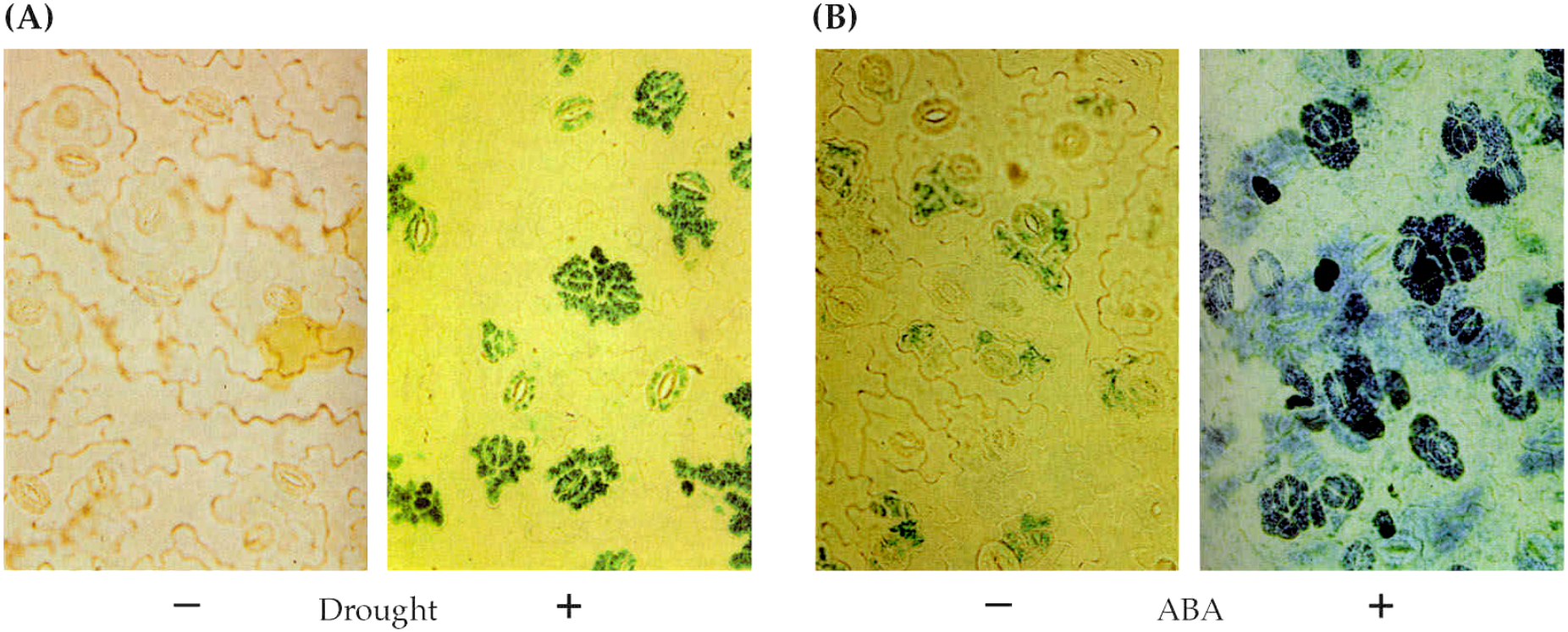


Fig. 7.40

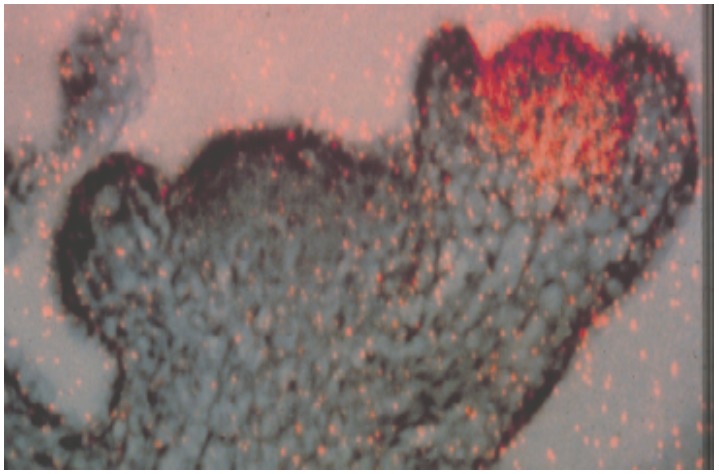
CDeT6-19: the promoter of a drought-responsive gene
ABA: abscisic acid
GUS: β-glucuronidase

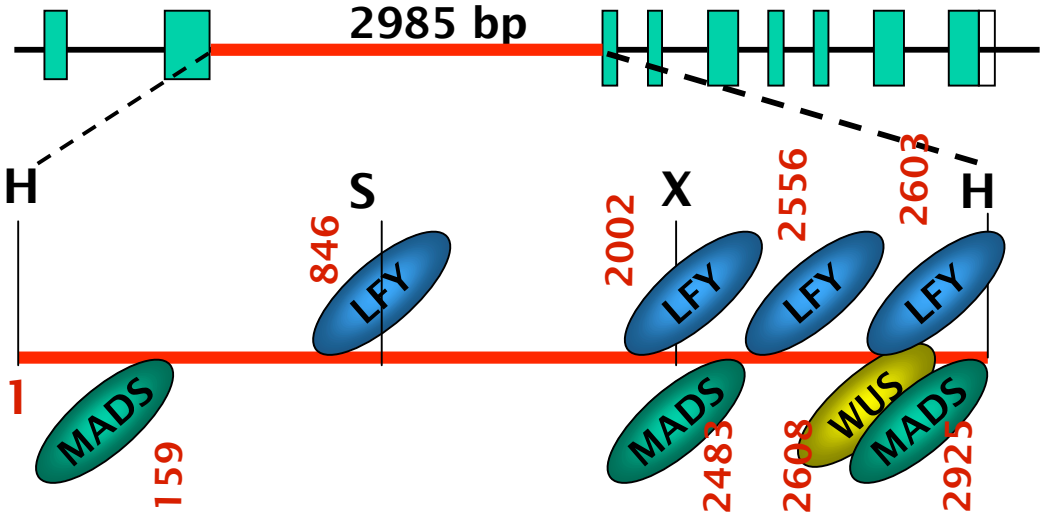
Certain genes are only expressed at specific stages and tissue

WT Arabidopsis flower

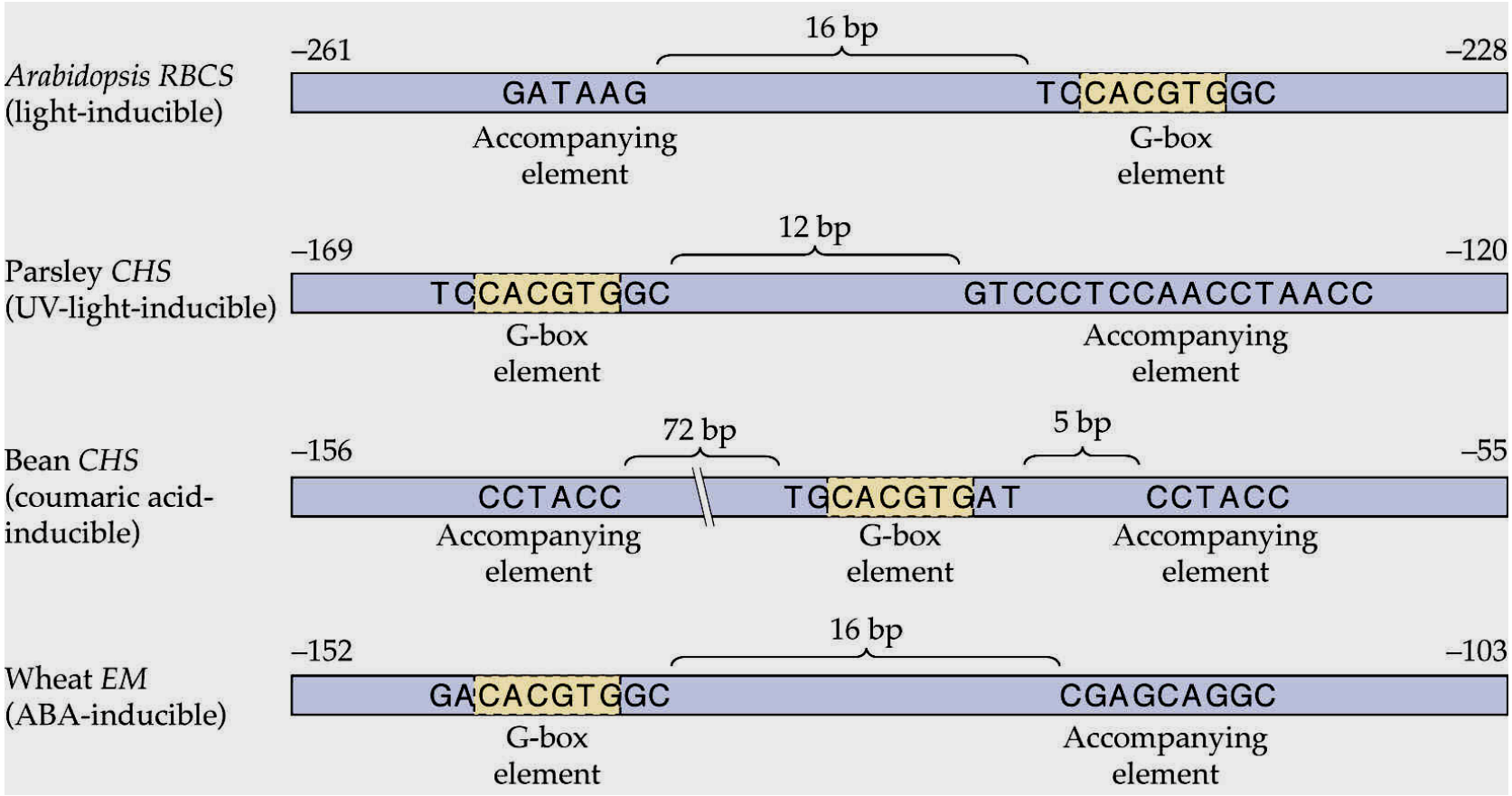
ag mutant flower

AG RNA expression





G-box (CCACGTGG): association with environmental stimuli

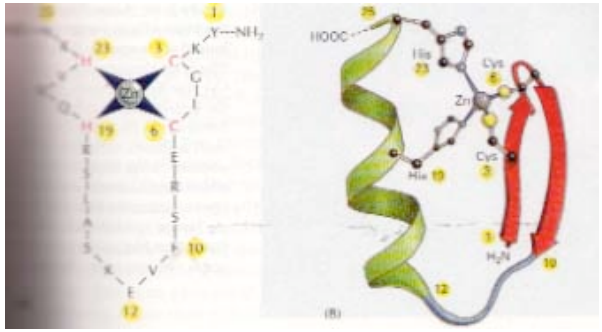


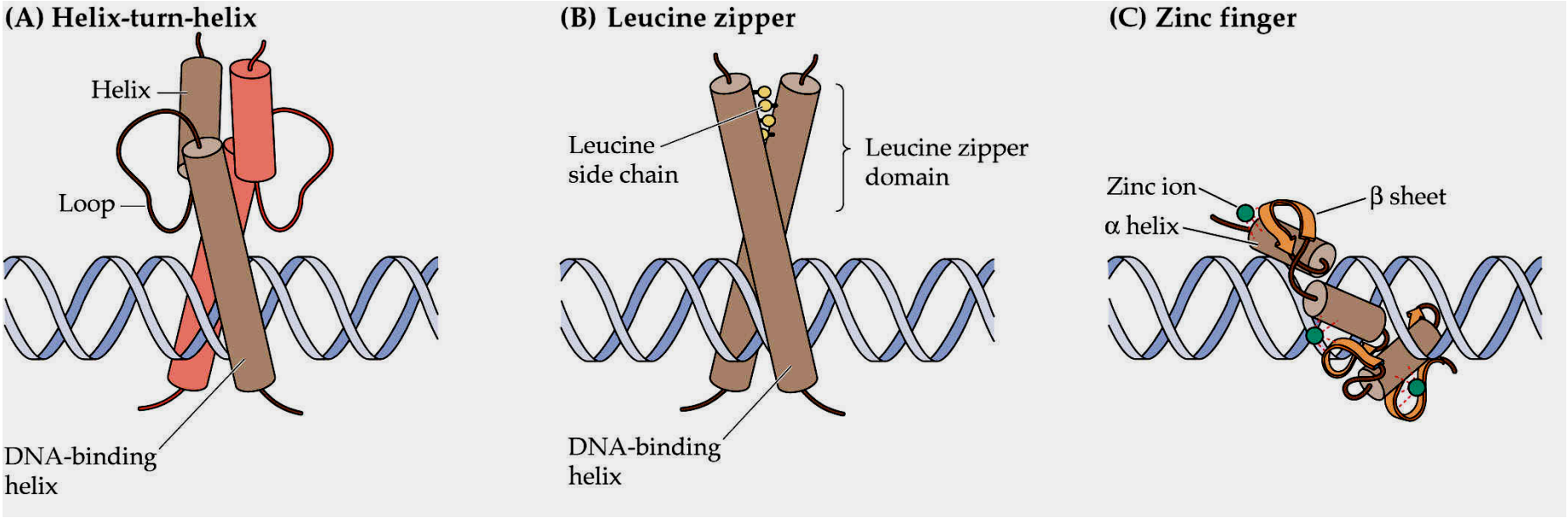
Trans-acting regulators: transcription factors

-Tx factors have DNA-binding domain and activation domain

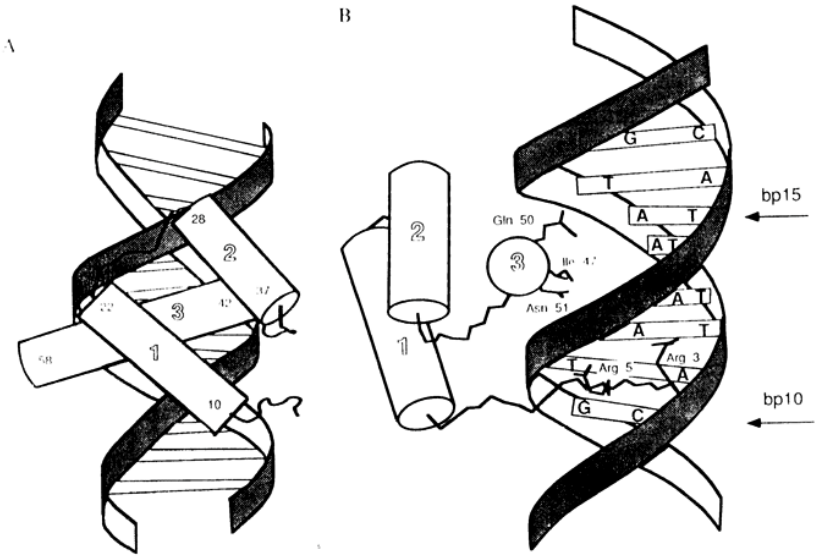
-Four major categories of Tx factors:

1. helix-turn-helix
2. basic leucine zippers
3. zinc fingers
4. high mobility group (HMG) box





		Helix I	Helix II	Turn	Helix III
	Class 1				
Maize	KN1	SKKKKKGKLPKEARQQLLSWWDQHYKWPYPSETQKVALAESTGLDLKQINNWFINQRKRHWKPS
Rice	OSH1	SKKKKKGKLPKDARQQLLNWWELHYKWPYPSESQKVALAESTGLDLKQINNWFINQRKRHWKPS
Maize	RS1	SKKKKKGKLPKEARQKLLHWWELHYKWPYPSETEKIALAESTGLDQKQINNWFINQRKRHWKPS
Maize	KNOX4	SKKKKKGKLPKEARQKLLHWWELHYKWPYPSETEKIALAEATGLDQKQINNWFINQRKRHWKPS
Arabidopsis	KNAT1	SKKKKKGKLPKEARQKLLTWWELHYKWPYPSESEKVALAESTGLDQKQINNWFINQRKRHWKPS
Maize	KNOX8	SKRKKKGKLPKEARQKLLHWWELHYKWPYPSETEKMALAETTGLDPKQINNWFINQRKRHWKPA
Maize	KNOX3	CKKRKKDKLPKEARQKLLSWWELHYRWPYPSEMEKIALAESTGLEQKQINNWFINQRKRHWKPS
Soybean	SBH1	MKKRKKGKLPKEARQQLLEWWNRHYKWPYPSESQKLALAESTGLDQKQINNWFINQRKRHWKPS
Arabidopsis	KNAT2	SKKKKKGKLPREARQALLDWWNVHNKWPYPTEGDKISLAEETGLDQKQINNWFINQRKRHWKPS
Maize	KNOX10	SRKKKKGKLPRDARQKLLHWWQLHYRWPYPSELEKAALAESTGLEAKQINNWFINQRKRHWKQA
Maize	LG3	LKKRKKGKLPKDARTVLLEWWNTHYRWPYPTEEDKVRLAAMTGLDPKQINNWFINQRKRHWKPS
Maize	KNOX5	LKKRKKGKLPKDARSALMDWWNTHYRWPYPTEEDKVRLAAMTGLDPKQINNWFINQRKRHWKPS
Maize	KNOX11	LKKRKKGKLPKDARSALMDWWNTHYRWPYPTEEDKVRLAAATGLDPKQINNWFINQRKRHWKPS
	Class 2	
Maize	KNOX1	LRKRRAGKLPGDTTSILKQWWQEHSKWPYPTEDDKAKLVEETGLQLKQINNWFINQRKRNWHNN
Maize	KNOX2	LRKRRAGKLPGDTASTLKAWWQAHSKWPYPTEEDKARLVQETGLQLKQINNWFINQRKRNWHNN
Maize	KNOX6	MRKRRAGKLPGDTASVLKAWWQAHSKWPYPTEDDKARLVQETGLQLKQINNWFINQRKRNWHSN
Maize	KNOX7	MRKRRAGKLPGDTASVLKAWWQAHSKWPYPTEDDKARLVQETGLQLKQINNWFINQRKRNWHSN
B. napus	BNHD1	MRKRRAGKLPGDTTTVLKNWWQQHCKWPYPTEDDKAKLVEETGLQLKQINNWFINQRKRNWHSN
	Other	
Arabidopsis	ATH1	QIWRPQRGLPEKSVSVLRNWMFQNFLHPYPKDSEKHLLAIRSGLTRSQVSNWFINARVRLWKPM
Human	PNX-1	LPKHATNVMRSWLFQHIGHPYPTEDEKKQIAAQTNLTLLQVNNWFINARRRILQPM



Homeobox proteins has a 60 amino acids homeobox

Some Tx factors do not have DNA-binding domain

(1) RNA Pol II

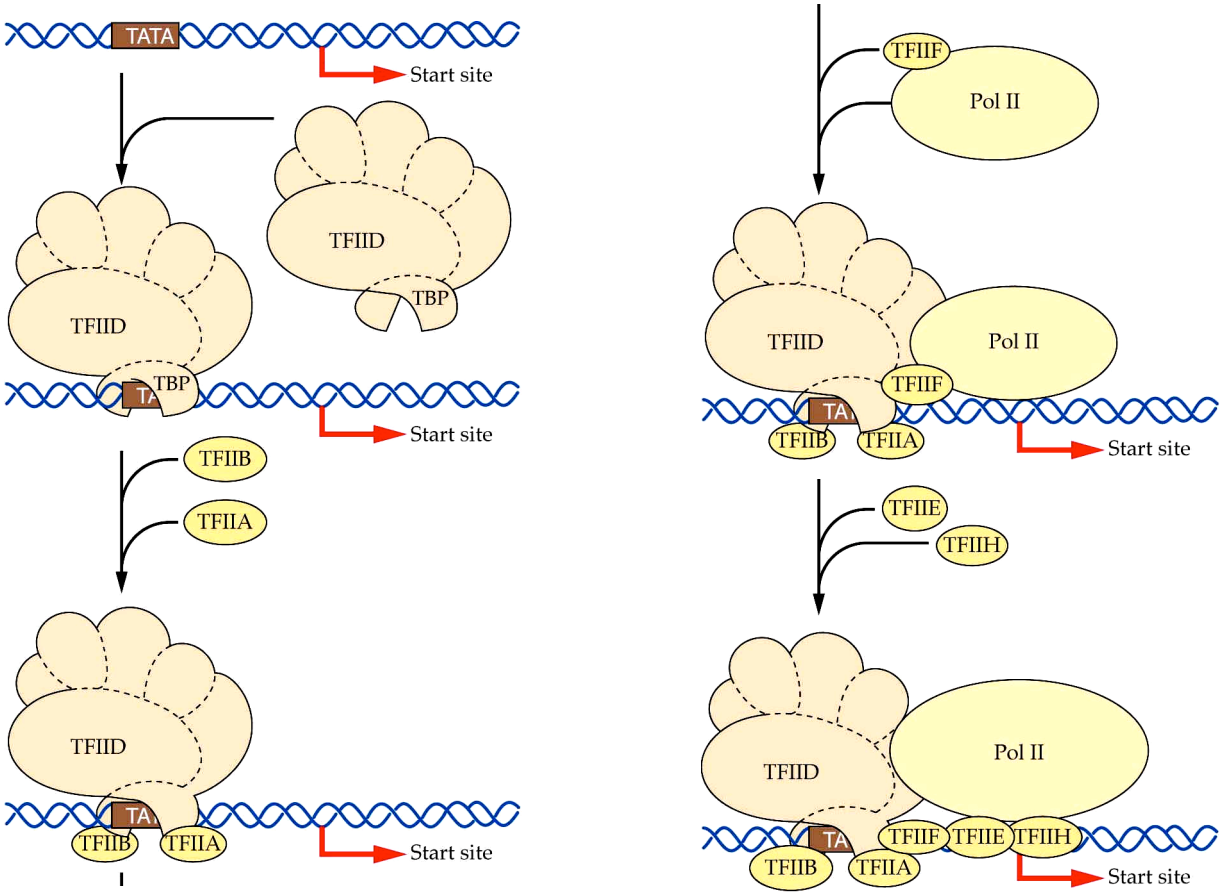


Fig. 7.45

(2) Transcription co-repressors Tup1

The repression of a-cell-specific genes in *S. cerevisiae* a cells