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 transposable elements:

**Snapdragon**  
Tam1  
Tam3

**Maize**  
Ac/Ds  
*Spm/dSpm*

Fig. 7.33
**Ac**: activator, autonomous, 4.6 kb long, encodes 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

Ac  Ds  C

Normal C gene expressing pigment product

(B) Colorless kernels

Ac activates Ds transposition

Ds can transpose into C

Disrupted (mutant) c gene

(C) Spotted kernels

Ac activates Ds transposition out of c during kernel development

Reversion of c mutation to C

Mutant c gene

Normal C gene

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 \textit{Ac} and \textit{Ds}-GUS T-DNAs

\textbf{a. Transposon T-DNAs}

\begin{tabular}{lll}
\textit{Ds} 1, 4: & 325 bp & 360 bp & 466 bp \\
\textit{Ds} 2, 5: & 378 bp & 281 bp & 466 bp \\
\textit{Ds} 3, 6: & 512 bp & 541 bp & 466 bp \\
\end{tabular}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1}
\caption{The structure of T-DNAs 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 \textit{Ac} element in each \textit{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. \textit{Ds} 1 and 4 contain only the first of the \textit{Ac} element’s 4 transcription start sites, while the others contain all of them with (\textit{Ds} 3 and 6) or without (\textit{Ds} 2 and 4) part of the element’s untranslated leader. \textit{Ds} constructs 4-6 contain the 35S core sequence upstream from the GUS gene, while \textit{Ds} constructs 1-3 do not. Abbreviations: RB: T-DNA right border; LB: T-DNA left border; GUS: \textbeta-glucuronidase; 35S and 19S: the promoters of the CaMV 35S and 19S transcripts; the Nae\textit{Ac} contains the \textOmega sequence of the tobacco mosaic; ALS: chlorsulfuron-resistant acetolactate synthase gene (Haughn et al., Mol.Gen.Genet. 211: 266-271 [1988]); hygro: the bacterial \textit{aph4} gene, confers hygromycin resistance; NPTII: bacterial neomycin phosphotransferase II gene; \textit{tms2}: the agrobacterial \textit{tms2} gene, confers sensitivity to auxin amides; Bam- and Nae-\textit{Ac}: deleted and promoter-substituted \textit{Ac} elements.}
\end{figure}
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

(A) 

- Drought +

(B) 

- ABA +

**Fig. 7.40**

CDeT6-19: the promoter of a drought-responsive gene

ABA: abscisic acid

GUS: $\beta$-glucuronidase
Certain genes are only expressed at specific stages and tissue

WT Arabidopsis flower  ag mutant flower  AG RNA expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>WT Arabidopsis</th>
<th>ag mutant</th>
<th>AG RNA expression</th>
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<td>WUS</td>
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<td>MADS 8</td>
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</tbody>
</table>
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
Homeobox proteins has a 60 amino acids homeobox
Some Tx factors do not have DNA-binding domain

(1) RNA Pol II

Fig. 7.45
The repression of α-cell-specific genes in S. cerevisiae α cells

(2) Transcription co-repressors Tup1

Tup1

Ssn6

2 MCM1

a-cell specific genes

1 72 706

Tup1 Ssn6 Binding Q-rich (97-118, 181-198) 7 WD