

PLNT2530 Plant Biotechnology
2025
Unit 8c

Factors Affecting Expression of Transgenes

Factors Affecting the Expression of Transgenes

It is often observed that transgenes become incorporated into chromosomes, but fail to express. This is referred to as **gene silencing**.

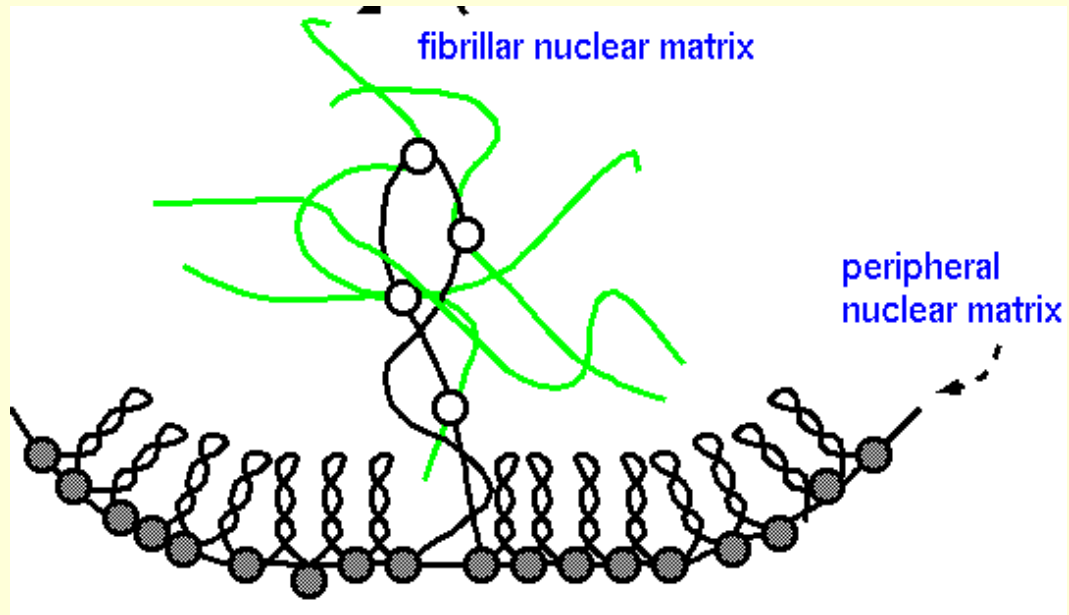
Two categories:

TGS - transcriptional gene silencing

PTGS - post-transcriptional gene silencing

Background - Chromatin Domains

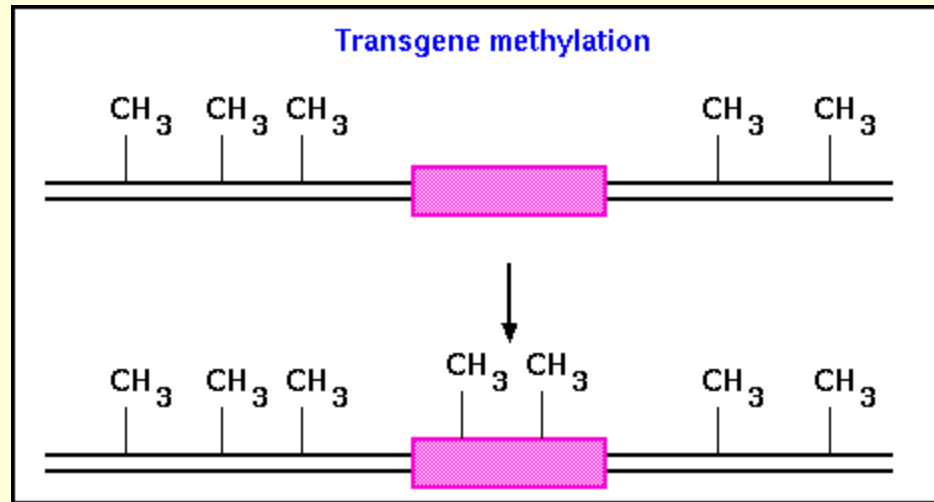
- Eukaryotic chromosomes are linear DNA molecules complexed with histone proteins. The protein/DNA complexes are referred to as **chromatin**.



- The nuclear matrix is a network of proteins on the inner surface of the nucleus, and in the interior.
- Chromatin is covalently attached to the peripheral nuclear matrix on the average of every 50 kb. The chromatin loops formed by these attachments are referred to as “domains”.
- Domains can coil and uncoil independently. Tightly coiled domains are not available for transcription. Domains that are extended into the interior associate with the fibrillar nuclear matrix, which contains the proteins needed for transcription. In this way, the cell can control which genes are available for transcription by coiling or uncoiling individual domains.

Background - Methylation of chromatin

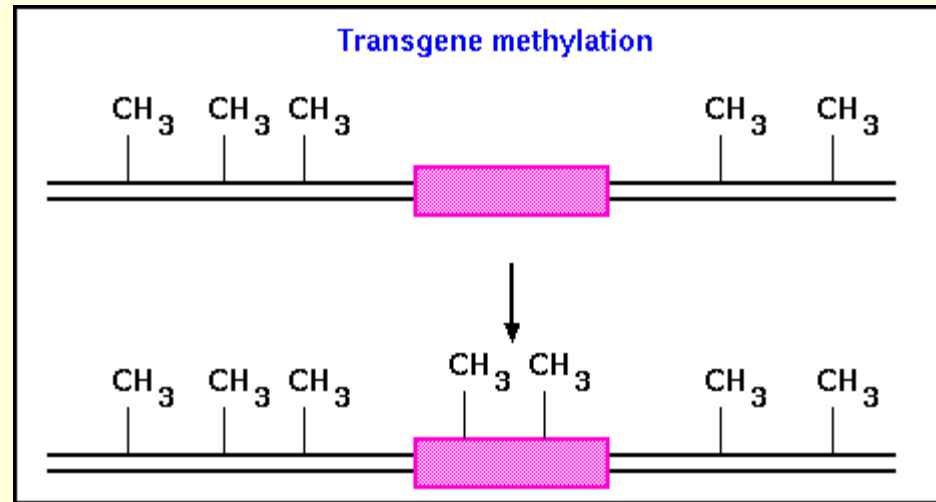
- Eukaryotes inactivate specific genes by methylation of cytosine residues, especially CpG.



- In plants, methylation tends to occur at CpG, CpHpG, and CpHpH, where H is any nucleotide other than G.
- By preventing transcription, methylation acts as a mechanism for selectively down-regulating genes.
- In embryos, methylation is often reversed. Subsequently during development, certain genes are methylated, and are no longer expressed in the adult.
- Methylation patterns are often inherited from one generation to the next.

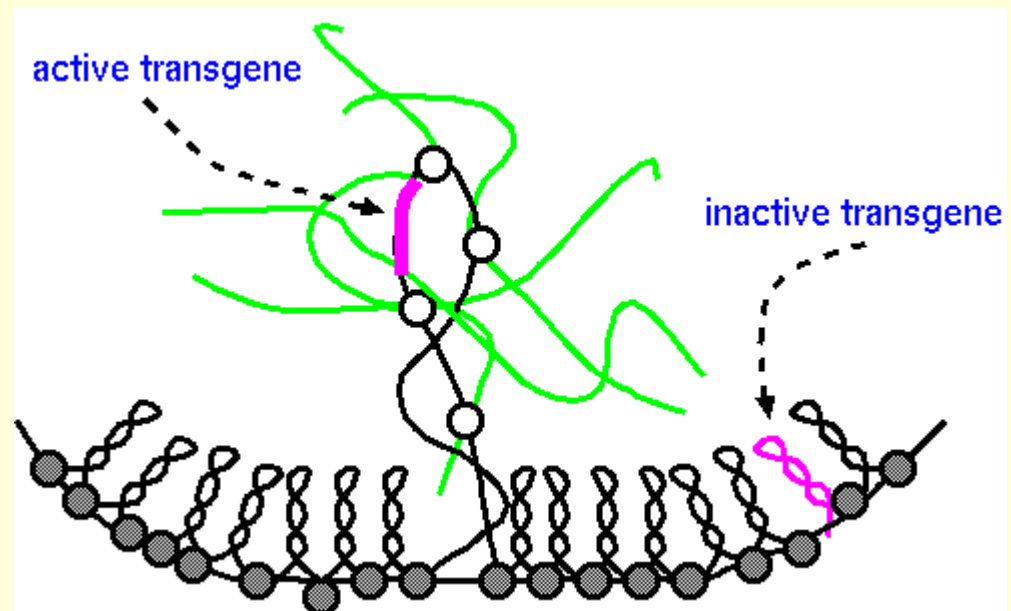
Transcriptional Gene Silencing (TGS)

Genes that insert into hypermethylated regions will usually become methylated, and thus inactive



Transgenes that insert into active chromatin domains will be expressed.

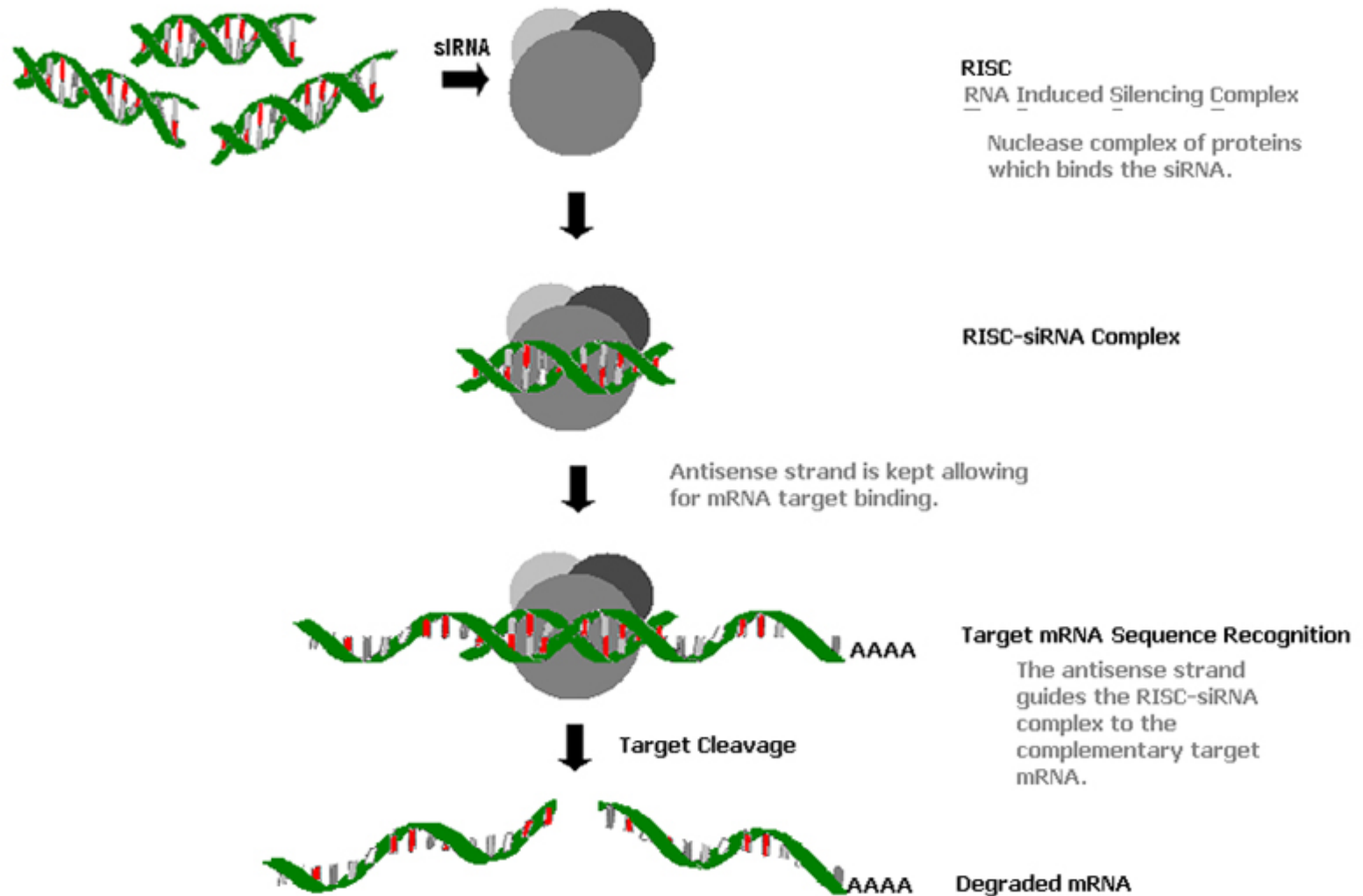
Transgenes that insert into inactive domains are not transcribed.



Post-Transcriptional Gene Silencing (PTGS)

- Found in all eukaryotes
- Mediated by small interference RNAs (siRNA)
- siRNAs are typically 21 - 24 nt in length
- Can be mediated by sense or antisense transcripts, or by double-stranded RNA
- Typically transmissible intercellularly. In plants, PTGS can be graft-transmissible
- In nature, PTGS is used by plants to inactivate viruses. It is therefore a natural defense mechanism.
- Foreign mRNAs from transgenes can often inactivate other copies of genes in plants with which the siRNAs share sequence similarity.
- PTGS is a very effective way of inactivating naturally-occurring genes in transgenic plants “gene knockout”.

Post-Transcriptional Gene Silencing (PTGS)



References

Fagard M, Vaucheret H (2000) (Trans)Gene Silencing in Plants:
How Many Mechanisms? *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 51:167-194.