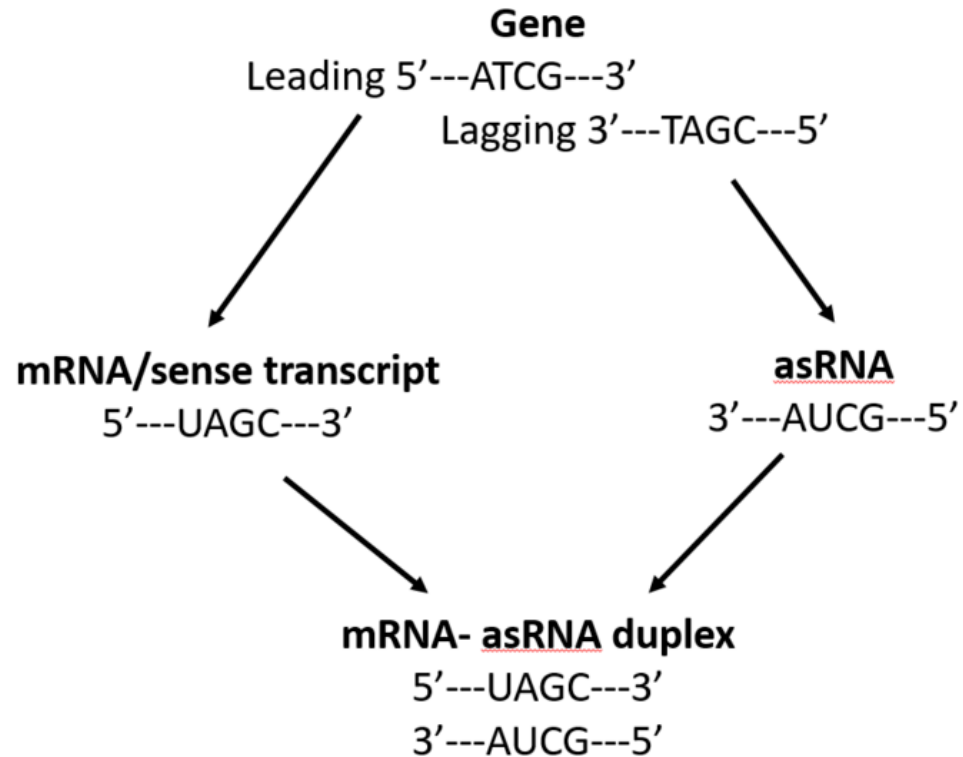


Gene silencing

- Suppression of a gene's expression at transcriptional or translational level
- Gene silencing can be used to study a gene's function by creating a mutant with reduced expression level
- Two methods for gene silencing
 - Antisense RNA
 - Small RNA

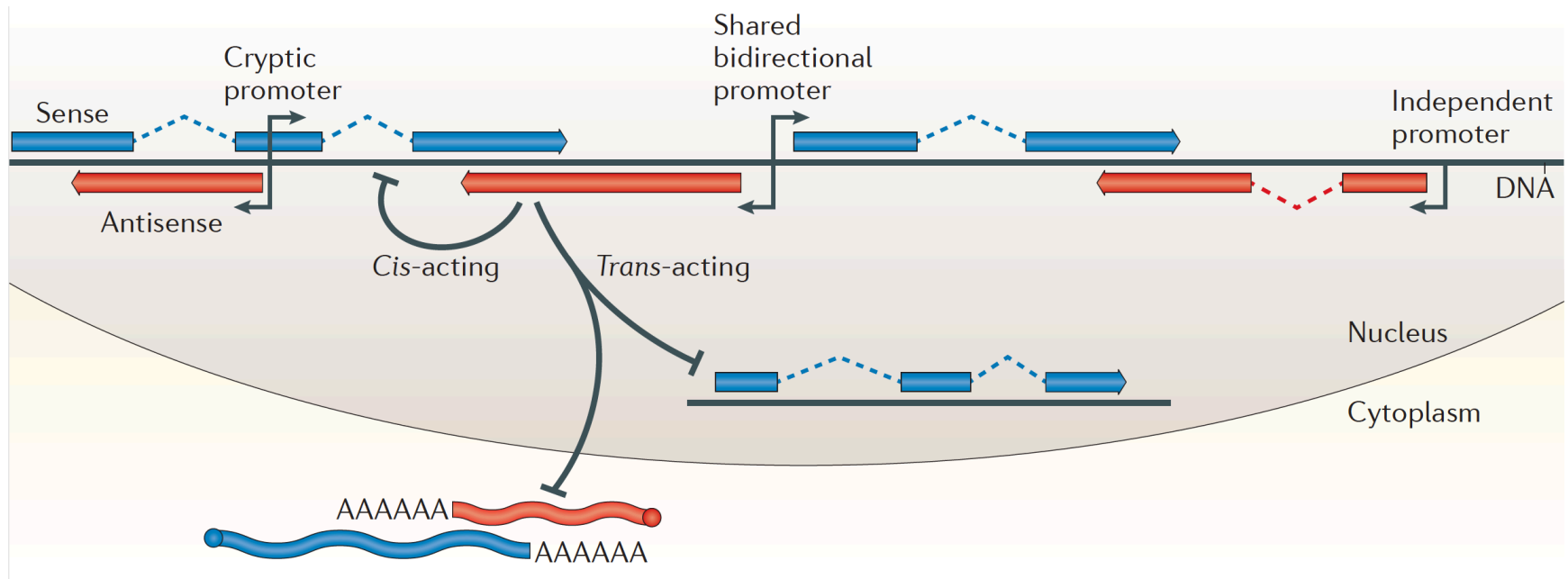
Antisense RNA

- **Antisense RNA (asRNA)** is long noncoding RNA (>200 nt) that does not code any protein
- **asRNA** is transcribed from the lagging strand of a gene and is complementary to a specific mRNA or sense transcript



Antisense RNA regulate gene expression

- Block translation or post-transcriptional regulation
- Block transcription initiation
 - One transcription negatively affects a second one in *cis*
 - Regulate transcription initiation by affecting **DNA methylation**

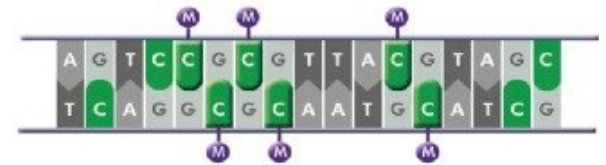


DNA epigenetic changes

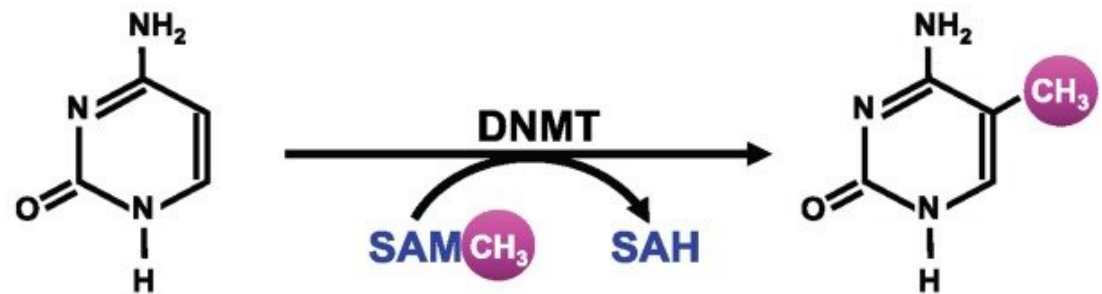
- No changing of DNA sequence
- Modification of DNA physical structure, like **DNA methylation** and histone modification
- Cytosine can be methylated at CG, CHG, and CHH sites

DNA Methylation

Methylating the cytosine of a CpG motif silences genes



DNA
methyltransferase
(DNMT)



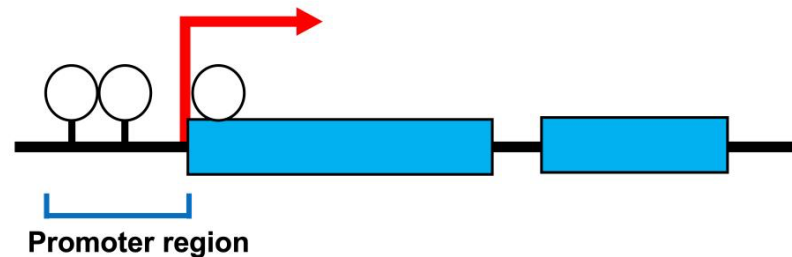
Cytosine

5' Methyl-cytosine

DNA methylation and gene silencing

- Methylation of cytosine in CG-rich regions located in or near gene promoters results in gene silencing and altered phenotype like cancer, stress tolerance, etc.

Genes that can be expressed



Genes inactivated by DNA methylation

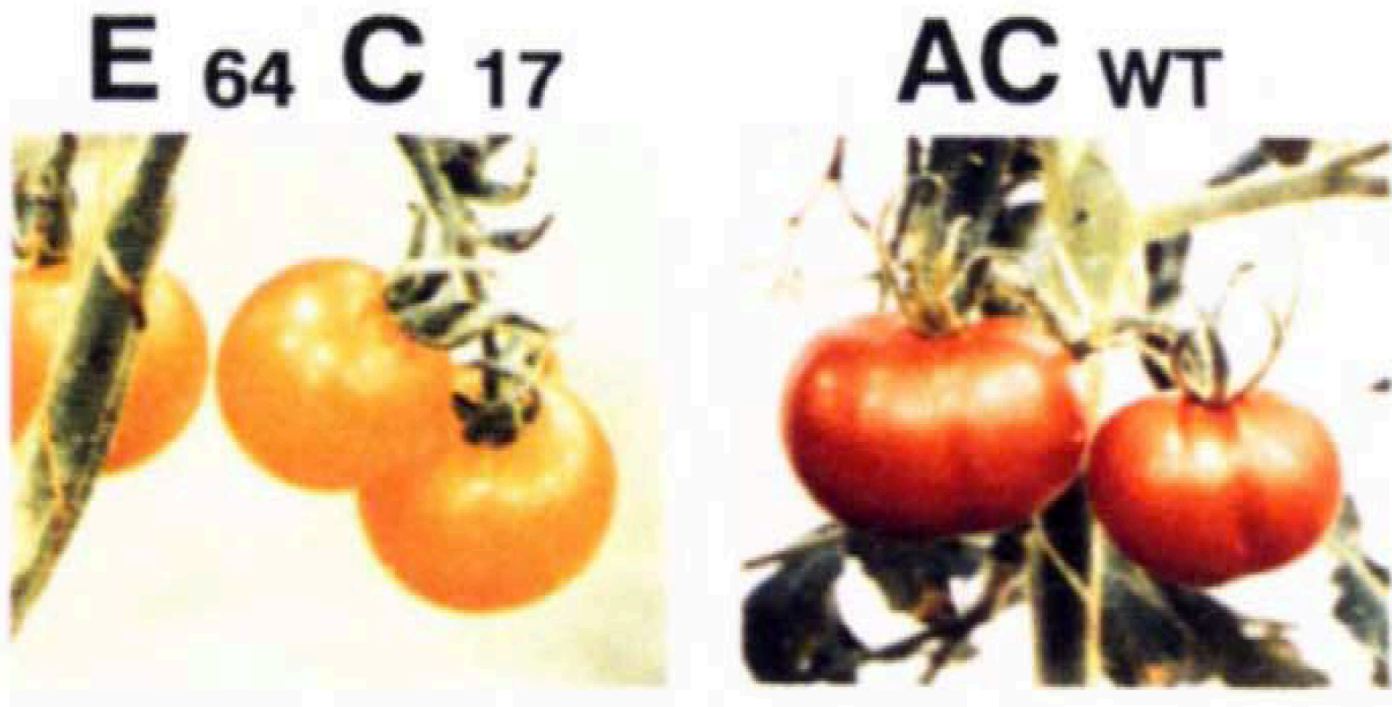


M Methylated

○ Unmethylated

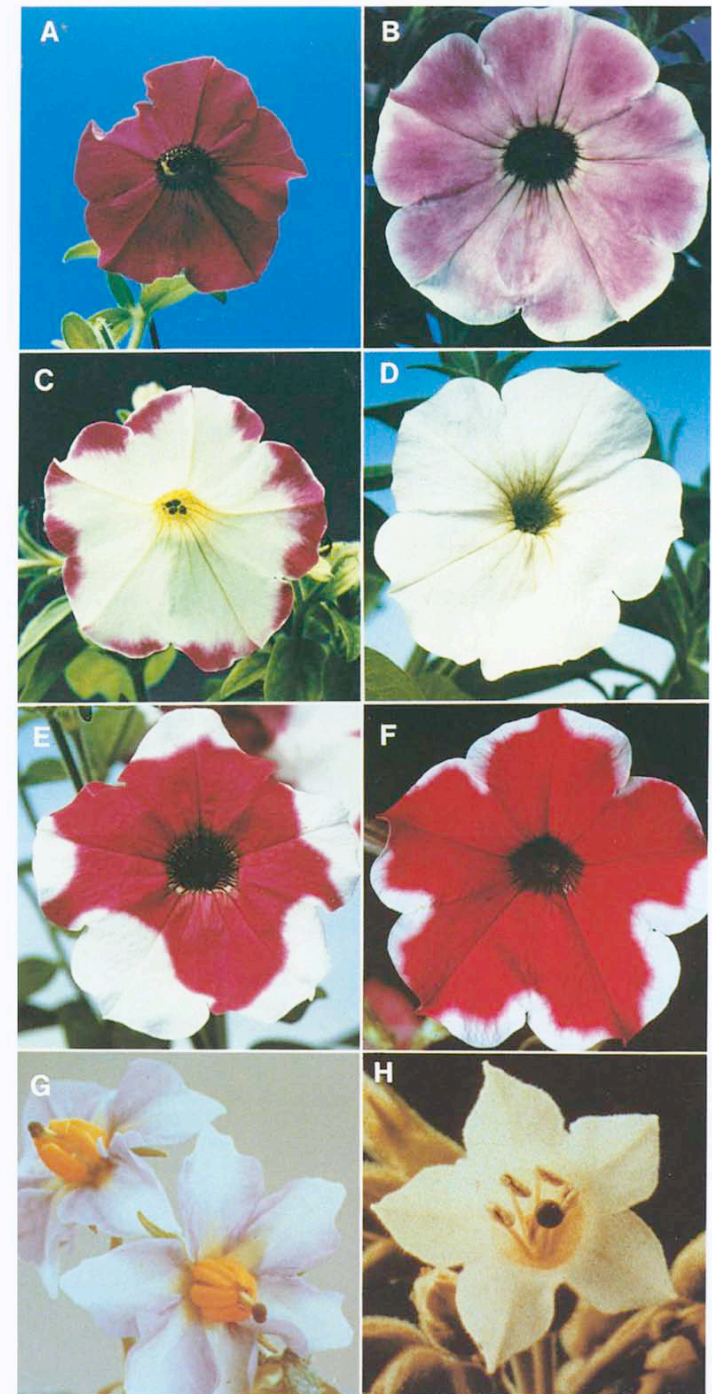
Antisense RNA to validate a gene's function in reverse genetics

- Genetic modified tomato plants expressing antisense RNA to a ripening related gene (pTOM5) have yellow fruit and reduced level of carotenoid



Antisense RNA in transformed ornamental plants

- Different phenotypes were observed in transformed plants, due to effects of promoters, antisense fragments, etc.

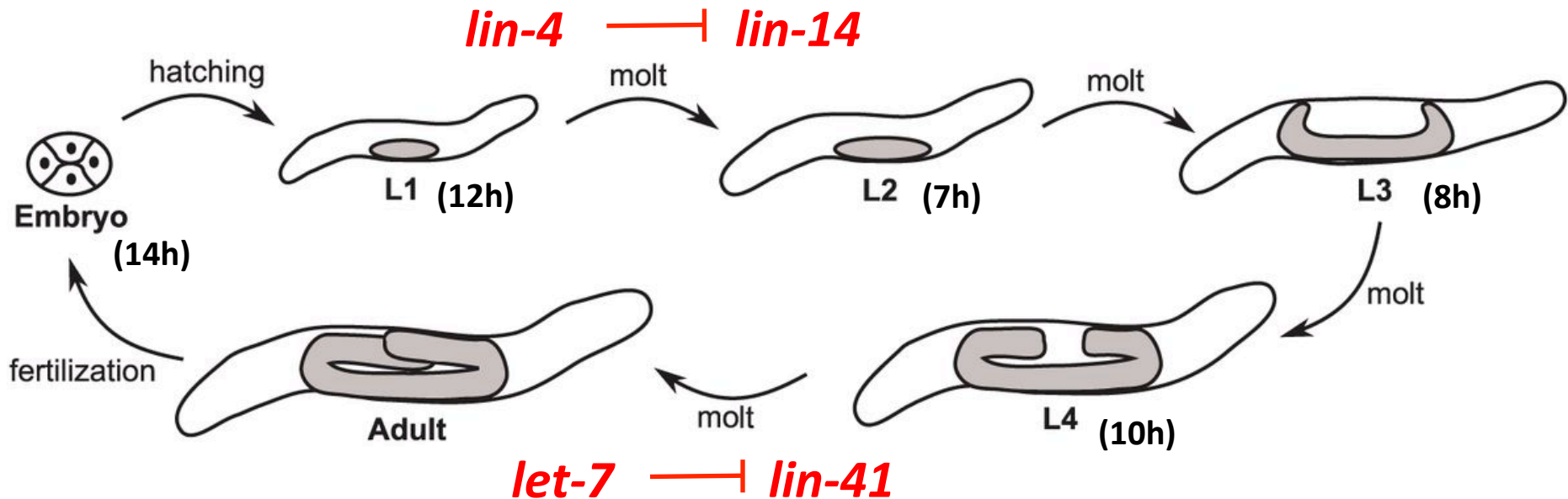


Small RNA

- A short (20-30 nt) RNA that does not code any protein
- Regulate expression of protein-coding genes

First identified small RNA in *C. elegans*

- *lin-4* and *lin-14*, induced mutants, show abnormal larva development from L1 to L2
- *lin-14* is a protein coding gene
- *lin-4* does not encode a protein, but 21 nt of *lin-4* RNA complementary to a repeated sequence element of *lin-14* mRNA

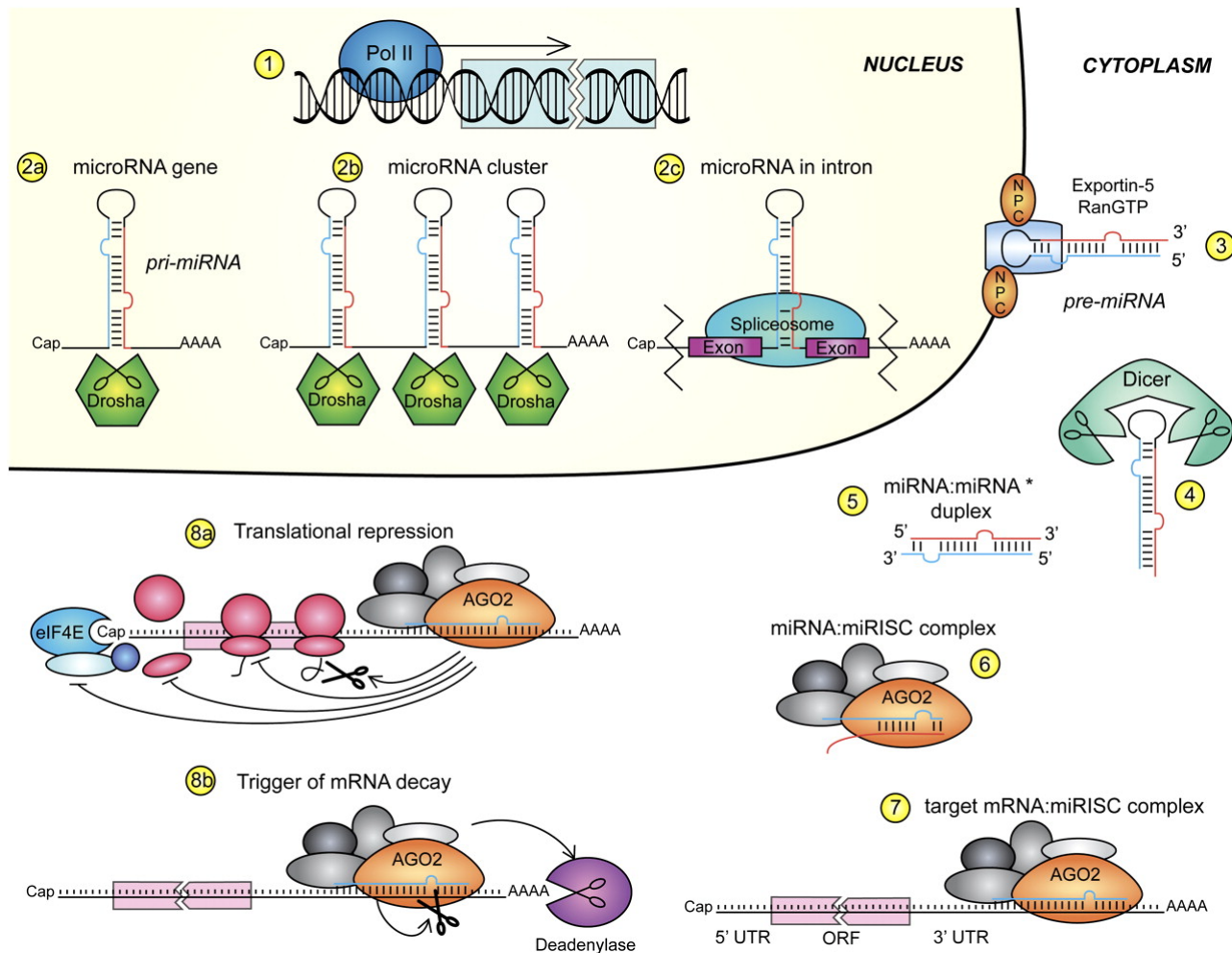


Small RNA identified in diverse species

- Hundreds of small RNAs and families were identified in diverse organisms
- In human, more than 5,300 genes involved in diverse biological functions/processes are targeted/regulated by those small RNAs
 - Cell fate (tissue development)
 - Cancer
- In plants, genes regulated by small RNA are involved in development in plants and abiotic stress

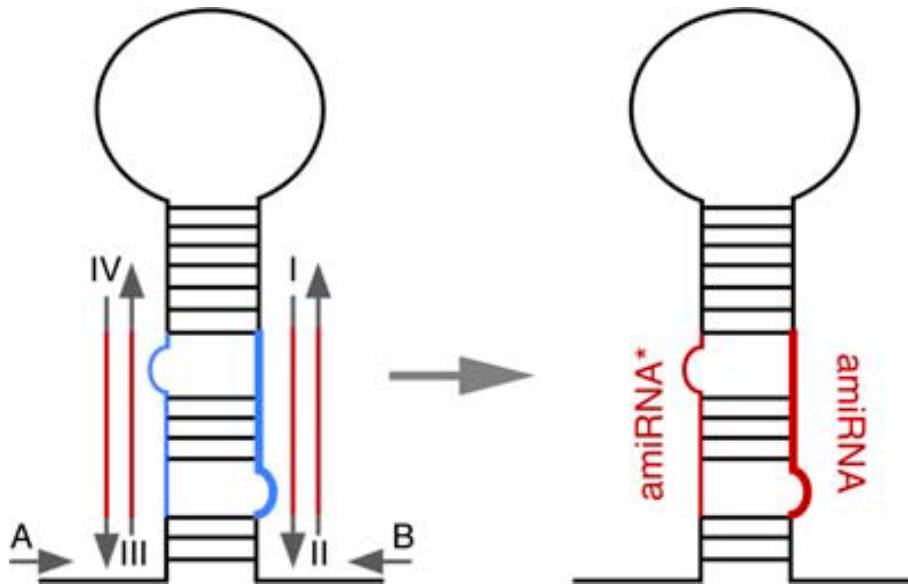
Small RNA regulates gene expression

<https://www.nature.com/news/video-animation-rna-interference-1.9673>



Artificial small RNA as a tool for a candidate gene silencing to study its function

- <http://wmd3.weigelworld.org/cgi-bin/webapp.cgi>
- This tool was initially implemented for *Arabidopsis thaliana*, but has now been extended to >90 additional species



Example 1: Gene silencing of FT using amiRNA

- amiR-ft-1 targets FT, a gene related to promotion of flowering
- Transgenic plants were generated by *Agrobacterium* mediated transformation



Wild type

ft-10

amiR-ft-1

*Example 2: Large-scale RNAi screening uncovers therapeutic targets in the parasite *Schistosoma mansoni**

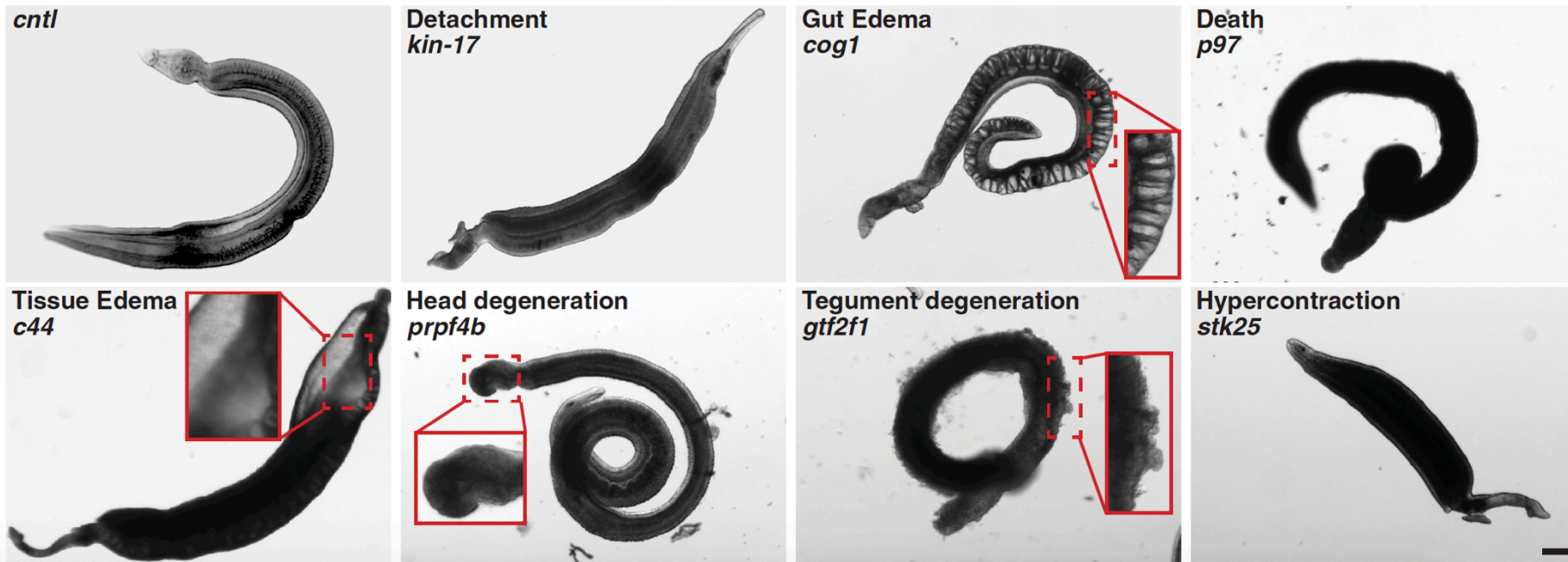
- *Schistosoma mansoni* parasites kill 250,000 people every year
- To discovery of new drug targets, a large-scale RNA interference (RNAi) screen in adult *Schistosoma mansoni* that examined the function of 2216 genes

Large-scale RNAi screening uncovers therapeutic targets in the parasite *Schistosoma mansoni*

- *Schistosoma mansoni* live in the veins (blood vessels) surrounding the host intestines and attach to the vascular endothelium to avoid being swept away in the blood and trapped in host organs
- Parasites attach to the substrate with a combination of their oral and ventral suckers
- **Substrate attachment** affect parasite vitality and can be used to predict in vivo survival

Large-scale RNAi screening uncovers therapeutic targets in the parasite *Schistosoma mansoni*

B



Large-scale RNAi screening uncovers therapeutic targets in the parasite *Schistosoma mansoni*

- 195 genes with fully penetrant attachment phenotypes, of which 121 possessed phenotypes in addition to attachment including tissue and intestinal edema, head and/or tegument degeneration, muscular hypercontraction, and death
- Gene Ontology analyses revealed that the dominant group of genes were those encoding components necessary for protein turnover via the ubiquitin-proteasome system (UPS)

Large-scale RNAi screening uncovers therapeutic targets in the parasite *Schistosoma mansoni*

- Searching literatures and the ChEMBL database to determine if any genes associated with attachment phenotypes encoded proteins targeted by existing **pharmacological agents**
- 205 compounds targeting 49 *S. mansoni* proteins; 14 compounds were examined for the activities on worms cultured in vitro by automated worm movement tracking or on the basis of parasite attachment
- The most potent effects on adult parasites were observed with CB-5083 and NMS-873, which inhibit the UPS component p97, led to death in vitro

What need to know about gene silencing for final exam

- What is gene silencing?
- What is antisense RNA?
- What is small RNA?
- What is DNA epigenetic change?
- How antisense RNA or small RNA regulate gene expression?

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