V12: Cell cycle – summary

(1) **Content of minitest #3:**

- Lecture V9 (slides 1-5),
- V10 (slides 1-13)
- V11 (slides 19-22, 24-25)

- Specified content from Papers 7 to 9:
  methods, results and discussion section related to the indicated figures.

V9: Cellular differentiation - development

In developmental biology, **cellular differentiation** is the process where a cell changes its **cell fate** from one cell type to another. Most commonly the cell changes to a **more specialized type**.

Differentiation occurs numerous times during the development of a multicellular organism as it changes from a simple zygote to a complex system of tissues and cell types.

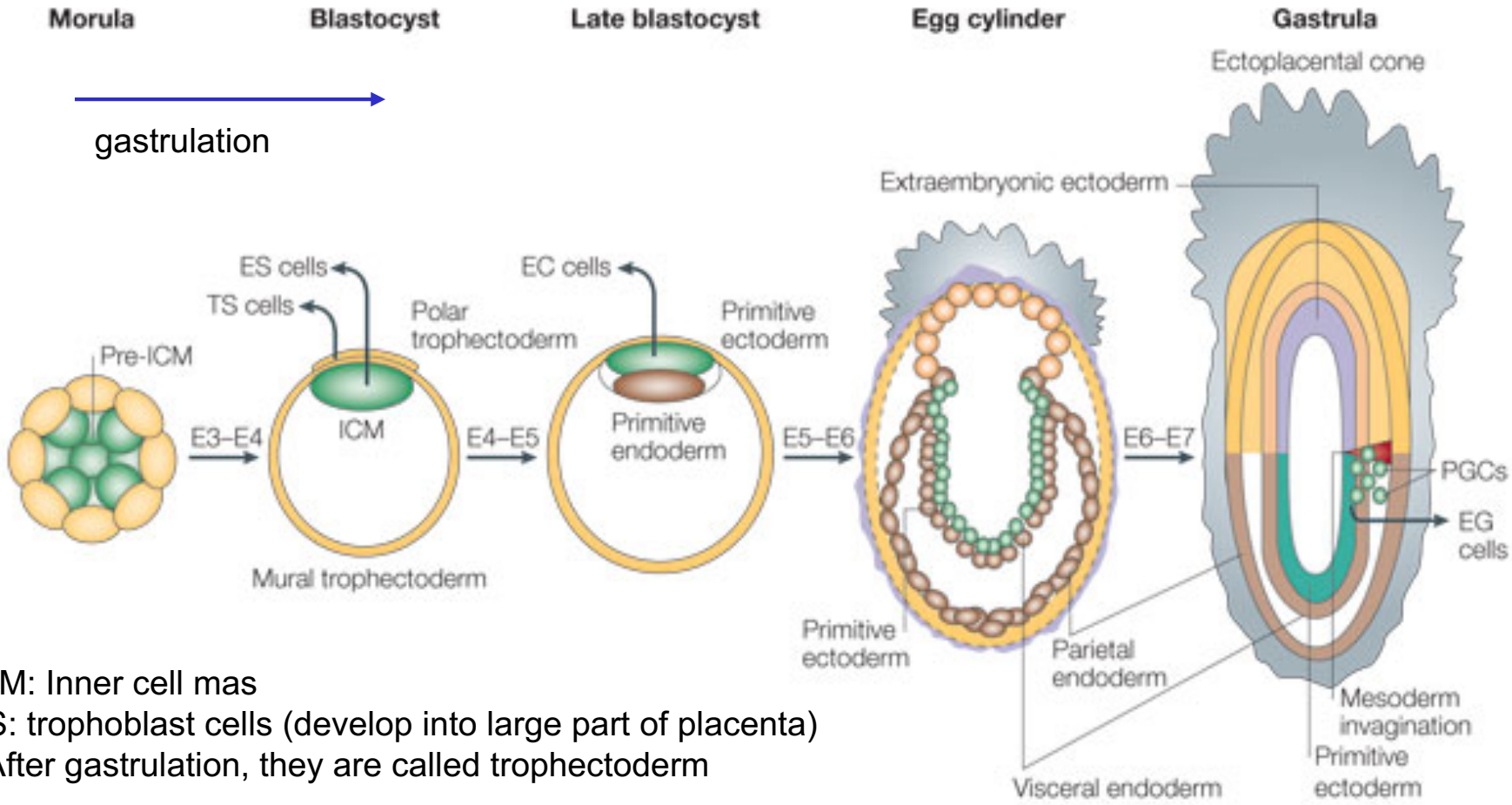
Differentiation continues in **adulthood** as adult stem cells divide and create fully differentiated daughter cells during tissue repair and during normal cell turnover.

Differentiation dramatically changes a cell's size, shape, membrane potential, metabolic activity, and responsiveness to signals.

These changes are largely due to highly controlled modifications in gene expression that are often controlled by **epigenetic** effects.

www.wikipedia.org

Embryonic development of mouse

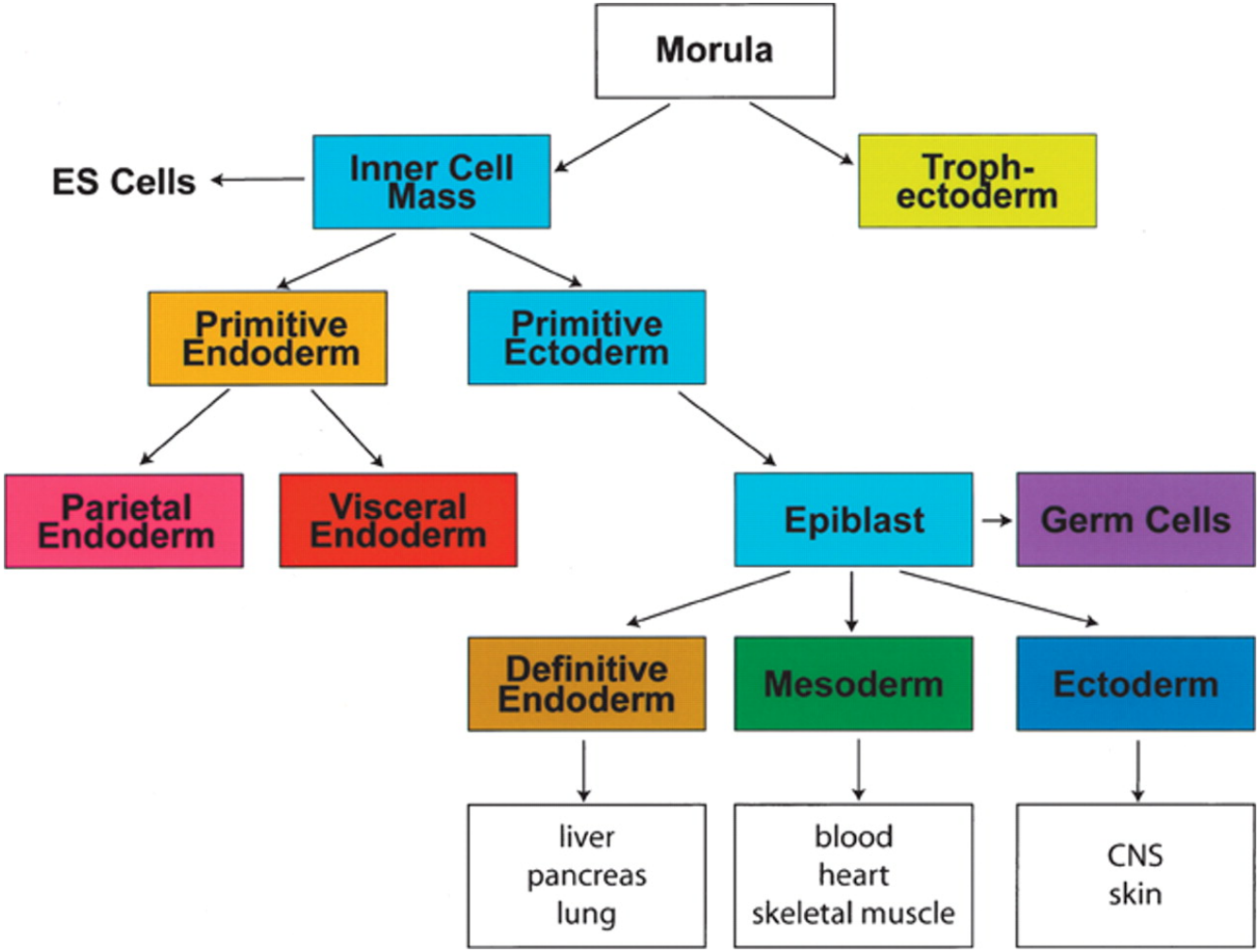


ICM: Inner cell mas
TS: trophoblast cells (develop into large part of placenta)
- After gastrulation, they are called trophectoderm

PGCs: primordial germ cells (progenitors of germ cells)
E3: embryonic day 3

Boiani & Schöler, Nat Rev Mol Cell Biol 6, 872 (2005)

Cell populations in early mouse development



Scheme of **early mouse development** depicting the relationship of early cell populations to the primary germ layers

Keller, Genes & Dev. (2005) 19: 1129-1155

Types of body cells

3 basic categories of cells make up the mammalian body:

germ cells (oocytes and sperm cells)
somatic cells, and
stem cells.

Each of the approximately 100 trillion (10^{14}) cells in an adult human has its own copy or copies of the genome except certain cell types, such as red blood cells, that lack nuclei in their fully differentiated state.

Most cells are **diploid**; they have two copies of each chromosome.

Cells differentiate to specialize for different functions.

Somatic cells make up most of the human body, such as skin and muscle cells.

www.wikipedia.org

Development controlled by transcriptional programs

Embryonic development is a complex process that remains to be understood despite knowledge of the complete genome sequences of many species and rapid advances in genomic technologies.

A fundamental question is how the unique gene expression pattern in each cell type is established and maintained during embryogenesis.

It is well accepted that the gene expression program encoded in the genome is executed by **transcription factors** that bind to cis-regulatory sequences and modulate gene expression in response to **environmental cues**.

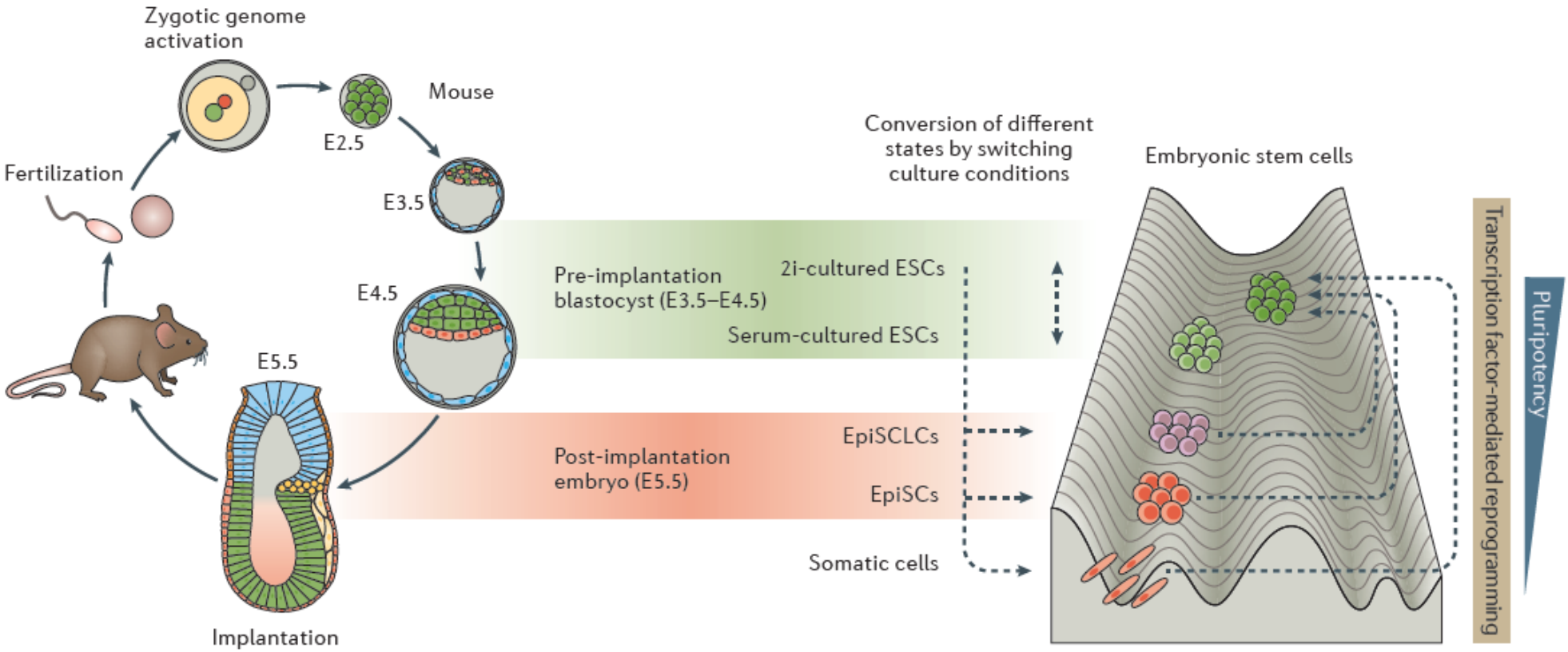
Xie et al., Cell 153, 1134-1148 (2013)

V10: Cellular differentiation - Epigenetics

E4.5 epiblast cells: represent ground-state pluripotency

Implantation: stage of pregnancy at which the blastocyst adheres to the wall of the **uterus**.

After implantation (E5.5): **epiblast cells** undergo a strong wave of epigenetic



Atlasi & Stunnenberg, *Nature Rev Genet* **18**, 643–658 (2017)

Epigenetic mechanisms

Epigenetics refers to **alternate phenotypic states** that are

not based on **differences in genotype**, and are potentially reversible,

but are generally stably maintained during cell division.

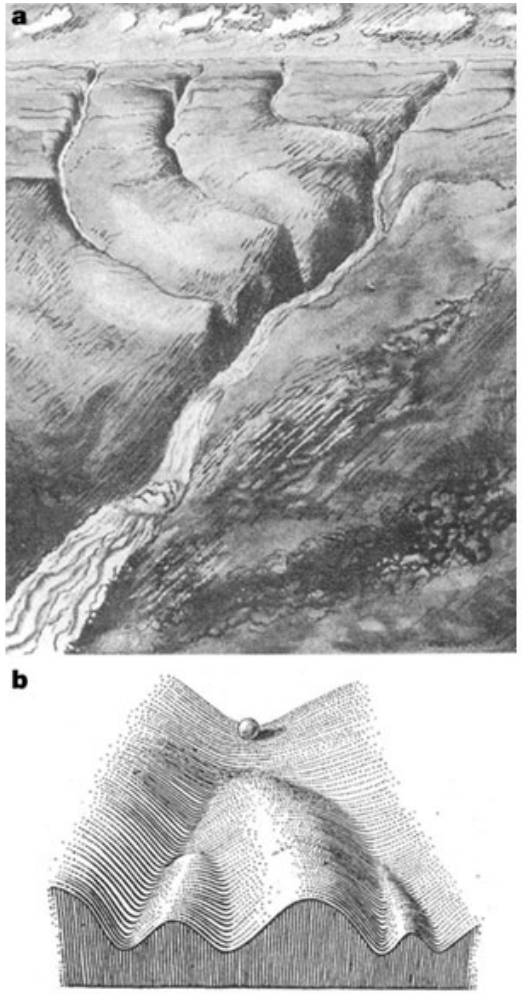
Examples: imprinting, twins, cancer vs. normal cells, differentiation, ...

Multiple mechanisms interact to collectively establish

- alternate states of chromatin structure (open – packed/condensed),
- **histone modifications**,
- composition of associated proteins (e.g. histones),
- transcriptional activity,
- activity of microRNAs, and
- in mammals, **cytosine-5 DNA methylation** at CpG dinucleotides.

Laird, Hum Mol Gen 14, R65 (2005)

Waddington's epigenetic landscape for embryology



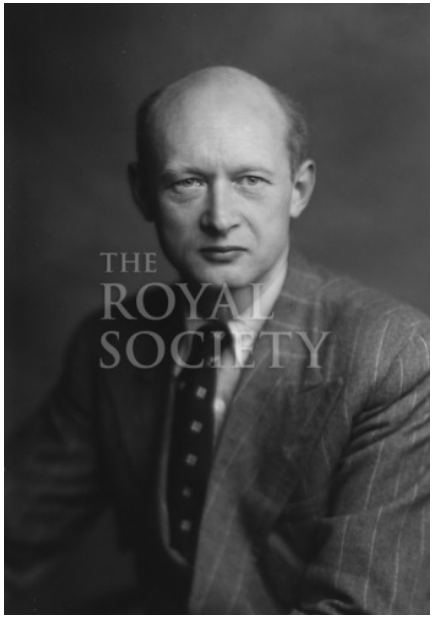
Slack, Nature Rev Genet 3, 889-895 (2002)

Waddington worked in **embryology**

a) is a painting by John Piper that was used as the frontispiece for Waddington's book *Organisers and Genes*.
It represents an epigenetic landscape.

Developmental pathways that could be taken by each cell of the embryo are metaphorically represented by the path taken by water as it flows down the valleys.

b) Later depiction of the epigenetic landscape. The ball represents a cell, and the bifurcating system of valleys represents bundles of trajectories in state space.

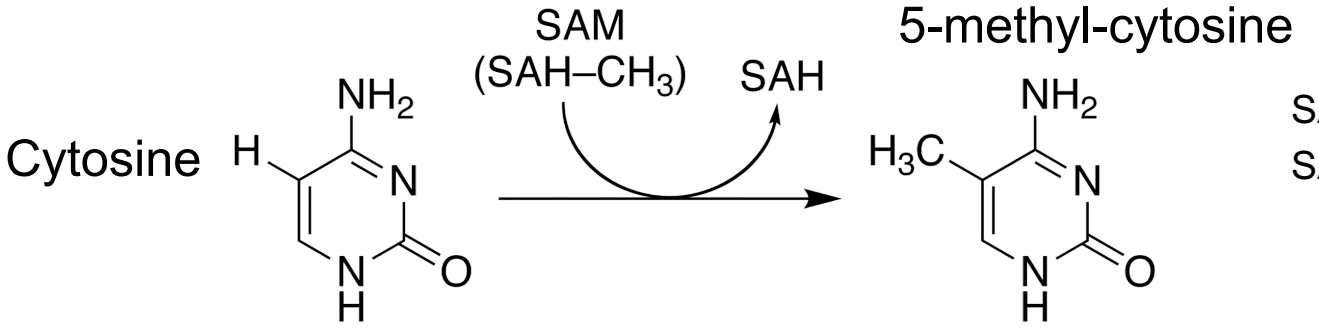


Conrad Hal Waddington (1905 – 1975)
pictures.royalsociety.org

Cytosine methylation

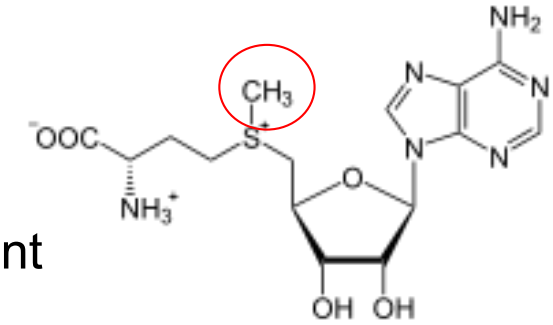
Observation: 3-6 % of all cytosines are methylated in human DNA.

This methylation occurs (almost) exclusively when cytosine is followed by a guanine base -> **CpG dinucleotide**.



SAM: S-adenosyl-methionine

SAH: S-adenosyl-homocysteine



Mammalian genomes contain much fewer (only 20-25 %) of the CpG dinucleotide than is expected by the G+C content (we expect 1/16 ≈ 6% for any random dinucleotide).

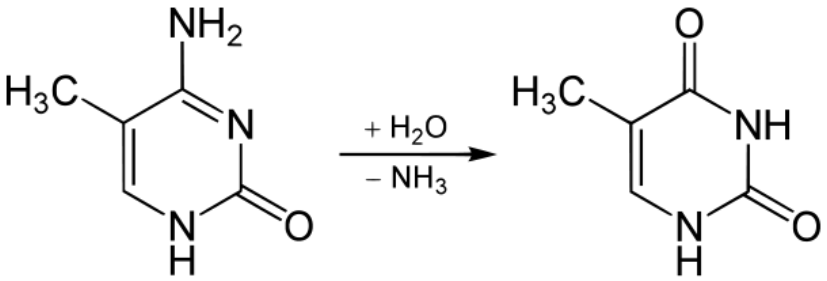
This is typically explained in the following way:

As most CpGs serve as targets of DNA methyltransferases, they are usually methylated …. (see following page)

Esteller, Nat. Rev. Gen. 8, 286 (2007)
www.wikipedia.org

Cytosine methylation

But 5-Methylcytosine can easily **deaminate** to **thymine**.

5-methyl-cytosine 

thymine

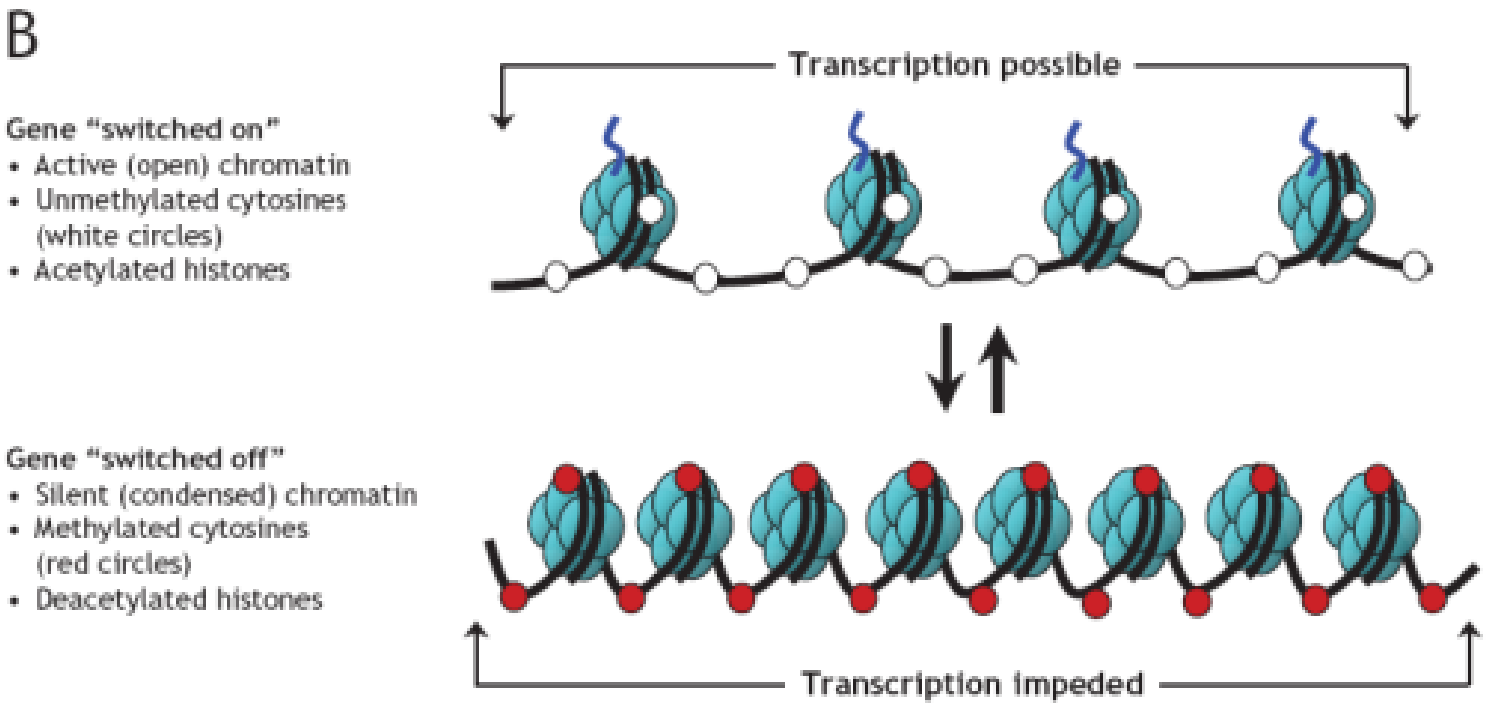
If this mutation is not repaired, the affected CpG is permanently converted to TpG (or CpA if the transition occurs on the reverse DNA strand).

Hence, methylCpGs represent **mutational hot spots** in the genome.
If such mutations occur in the germ line, they become heritable.

A constant loss of CpGs over thousands of generations
can explain the low frequency of this
special dinucleotide in the genomes of human and mouse.

Esteller, Nat. Rev. Gen. 8, 286 (2007)
www.wikipedia.org

chromatin organization affects gene expression



Schematic of the reversible changes in chromatin organization that influence gene expression:

genes are expressed (switched on) when the chromatin is **open** (active), and they are inactivated (switched off) when the chromatin is **condensed** (silent).

White circles = unmethylated cytosines;

red circles = methylated cytosines.

Rodenhiser, Mann, CMAJ 174, 341 (2006)

Altered DNA methylation upon cancerogenesis

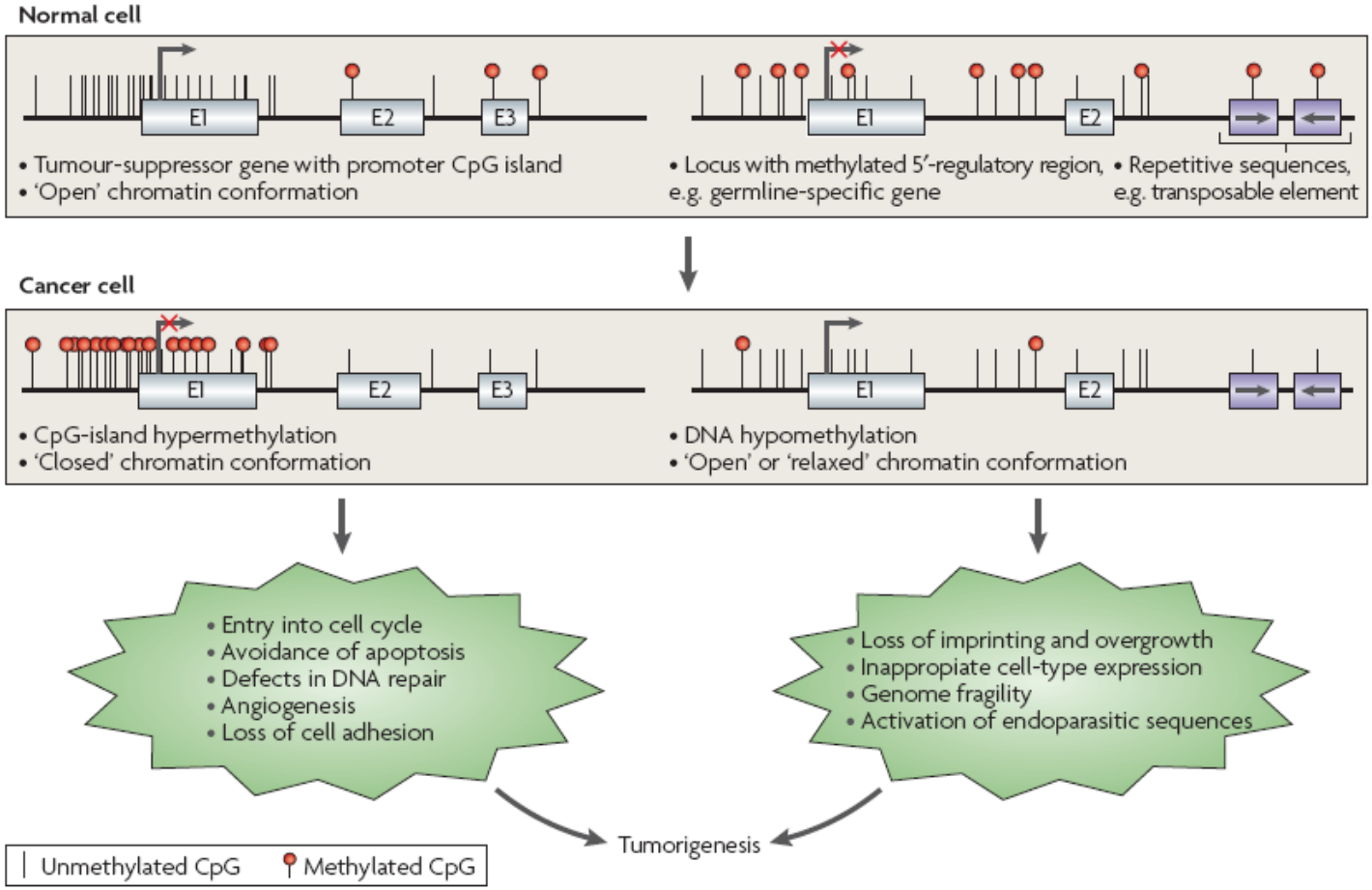


Figure 1 | **Altered DNA-methylation patterns in tumorigenesis.** The hypermethylation of CpG islands of tumour-suppressor genes is a common alteration in cancer cells, and leads to the transcriptional inactivation of these genes and the loss of their normal cellular functions. This contributes to many of the hallmarks of cancer cells. At the same time, the genome of the cancer cell undergoes global hypomethylation at repetitive sequences, and tissue-specific and imprinted genes can also show loss of DNA methylation. In some cases, this hypomethylation is known to contribute to cancer cell phenotypes, causing changes such as loss of imprinting, and might also contribute to the genomic instability that characterizes tumours. E, exon.

Esteller, Nat. Rev. Gen. 8, 286 (2007)

Genomic Imprinting: Mono-allelic expression; one allele (either from the mother or the father) is silenced.

Typically, this is implemented by methylating the silenced allele.

The human genome contains ca. 8% of **retroviral sequences**. Typically, these are also silenced by DNA methylation.

DNA methylation

Typically, unmethylated clusters of CpG pairs are located in
tissue-specific genes and in essential **housekeeping genes.**

(House-keeping genes are involved in routine maintenance roles and are expressed in most tissues.)

These clusters, or **CpG islands**, are targets for proteins
that bind to unmethylated CpGs and initiate gene transcription.

In contrast, **methylated CpGs** are generally associated with silent DNA,
can block methylation-sensitive proteins and can be easily mutated.

The **loss** of normal DNA methylation patterns is the
best understood epigenetic cause of **disease.**

In animal experiments, the removal of genes that encode DNMTs is lethal;
in humans, overexpression of these enzymes has been linked
to a variety of cancers.

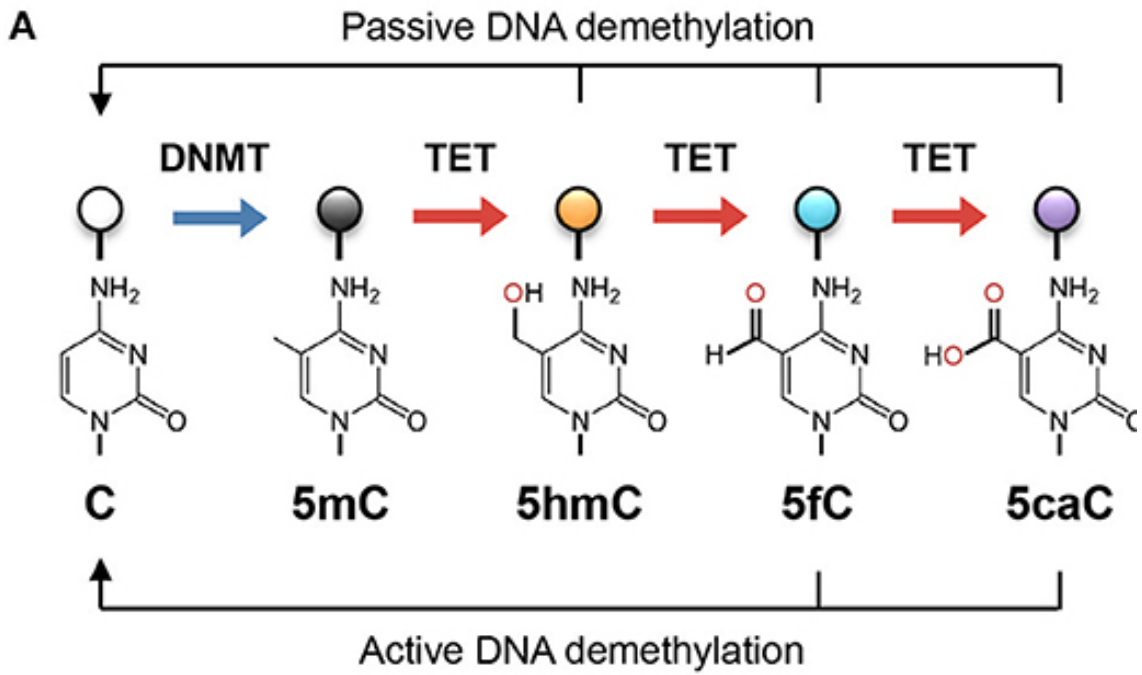
Rodenhiser, Mann, CMAJ 174, 341 (2006)

Higher forms of methylation – Tet enzymes

Unmodified cytosine (C) is methylated by DNA methyltransferases (DNMTs) at the 5 position to become 5-methylcytosine (5mC).

TET proteins oxidize 5mC into 5-hydroxymethylcytosine (5hmC), a stable epigenetic mark, and subsequently to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC).

TET can demethylate DNA via replication-dependent (passive) or replication-independent (active) mechanisms.



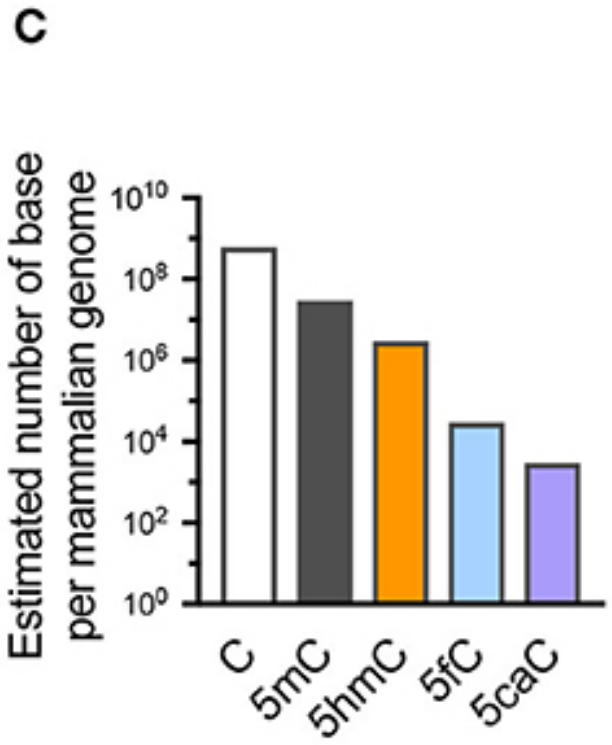
Lio & Rao, Front. Immunol. (2019)

Higher forms of methylation – abundance

The approximate abundance of unmodified and modified cytosines in the haploid human/mouse genome.

About 5% of cytosine is methylated (5mC); in most cells, the vast majority of 5mC is present at CG dinucleotides although it is low at CpG islands.

5hmC amounts to about 1-10% of 5mC (estimated at 10% here as in embryonic stem cells),
while the levels of 5fC and 5caC are each about an order of magnitude lower than the previous oxidative modification.



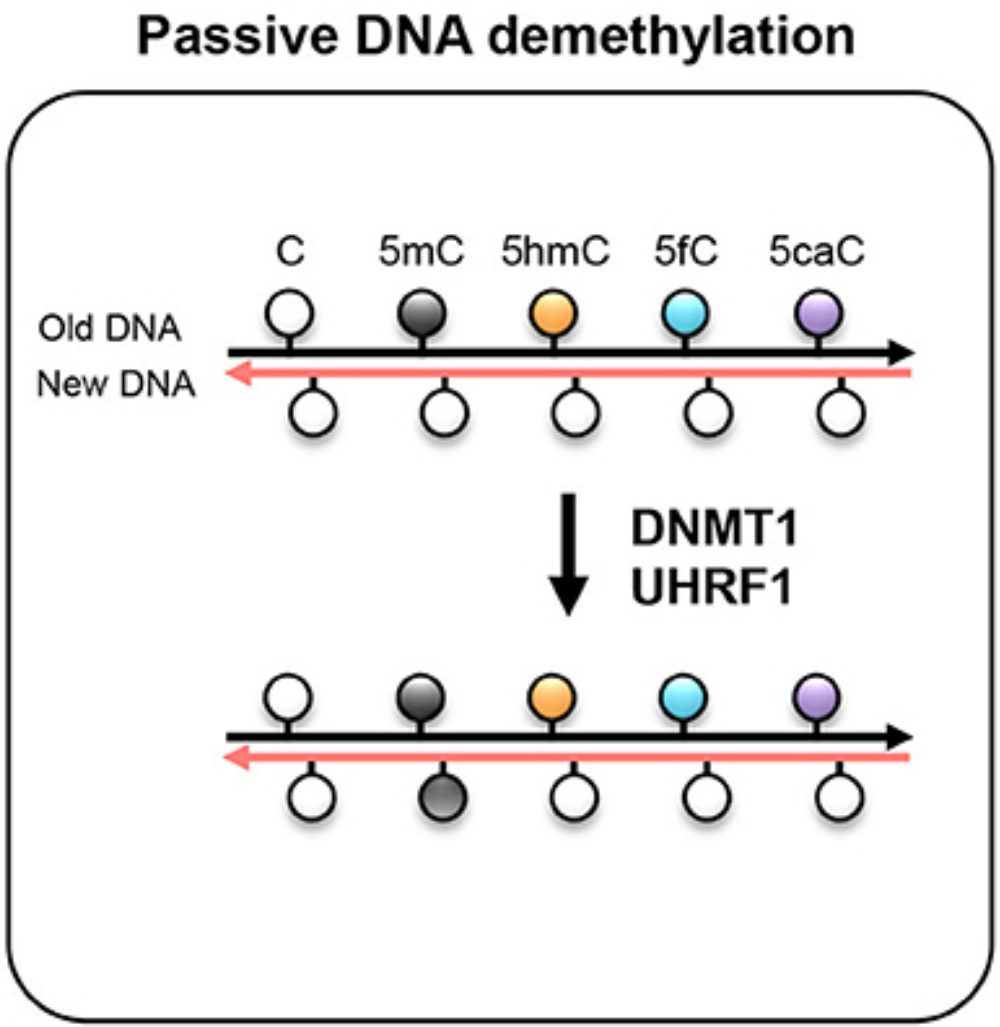
Lio & Rao, Front. Immunol. (2019)

Passive DNA methylation

The DNMT1/UHRF1 complex recognizes 5mC at the hemi-methylated CpG motif during DNA replication and methylates the unmodified cytosine on the newly synthesized DNA strand.

However, the oxidized methylcytosines 5hmC, 5fC, and 5caC are not recognized by DNMT1/UHRF1, resulting in unmodified cytosine on the new DNA strand.

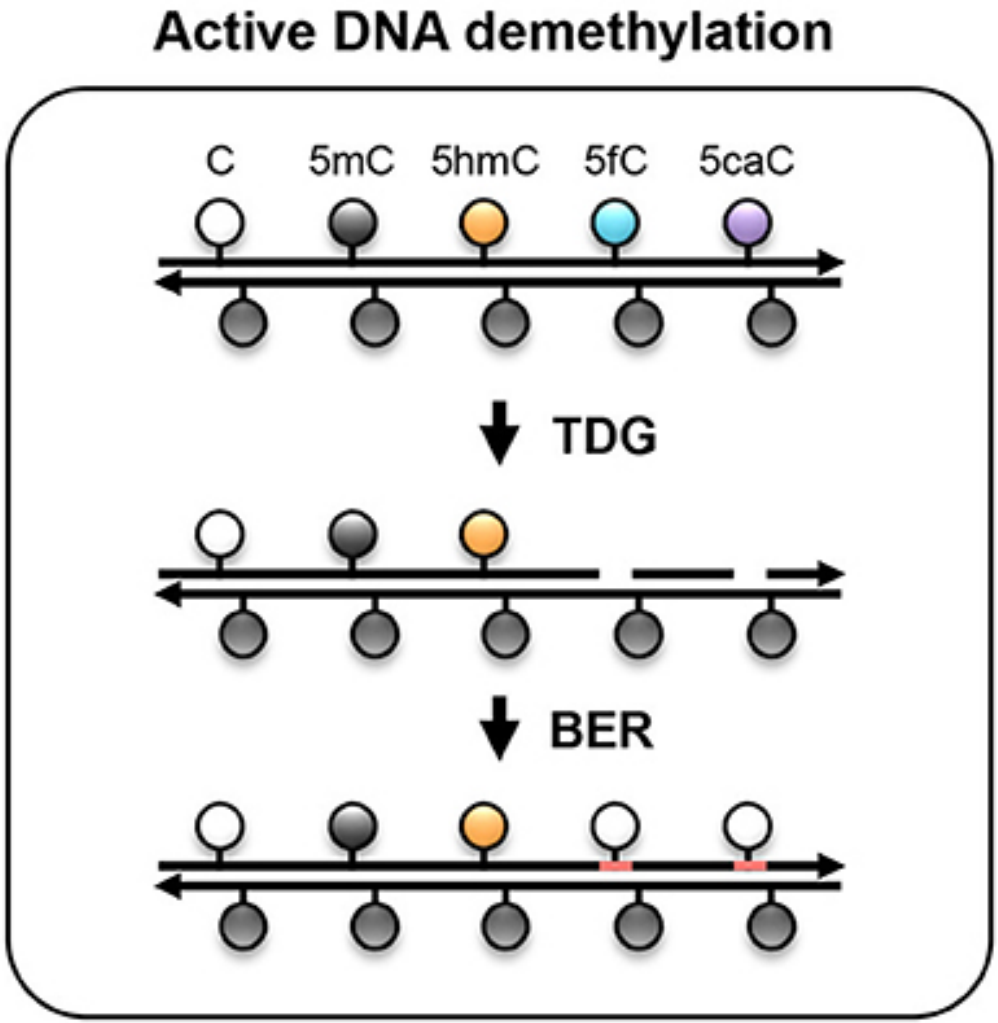
Further DNA replication in the presence of continuing TET activity will result in progressive dilution of 5mC in the daughter cells.



Lio & Rao, Front. Immunol. (2019)

Active DNA methylation

While 5hmC is stable and persists in the genome, 5fC and 5caC can be recognized and **excised** by thymine DNA glycosylase (TDG),
and the resulting abasic sites are repaired as unmodified C by base excision repair (BER).



Lio & Rao, Front. Immunol. (2019)

V11 miRNAs

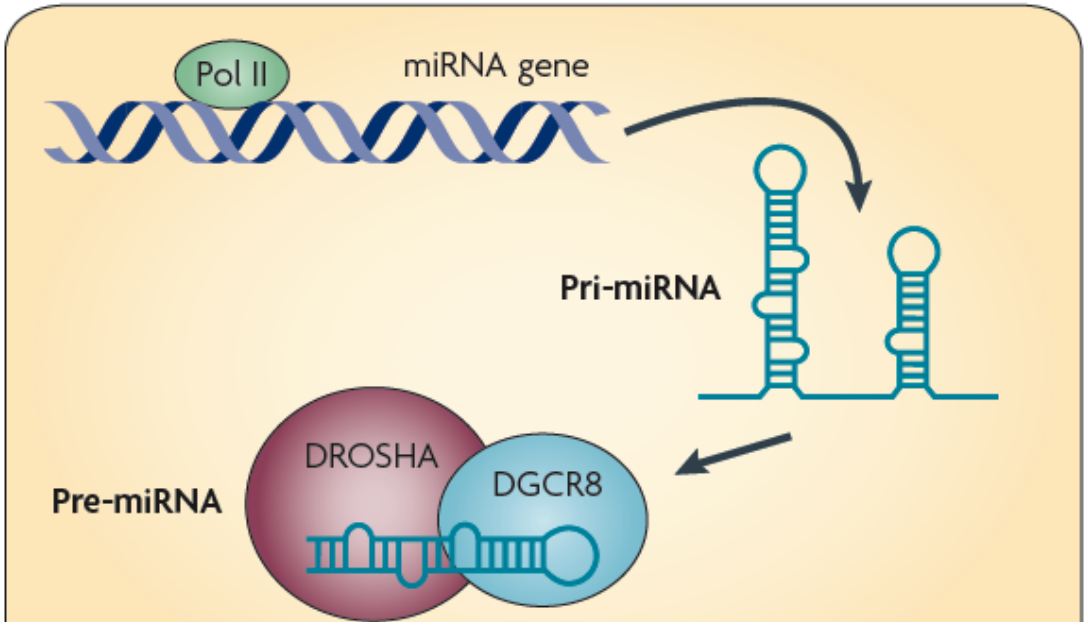
microRNAs (miRNA) are **single-stranded RNA** molecules of 21-23 nucleotides in length.

miRNAs have a crucial role in regulating gene expression.

Remember: miRNAs are encoded by DNA but not translated into protein (non-coding RNA).

www.wikipedia.org

Overview of the miRNA network



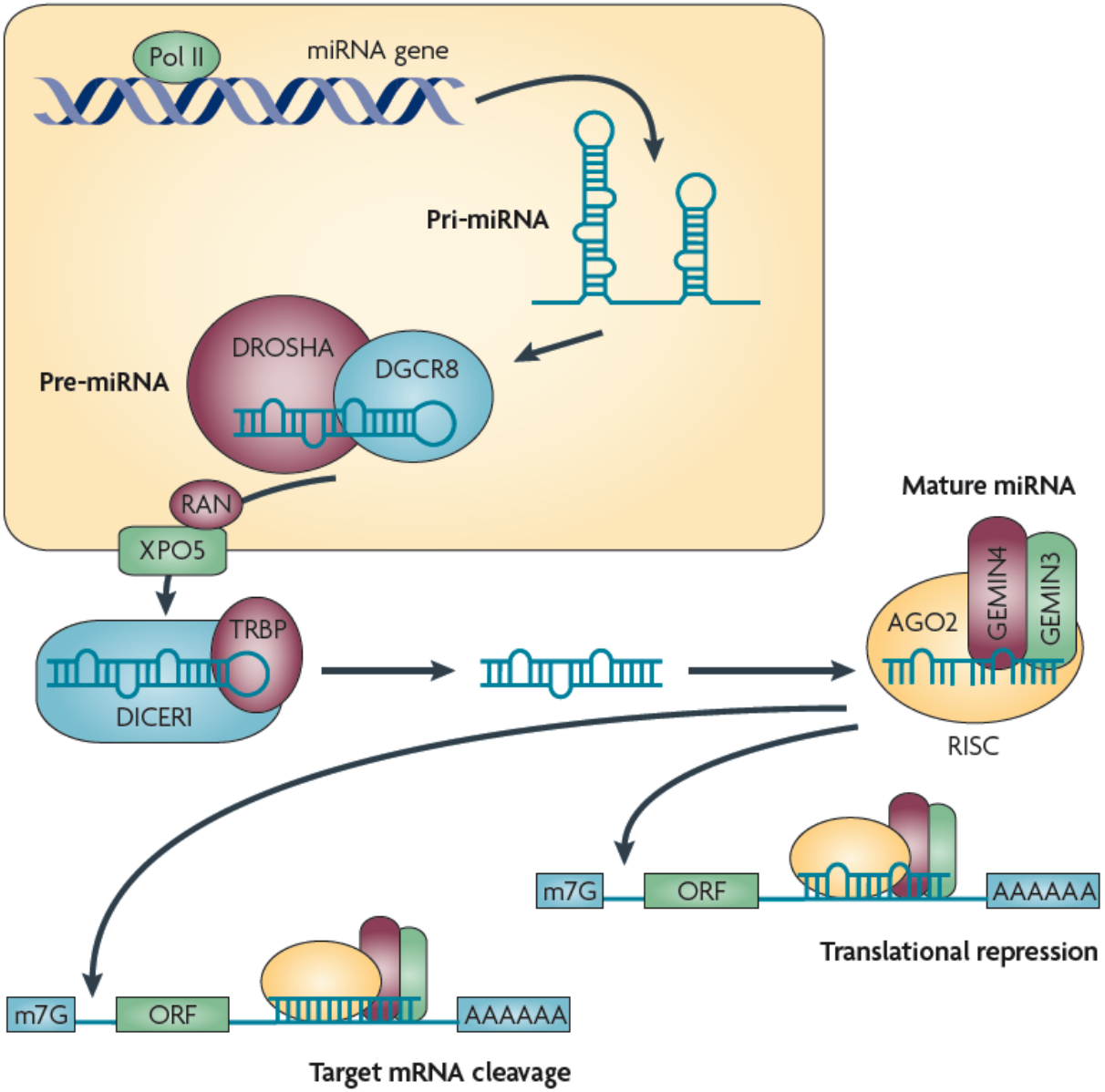
RNA polymerase II (Pol II) produces a 500–3,000 nucleotide transcript, called the primary microRNA **(pri-miRNA)**.

pri-miRNA is then cropped to form a **pre-miRNA** hairpin of ~60–100 nucleotides in length by a multi-protein complex that includes the protein **DROSHA**.

AA, poly A tail;
m7G, 7-methylguanosine cap;
ORF, open reading frame.

Ryan et al. Nature Rev. Cancer (2010) 10, 389

Overview of the miRNA network

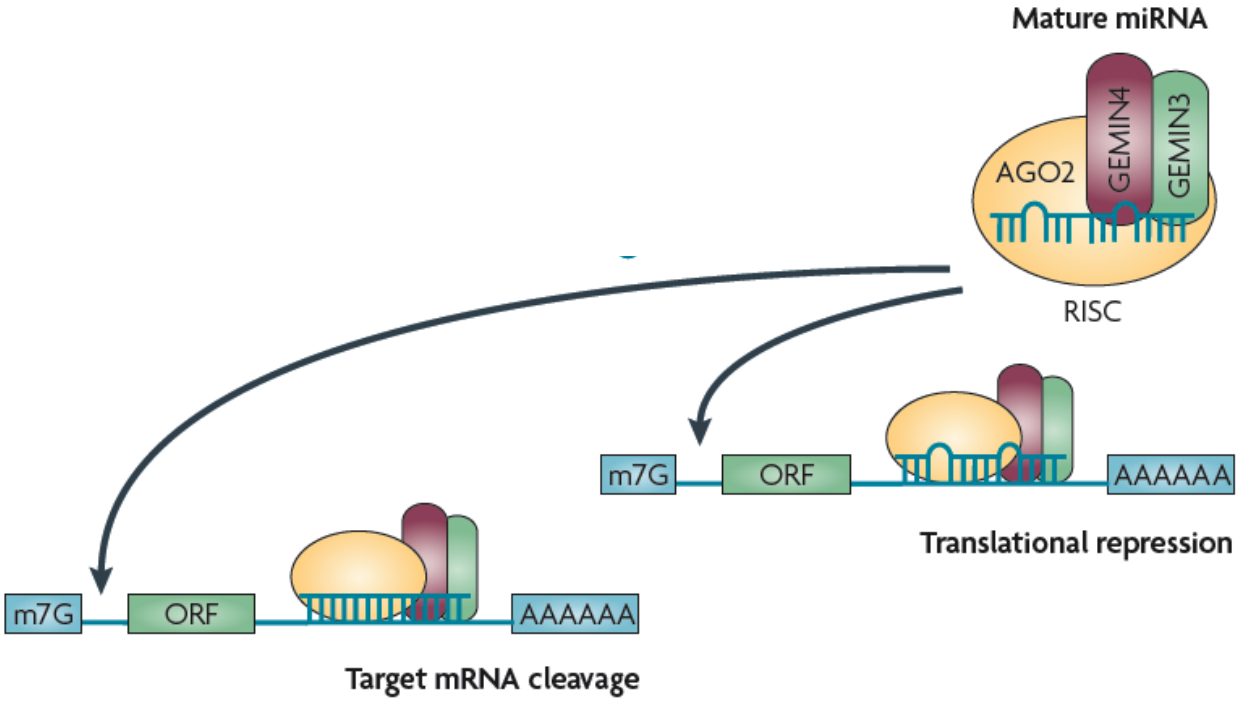


This double-stranded **pre-miRNA** hairpin structure is **exported** from the nucleus by RAN GTPase and exportin 5 (XPO5).

Finally, the pre-miRNA is cleaved by the protein **DICER1** to produce two miRNA strands:
- a mature miRNA sequence, approximately 20 nt in length,
- and its short-lived complementary sequence, which is denoted miR.

Ryan et al. Nature Rev. Cancer (2010) 10, 389

Overview of the miRNA network



The thermodynamic stability of the miRNA duplex termini and the identity of the nucleotides in the 3′ overhang determines which of the single strand miRNA is incorporated into the RNA-inducing silencing complex (**RISC**).

The RISC complex is then targeted by the miRNA to the target 3′ untranslated region of a mRNA sequence to facilitate **repression** and **cleavage**.

The main function of miRNAs is to down-regulate gene expression of their target mRNAs.

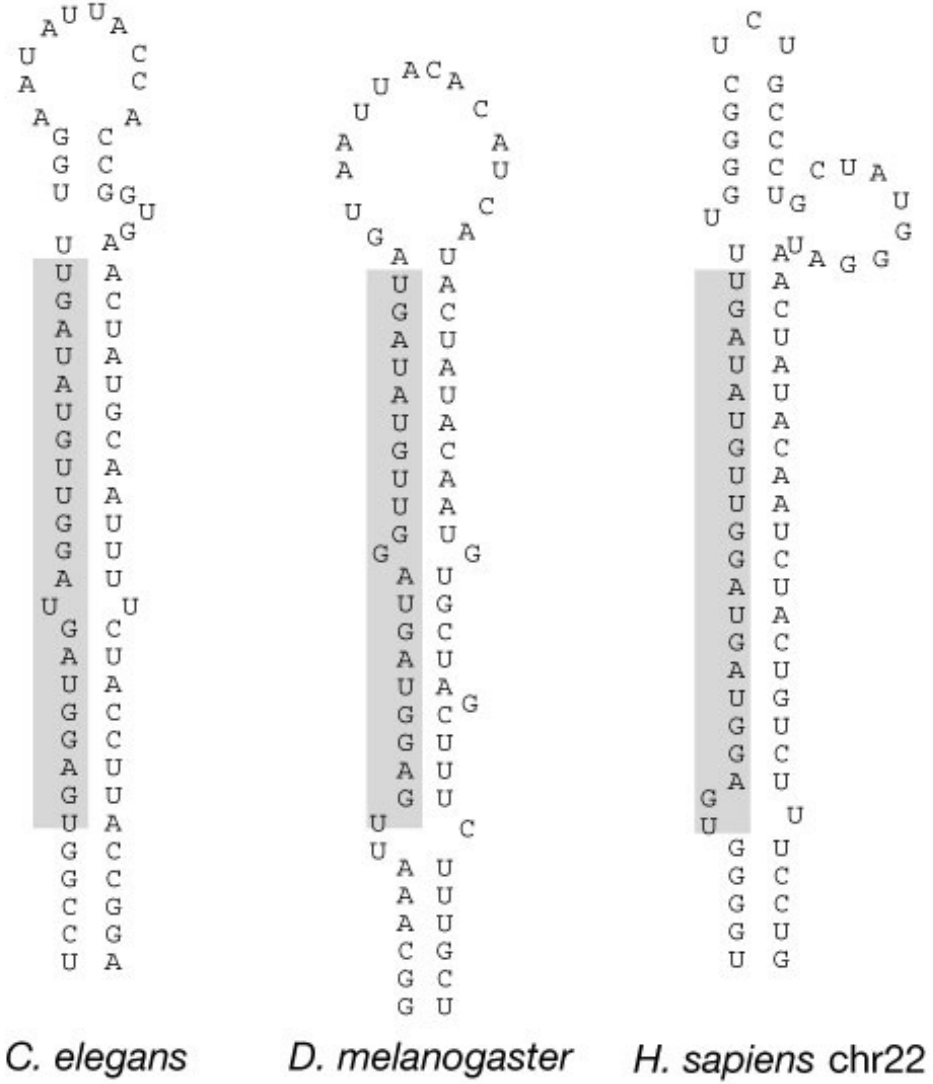
Ryan et al. Nature Rev. Cancer (2010) 10, 389

discovery of let7

The first two known microRNAs, lin-4 and let-7, were originally discovered in the nematode *C. elegans.*
There, they control the timing of stem-cell division and differentiation.

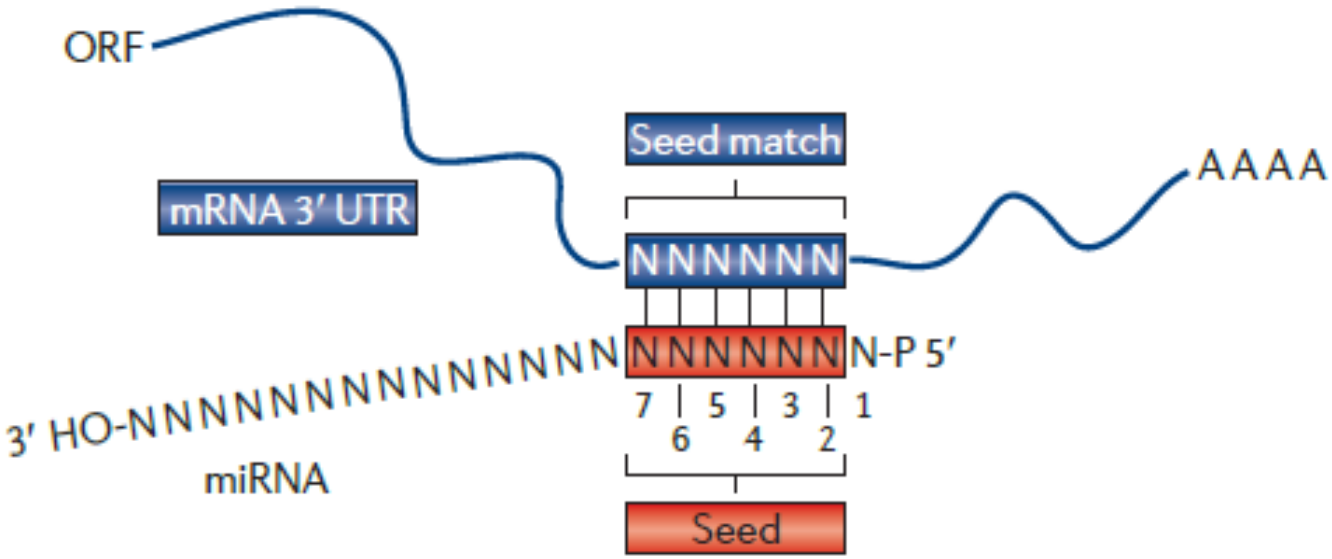
let-7 was subsequently found as the first known human miRNA.

let-7 and its family members are **highly conserved** across species in sequence and function.
Misregulation of let-7 leads to a less differentiated cellular state and the development of cell-based diseases such as cancer.



Pasquinelli et al. Nature (2000) 408, 86
www.wikipedia.org

miRNAs recognize targets by Watson-Crick base pairing



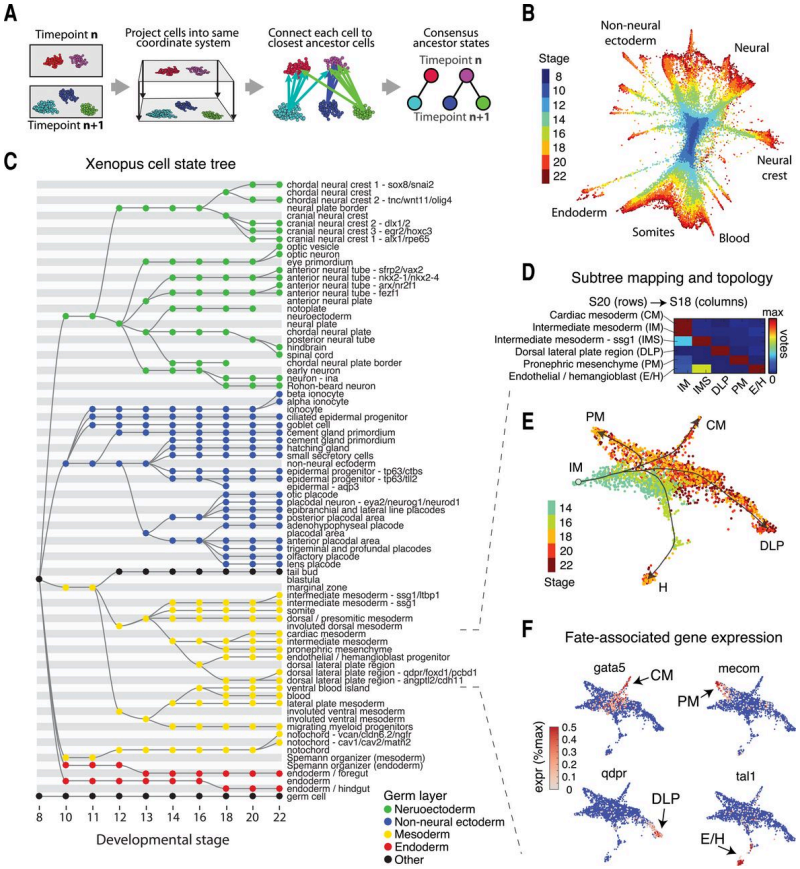
Animal miRNAs recognize **partially complementary** binding sites which are generally located in 3' UTRs of mRNA.

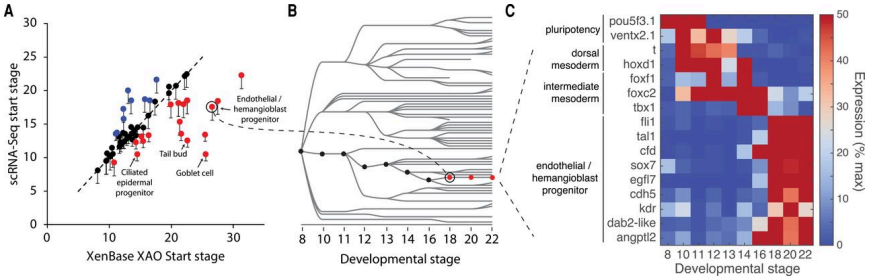
Complementarity to the 5' end of the miRNA – the "**seed**" sequence containing nucleotides 2-7 – is a major determinant in target recognition and is sufficient to trigger silencing.

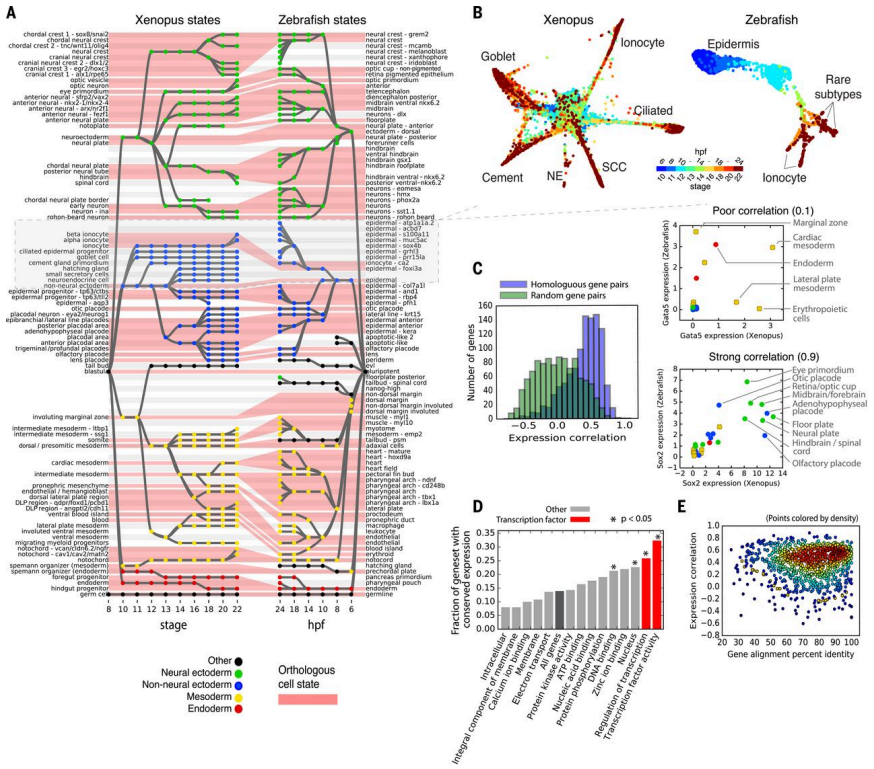
Huntzinger, Izaurralde, Nat. Rev. Genet. 12, 99 (2011)

Content from paper 7 that is relevant for mini test #3

ONLY: methods and results related to Figs 2, 3, 4

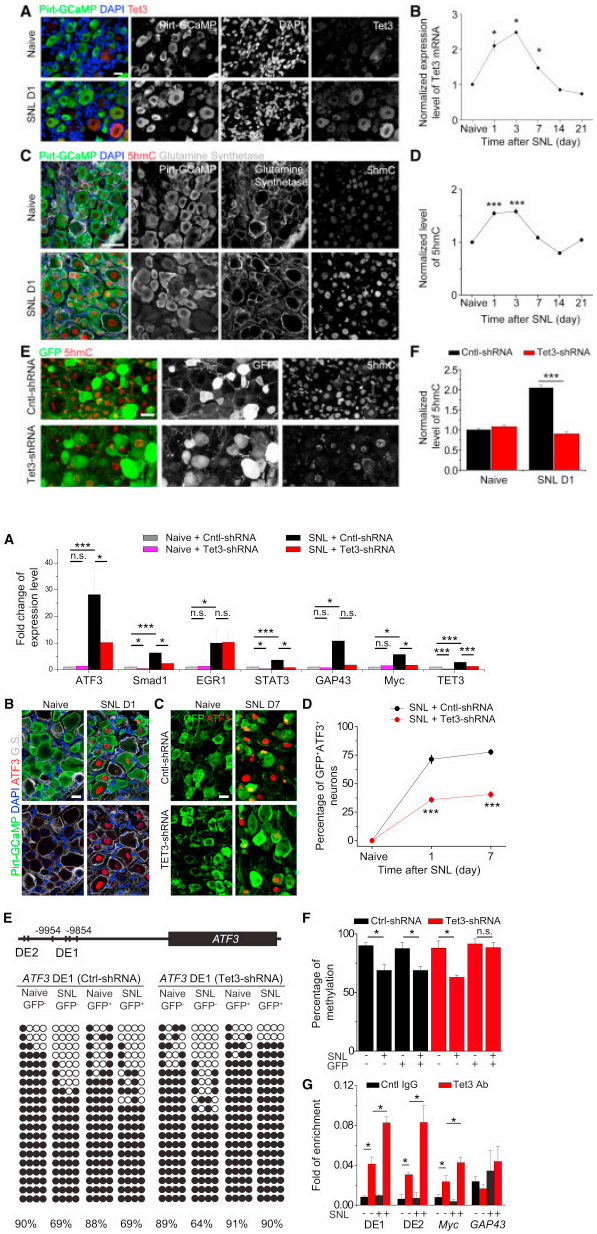


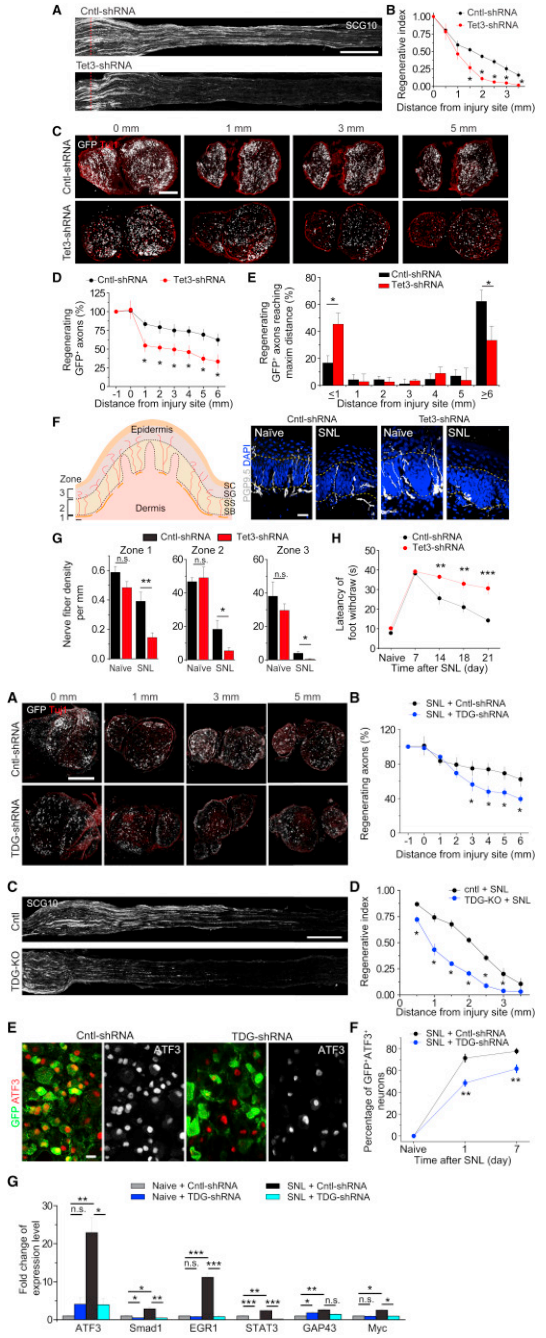




Content from paper 8 that is relevant for mini test #3

ONLY: methods and results related to Figs 1, 2, 3, 4





Content from paper 9 that is relevant for mini test #3

ONLY: methods and
results related
to Figs 1, 2, 4