

20.109

LABORATORY FUNDAMENTALS IN
BIOLOGICAL ENGINEERING

MODULE 2

EXPRESSION ENGINEERING

Lecture # 1

Leona Samson

March 10th 2009

Snapshot of the next four weeks

We will eliminate the expression of various genes using

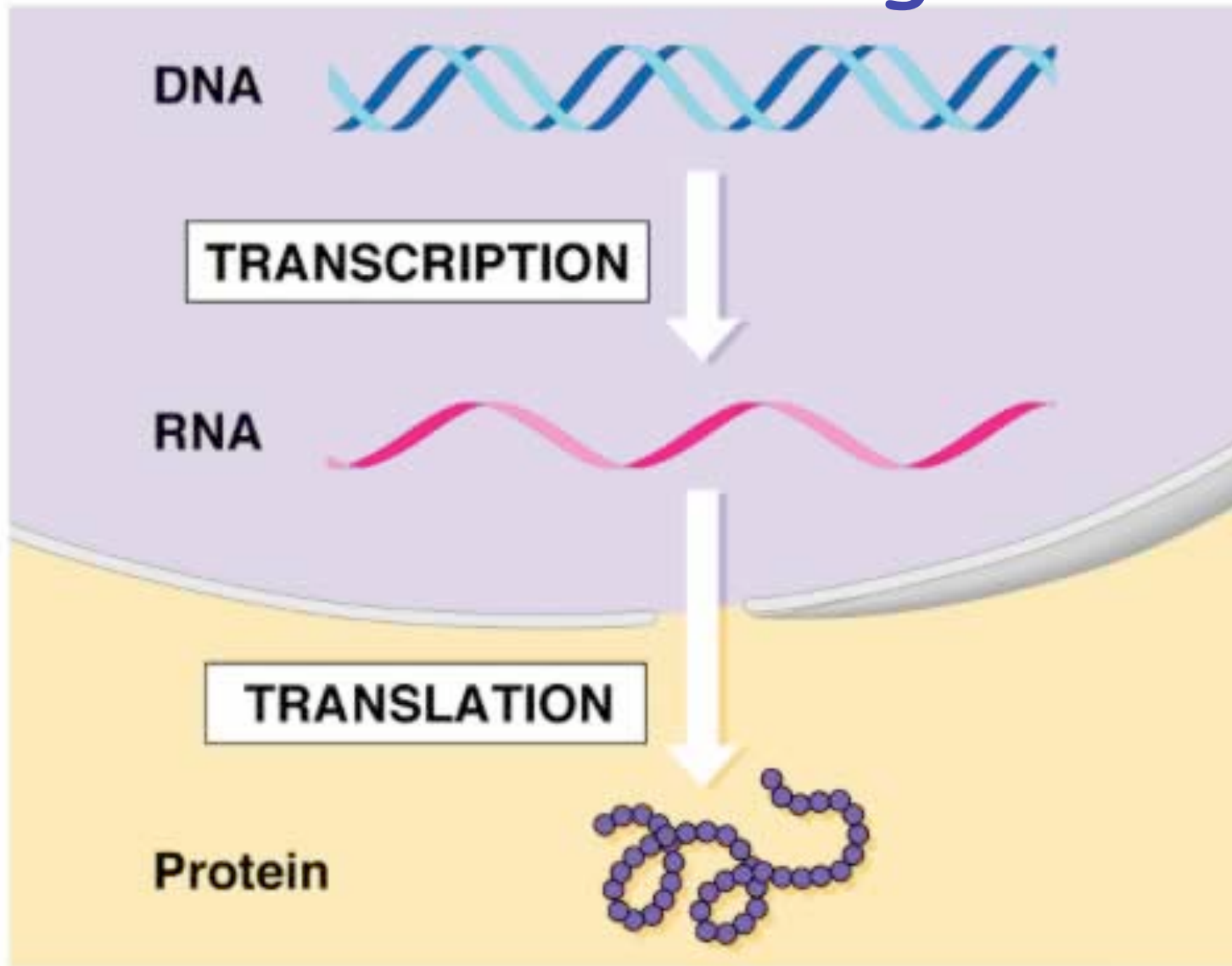
- (i) RNA interference technology
- (ii) Cultured mouse ES cells
- (iii) Chemiluminescent proteins
- (iv) DNA microarrays

The use of RNA Interference
RNAi
to modulate gene expression

How are genes expressed?

Why do we want to be able to
modulate gene expression?

The Central Dogma



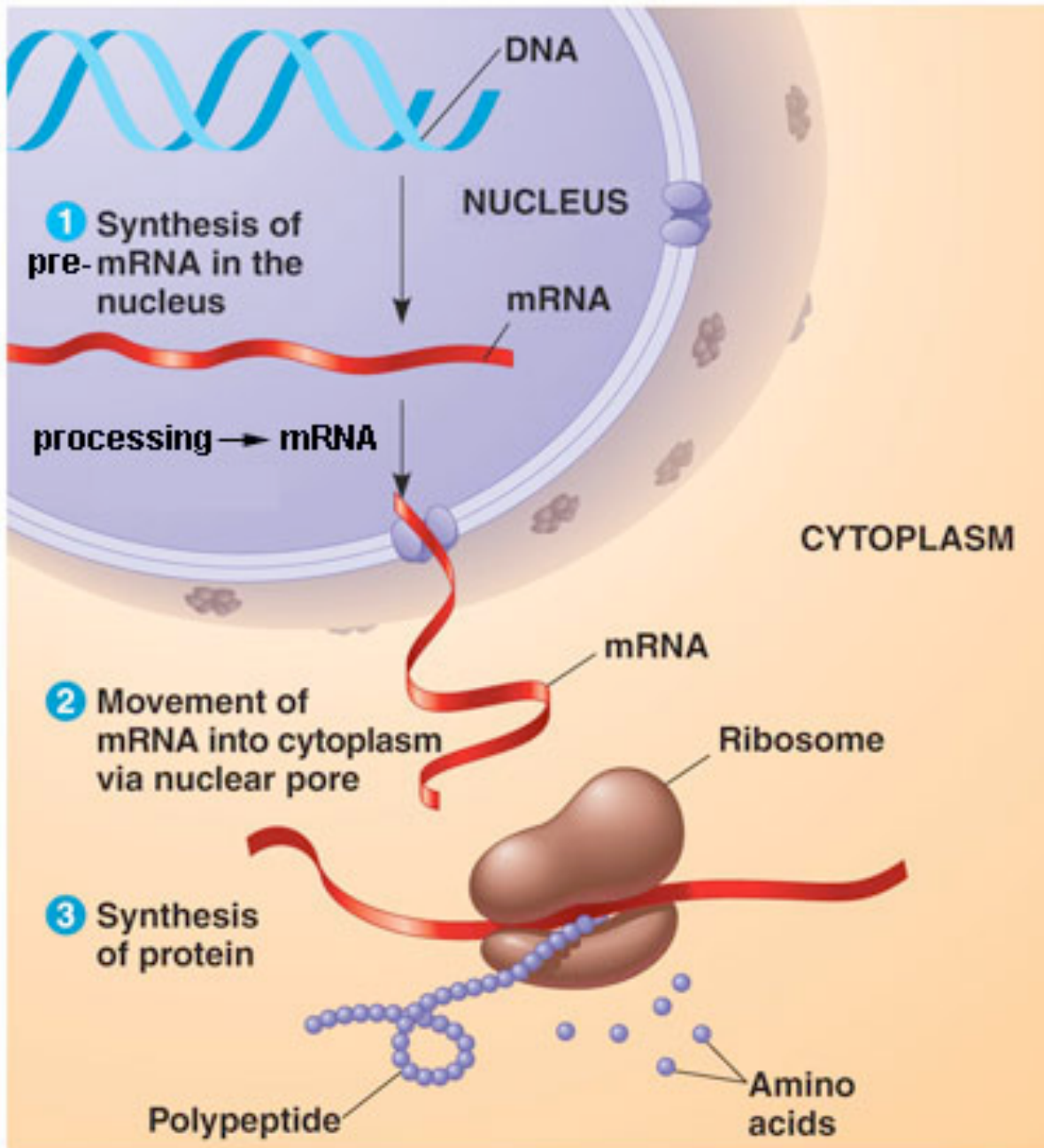
DNA

makes

RNA

makes

Protein



DNA

makes

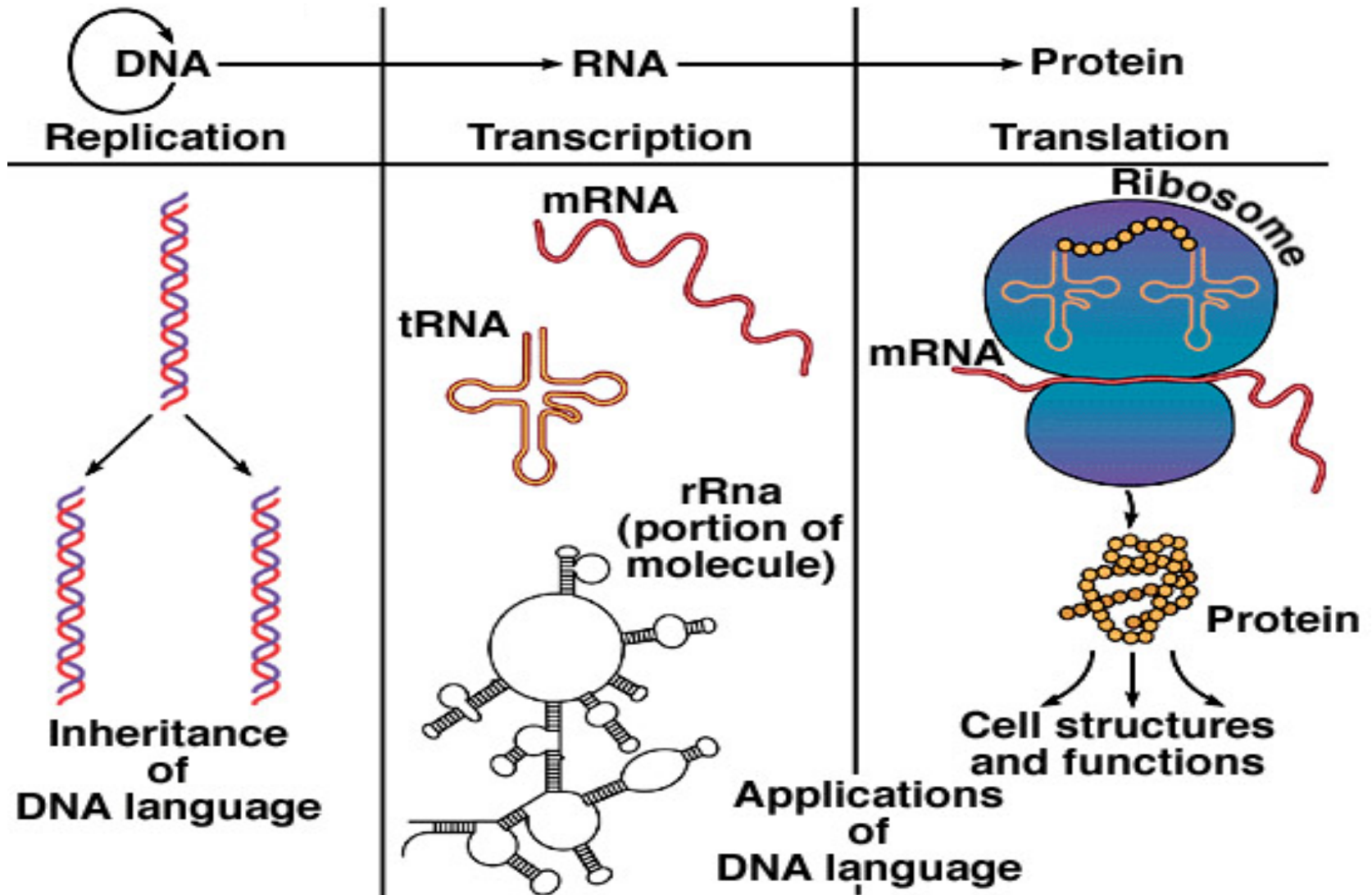
RNA

makes

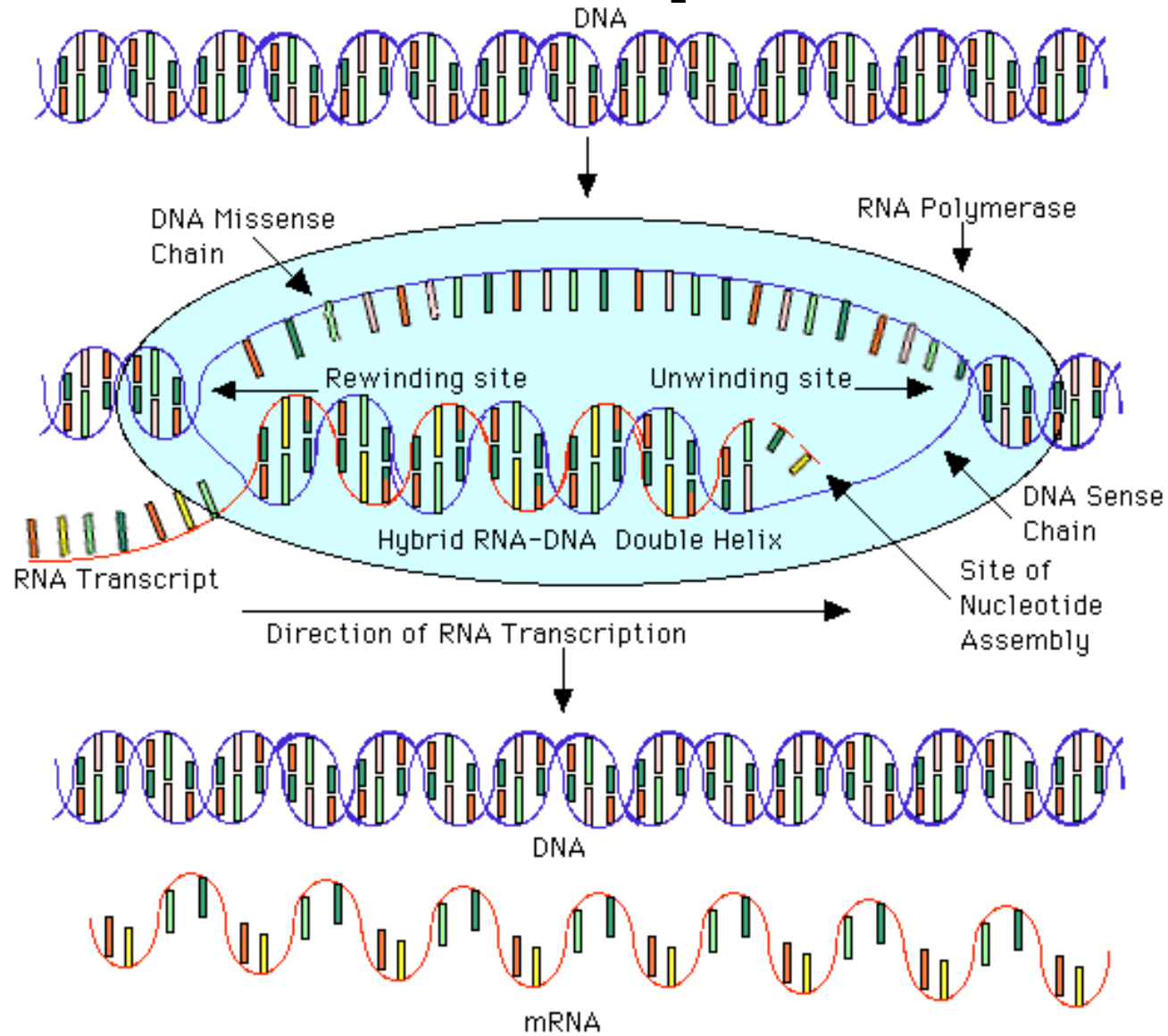
Protein

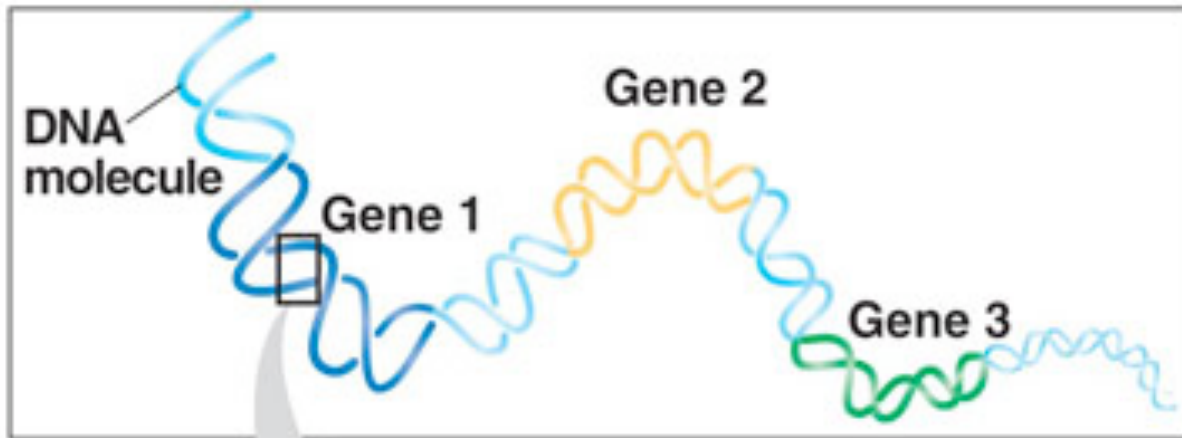
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Central Dogma of Molecular Biology



Transcription





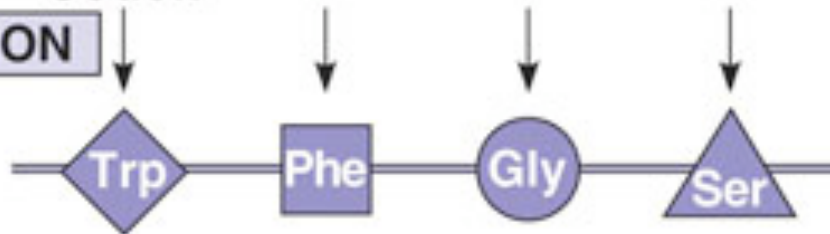
TRANSCRIPTION

mRNA



TRANSLATION

Protein



Amino acid

DNA

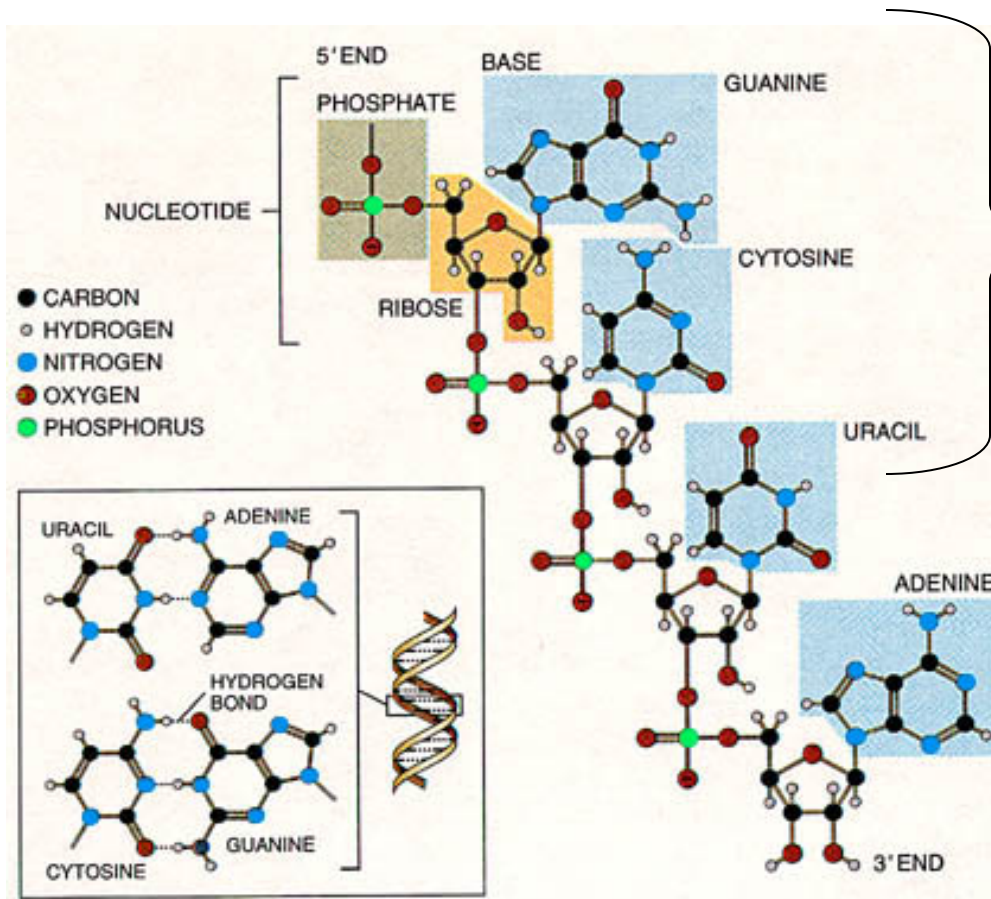
makes

RNA

makes

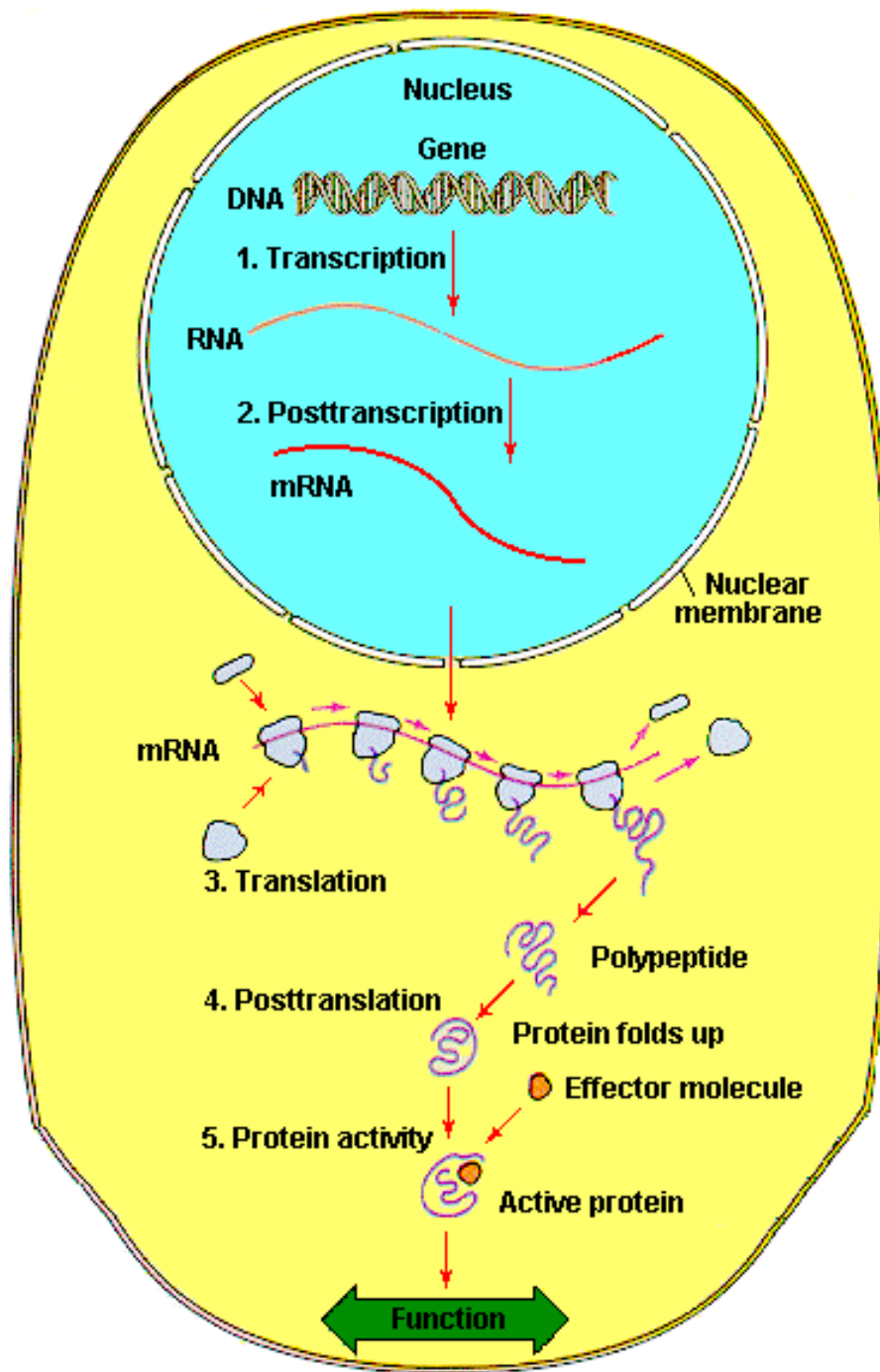
Protein

What is mRNA?



GCU = Ala

		2nd base in codon					
		U	C	A	G		
1st base in codon	U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr STOP STOP	Cys Cys STOP Trp	U C A G	3rd base in codon
	C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G	
	A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G	
	G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G	



DNA

makes

RNA

makes

Protein

"Genes" were first described by their mutant phenotype...

e.g., Mendel described inherited properties like wrinkled versus smooth peas... later Bateson coined the word "gene" to account for these phenotypic traits. Genes were said to be inherited in a Mendelian fashion.



1940's

Beadle and Tatum's classic experiment with moulds established the "one gene one enzyme" hypothesis

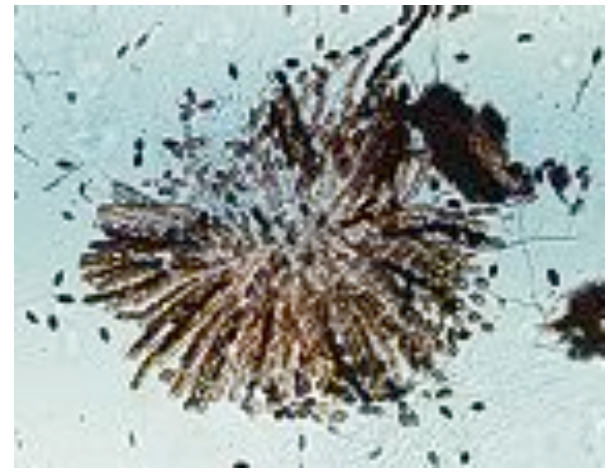
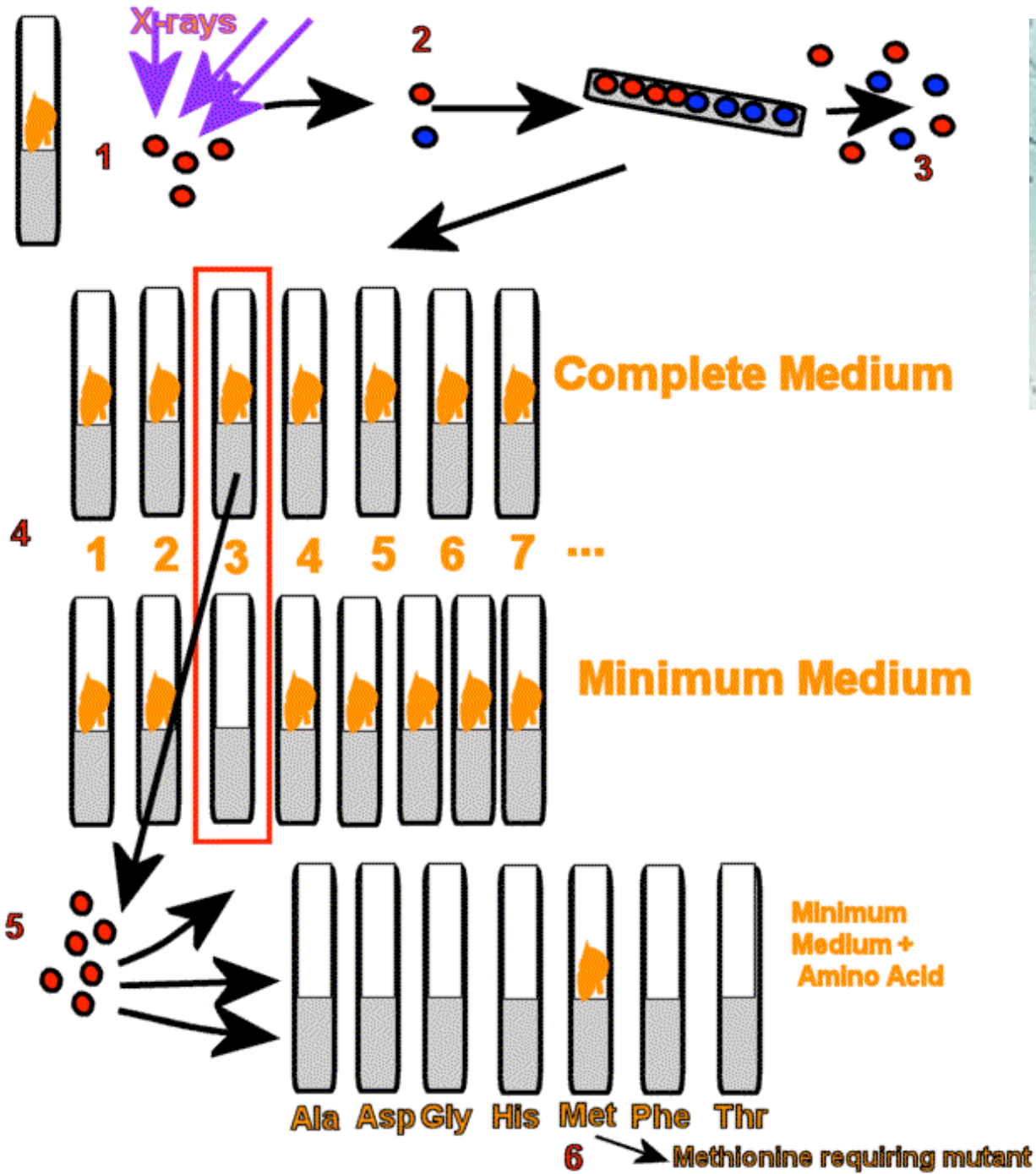


NOBEL PRIZE - 1958

In the 1940's Beadle and Tatum mutated genes to analyze biochemical pathways

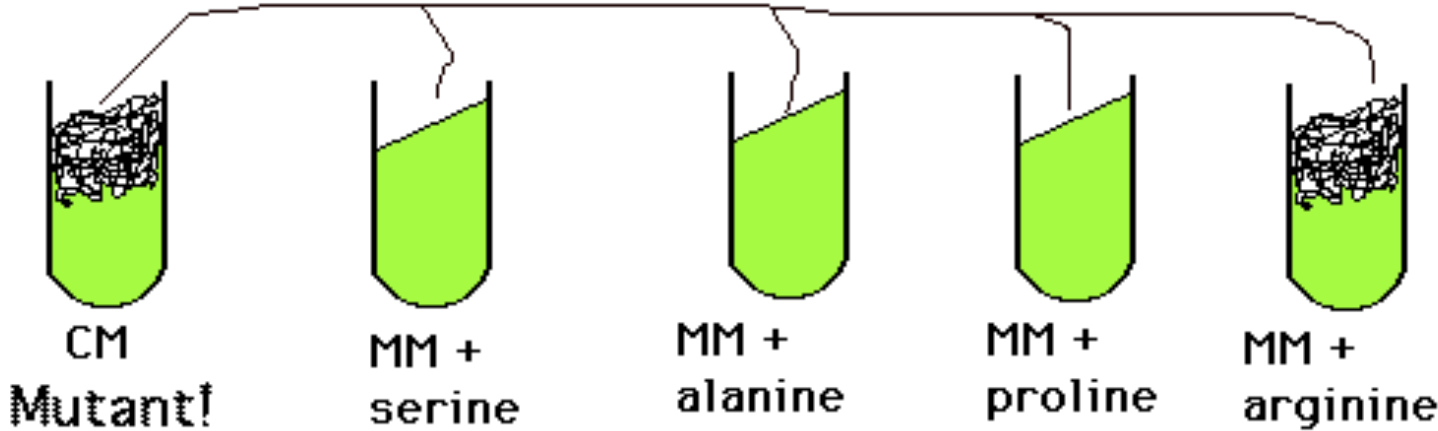
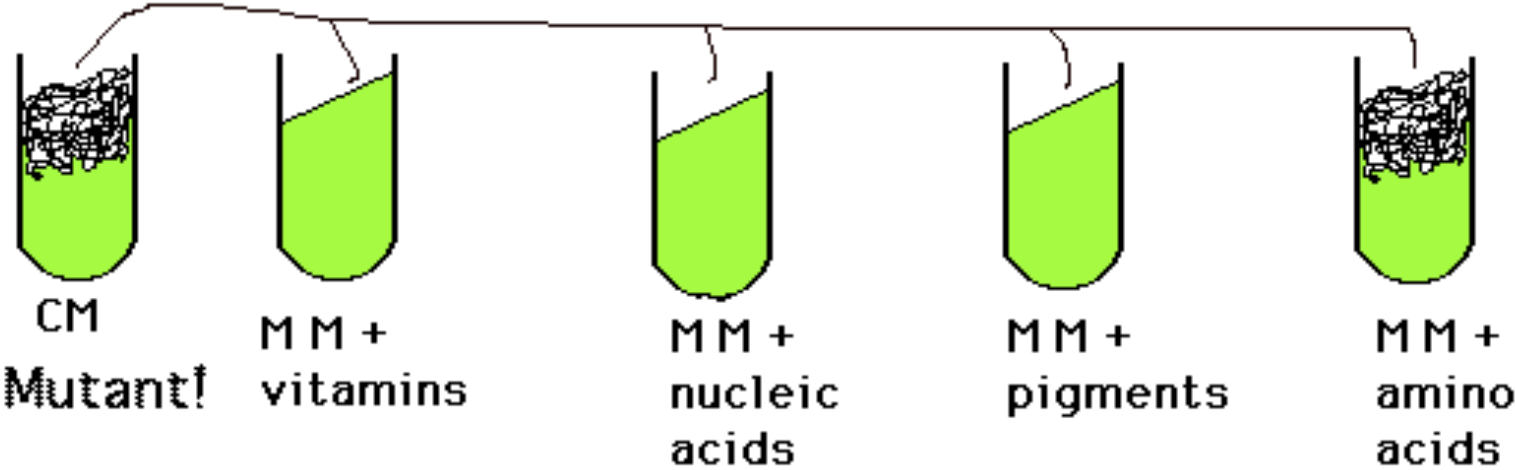
Mutagens?

X-rays, Nitrogen Mustards



Neurospora mould

The classic experiment was actually done with Arginine requiring mutants



CM = Complete Media MM = Minimal Media

Precursor

Enzyme A



Ornithine

Enzyme B

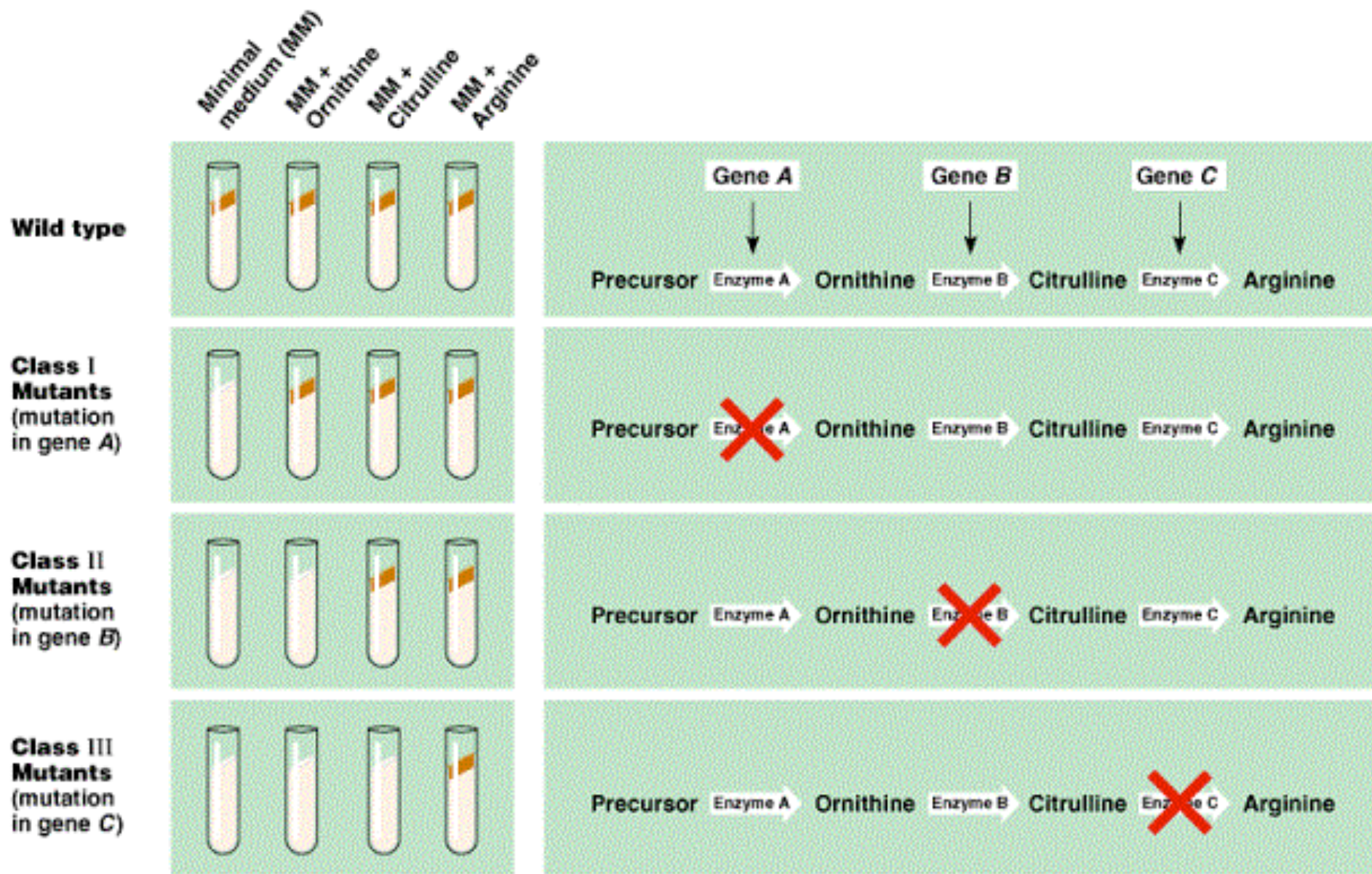


Citrulline

Enzyme C



Arginine



(a) Experiment

(b) Interpretation



1940's
Beadle and Tatum's classic experiment with moulds established the "one gene one enzyme" hypothesis

Eliminating the expression of a gene is one of the most powerful tools in biology

We can now engineer the alteration and even deletion of specific genes to probe their biological function

Forward Genetics:

Phenotype → **Genotype**

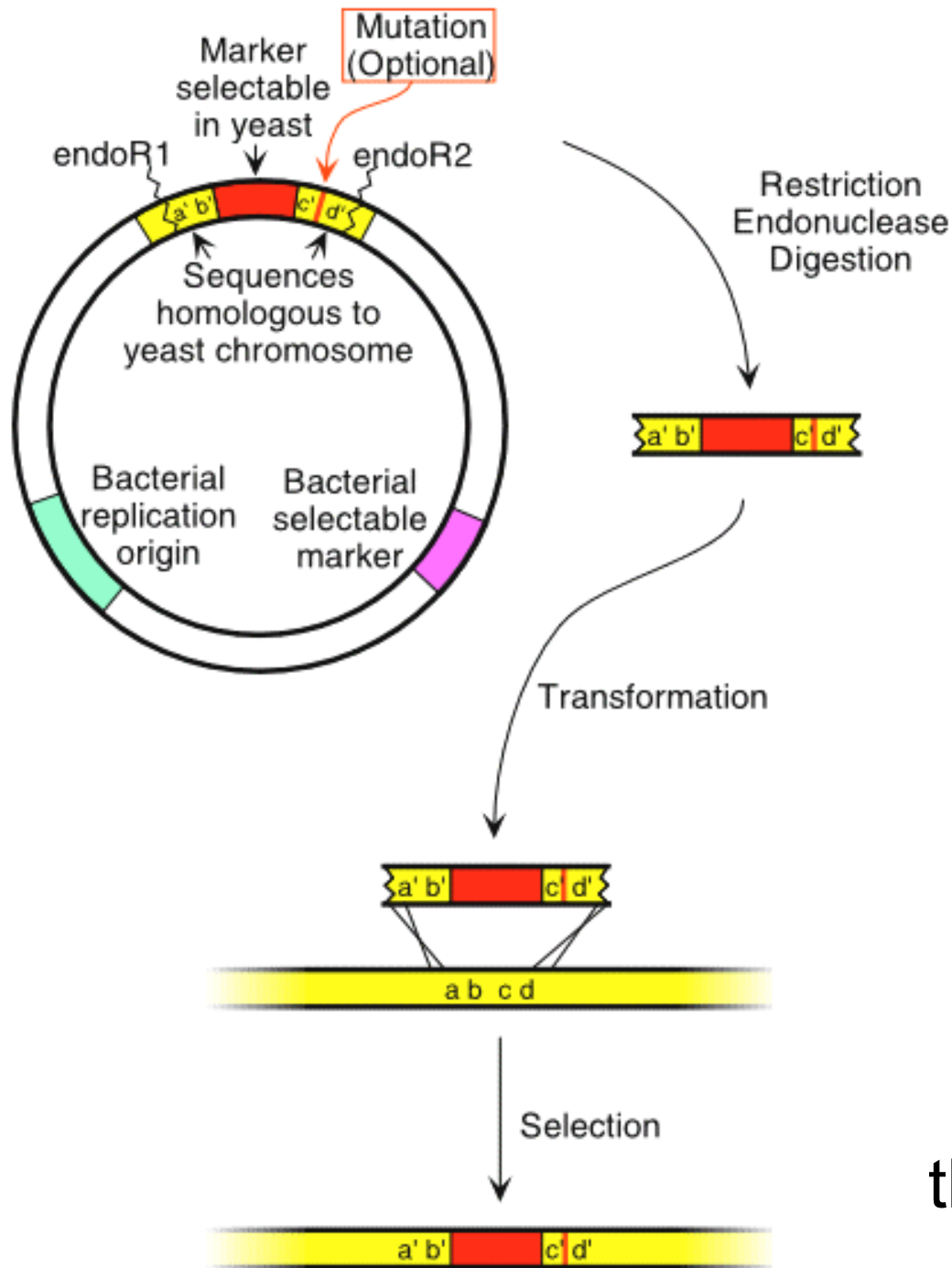
Reverse Genetics

Genotype → **Phenotype**

The most common method for
Reverse Genetics

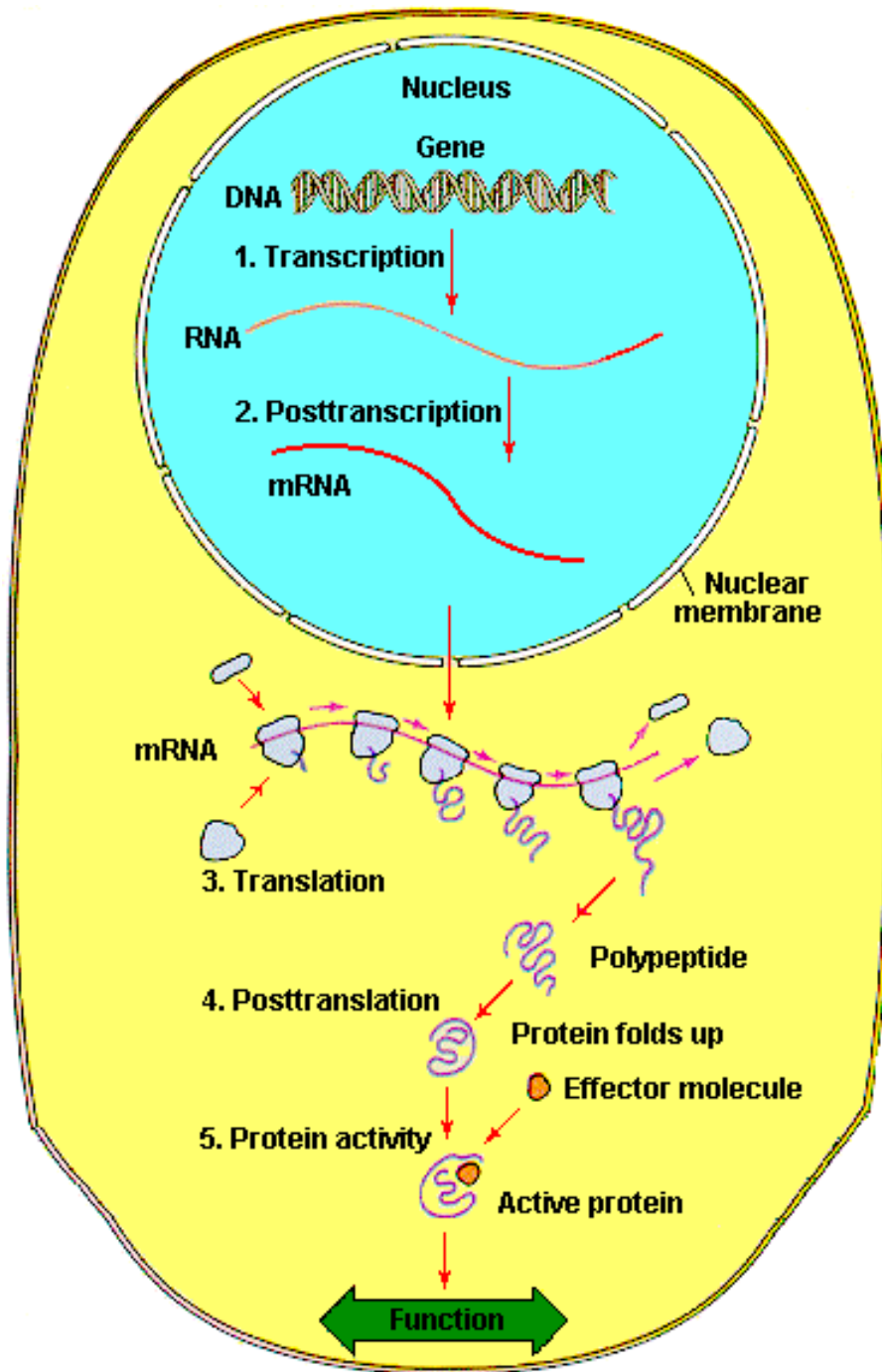
has been

Targeted Gene Deletion



An example for yeast cells.....
 but this fundamental approach works in virtually all organisms....

Homologous Recombination inside the cell does the work!!



Mammals
are diploid!

Have to
knock out
both genes
to test the
null
phenotype

KILLING THE MESSENGER: SHORT RNAS THAT SILENCE GENE EXPRESSION

Derek M. Dykxhoorn, Carl D. Novina* and Phillip A. Sharp*‡*

Short interfering RNAs can be used to silence gene expression in a sequence-specific manner in a process that is known as RNA interference. The application of RNA interference in mammals has the potential to allow the systematic analysis of gene expression and holds the possibility of therapeutic gene silencing. Much of the promise of RNA interference will depend on the recent advances in short-RNA-based silencing technologies.

Instead of “knocking out” two genes....

We can now “knock down” the mRNA that is produced by those two genes using

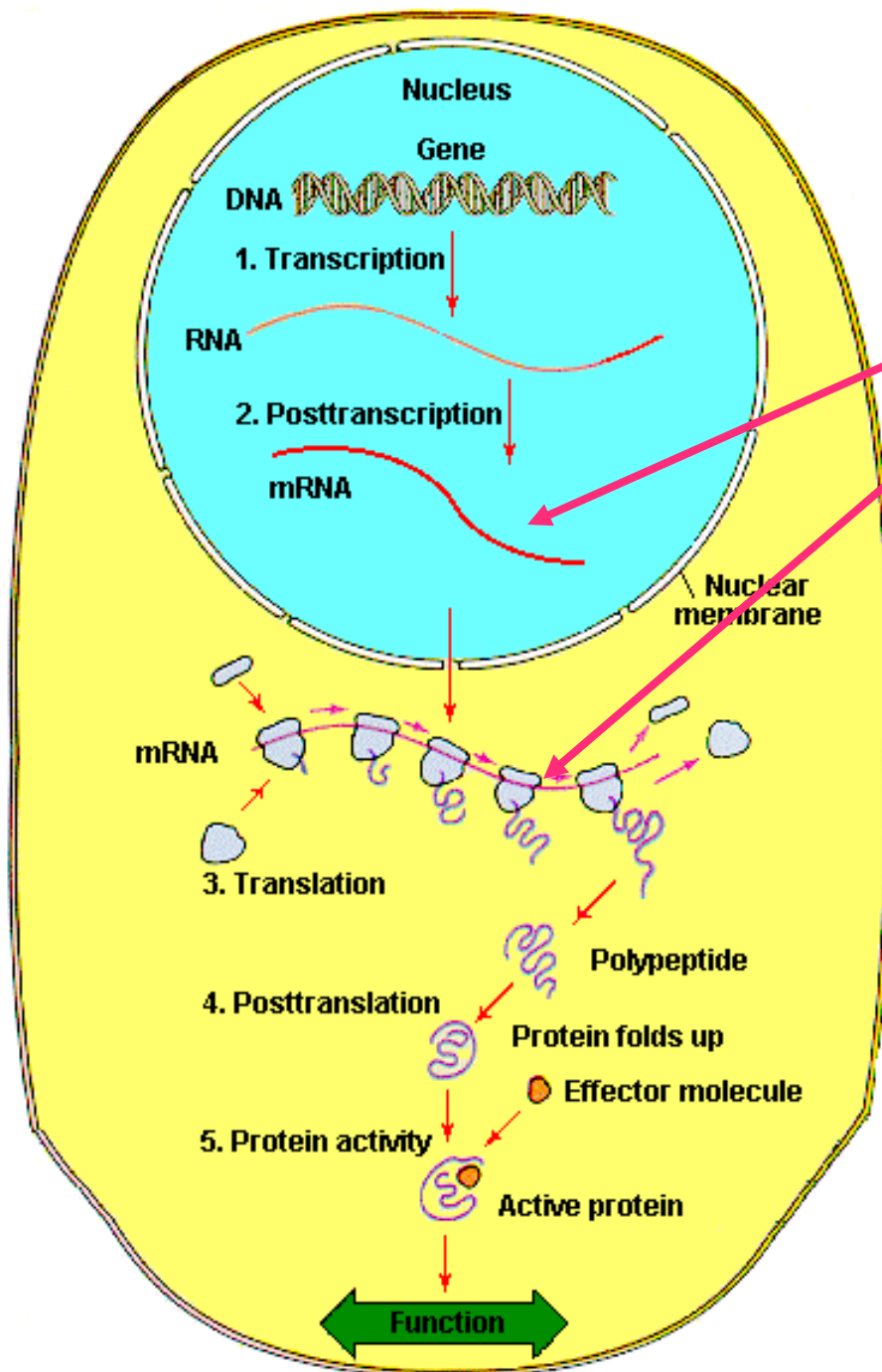
RNA interference (RNAi)

So what is RNAi?

RNA interference

And what are
siRNAs???

Short interfering RNAs

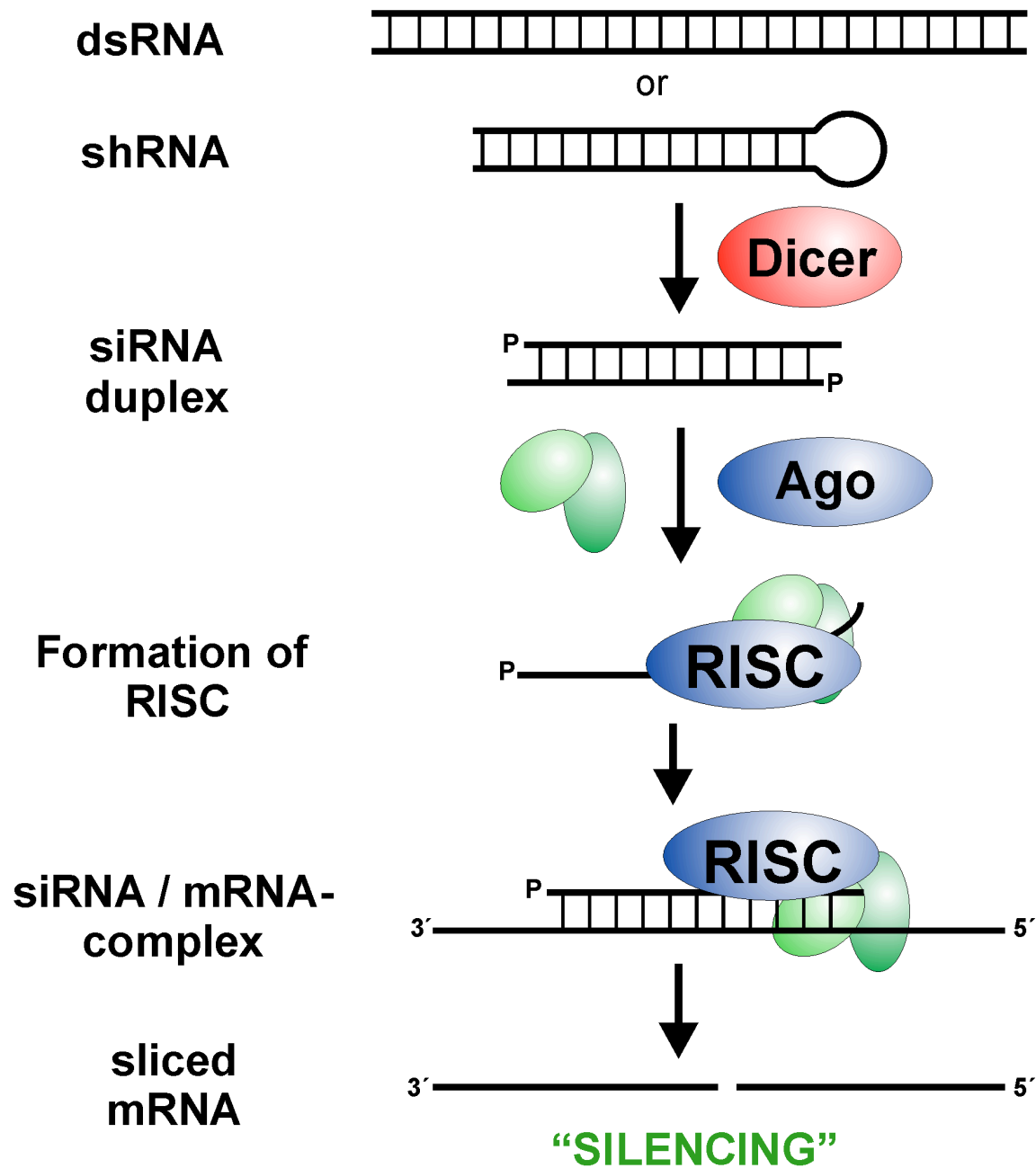


siRNAs will attack gene expression at the mRNA transcript level.....

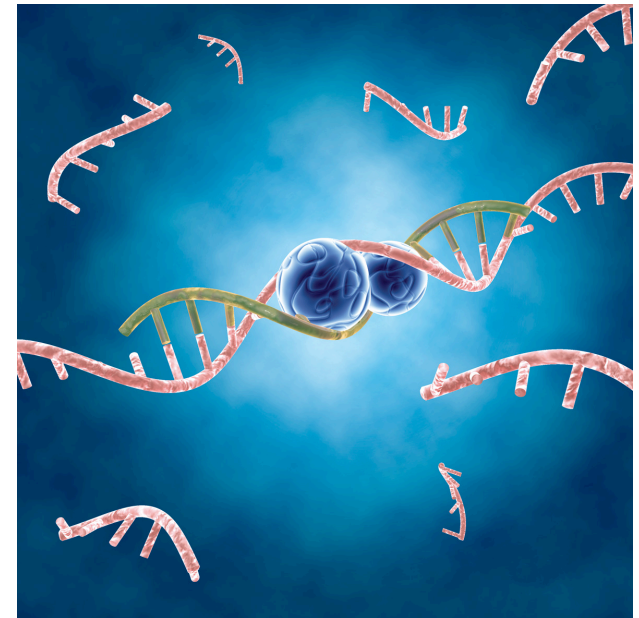
This means you get

- LESS mRNA
- LESS protein, and so less protein activity

You will measure Both of these

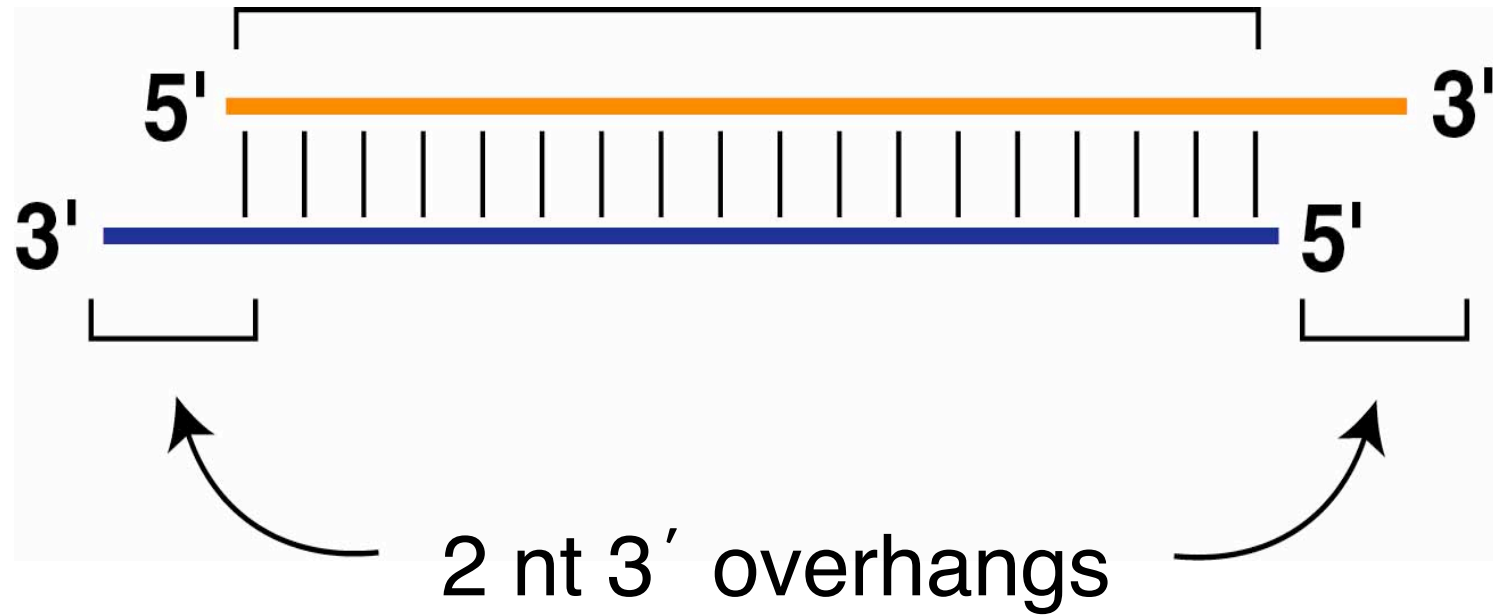


RISC = RNA induced silencing complex



siRNAs have a defined structure

19 nt duplex



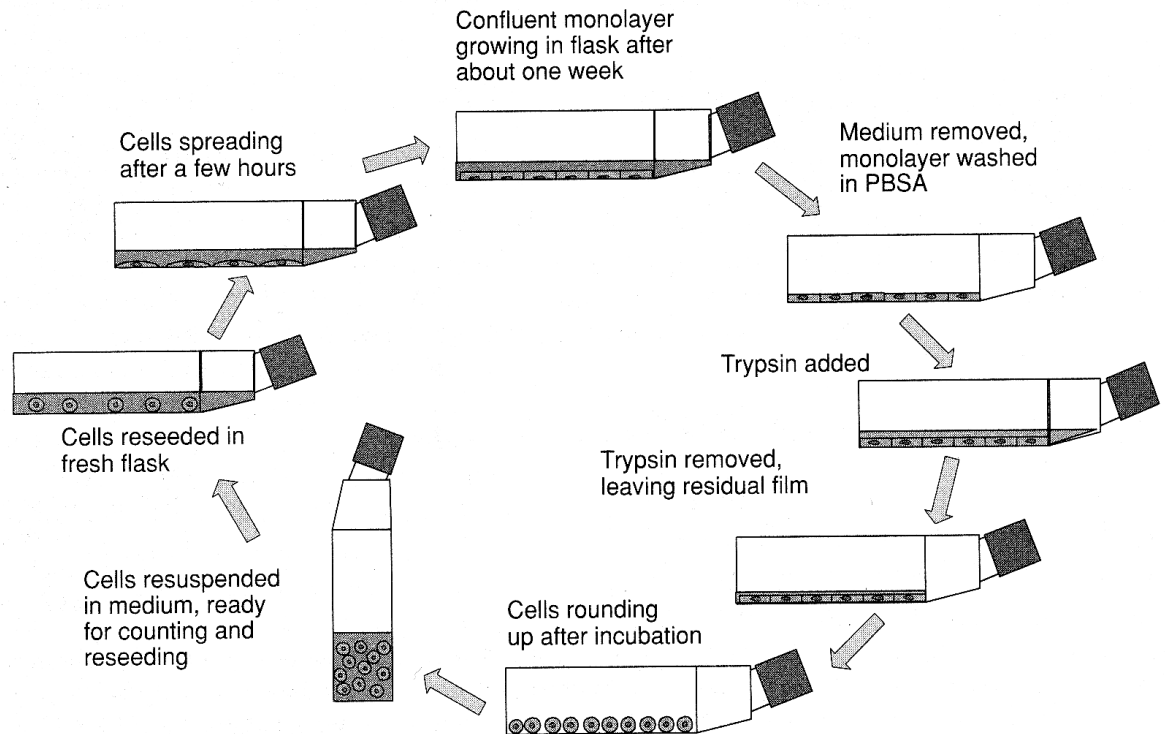
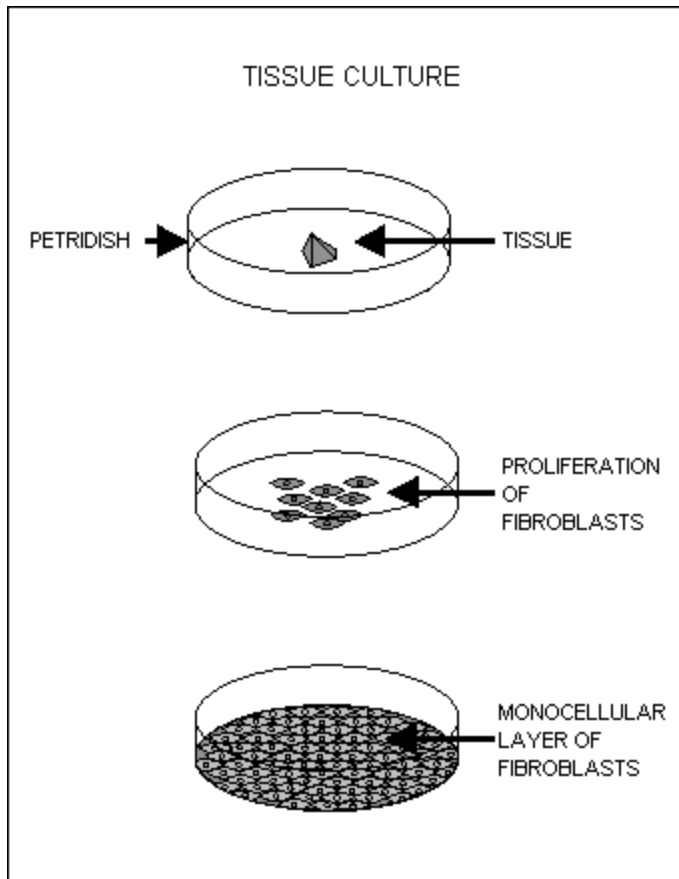
"We will eliminate the expression of various different genes using RNAi technology, mammalian cells, chemiluminescent proteins and DNA microarrays"

What cells? Mouse Embryonic Stem (ES) Cells

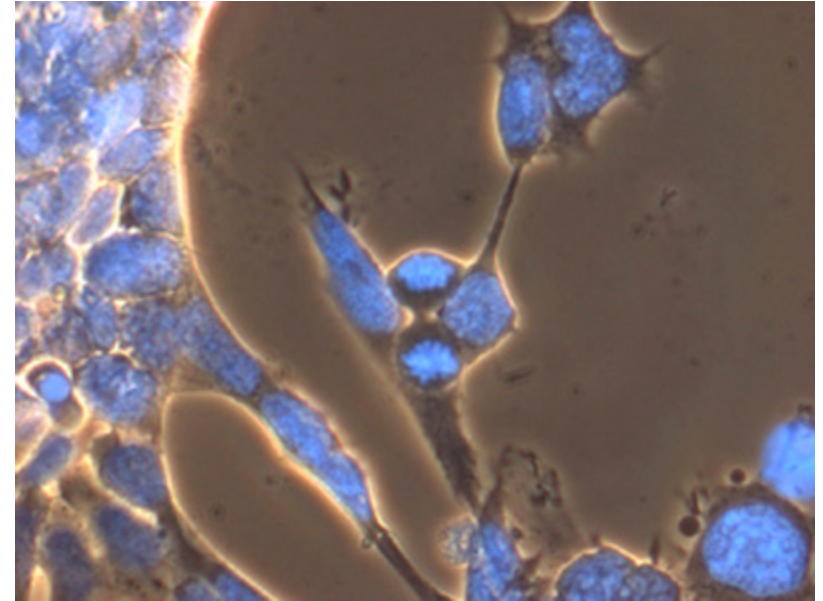
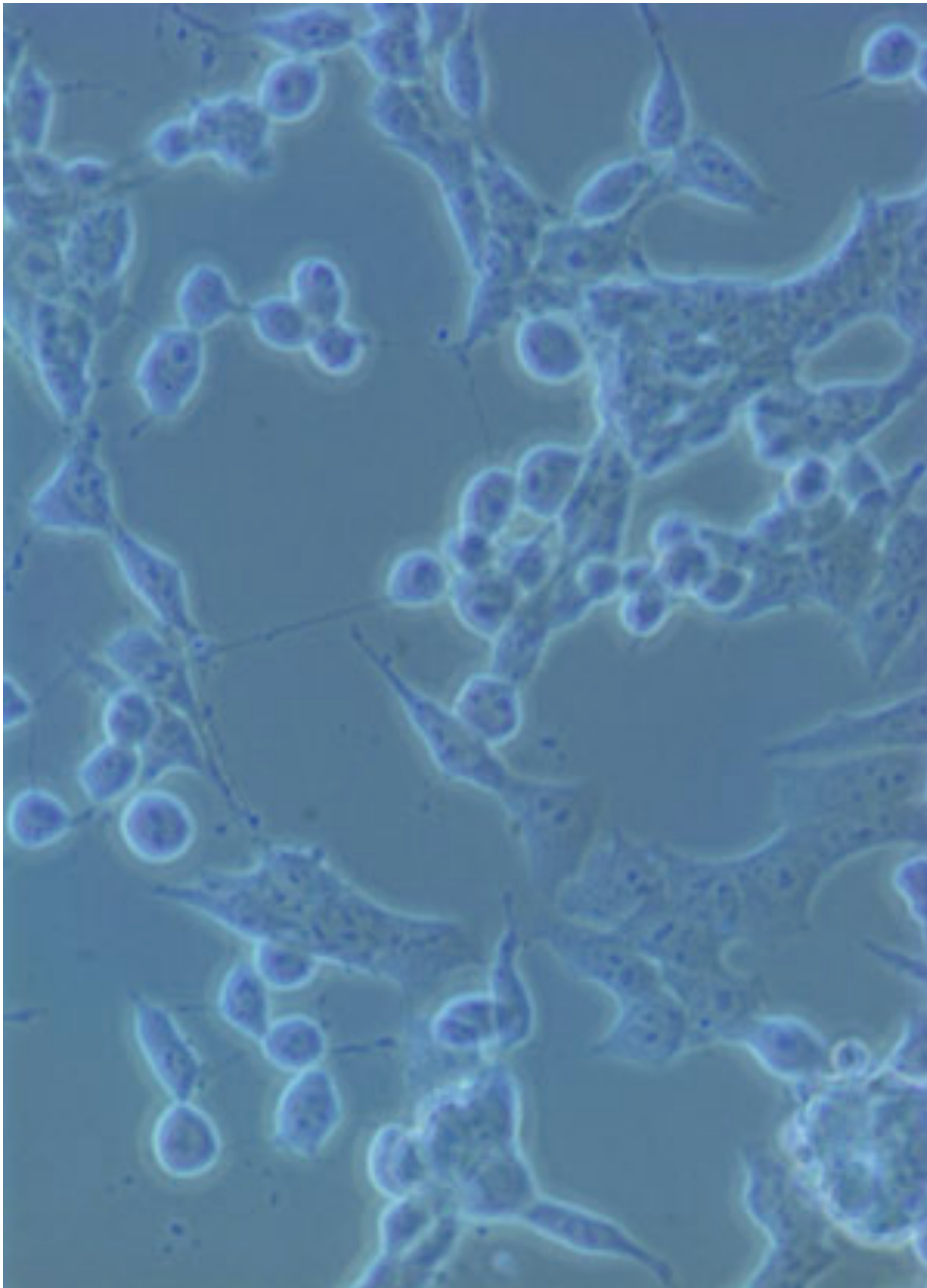
What genes? *Renilla luciferase*

Additional genes

How do you grow mammalian cells?



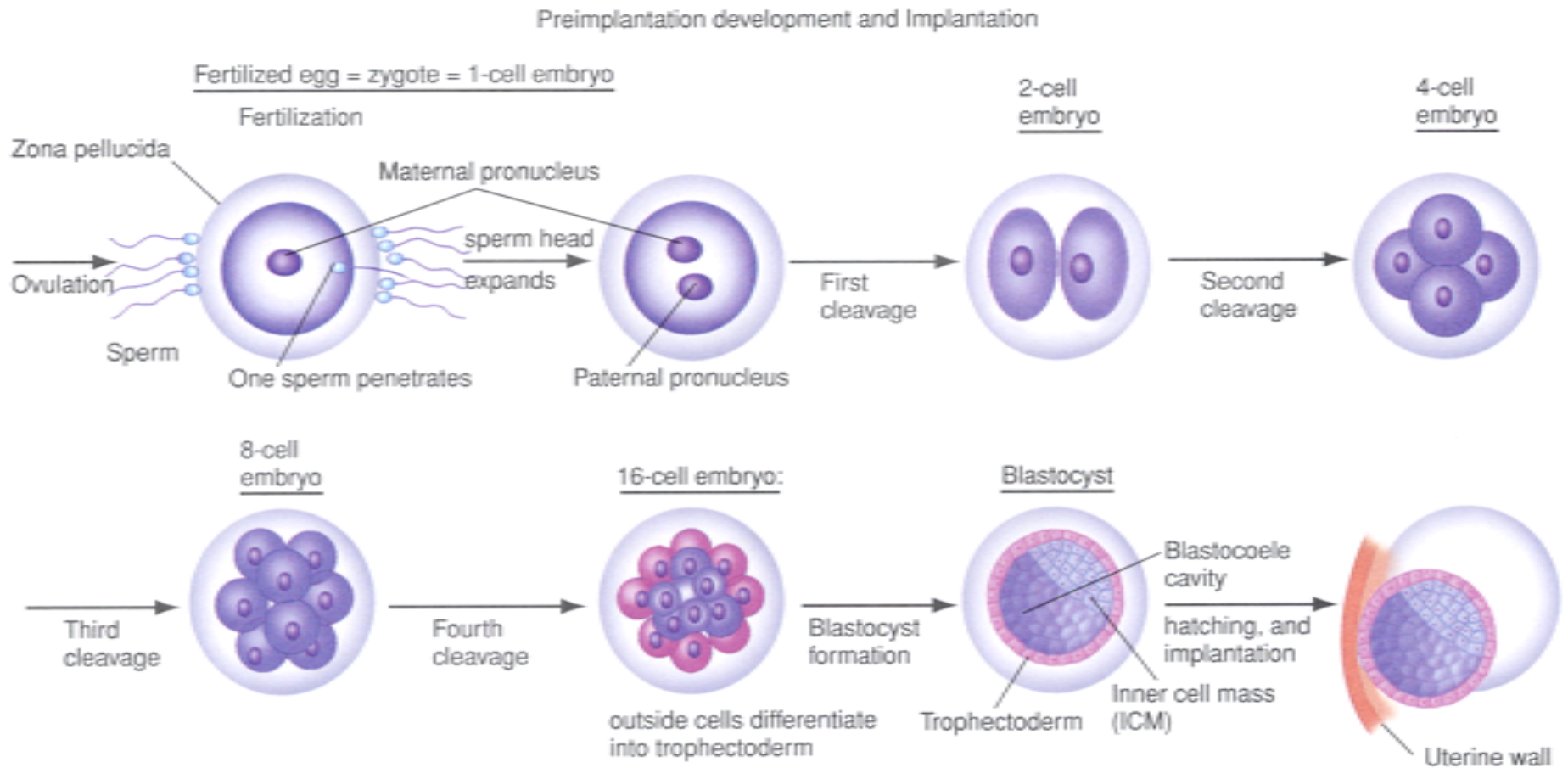
From Freshney's "Culture of Mammalian Cells"



Mouse embryonic stem cells,

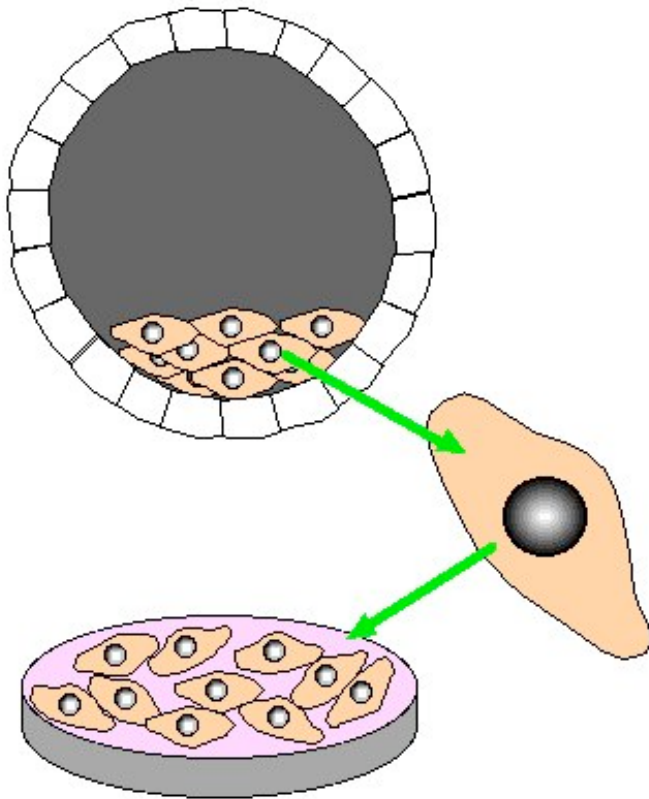
The mouse embryonic stem cells (above) have been treated with a stain that makes DNA fluoresce, causing nuclei to appear blue.

Some background information about mammalian embryos - from fertilization to implantation



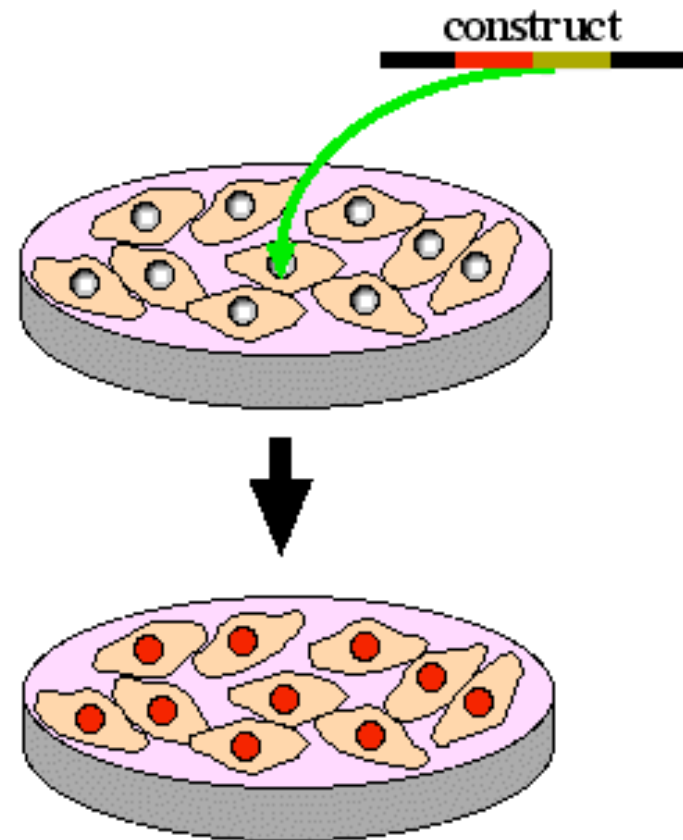
The Preimplantation stage lasts 4-5 days in mice

Preimplantation
blastocyst from an
embryo that would
produce a mouse with
GREY FUR



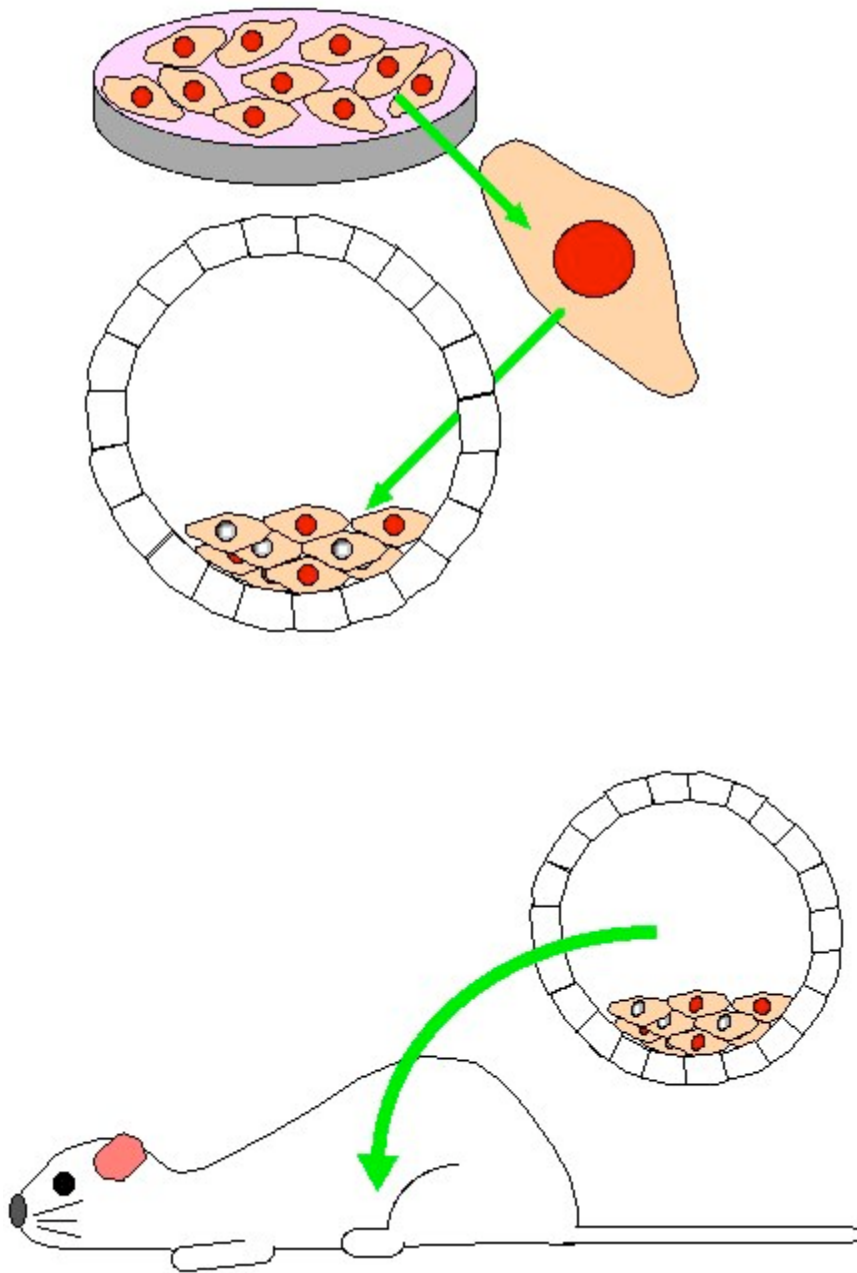
Can remove **totipotent**
EMRYONIC STEM
CELLS (ES cells) and
culture in vitro

Specifically replace your
gene of interest (α or β -
globin genes) with a
mutated version of that
gene in cultured ES cells

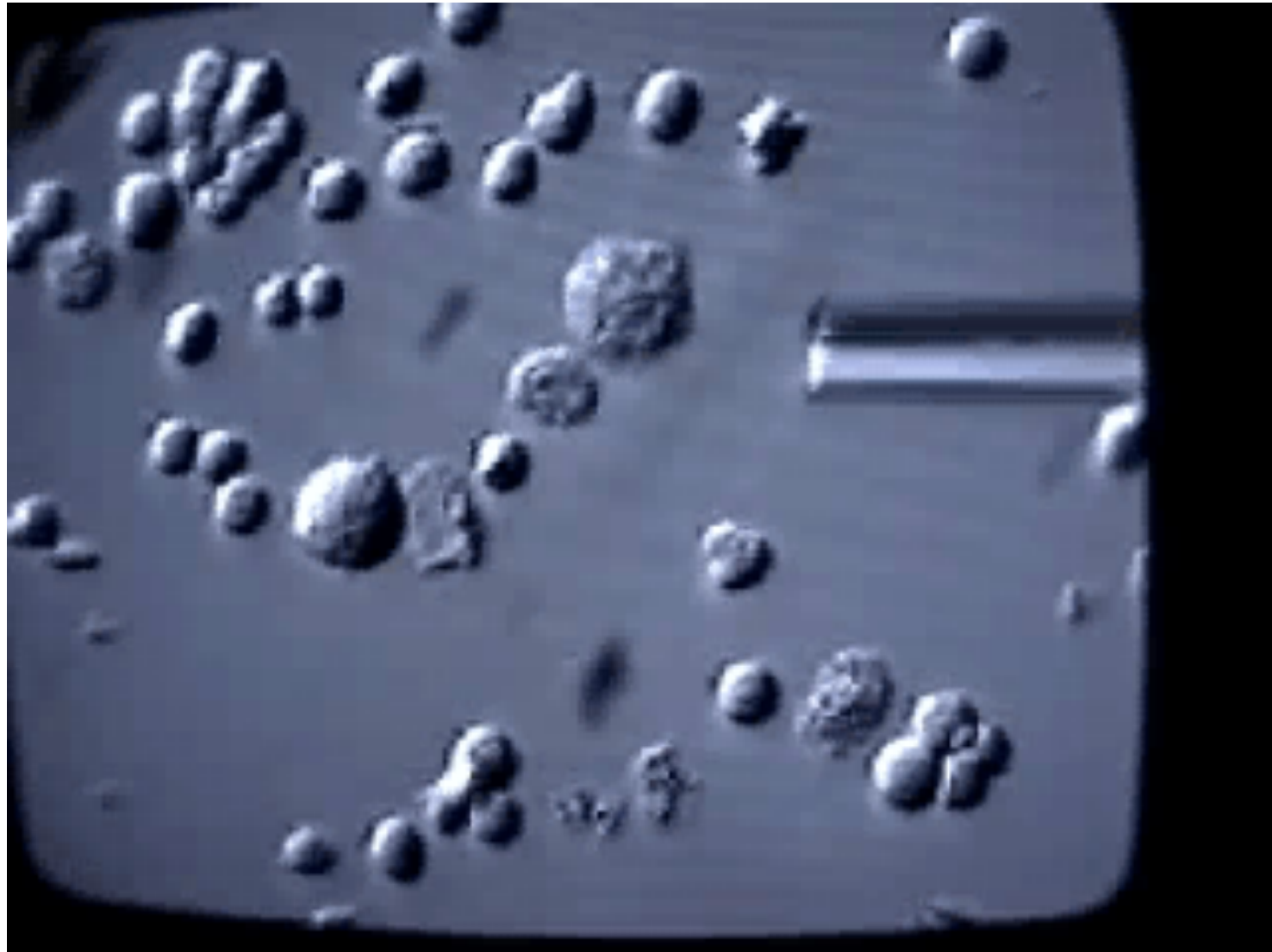


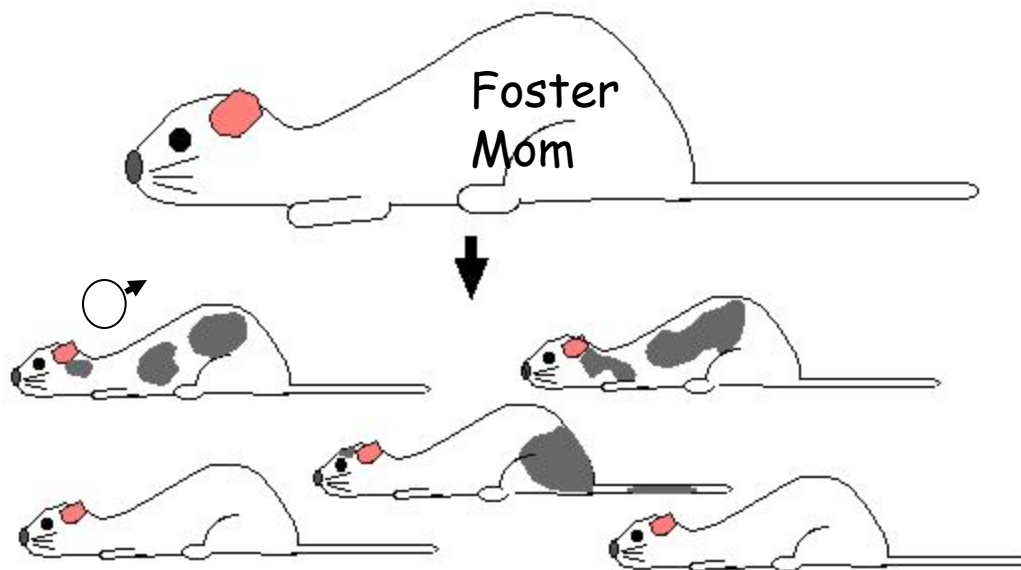
Select for the genetically
altered cells you want

Now you inject the genetically modified ES cells (originally from a blastocyst for a mouse with GREY FUR) and inject into a new blastocyst that would normally give rise to a mouse with WHITE FUR



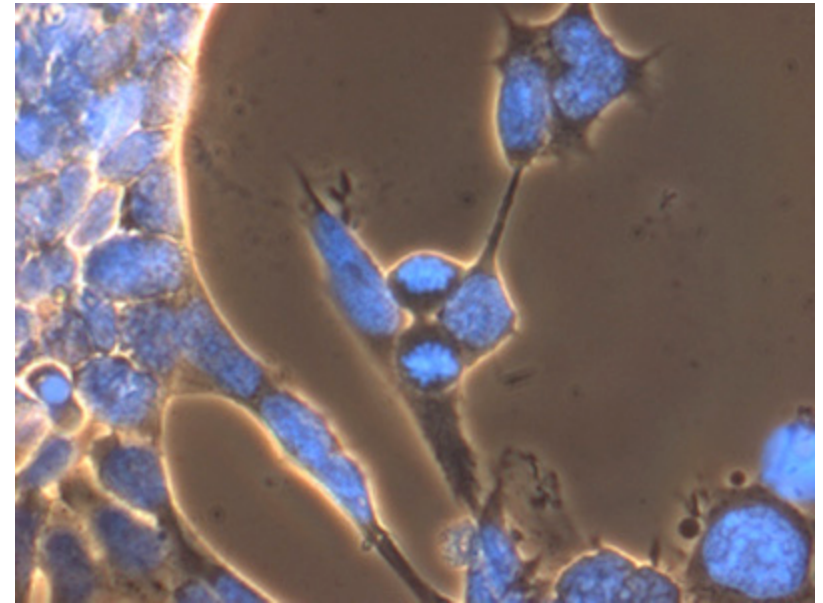
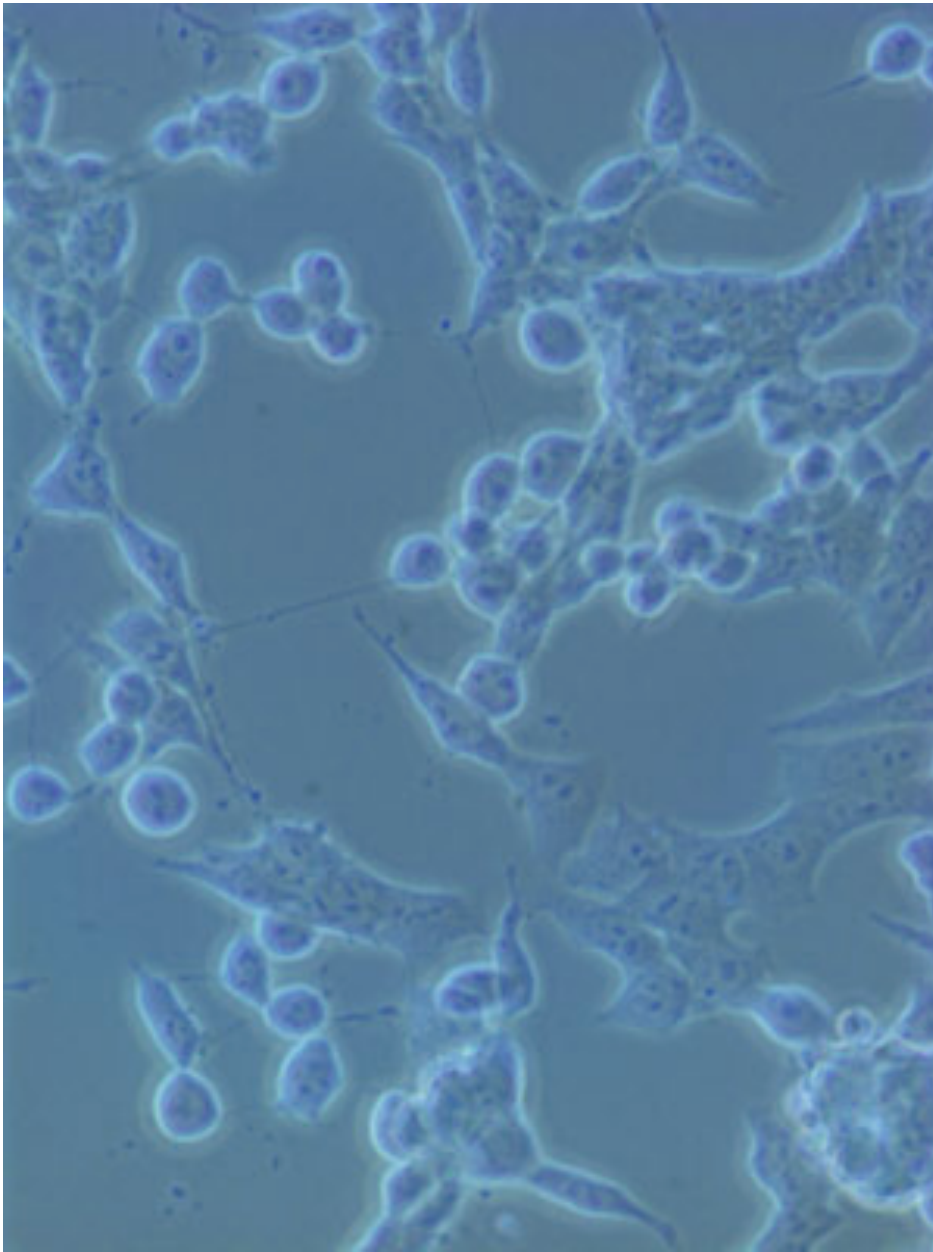
The blastocyst, now containing two types of **totipotent** embryonic stem cells, is implanted into a foster mother; she will give birth the chimeric offspring





Some mice are Chimeric

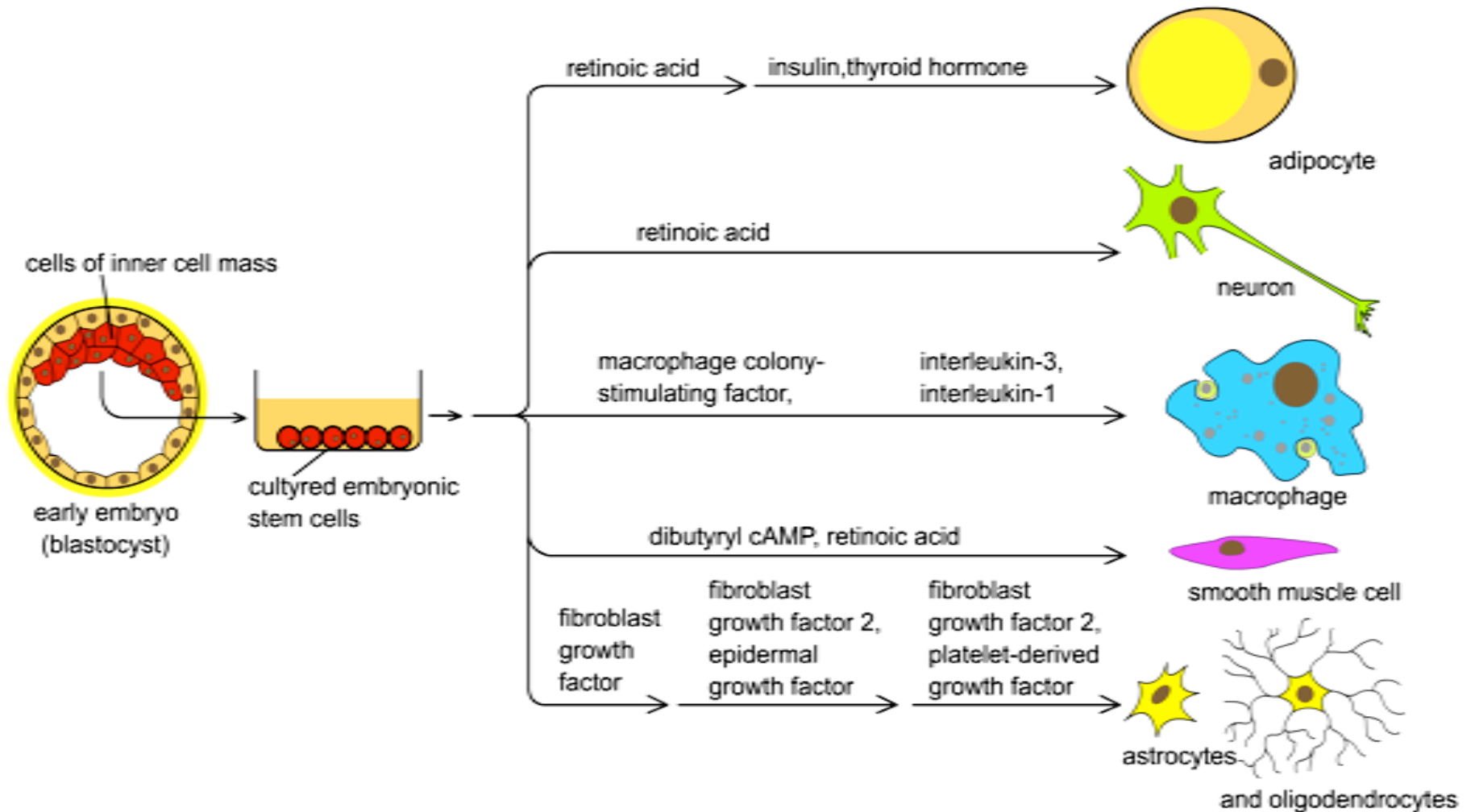




Mouse embryonic stem cells,

These mouse embryonic stem cells (above) have been treated with a stain that makes DNA fluoresce, causing nuclei to appear blue.

Mouse ES Cells can also be induced to differentiate in culture



"We will eliminate the expression of various different genes using RNAi technology, mammalian cells, chemiluminescent proteins and DNA microarrays"

What cells? Mouse Embryonic Stem (ES) Cells

What genes? *Renilla luciferase*

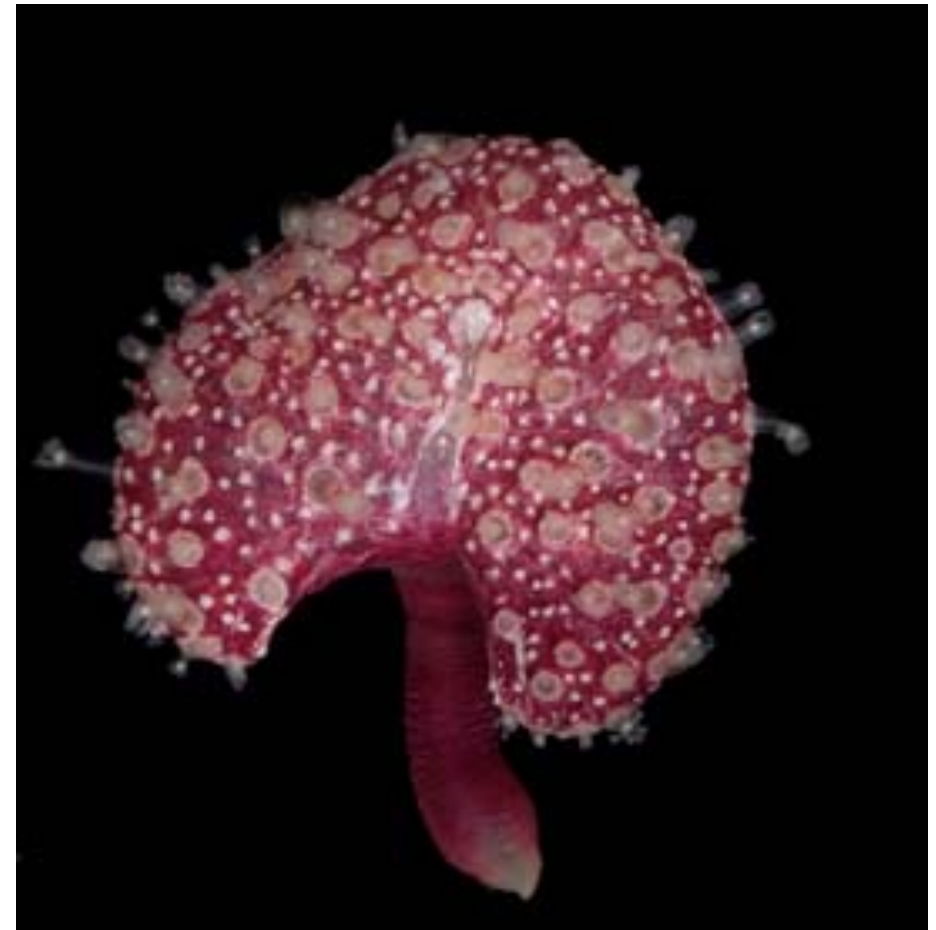
Additional genes

Renilla reniformis

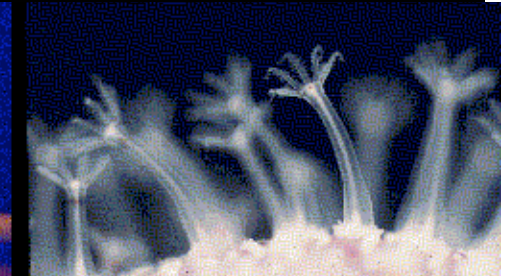
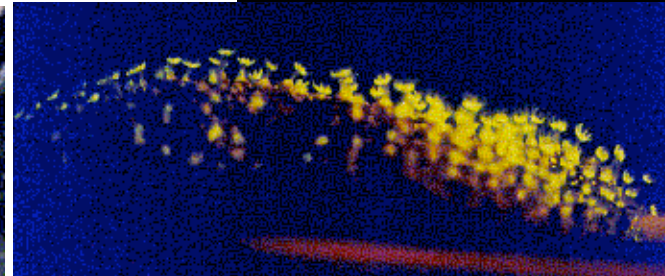
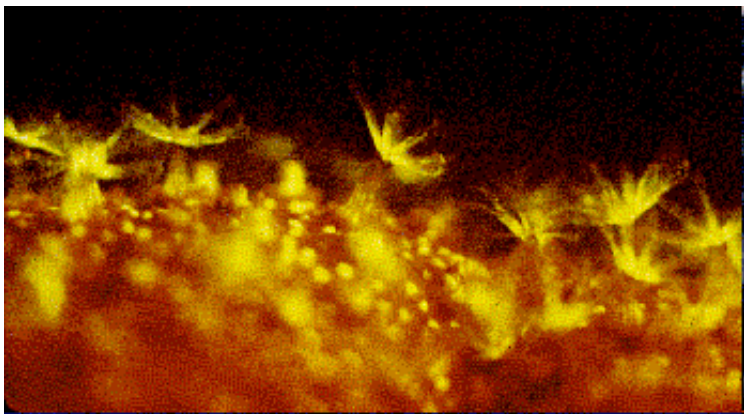
The Sea Pansy

The species is well known for its ability to bioluminesce.

Figure 1. *Renilla reniformis*, live specimen (S427), whole colony (approximately 22 mm in diameter).



Renilla Luciferase



Renilla reniformis, the Sea Pansy, is a soft coral. The organism is a colony of polyps each of which is bioluminescent at the sites identified by the characteristic green fluorescence (photos by James M. Anderson).

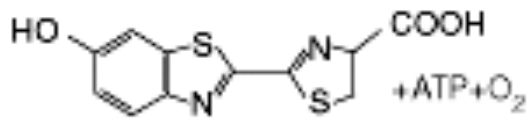
© 1997 J. E. Wampler



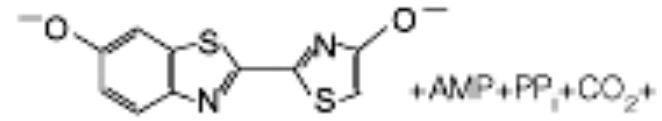
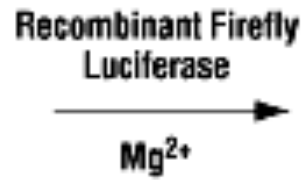
Fireflies or **lightning bugs** make light within their bodies. This process is called **bioluminescence** and is shared by many other organisms, mostly sea-living or marine organisms. Fireflies light up to attract a mate. To do this, the fireflies contain specialized cells in their abdomen that make

Firefly Luciferase

light



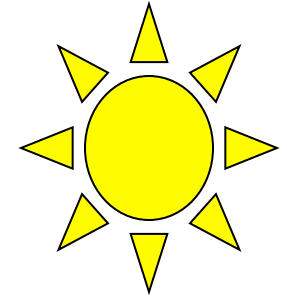
Beetle Luciferin



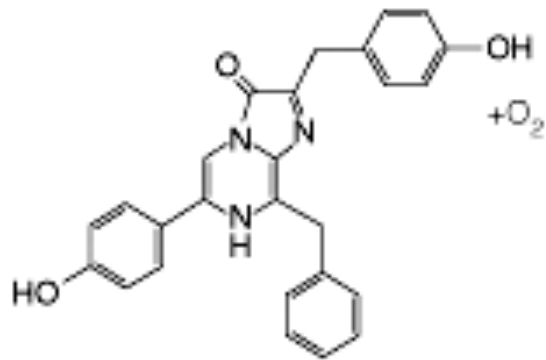
Oxyluciferin



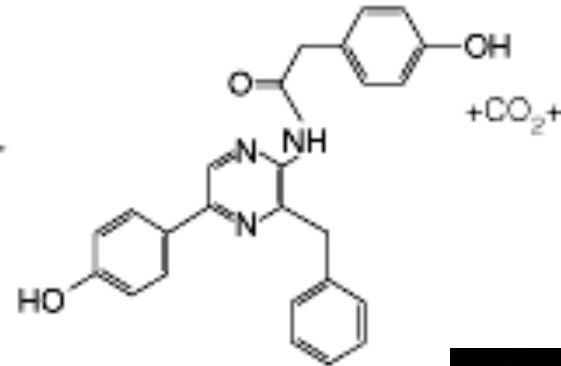
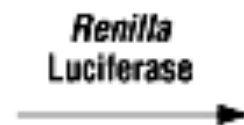
Light



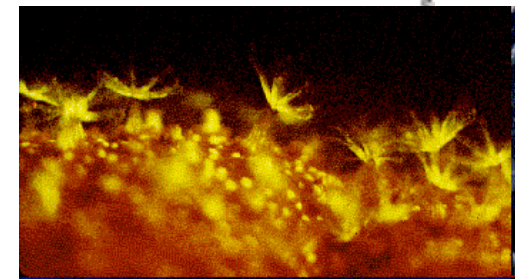
Light

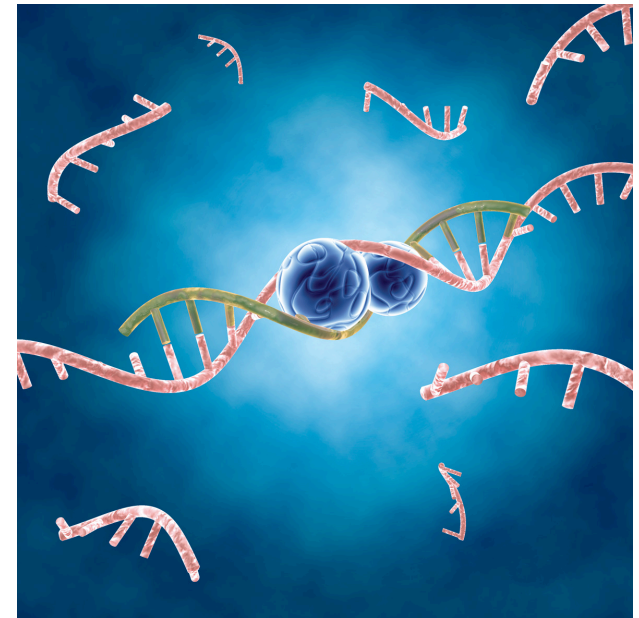
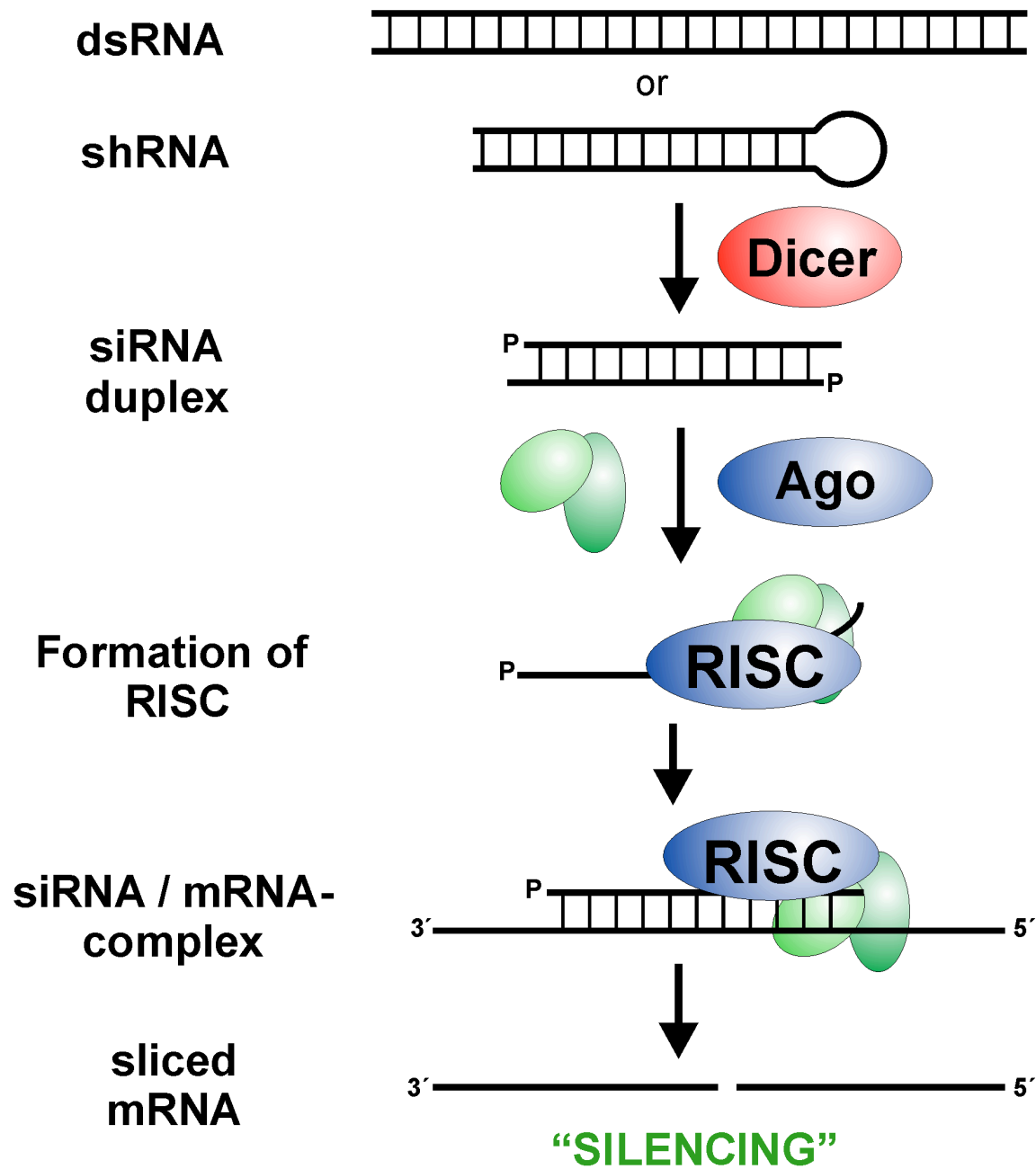


Coelenterazine



Coelenteramide





Snapshot of the next four weeks

We will eliminate the expression of various genes using

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