

# Module 2: Expression Engineering

20.109

Lecture 1

October 11th, 2007

# Scientific Endeavors...

Images of butterfly collection and evolving hominid skulls  
removed due to copyright restrictions.

## Is a butterfly collection a science experiment?

*“Charles Darwin was once asked if he thought that natural historians should collect data without the prejudice of a preformed hypothesis, or whether they should be observing nature with a particular theory in mind. In a stinging reply to friend, the economist Henry Fawcett, Darwin wrote that they may as well “go into a gravel-pit and count the pebbles and describe the colors.”*

From Erren, T. C., et al. “Comparing Neanderthal and Human Genomes.” *Science* 315, no. 5819 (3 March, 2007): 1664.

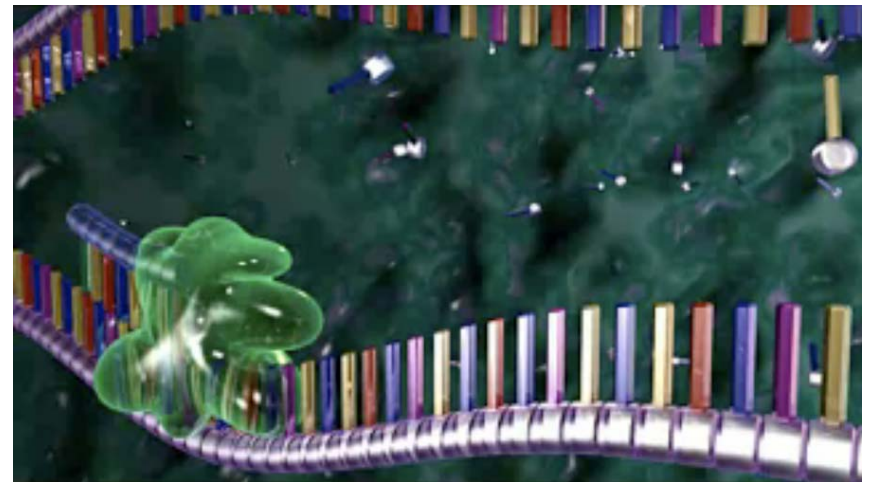
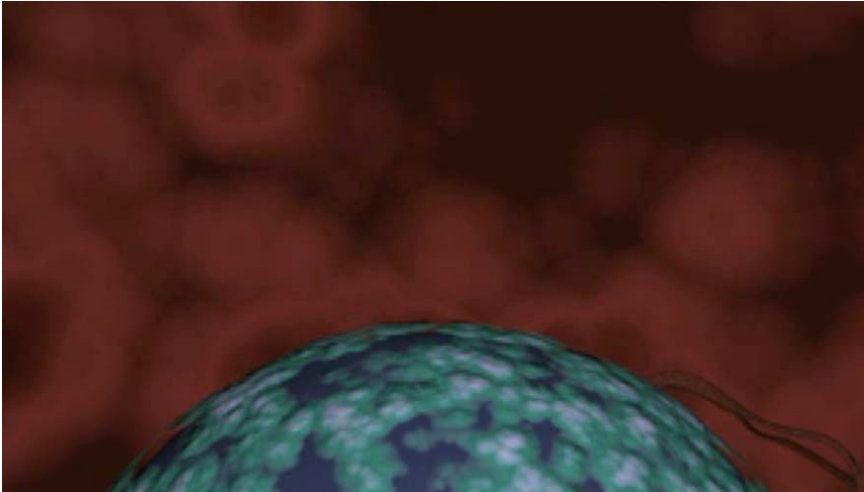
The dynamic proteome, not the static genome,  
determines an organism's phenotype.

For instance, the caterpillar and tadpole share genomes  
with the butterfly and the frog, but their very different  
proteomes create very different organisms.

Images removed due to copyright restrictions.

# Eukaryotic Gene Expression

## *Obstacles to expression*



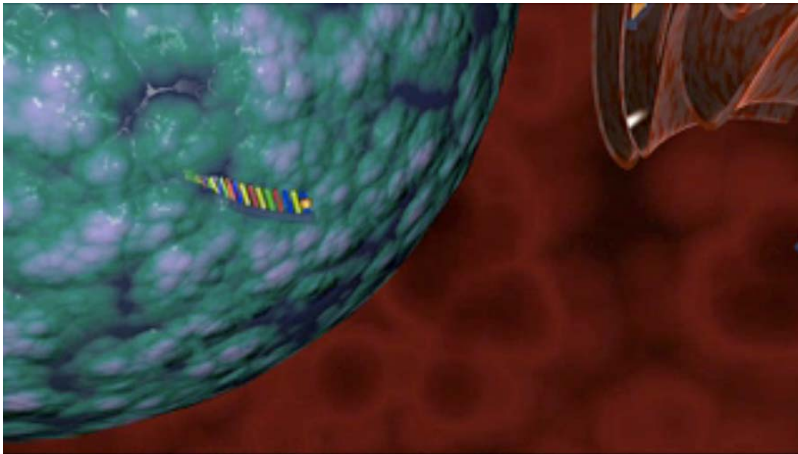
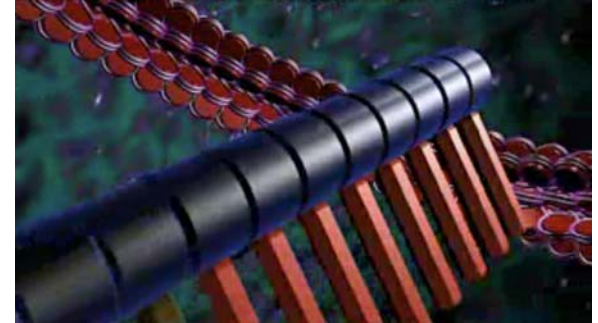
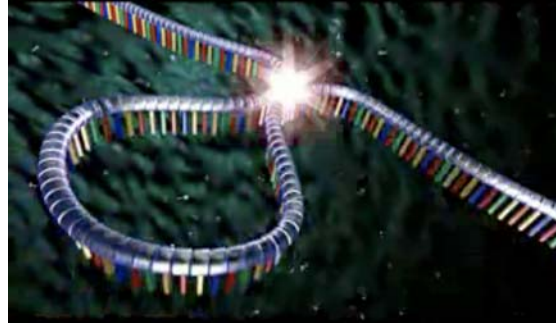
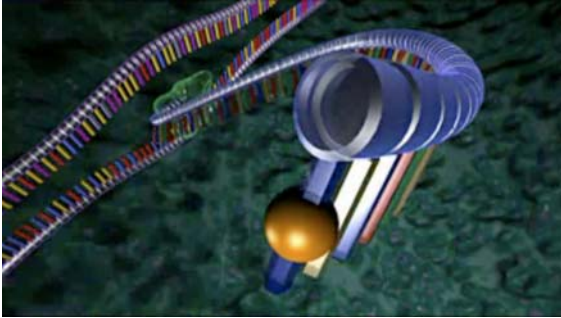
## *Transcription*

Courtesy of Arkitek Studios. Used with permission.

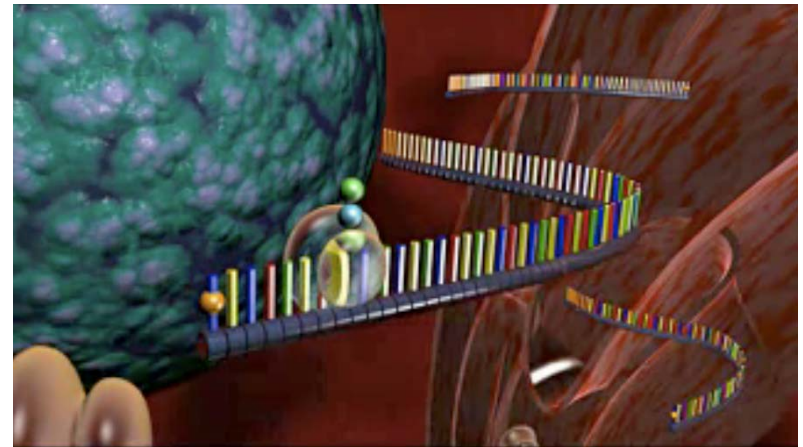
Source: RNAi Interface animation for nature.com (<http://www.nature.com/focus/rnai/animations/index.html>)

# Eukaryotic Gene Expression

## *mRNA processing*



## *Export*

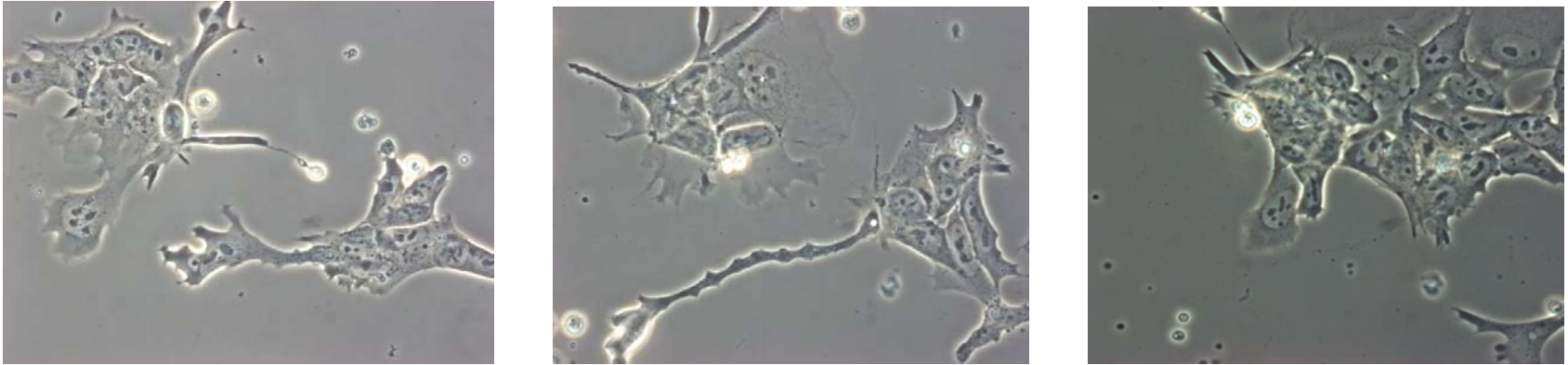


## *Translation*

Courtesy of Arkitek Studios. Used with permission.

Source: RNAi Interface animation for nature.com (<http://www.nature.com/focus/rnai/animations/index.html>)

# DNA---> RNA--> Protein



Movie source: [http://www.exploratorium.edu/imaging\\_station/gal\\_media/stemcells/mouse\\_embryonic\\_1/mouse\\_embryonic\\_1.mov](http://www.exploratorium.edu/imaging_station/gal_media/stemcells/mouse_embryonic_1/mouse_embryonic_1.mov)

Credit: Kristina Yu. Courtesy of The Exploratorium.

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This movie was taken over a 12-hour period.

1. To  $\Delta$  cell's behavior, genome engineering too slow
2. To understand cell's behavior, we need a way to study them

# Expression Engineering Pressure Points

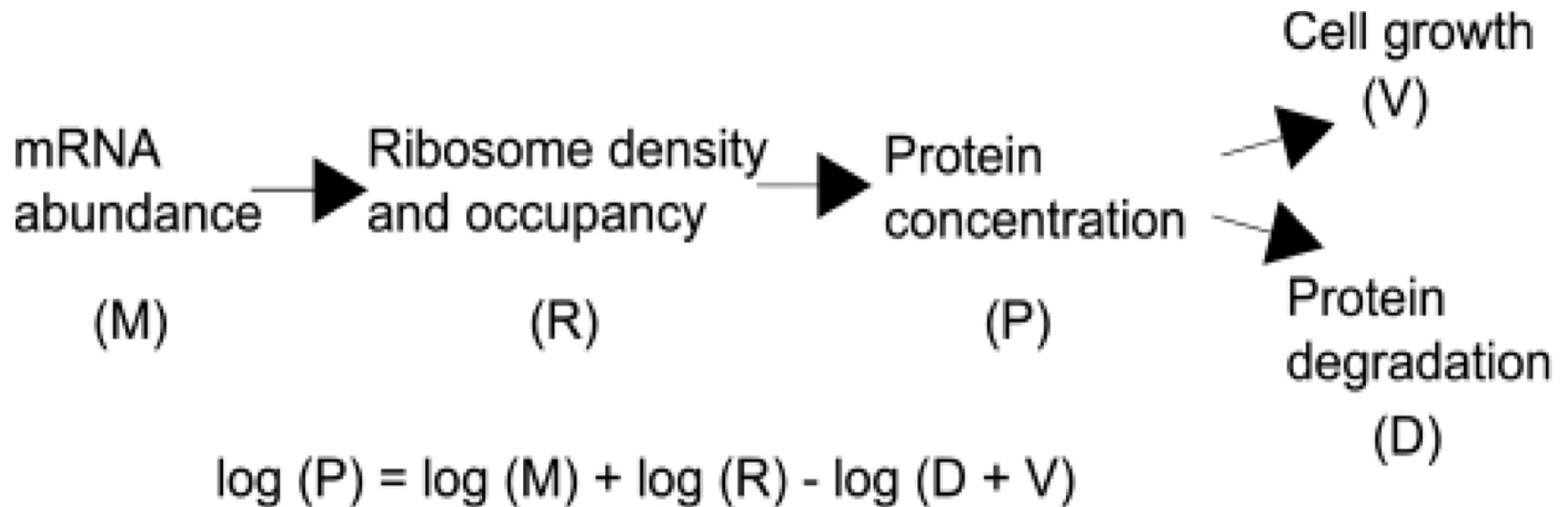


Figure courtesy of National Academy of Sciences, U. S. A. Used with permission.

Source: Belle, A., et al. "Quantification of protein half-lives in the budding yeast proteome." *PNAS* 103 no. 35 (2006): 13004-9. DOI: 10.1073/pnas.0605420103. Copyright © 2006 National Academy of Sciences, U.S.A.



# Expression Engineering Pressure Points

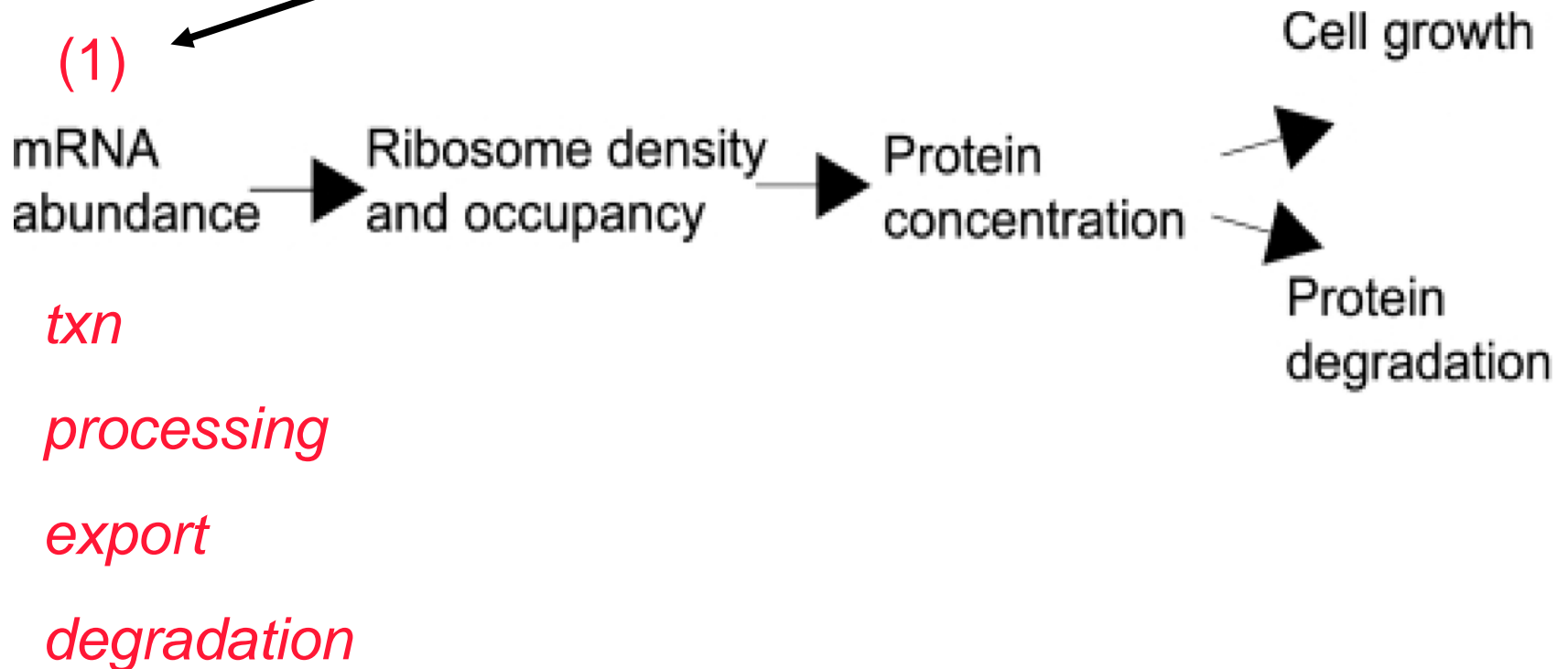


Figure courtesy of National Academy of Sciences, U. S. A. Used with permission.

Source: Belle, A., et al. "Quantification of protein half-lives in the budding yeast proteome." *PNAS* 103 no. 35 (2006): 13004-9. DOI: 10.1073/pnas.0605420103. Copyright © 2006 National Academy of Sciences, U.S.A.

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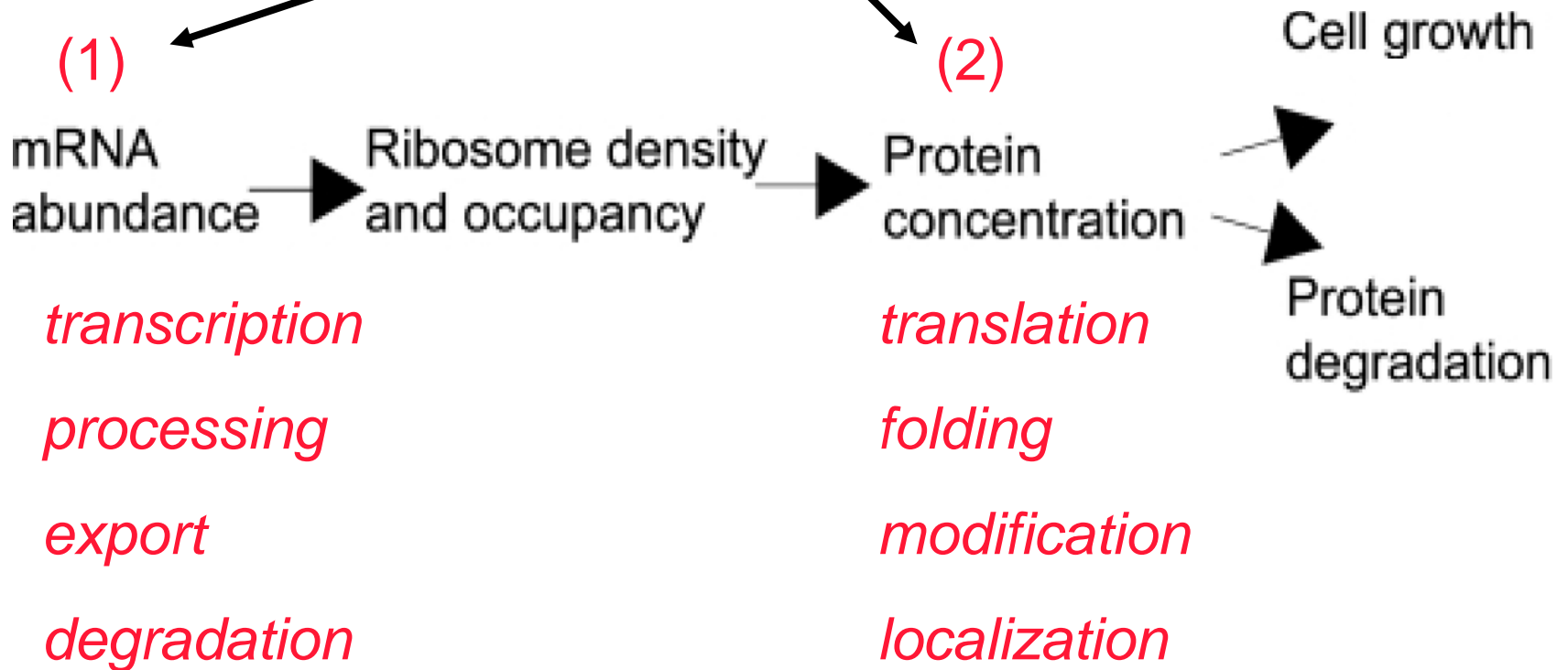


Figure courtesy of National Academy of Sciences, U. S. A. Used with permission.

Source: Belle, A., et al. "Quantification of protein half-lives in the budding yeast proteome." *PNAS* 103 no. 35 (2006): 13004-9. DOI: 10.1073/pnas.0605420103. Copyright © 2006 National Academy of Sciences, U.S.A.

# Expression Engineering Pressure Points

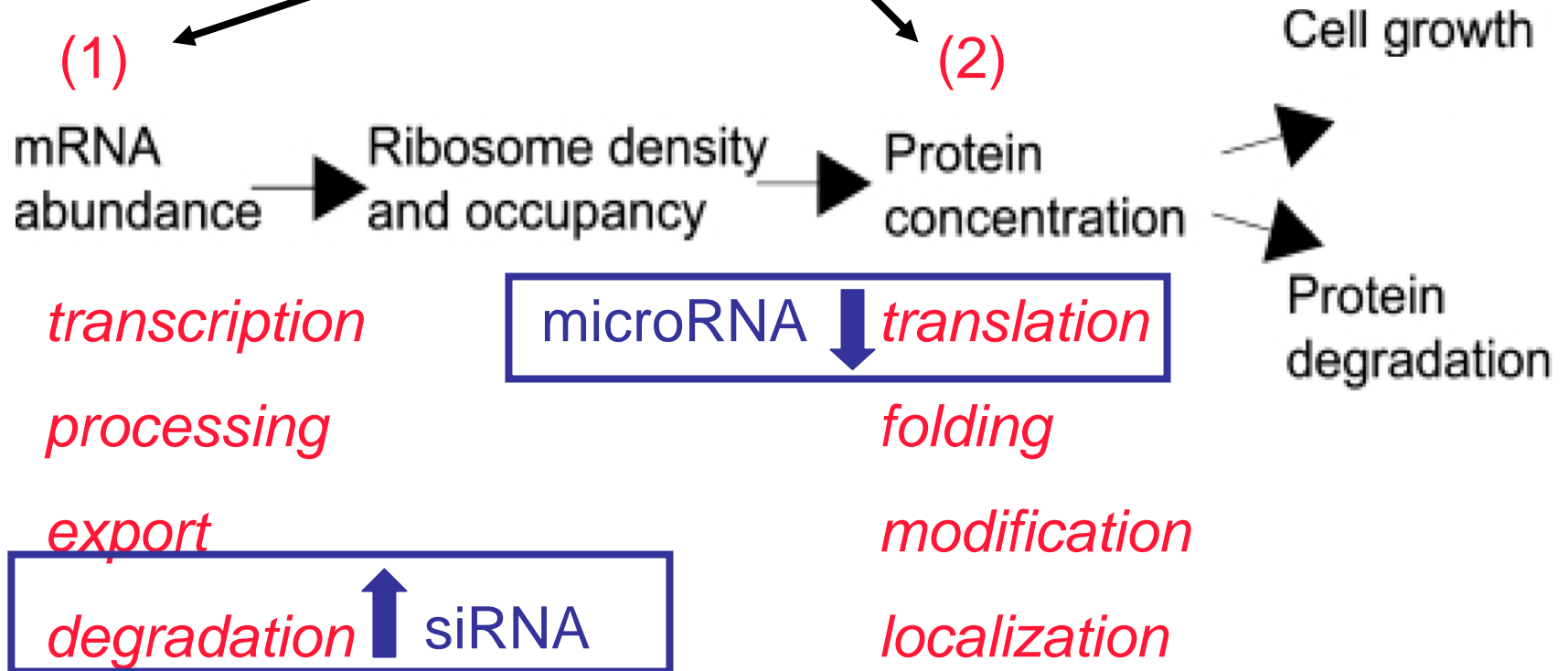


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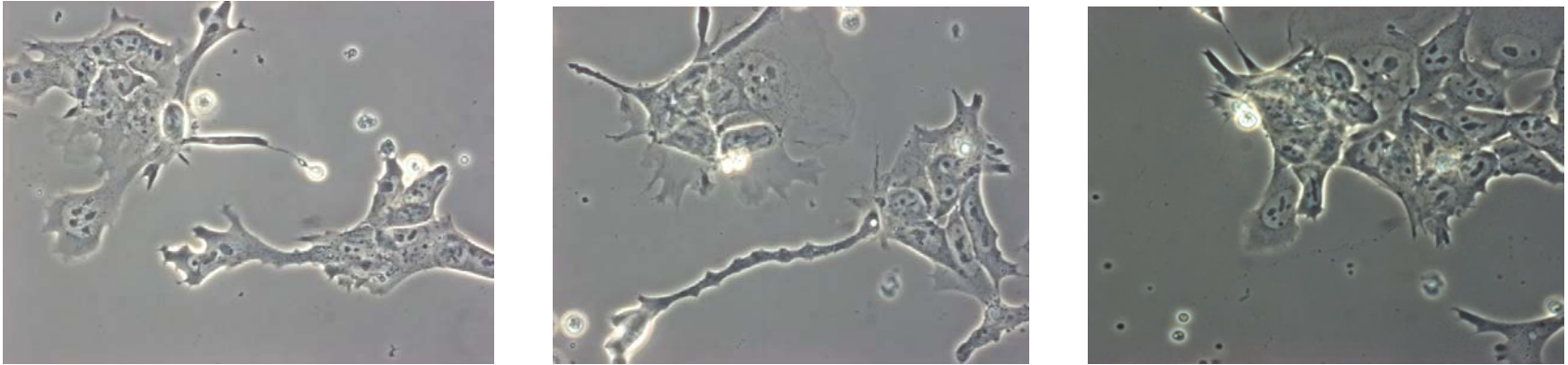
Source: Belle, A., et al. "Quantification of protein half-lives in the budding yeast proteome." *PNAS* 103 no. 35 (2006): 13004-9. DOI: 10.1073/pnas.0605420103. Copyright © 2006 National Academy of Sciences, U.S.A.

# Why care about gene expression?

## Why work on a module called “expression engineering?”

1. Disease from mis-expression
2. Expression is part of cellular reactions to environment
3. Knowledge is good
4. Make things that are useful from gene expression regulation
5. Silencing/timing of gene expression regulate growth and development

# DNA---> RNA--> Protein



Movie source: [http://www.exploratorium.edu/imaging\\_station/gal\\_media/stemcells/mouse\\_embryonic\\_1/mouse\\_embryonic\\_1.mov](http://www.exploratorium.edu/imaging_station/gal_media/stemcells/mouse_embryonic_1/mouse_embryonic_1.mov)

Credit: Kristina Yu. Courtesy of The Exploratorium.  
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This movie was taken over a 12-hour period.

1. To  $\Delta$  cell's behavior, genome engineering too slow
2. To understand cell's behavior, we need a way to study them

# How do you grow mammalian cells?

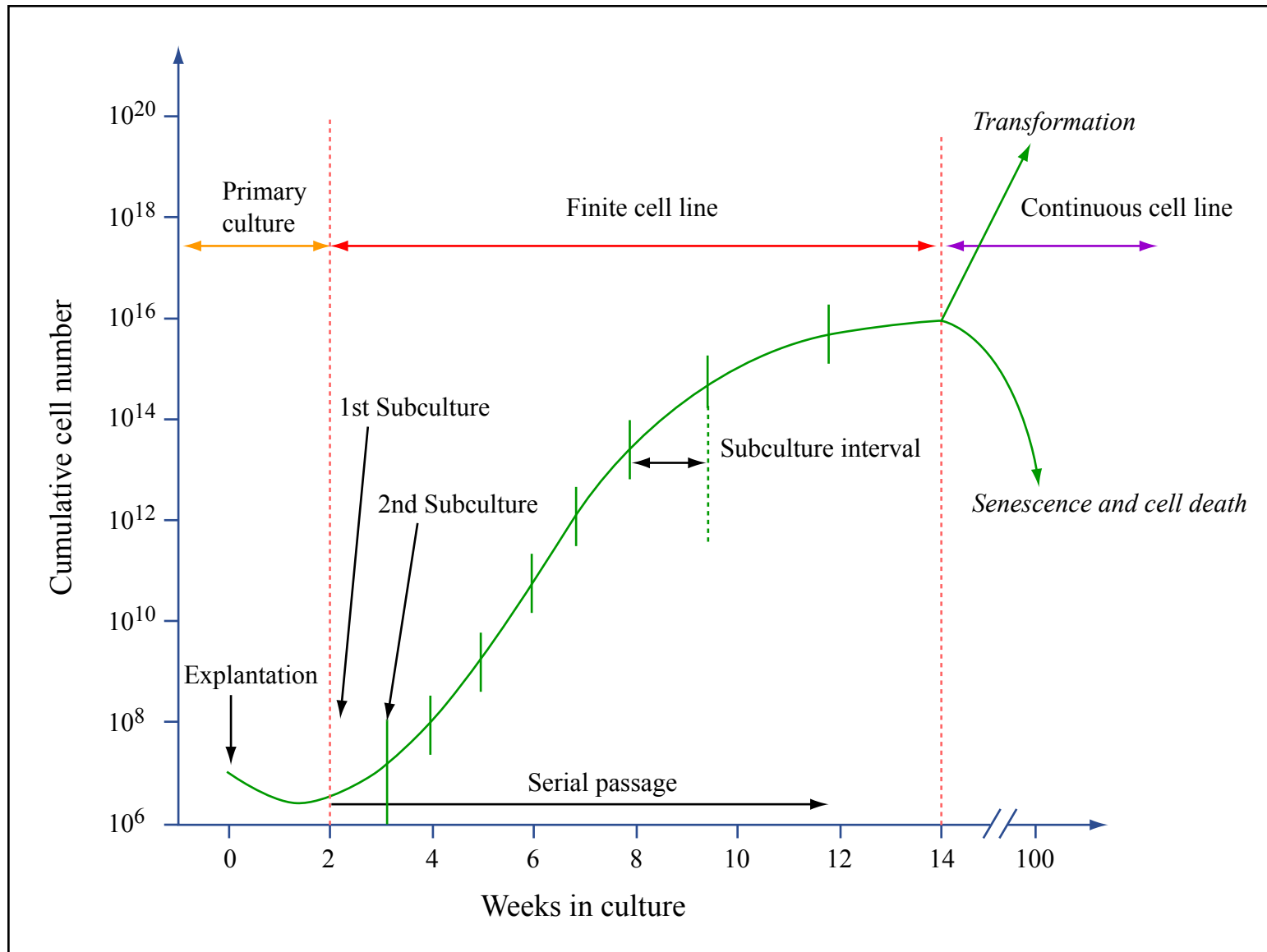
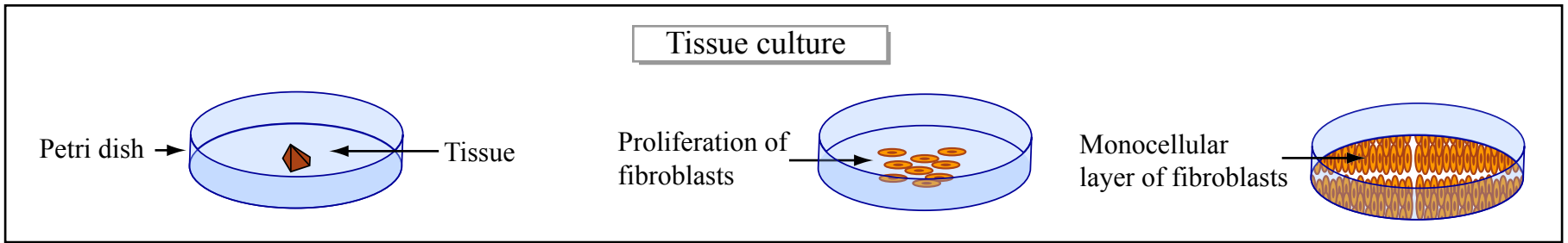
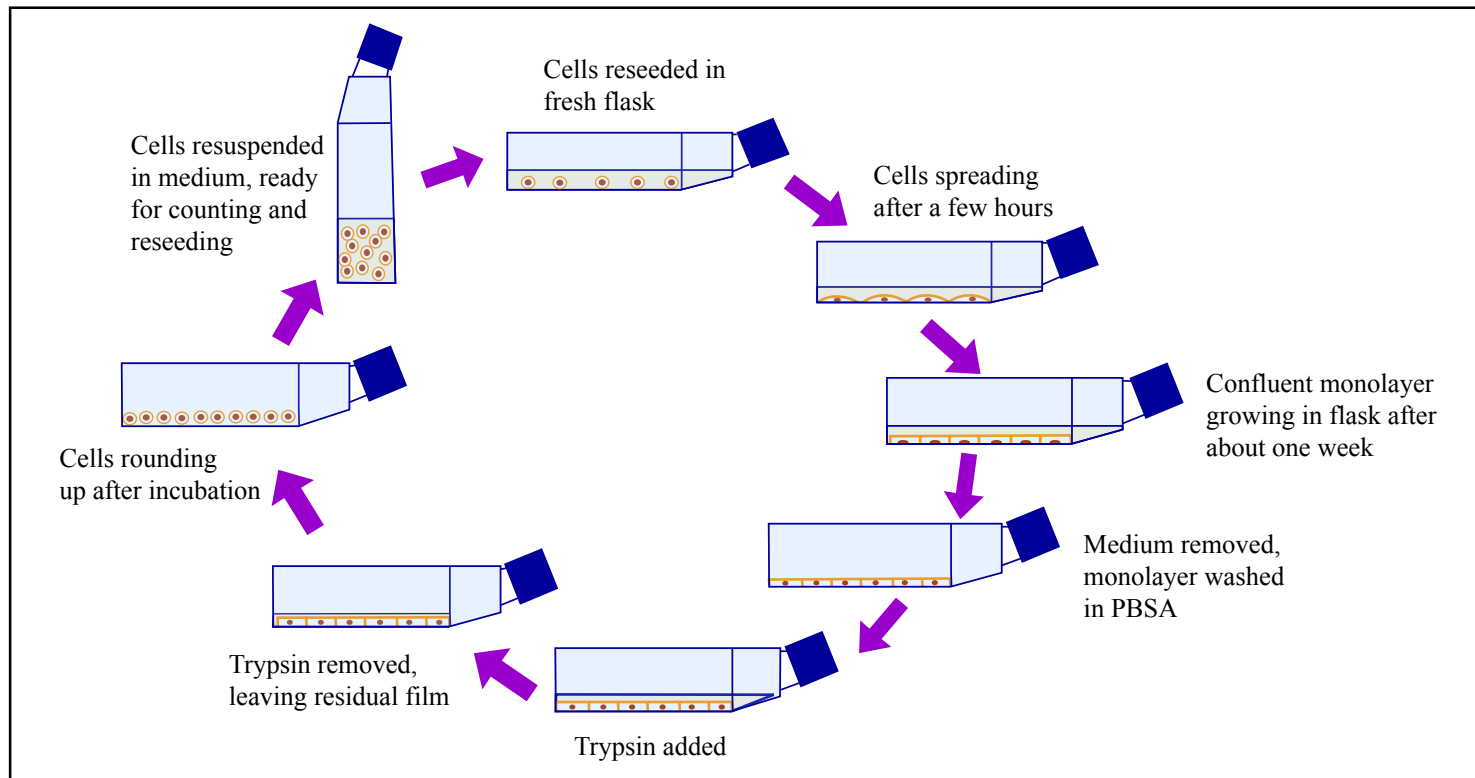


Figure by MIT OpenCourseWare. Adapted from Freshney's *Culture of Mammalian Cells*.

# How do you grow mammalian cells?



Figures by MIT OpenCourseWare. Adapted from Freshney's *Culture of Mammalian Cells*.



# How do you grow mammalian cells?

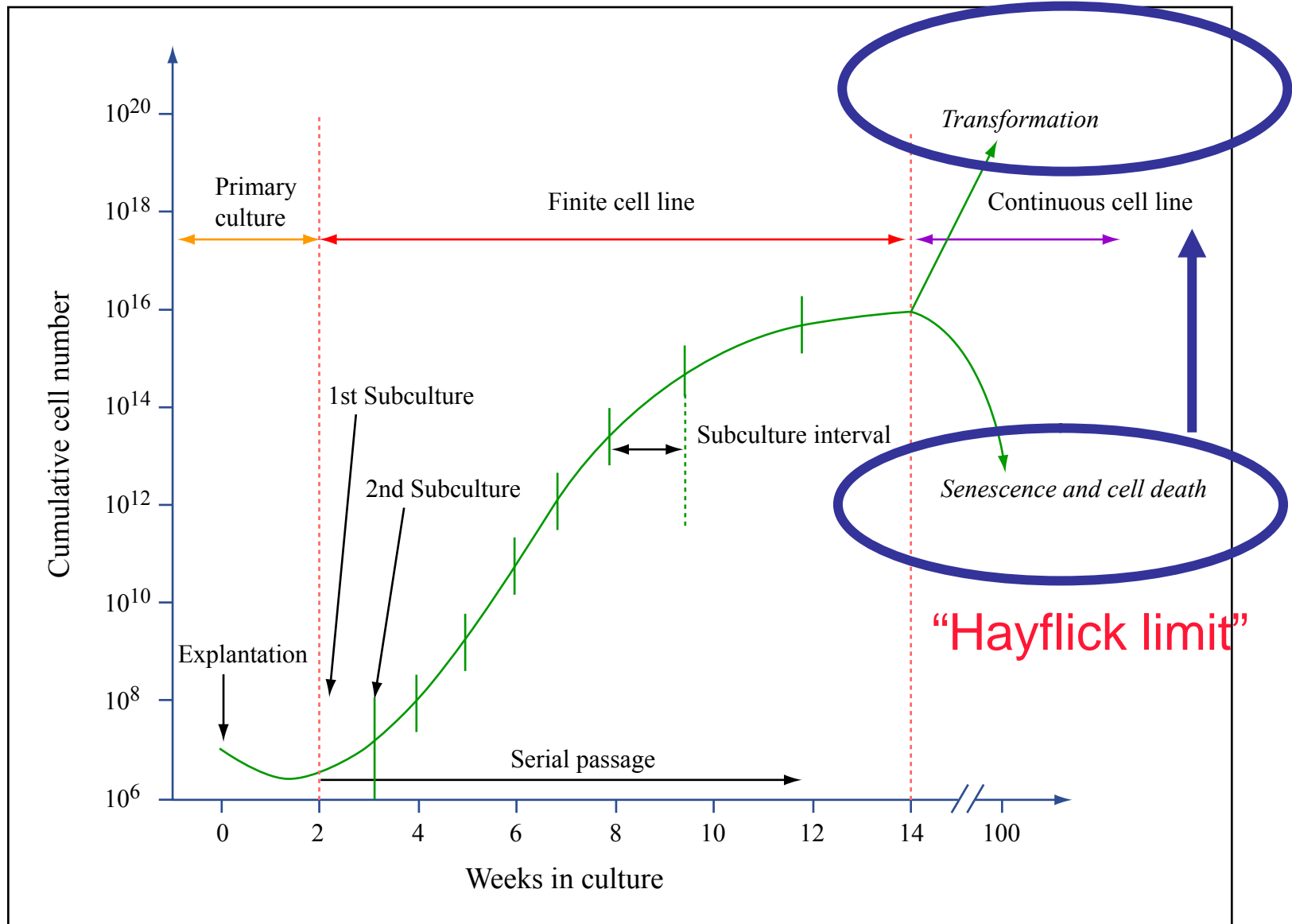


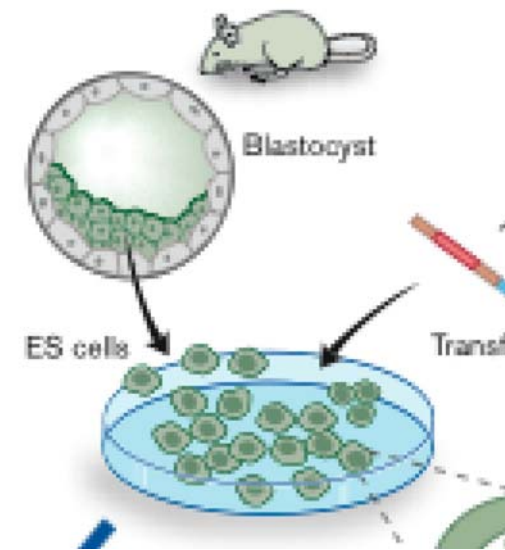
Figure by MIT OpenCourseWare. Adapted from Freshney's *Culture of Mammalian Cells*.



# Culturing MES cells



are cultivated from mouse pre-implantation embryos (blastocysts).



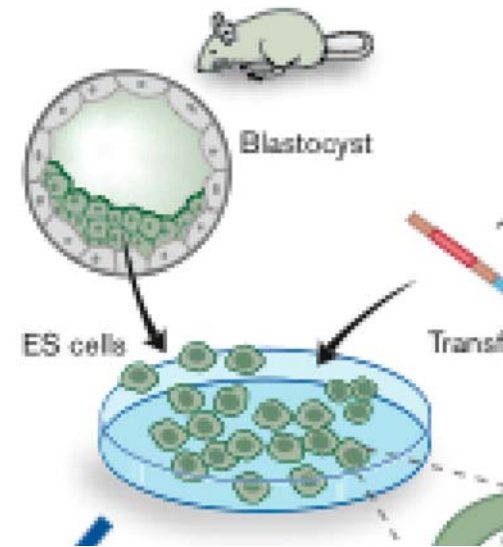
[http://nobelprize.org/nobel\\_prizes/medicine/laureates/2007/index.html](http://nobelprize.org/nobel_prizes/medicine/laureates/2007/index.html)

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Photos of the laureates removed due to copyright restrictions.  
Mario R Capecchi, Sir Martin J. Evans, and Oliver Smithies.

# Culturing MES cells

Embryonic stem (ES) cells are cultivated from mouse pre-implantation embryos (blastocysts).



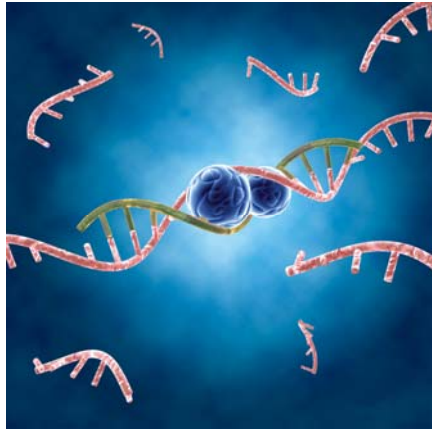
Set of six images removed due to copyright restrictions.

See [http://firstivf.net/laboratory\\_tour.htm](http://firstivf.net/laboratory_tour.htm).

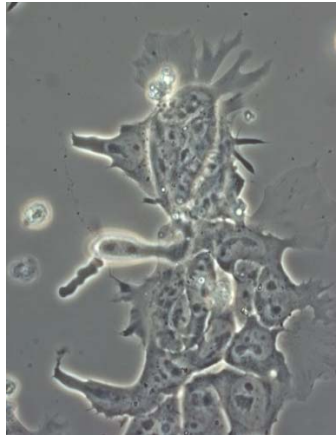
Photos of the laureates removed due to copyright restrictions.  
Mario R Capecchi, Sir Martin J. Evans, and Oliver Smithies.

# Expression Engineering Experiment

Day 1

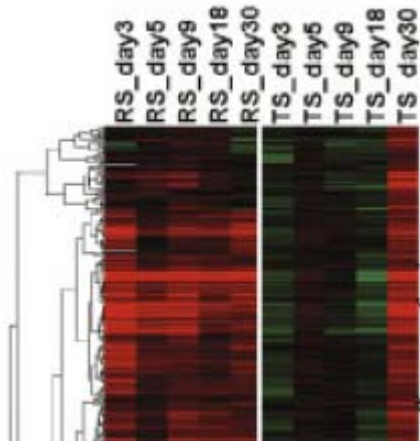


Day 2

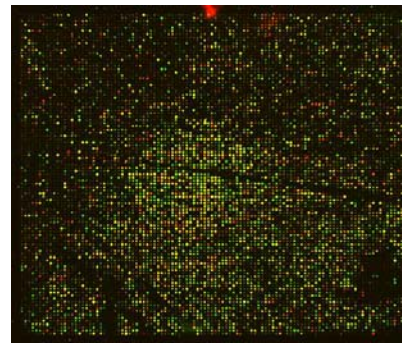


Day 3

Image of glowing luciferase in a microcentrifuge tube, removed due to copyright restrictions.



Day 6



Day 5

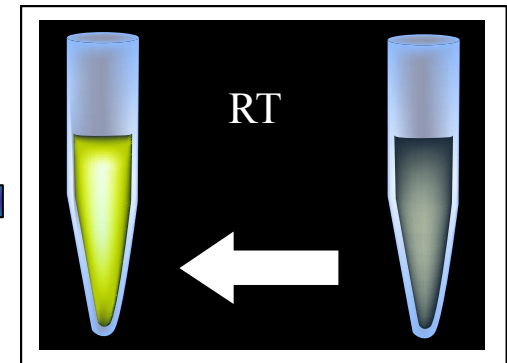


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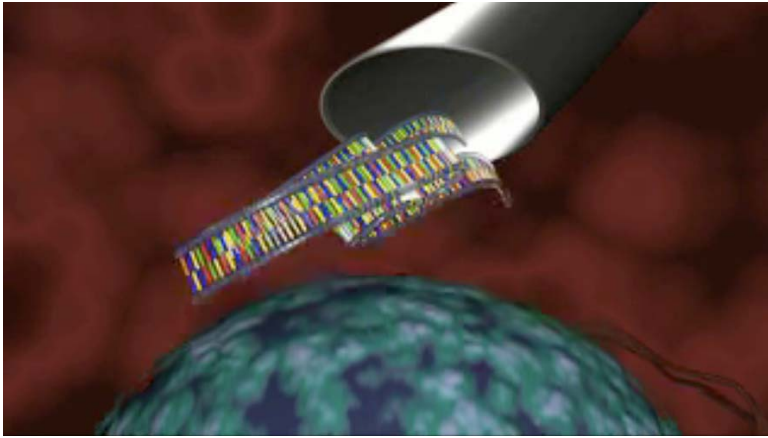
Day 4

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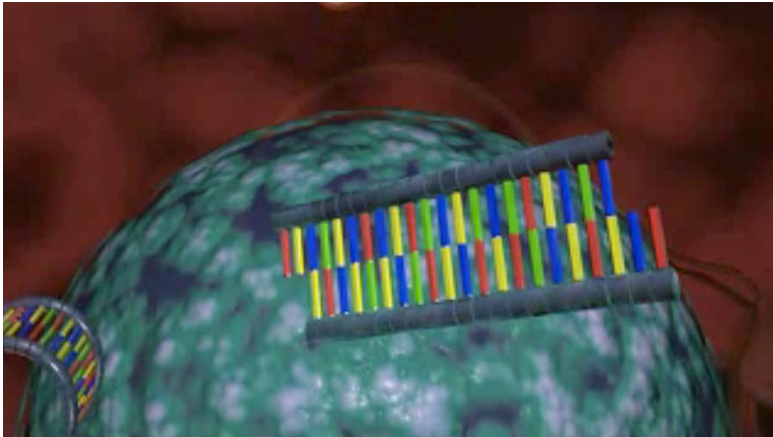
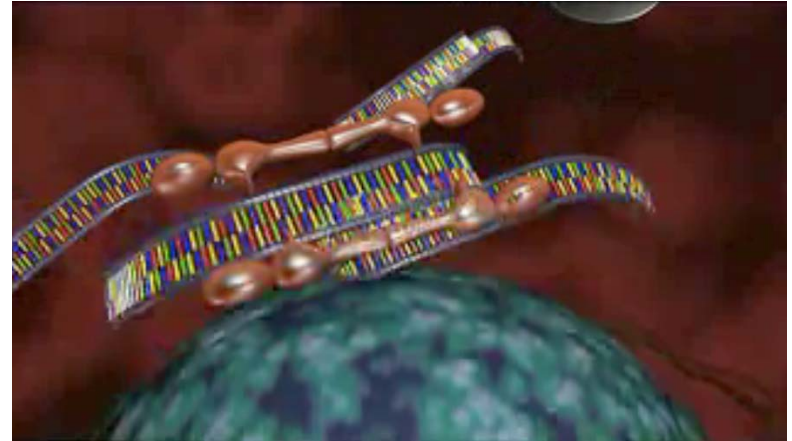
Cite as: Natalie Kuldell. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (<http://ocw.mit.edu>), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].

# RNA interference vocabulary

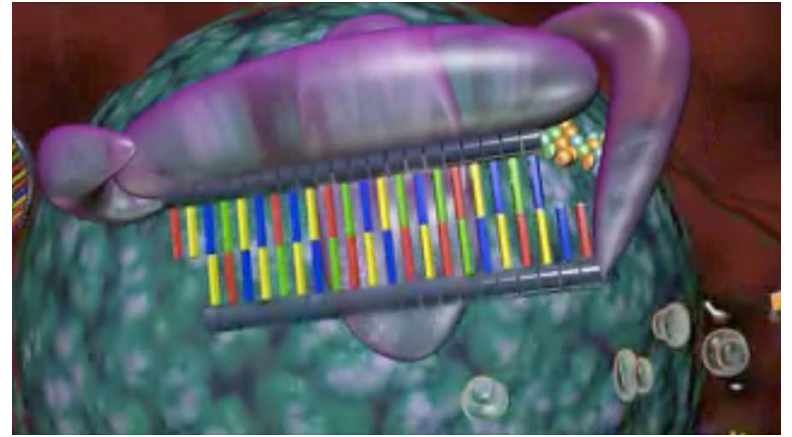
Transfection



Dicer



siRNA



RISC

Courtesy of Arkitek Studios. Used with permission.

Source: RNAi Interface animation for nature.com (<http://www.nature.com/focus/rnai/animations/index.html>)

# Expression Engineering Experiment

## Lecture 1

- intro to cell culture
- intro to gene exp'n/RNAi

## Lecture 2

- transfection
- luciferase

## Lecture 3

- off-target/nonspecific RNAi

## Lecture 4

- Writing lecture  
(Neal Lerner)

## Lecture 5

- measuring gene express'n

## Lecture 6

- microarray analysis  
(Rebecca Fry)

## Lecture 7

- high throughput technologies  
or RNAi applications (no lab)

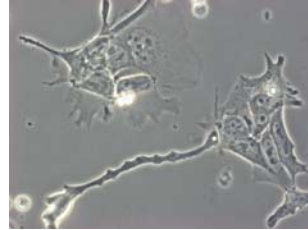
## Lecture 8

- review of your data

# Summary

1. Eukaryotic gene expression

2. Cell culture basics



2a. cells in the lab

2b. cells reaction times require  $\Delta$  protein

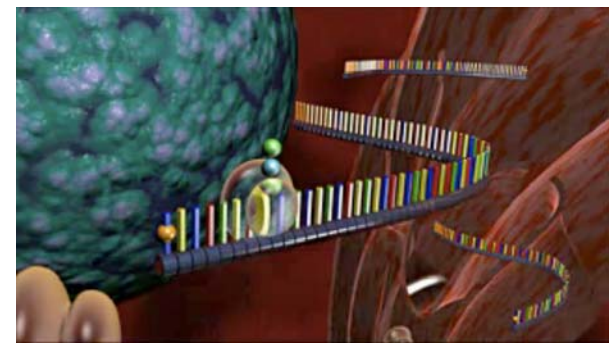
3. RNAi features

21bp siRNAs

Dicer

RISC

bind and destroy target mRNA



Courtesy of Arkitek Studios. Used with permission.

Source: RNAi Interface animation for nature.com

(<http://www.nature.com/focus/rnai/animations/index.html>)

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<http://www.exploratorium.edu>.

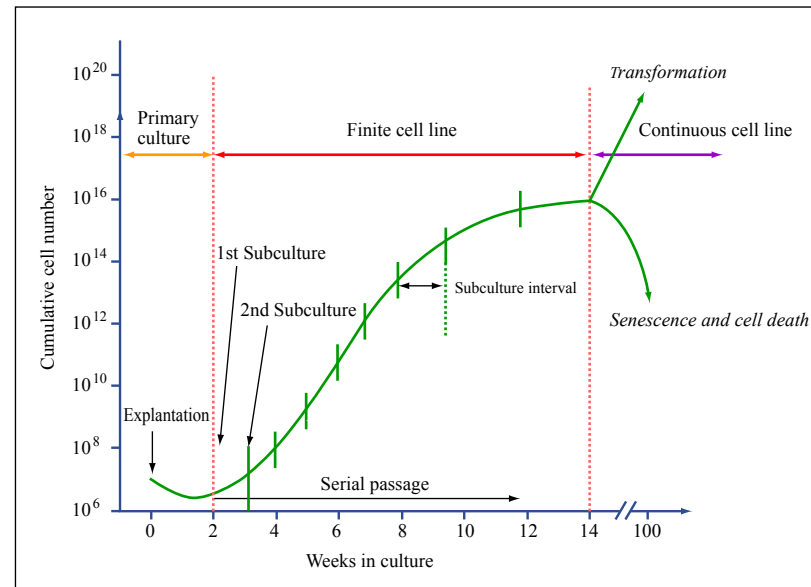


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