

Chapter 5 Topics

1. Animal Life Cycles (We are kind of ignoring plants and many other groups!!! 😞)
2. Structure of DNA in Eukaryotes and Prokaryotes!
(*and HGT*)
3. Replication of DNA (*copying of DNA*) in mitosis
4. Making proteins!
 - Transcription-going from DNA to mRNA
 - Translation-going from mRNA to protein
5. **Gene regulation!**
6. **Sizing up the Genome**
7. Mutations
8. Mitosis and Meiosis (*sexual reproduction*)
9. Mendel and Punnett Squares

Now we get to the fun stuff!

5. Gene regulation!

Gene regulation in Eukaryotes is so important when we look at the diversity of life on earth.

What is gene regulation?

How are some genes turned on and expressed (making proteins) while others are turned off (not making proteins)?

How do some genes make proteins only at certain times in certain cells?

Obviously not every cell is always making everything it has the instructions for!
(housekeeping genes)

What are the different ways genes are regulated?

1. Epigenetic modifications
2. Transcription factors
3. Post-transcriptional modification or processing
4. By affecting whether the mRNA is translated into a protein (microRNA can block)
5. By modifying the polypeptide or protein after it has been made (**post-translational** level)

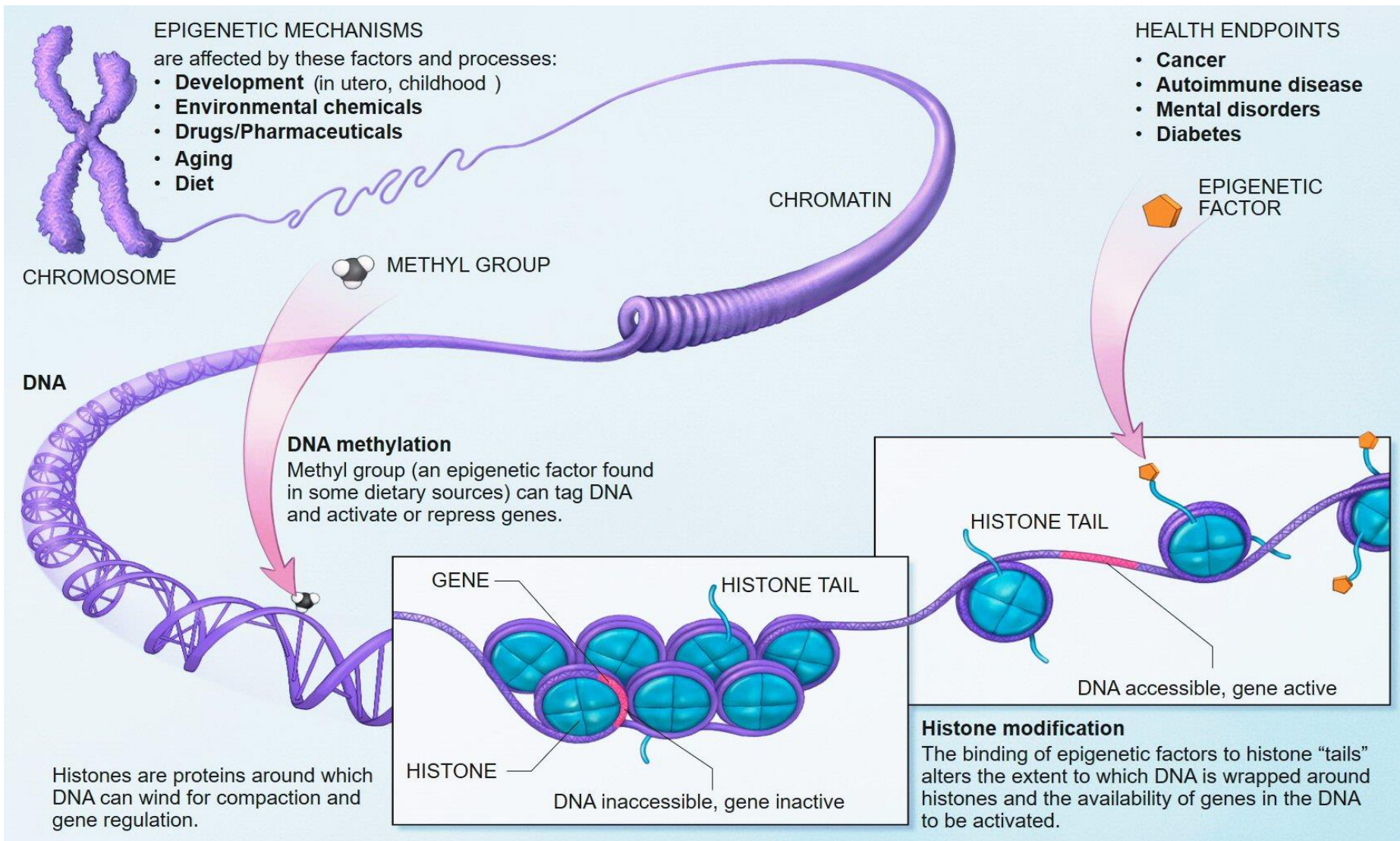
(FYI your text categorizes these slightly differently)

1. Through epigenetic modifications

histone modification affects coiling of DNA or
methylation (addition of methyl groups) “covers” the DNA

Access to the DNA “from above” is blocked so mRNA cannot be made.

“epigenetic” means above the genome



Wikipedia!

Cool Epigenetic facts!

Epigenomic modifications or tags generally remain as cells divide or go through mitosis.

Parts of the genome may be shut down as cells specialize during development.

Environmental influences, such as your diet and exposure to pollutants and level of exercise and stress, can impact your epigenome!

Tags may also persist through **meiosis** (to next generation when gametes are made).

So parents may pass their tags on to their offspring!

Do these epigenetic effects sound Lamarckian?

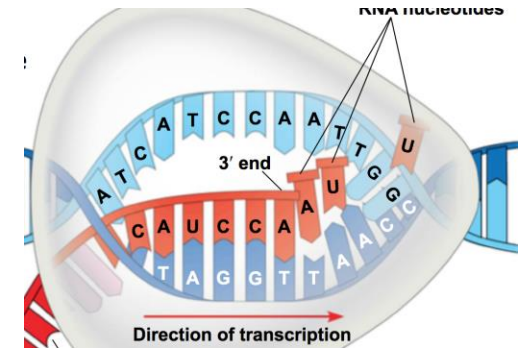
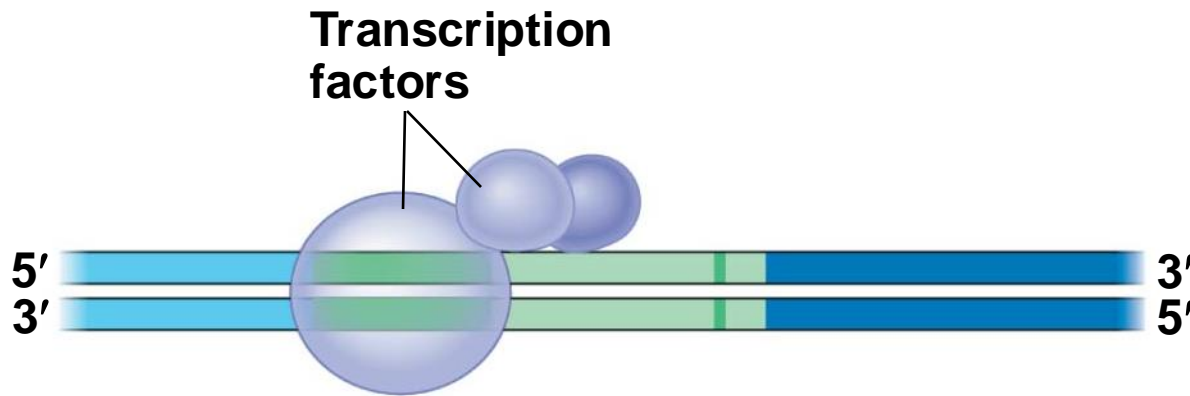
What are the different ways genes are regulated?

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Regulation may occur(continued)

2. Through proteins called **transcription factors** that trigger making of mRNA

Remember that gray blob of RNA polymerase? How did it know where to attach? Transcription factors!



Transcription factors bind to DNA.

In Eukaryotes **transcription factors** (which are proteins) bind first at a spot called the promoter triggering the making of the **mRNA**

It might be that genes are turned on or off (transcribed) based on these transcription factors (your text says like a light switch).

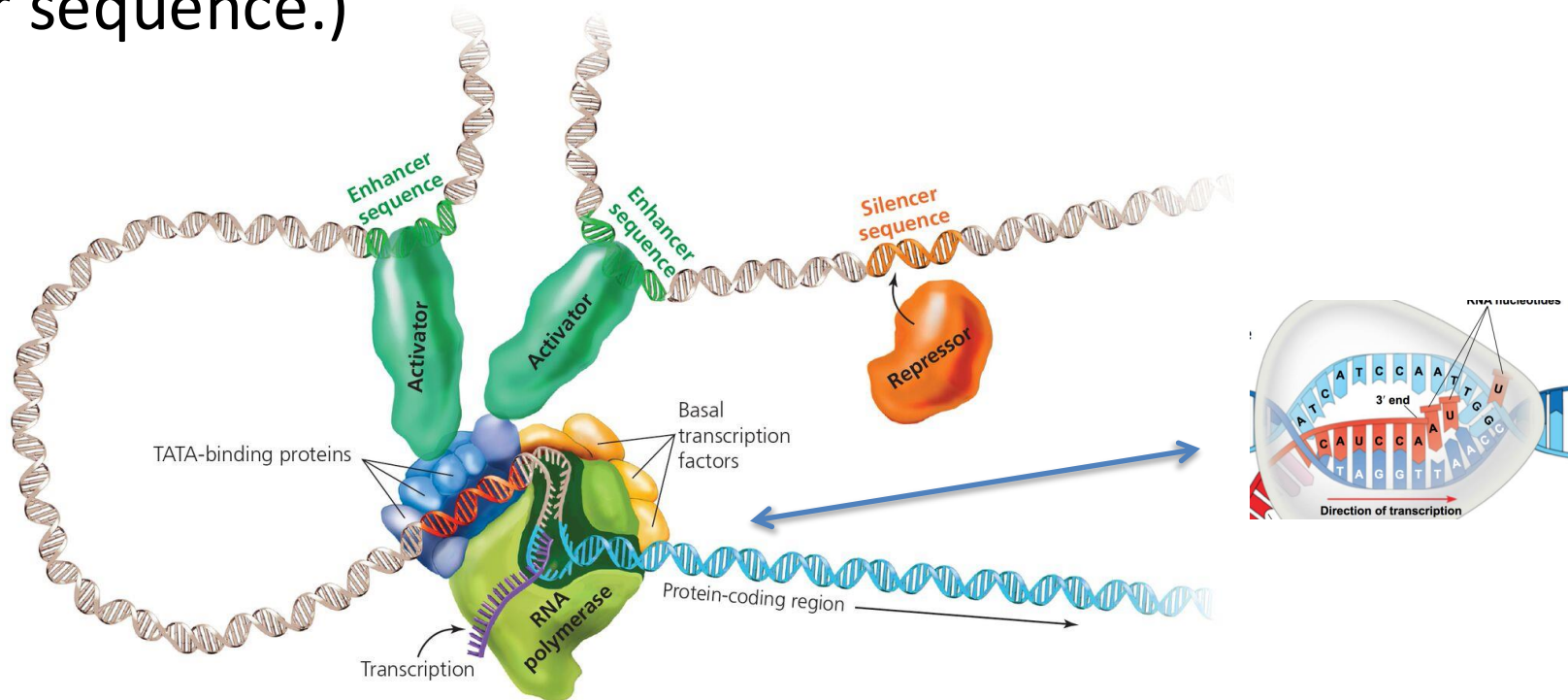
More info but not required...

Binding sites (places where transcription factors attach) for these transcription factors can be close to the gene of interest or far away from the gene (how crazy is that?).

Also note that the gene control region (or locus control region) is just the part of the genome that these transcription factors bind to.

And even more info but not required...
(This is an image from your text!)

The dark green blobs (activators) and the orange blob (repressor) and the yellow blobs (basal transcription factors) are **all transcription factors**. (Activators activate and attach to an enhancer sequence, repressors repress and attach to a silencer sequence.)



Take away...there are a lot of different kinds of transcription factors!

Their presence clues the RNA polymerase to the fact that transcription needs to happen at that place on that chromosome.

Absence means no transcription happens.

So they regulate genes!

1. Epigenetic modifications
2. Through proteins called transcription factors that trigger making of mRNA
3. **Post-transcriptional modification or RNA processing**

Regulation may occur

By affecting how mRNA is **processed after it is transcribed**

In Eukaryotes we actually first make a

“pre-mRNA”

“an RNA transcript”

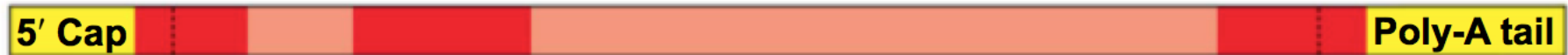
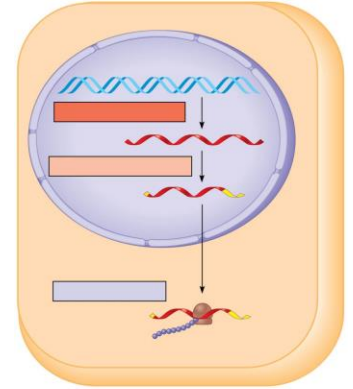
“primary RNA transcript”

“immature RNA transcript”

In Eukaryotes this initial RNA sequence or **pre-mRNA** needs to go through **post-transcriptional modification** before becoming a mature mRNA!

What happens?

- Ends modified (5' cap, poly-A tail added)

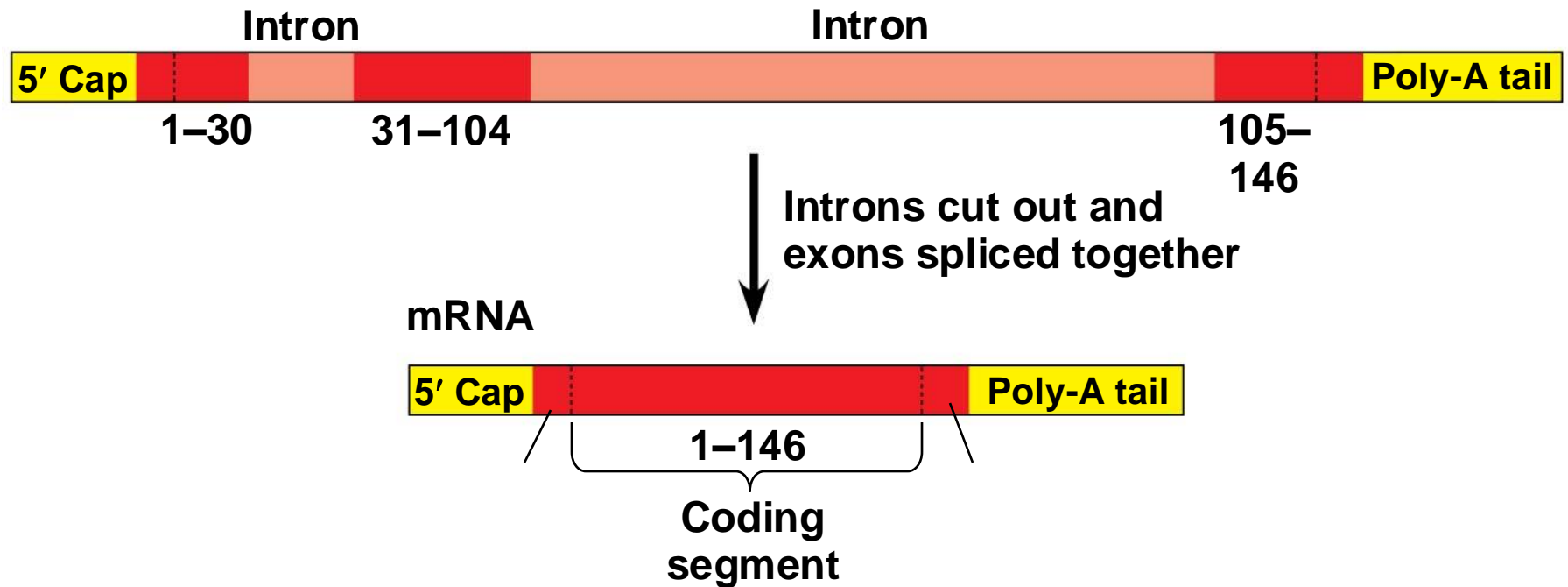


(Why? Seems to help export process, may reduce degradation at ends, may help ribosome grab.)

- Interior sections or **introns** cut out and trashed and **exons** kept and spliced together=RNA splicing.



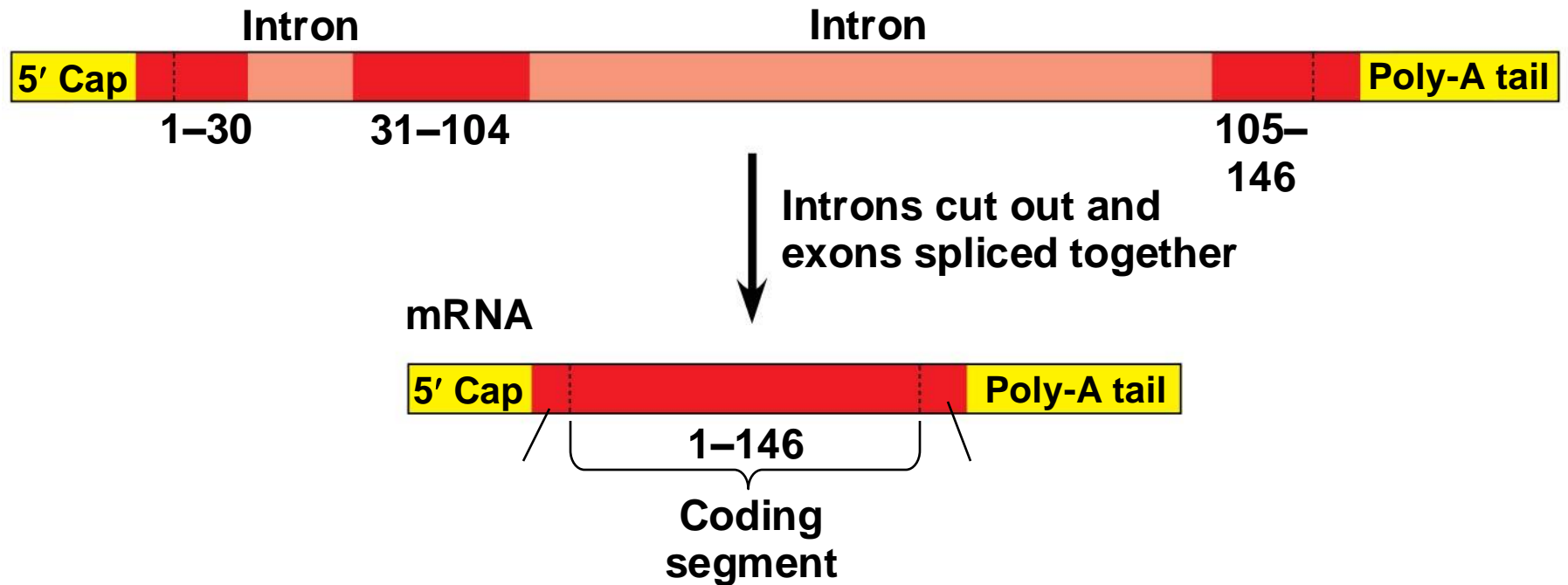
RNA splicing



So....the mRNA molecule that enters cytoplasm is a very abridged version!

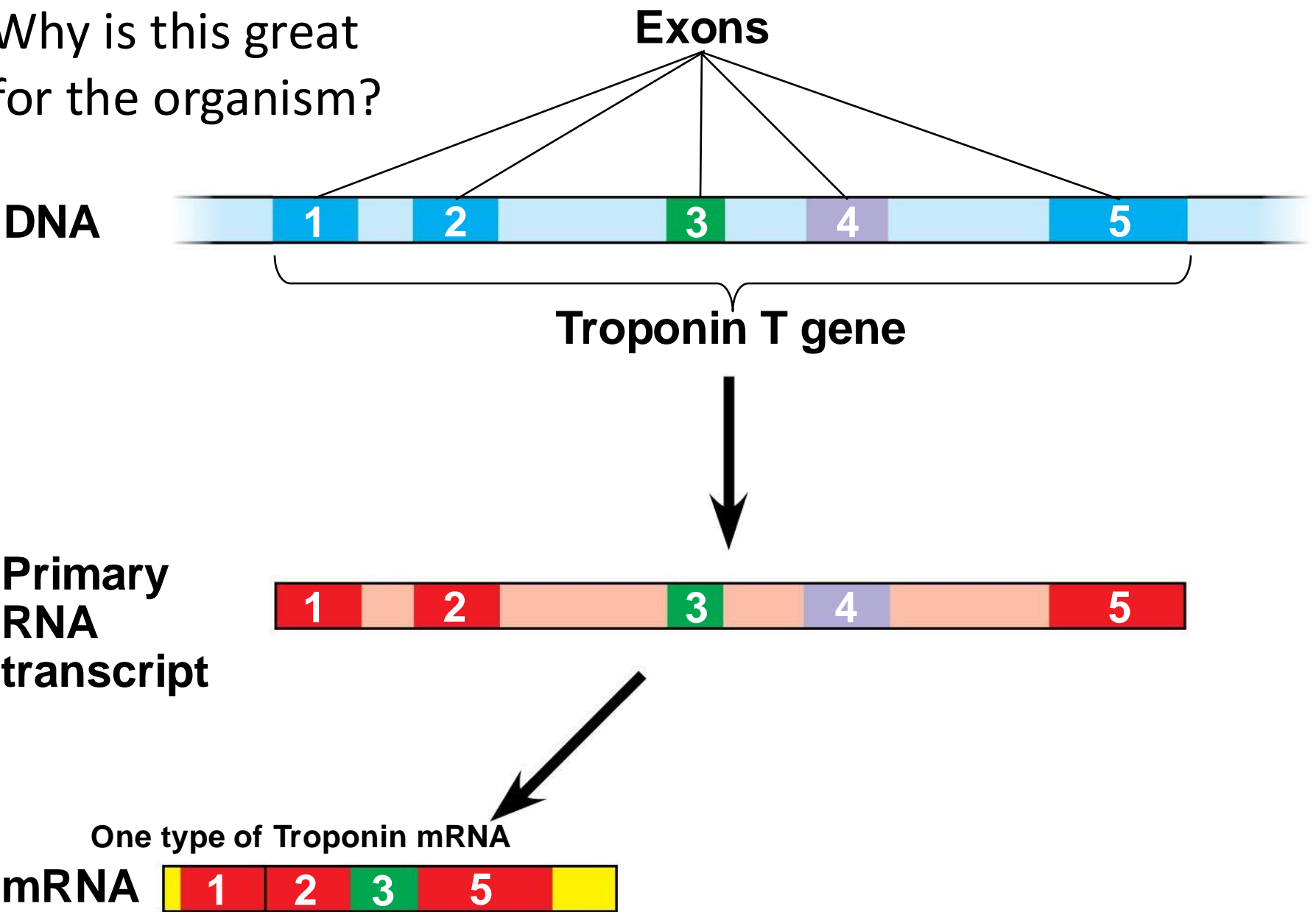
RNA splicing is really amazing because.....

A single gene can encode more than one kind of polypeptide or protein –depends on which sections treated as exons!

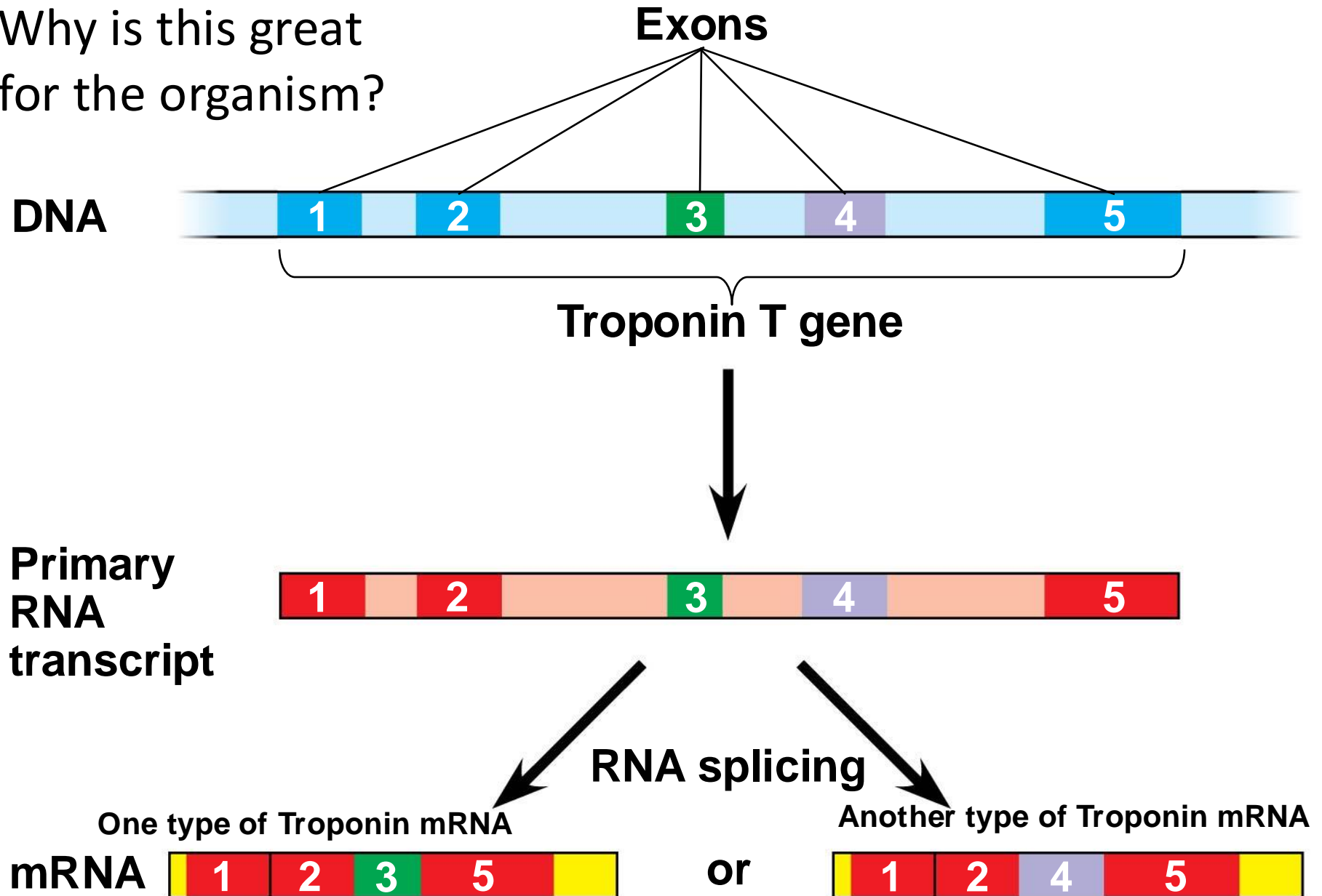


So lets say you were going to make a protein out of only two of the exons above rather than the three...how many different proteins could you potentially make?

Why is this great
for the organism?



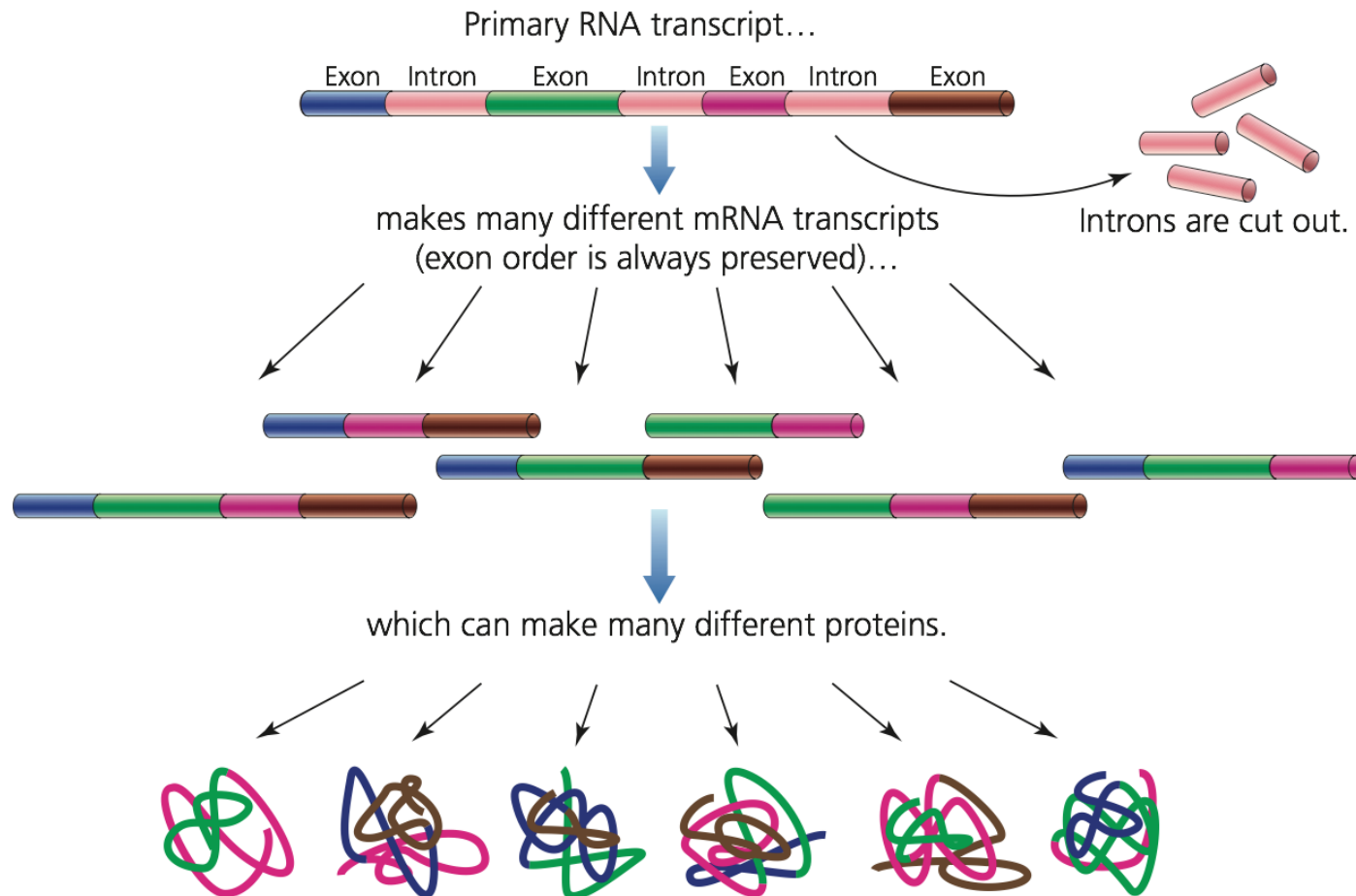
Why is this great
for the organism?



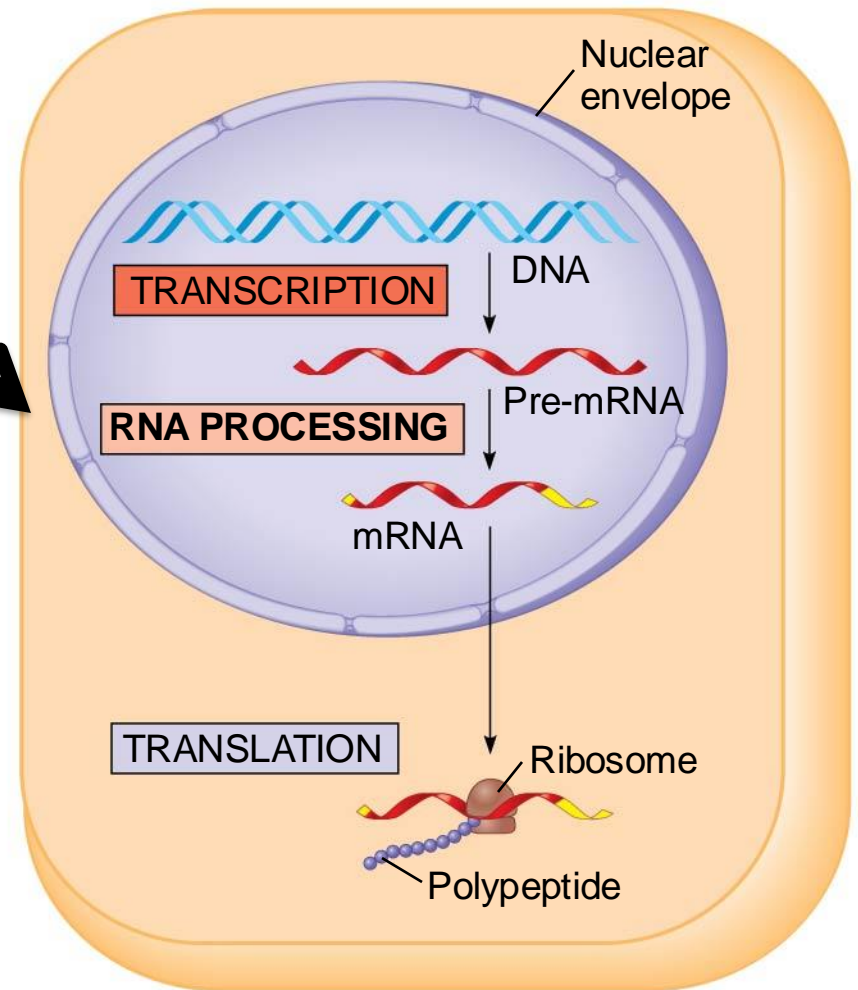
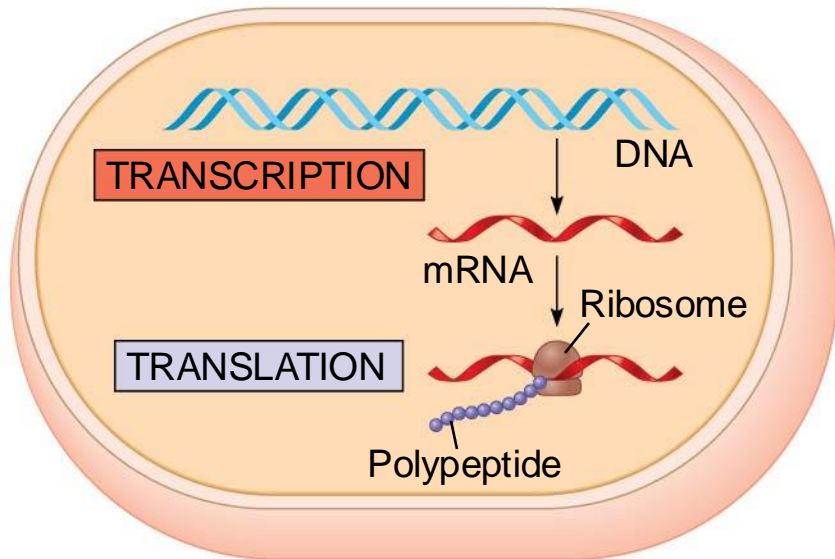
FYI..."Troponins are a group of proteins found in skeletal and heart (cardiac) muscle fibers that regulate muscular contraction." Wikipedia!

RNA splicing can create multiple proteins from a single gene (and these proteins can have very, very different jobs).

In other words.... protein products are much more diverse than number of genes in Eukaryotes.



Where is “post-transcriptional modification” or “RNA processing” happening?



A research article on alternative RNA splicing....

Check it out!

Adv Exp Med Biol. Author manuscript; available in PMC 2015 Apr 7.

PMCID: PMC4387867

Published in final edited form as:

NIHMSID: NIHMS47396

Adv Exp Med Biol. 2007; 623: 50–63.

PMID: 18380340

doi: 10.1007/978-0-387-77374-2_4

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Complex Alternative Splicing

Jung Woo Park and Brenton R. Graveley

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The publisher's final edited version of this article is available at [Adv Exp Med Biol](#)

See other articles in PMC that [cite](#) the published article.

Abstract

Go to: 

Alternative splicing is a powerful means of controlling gene expression and increasing protein diversity. Most genes express a limited number of mRNA isoforms, but there are several examples of genes that use alternative splicing to generate hundreds, thousands, and even tens of thousands of isoforms. Collectively such genes are considered to undergo complex alternative splicing. The best example is the *Drosophila* Down syndrome cell adhesion molecule (Dscam) gene, which can generate 38,016 isoforms by the alternative splicing of 95 variable exons. In this review, we will describe several genes that use complex alternative splicing to generate large repertoires of mRNAs and what is known about the mechanisms by which they do so.

Introduction

Go to: 

Alternative splicing affords eukaryotes with the opportunity to produce multiple proteins from a single gene ¹⁻³. This allows organisms to maximize the coding capacity of their genomes. To illustrate this, let us consider two different ways in which evolution can produce two highly related proteins starting from a single gene. Take, for instance, a representative human gene that contains ~10 exons, produces a single mRNA iso-form, and encompasses ~28,000 bp ^{4,5}. At least two different scenarios can give rise to a new variant of the encoded protein that contains an additional 10 amino acids. First, the gene could be duplicated and diverge such that either a pre-existing exon is extended by 30 nucleotides or a new 30 nt exon is created. Alternatively, a single 30 nucleotide cassette exon could be inserted into, or arise within an intron in the original gene allowing for two mRNA isoforms that either contain or lack the exon to now be produced. While these two scenarios have a similar outcome - the production of a new protein 10 amino acids longer than the original protein - they can have drastically different consequences on the size of the genome. Gene duplication requires expanding the genome by at least 28,000 bp. In contrast, creating the same protein by simply adding an alternative exon to the original gene would only increase the size of the genome by ~30 nt. The difference in efficiency between gene duplication or the evolution of new alternative exons becomes more pronounced as the number of new isoforms increases. For example, in the same amount of genome space required to generate a single new isoform of our hypothetical gene by gene duplication, hundreds of new isoforms could be created by evolving new alternative exons. Thus, alternative splicing is an extremely economic means of increasing protein diversity.

Alternative splicing is prevalent in metazoan genomes. For example, current estimates suggest that at least 42% of *Drosophila* genes ⁶ and over two thirds of mouse and human genes ⁷ encode alternatively spliced pre-mRNAs. These numbers have been increasing at a brisk pace over the past several years and are likely to still be underestimates as many low abundance, tissue-specific or developmentally regulated isoforms almost certainly remain to be characterized. Thus, it is now fair to say that the majority of metazoan genes encode alternatively spliced pre-mRNAs. Alternative splicing is clearly the rule, not the exception.

So far we have talked about...

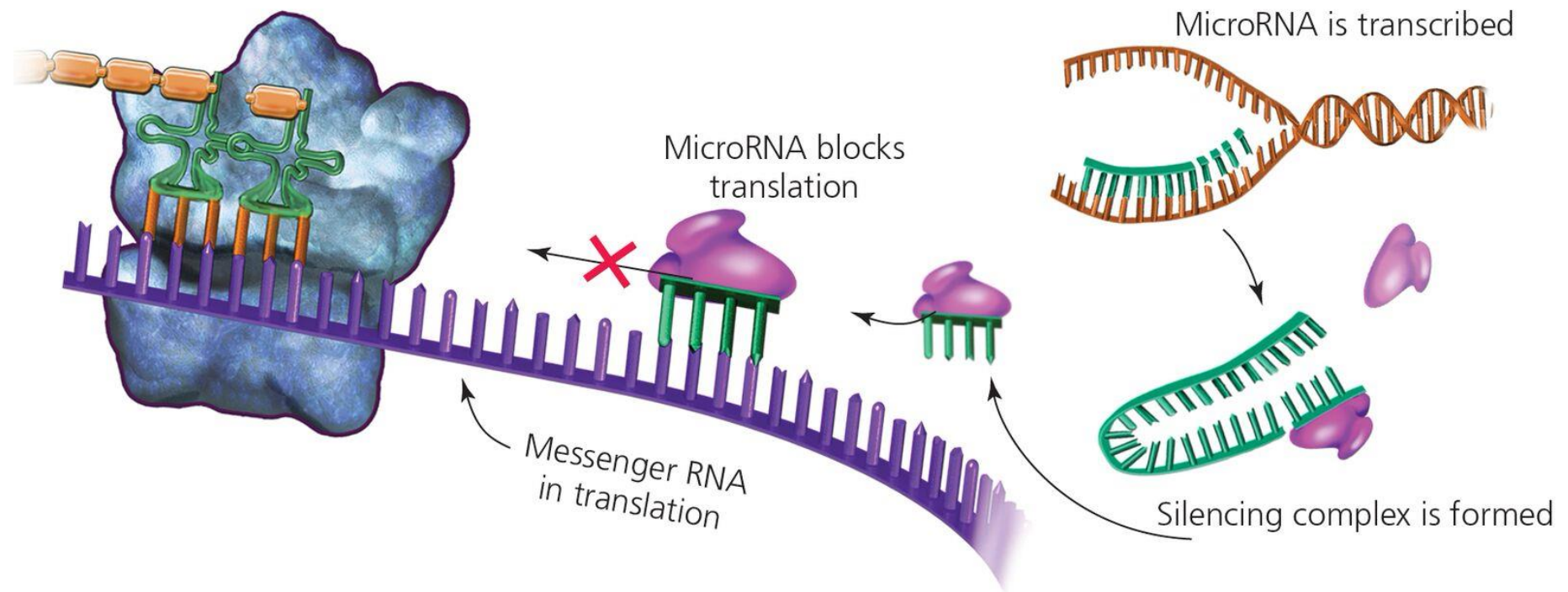
1. Epigenetic modifications
2. Transcription factors
3. Post-transcriptional modification or processing

Now, once you have your mature mRNA gene regulation may also occur

4. By affecting whether or not that mRNA is actually translated into a protein

How does this happen?

microRNA out in the cytoplasm can block translation



Equivalent of our red ribbon in previous images.



MicroRNAs: From Mechanism to Organism

Philipp J. Dexheimer and Luisa Cochella*

Research Institute of Molecular Pathology (IMP), Vienna BioCenter (VBC), Vienna, Austria

MicroRNAs (miRNAs) are short, regulatory RNAs that act as post-transcriptional repressors of gene expression in diverse biological contexts. The emergence of small RNA-mediated gene silencing preceded the onset of multicellularity and was followed by a drastic expansion of the miRNA repertoire in conjunction with the evolution of complexity in the plant and animal kingdoms. Along this process, miRNAs became an essential feature of animal development, as no higher metazoan lineage tolerated loss of miRNAs or their associated protein machinery. In fact, ablation of the miRNA biogenesis machinery or the effector silencing factors results in severe embryogenesis defects in every animal studied. In this review, we summarize recent mechanistic insight into miRNA biogenesis and function, while emphasizing features that have enabled multicellular organisms to harness the potential of this broad class of repressors. We first discuss how different mechanisms of regulation of miRNA biogenesis are used, not only to generate spatio-temporal specificity of miRNA production within an animal, but also to achieve the necessary levels and dynamics of expression. We then explore how evolution of the mechanism for small RNA-mediated repression resulted in a diversity of silencing complexes that cause different molecular effects on their targets. Multicellular organisms have taken advantage of this variability in the outcome of miRNA-mediated repression, with differential use in particular cell types or even distinct subcellular compartments. Finally, we present an overview of how the animal miRNA repertoire has evolved and diversified, emphasizing the emergence of miRNA families and the biological implications of miRNA sequence diversification. Overall, focusing on selected animal models and through the lens of evolution, we highlight canonical mechanisms in miRNA biology and their variations, providing updated insight that will ultimately help us understand the contribution of miRNAs to the development and physiology of multicellular organisms.

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Or finally ...

5. By modifying the polypeptide or protein **after** it has been made (**post-translational** level)

What are these changes made to the polypeptide after it is made (“Post Translational Modifications”)

It will coil and fold due to its primary structure (its amino acid sequence)

- Groups are **added** (sugars, lipids, phosphate groups)
- Parts might be **removed** (e.g. amino acids from leading end or middle EX. Insulin is formed after a chunk of a.a. are taken out of its middle.)
- Several polypeptides may be joined together (e.g. hemoglobin has several subunits that are joined together)

Post Translational Modifications

These modifications take the chain of amino acids made by the ribosome (remember polypeptide-is just the chain of amino acids)...

And makes it into a **protein or a mature protein** (which is the finished product)



Review

Protein Language: Post-Translational Modifications Talking to Each Other

Lam Dai Vu ^{1, 2, 3, 4}, Kris Gevaert ^{3, 4, 5} , Ive De Smet ^{1, 2, 5}  

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<https://doi.org/10.1016/j.tplants.2018.09.004>

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PTM Crosstalk Adds More Complexity to Cellular Signaling

Plants are constantly exposed to environmental changes and developmental cues that require fast cellular sensing and response mechanisms. To decipher these mechanisms, the genome, transcriptome, and proteome have been explored in depth, each adding an additional level of complexity to signaling networks. In addition, PTMs of proteins are diverse, with 461 different types of modified amino acid registered for eukaryotic proteins in UniProt; these PTMs affect protein activity, stability, localization, and interactions [1]. This diversity, together with the dynamic nature of many PTMs, is an advantage at the protein level, especially since PTMs are often considered as switchboxes for cellular signaling.

Proteins originating from the same gene often harbor various PTMs, resulting in so-called 'proteoforms' (Box 1) [2], each of which might exhibit different activities or functions. In this way, responses to even slight changes in the environment can be precisely fine-tuned, without much, if any, evolutionary cost for the organism [3]. For

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Up-regulation = increased expression of one or more genes and the proteins encoded by those genes.

Down-regulation = decreased expression of one or more genes and the proteins encoded by those genes.

Wrap up importance of Gene regulation

Why do we care about gene regulation?

What does it do for us?

Prokaryotes regulate their genes but nearly as much as Eukaryotes

Reflect on why do you think all these controls evolved?

Hint: While there are single celled eukaryotes, many are multicellular!

“Control” and “Management” of cells becomes very important when you are multicellular. Why?

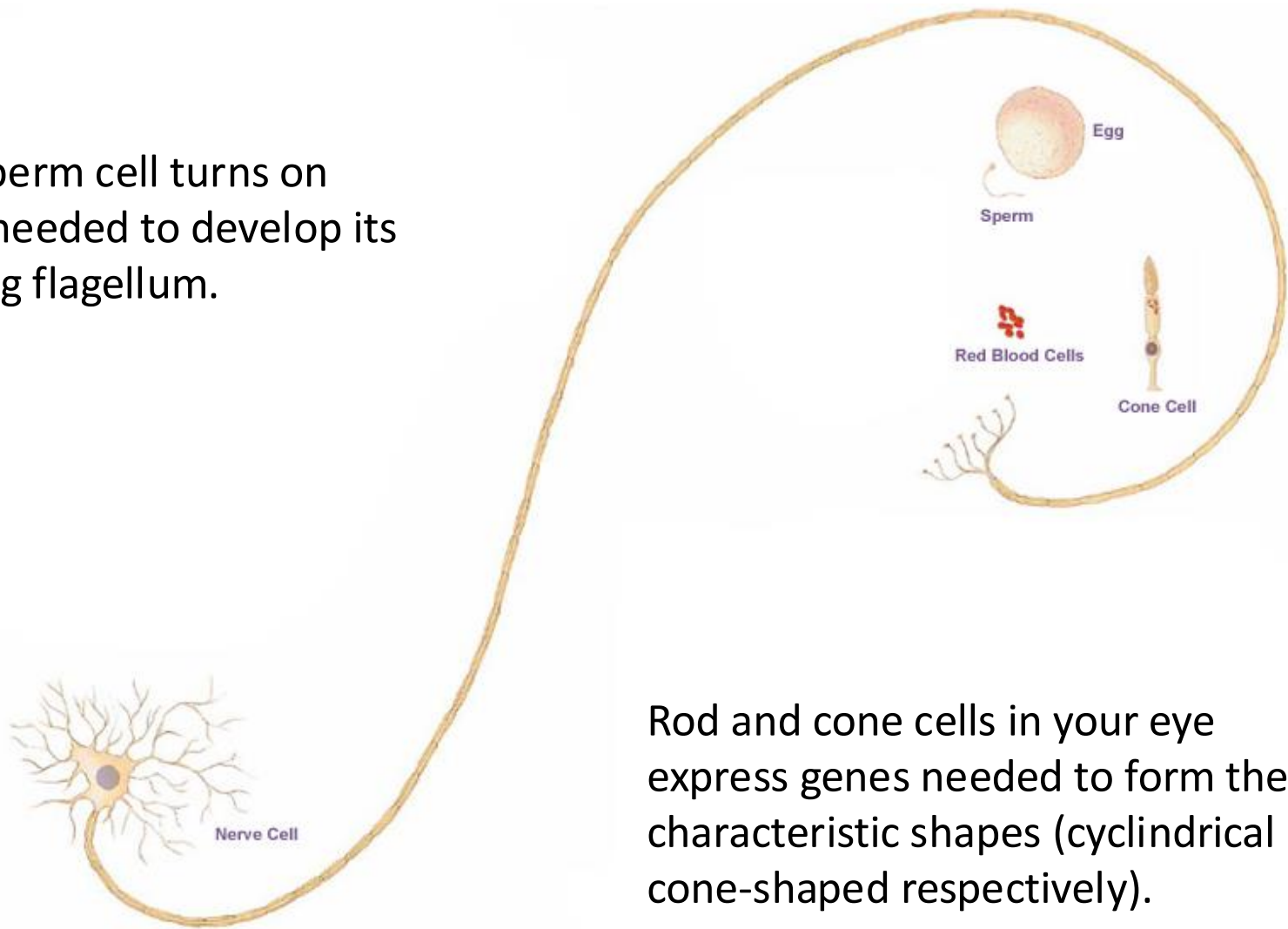
Multicellular organisms have cells that go through mitosis and specialize into different populations of cells called tissues that have different jobs.

These different jobs mean they express different genes.

- Liver cells make loads of enzymes to break down drugs and toxins.
- Certain immune cells produce antibodies to help fight infections.

Different patterns of gene expression mean cells in multicellular adult organisms differ in structure (or shape)

Each sperm cell turns on genes needed to develop its wagging flagellum.



Rod and cone cells in your eye express genes needed to form their characteristic shapes (cylindrical and cone-shaped respectively).

Different patterns of gene expression mean cells differ in number of mitochondria....

- Heart muscle cells contains several thousand mitochondria—around 25 percent of the cell's volume.
- Cells that don't need much energy, like skin cells, contain only a few hundred mitochondria.

And where did those mitochondria come from????

Different patterns of gene expression mean cells differ in lifespan and thus go through mitosis at different rates!

Gene expression signatures of human cell and tissue longevity
Inge Seim^{1,2}, Siming Ma¹ and Vadim N Gladyshev¹

Table 1. Summary of human cells and tissues used in the study

<i>Tissue/cell type</i>	<i>Germ layer</i>	<i>Estimated turnover (days)</i>
Adipose tissue	Mesoderm	2,448
Adrenal gland	Ectoderm	455
Bone marrow	Mesoderm	3.2
(CD14 ⁺) monocytes	Mesoderm	2
Colon	Endoderm	3.5
Endometrium	Mesoderm	13
Esophagus	Endoderm	10
Heart muscle	Mesoderm	25,300
Keratinocytes (skin epidermis)	Ectoderm	64
Kidney	Mesoderm	270
Liver	Endoderm	327
Lung	Endoderm	200
Neuron (neocortex)	Ectoderm	32,850
Osteoblasts (bone)	Mesoderm	8.3
Rectum	Endoderm	3.5
Salivary gland	Ectoderm	60
Skeletal muscle	Mesoderm	5,510
Smooth muscle	Mesoderm	67.5
Spleen	Mesoderm	7.8
Thyroid gland	Endoderm	3,180
Urinary bladder	Endoderm	49

See Supplementary Table S1 for further details.

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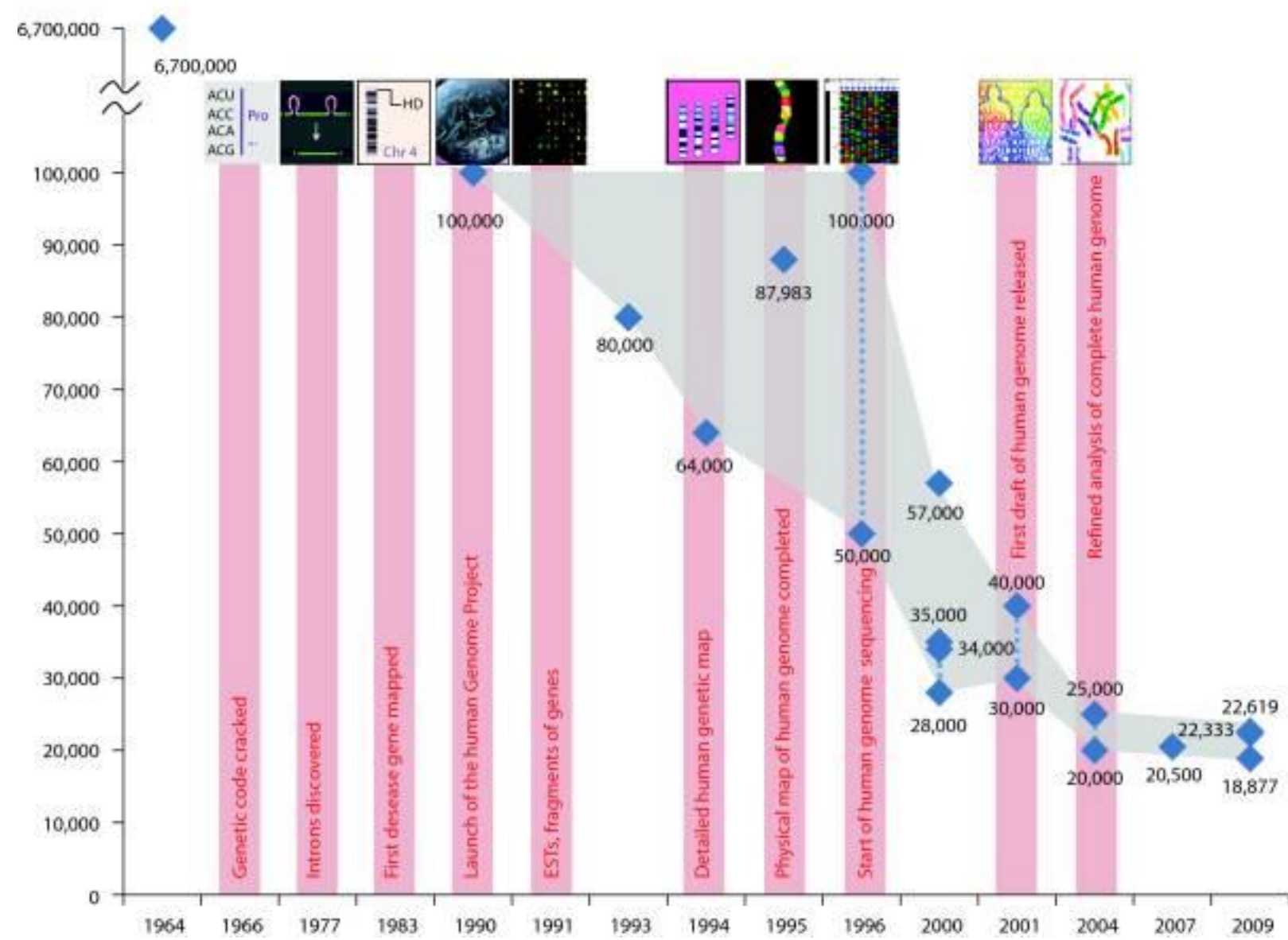
Sizing up the Genome

What is in your genome?

“Exploring the Landscape of the Genome”

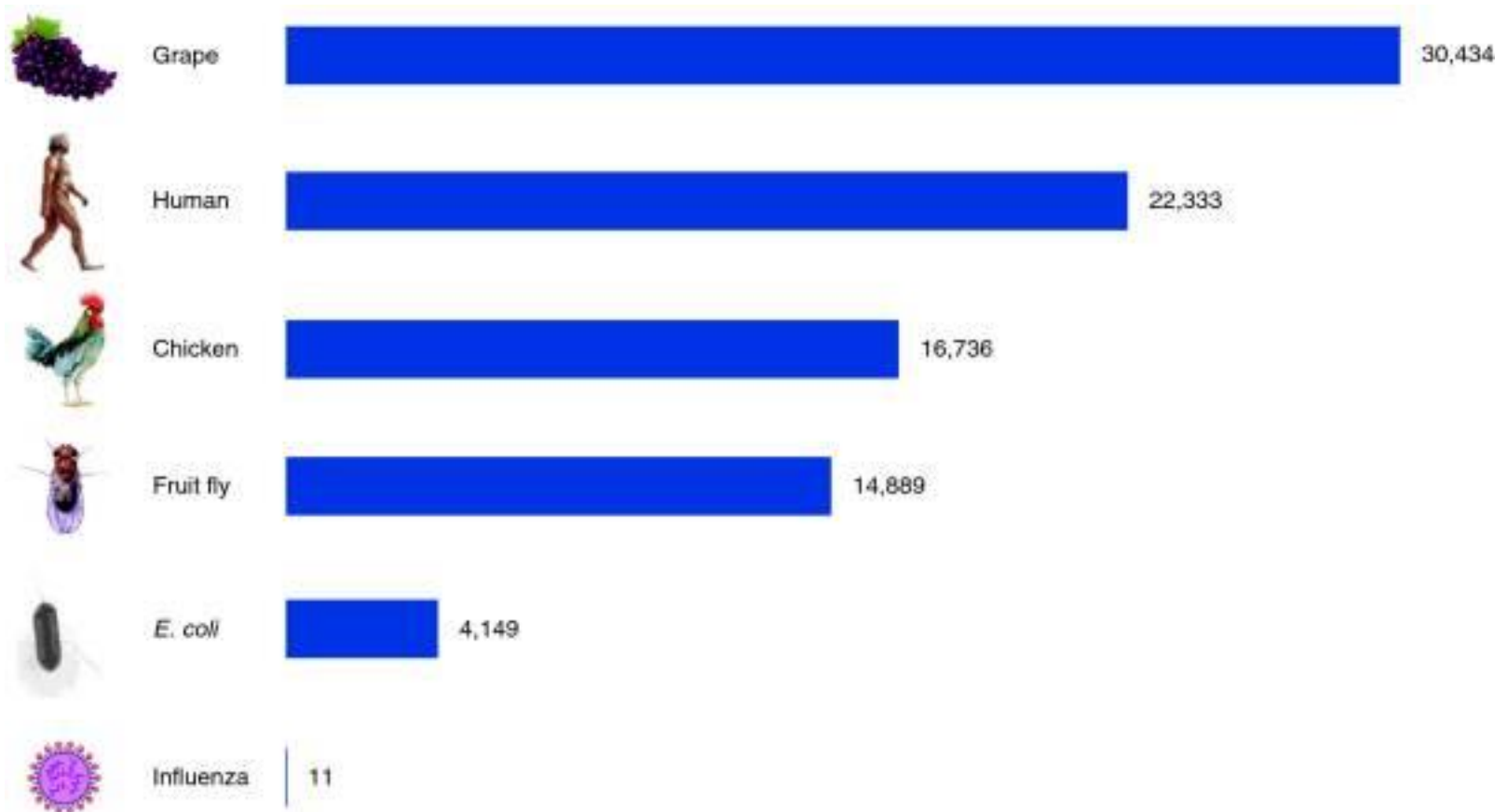
Estimates of number of genes in **human** genome

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2898077/#lpo=96.8750>



Where do we fit in terms of other life on earth (number of genes)?

2010 paper said..“Between a chicken and grape....”



3 DOMAINS (great branchings)!

How about total genome size relative to number of genes?

What generalizations you make?

Mb = million base pairs

Table 18.1 Genome Sizes and Estimated Numbers of Genes*

Organism	Haploid Genome Size (Mb)
Bacteria	
<i>Haemophilus influenzae</i>	1.8
<i>Escherichia coli</i>	4.6
Archaea	
<i>Archaeoglobus fulgidus</i>	2.2
<i>Methanosarcina barkeri</i>	4.8
Eukaryotes	
<i>Saccharomyces cerevisiae</i> (yeast, a fungus)	12
<i>Caenorhabditis elegans</i> (nematode)	100
<i>Arabidopsis thaliana</i> (mustard family plant)	120
<i>Drosophila melanogaster</i> (fruit fly)	165
<i>Oryza sativa</i> (rice)	430
<i>Zea mays</i> (corn)	2,300
<i>Mus musculus</i> (house mouse)	2,600
<i>Ailuropoda melanoleuca</i> (giant panda)	2,400
<i>Homo sapiens</i> (human)	3,000
<i>Fritillaria assyriaca</i> (lily family plant)	124,000

*Some values given here are likely to be revised as genome sizes are refined. ND = not determined.

What is the relationship between number of genes and genome size?

Divide the number of genes by genome size for three of the organisms in this table.

Mb = million base pairs

Table 18.1 Genome Sizes and Estimated Numbers of Genes*

Organism	Haploid Genome Size (Mb)	Number of Genes
Bacteria		
<i>Haemophilus influenzae</i>	1.8	1,700
<i>Escherichia coli</i>	4.6	4,400
Archaea		
<i>Archaeoglobus fulgidus</i>	2.2	2,500
<i>Methanosarcina barkeri</i>	4.8	3,600
Eukaryotes		
<i>Saccharomyces cerevisiae</i> (yeast, a fungus)	12	6,300
<i>Caenorhabditis elegans</i> (nematode)	100	20,100
<i>Arabidopsis thaliana</i> (mustard family plant)	120	27,400
<i>Drosophila melanogaster</i> (fruit fly)	165	13,900
<i>Oryza sativa</i> (rice)	430	40,600
<i>Zea mays</i> (corn)	2,300	32,000
<i>Mus musculus</i> (house mouse)	2,600	22,000
<i>Ailuropoda melanoleuca</i> (giant panda)	2,400	21,000
<i>Homo sapiens</i> (human)	3,000	<21,000
<i>Fritillaria assyriaca</i> (lily family plant)	124,000	ND

*Some values given here are likely to be revised as genome analysis continues. ND = not determined.

Gene Density

Table 18.1 Genome Sizes and Estimated Numbers of Genes*

Organism	Haploid Genome Size (Mb)	Number of Genes	Genes per Mb
Bacteria			
<i>Haemophilus influenzae</i>	1.8	1,700	940
<i>Escherichia coli</i>	4.6	4,400	950
Archaea			
<i>Archaeoglobus fulgidus</i>	2.2	2,500	1,130
<i>Methanosarcina barkeri</i>	4.8	3,600	750
Eukaryotes			
<i>Saccharomyces cerevisiae</i> (yeast, a fungus)	12	6,300	525
<i>Caenorhabditis elegans</i> (nematode)	100	20,100	200
<i>Arabidopsis thaliana</i> (mustard family plant)	120	27,400	228
<i>Drosophila melanogaster</i> (fruit fly)	165	13,900	84
<i>Oryza sativa</i> (rice)	430	40,600	95
<i>Zea mays</i> (corn)	2,300	32,000	14
<i>Mus musculus</i> (house mouse)	2,600	22,000	11
<i>Ailuropoda melanoleuca</i> (giant panda)	2,400	21,000	9
<i>Homo sapiens</i> (human)	3,000	<21,000	7
<i>Fritillaria assyriaca</i> (lily family plant)	124,000	ND	ND

*Some values given here are likely to be revised as genome analysis continues. Mb = million base pairs. ND = not determined.

Mb = million base pairs

TABLE 5.2 Variation in Genome Size and Complexity

Organism	Number of Chromosomes	Megabases in Genome	Approximate Number of Protein-Coding Genes
<i>N. deltocephalinicola</i> (bacteria)	1	0.112	137
<i>E. coli</i> (bacteria)	1	4.6	4,300
<i>S. cerevisiae</i> (yeast)	16	12.1	6,700
<i>C. elegans</i> (nematode)	6	100	20,000
<i>A. thaliana</i> (Thale cress)	5	120	27,000
<i>D. melanogaster</i> (fly)	4	180	14,000
<i>N. vectensis</i> (sea anemone)	15	450	27,000
<i>C. familiaris</i> (dog)	39	2,400	20,000
<i>M. musculus</i> (mouse)	20	2,600	19,900
<i>H. sapiens</i> (humans)	23	3,000	20,000
<i>P. abies</i> (Norway spruce)	12	20,000	28,300

So what is in a typical Eukaryotic genome?

We will use us as an example!

CODING Regions (make stuff)

- 1.2% codes for proteins
- Other parts are transcribed into rRNAs and tRNAs

Lots of NONCODING Regions!

- Gene control region-does something important
- Pseudogenes-no longer make a functional protein (17,032 of these in us)
- mobile genetic elements-copy themselves and insert themselves

We used to think of noncoding stuff as junk DNA but now rethinking this...

Much of it is “highly conserved” over long periods of time-suggesting?

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- 9. Mendel and Punnett Squares**