MCDB 4100
Experimental Design & CRISPR Mutagenesis in *Xenopus*

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Office hours: TBD depending on your response

Location: TBD

Let's Get to Know Each Other

Everybody take a minute and in order, state

- Your name
- Favorite fruit
- Most recent movie you saw in a movie theater
- Something you fear
- Something you are proud of (about yourself)

Course Info

http://virtuallaboratory.colorado.edu/MutatingXenopus/

Learning Outcomes

1) Become a more inquisitive individual and a critical thinker

2) Understand the scientific process and become science literate

3) Learn about major concepts and advances in molecular biology (PCR, plasmid construction, DNA isolation, RNA synthesis, experimental analysis of embryonic phenotypes, and CRISPR-Cas9 technology

4) Troubleshoot problems and come up with alternative solutions

5) Learn how to read a research article and evaluate it critically

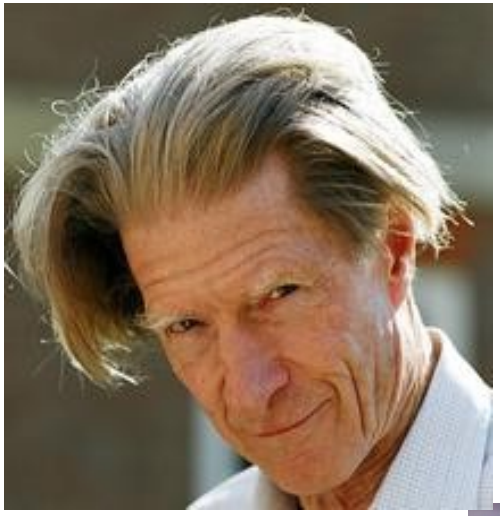
6) Learn how to design experiments and write a scientific proposal

7) Develop both oral and written science communication skills

Top 10 Skills Employers Most Want

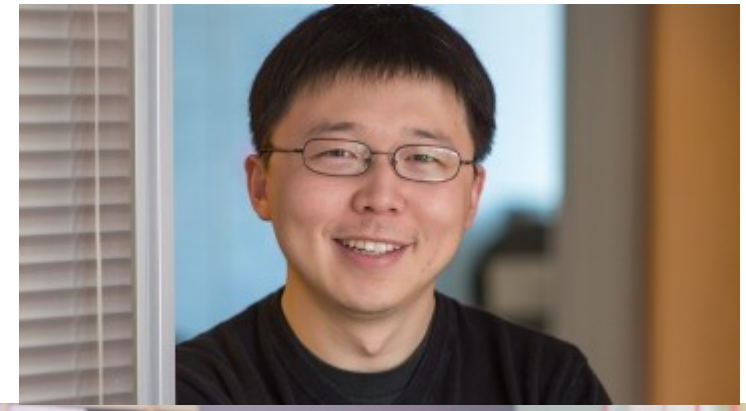
1. Ability to work in a **team**
2. Ability to **make decisions** and **solve problems**
3. Ability to **plan**, **organize** and **prioritize** work
4. Ability to **communicate verbally** with people inside and outside an organization
5. Ability to **obtain and process information**
6. Ability to **analyze quantitative data**
7. **Technical knowledge** related to the job
8. **Proficiency with computer software** programs
9. Ability to **create and/or edit written reports**
10. Ability to **sell and influence others**

- Survey by The National Association of Colleges and Employers (NACE)



Sir John Gurdon

Dr. Feng Zhang



Dr. Jennifer Doudna

George Takei

Simone Biles

Need to do

Required reading: original research articles, reviews, book chapters, assignments and lab manual

Read the materials **before** Monday lectures and lab sessions.

Before each lab session, you will

Meet in Room B436.

Receive your lab notebook from your instructor.

Using a **pen**, write down what you will be doing in the lab session in your lab notebook.

Move to the lab (B425) to start the lab session.

What to bring

Your full attention and enthusiasm

Always bring a ballpoint pen (no pencils please)

Optional: your personal notebook

For the first few weeks, you will need a laptop or mobile device with wireless capabilities

Let me know if you don't have access to one, I'll make arrangements

Dress code: NO open-toed shoes

What you will be given

Personal Protective Equipment (PPE): A lab coat, safety googles

and sharpies (These will remain in the lab)

A lab notebook (with about 100 pages, sequentially numbered) The lab notebook cannot be taken home.

Abbreviated Lab Rules

KEEP YOURSELF SAFE.

KEEP YOUR FRIENDS SAFE.

KEEP YOUR THINGS SAFE.

A) **STOP** and **ASK** if you are uncertain of ANYTHING.

B) **NO FOOD or DRINKS** in the lab.

C) Follow the **dress code** and all **safety** rules and guidelines.

D) Keep your **bench clean and tidy**.

E) **Wash hands** frequently.

F) **STOP** and **ASK** if you are uncertain of ANYTHING.

Grading

This class requires your active participation throughout the semester.

You will not be graded on regurgitation of facts.

First presentation:	15%
Reading, nota bene contributions, in-class/in-lab discussions:	20%
Quizzes, Lab books and in-lab activities:	30%
Survey / questionniare points	5%
Final project (video+written or oral presentation)	30%

As a courtesy to me, don't approach me with “How do I get an A?”,

try “How can I learn more?” “I don't understand this, help me figure this out”, “I am curious about XYZ, where can I find more info on XYZ?

Flowchart of what you will be doing

Pick a gene of interest

Design oligos to clone sgRNA for CRISPR-Cas9 mutagenesis

Clone sgRNA constructs and transcribe sgRNA

Inject sgRNA+Cas9 enzyme to mutate your gene of interest

Confirm mutation

Carry out genotype and phenotype analysis

Present your rationale and your results

Critical Thinking

results in well-reasoned decisions with an emphasis on whether the information is reliable, relevant, clear, free of unreasoned assumptions and biases,

interpretation is logical and objective

As opposed to undisciplined thinking when one simply acquires and retains information, critical thinking requires information to be sought and assessed in particular ways and requires habitual use of thinking skills.

Critical thinkers

promote curiosity

clearly formulate questions and problems

reach well reasoned conclusions

encourage objectivity

use skepticism

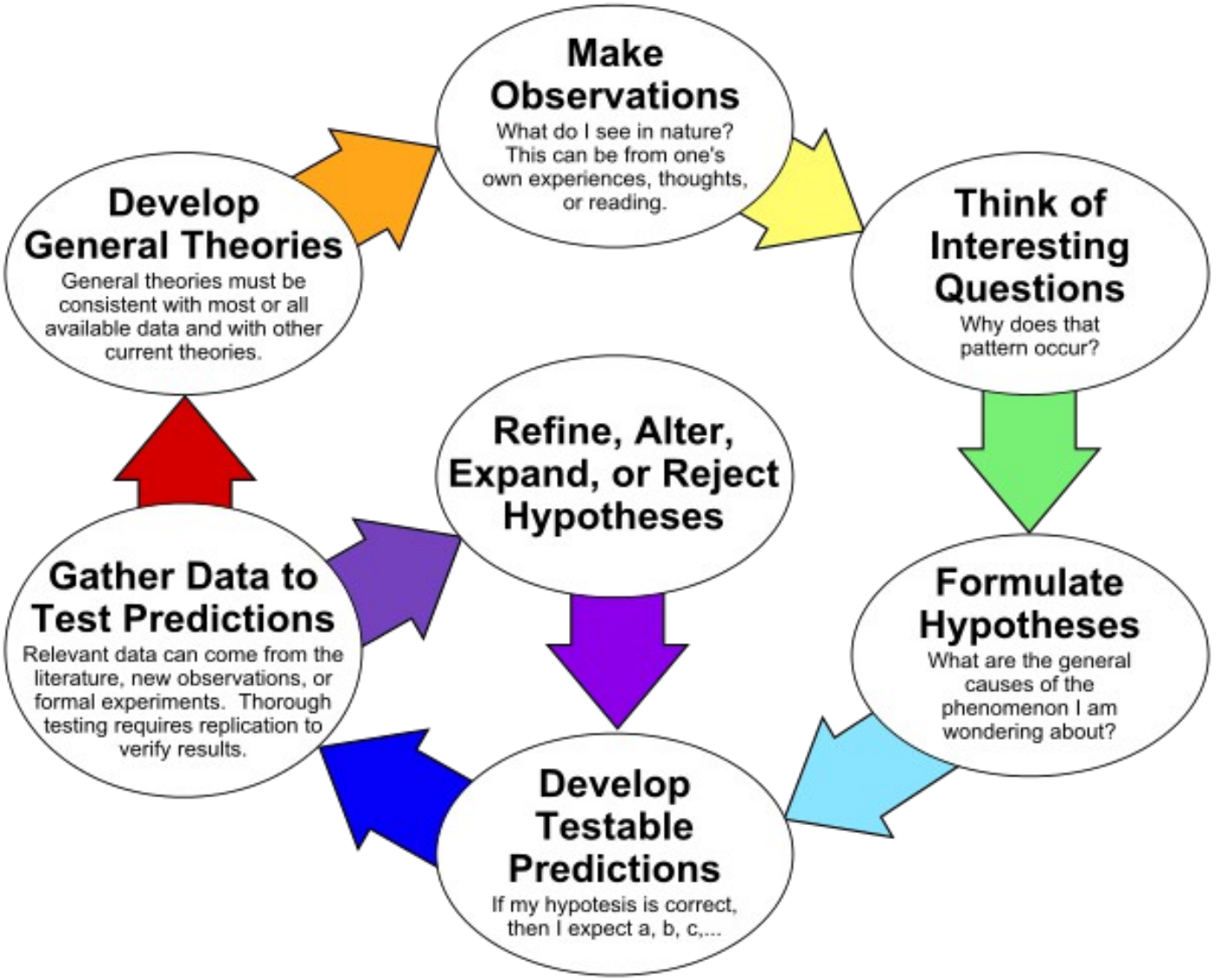
assess the relevance of information

think open-mindedly about information

common obstacles to critical thinking: arrogance, unwillingness to listen, lack of respect for reason, laziness, black and white thinking

http://idea.ucr.edu/documents/flash/scientific_method/story.htm

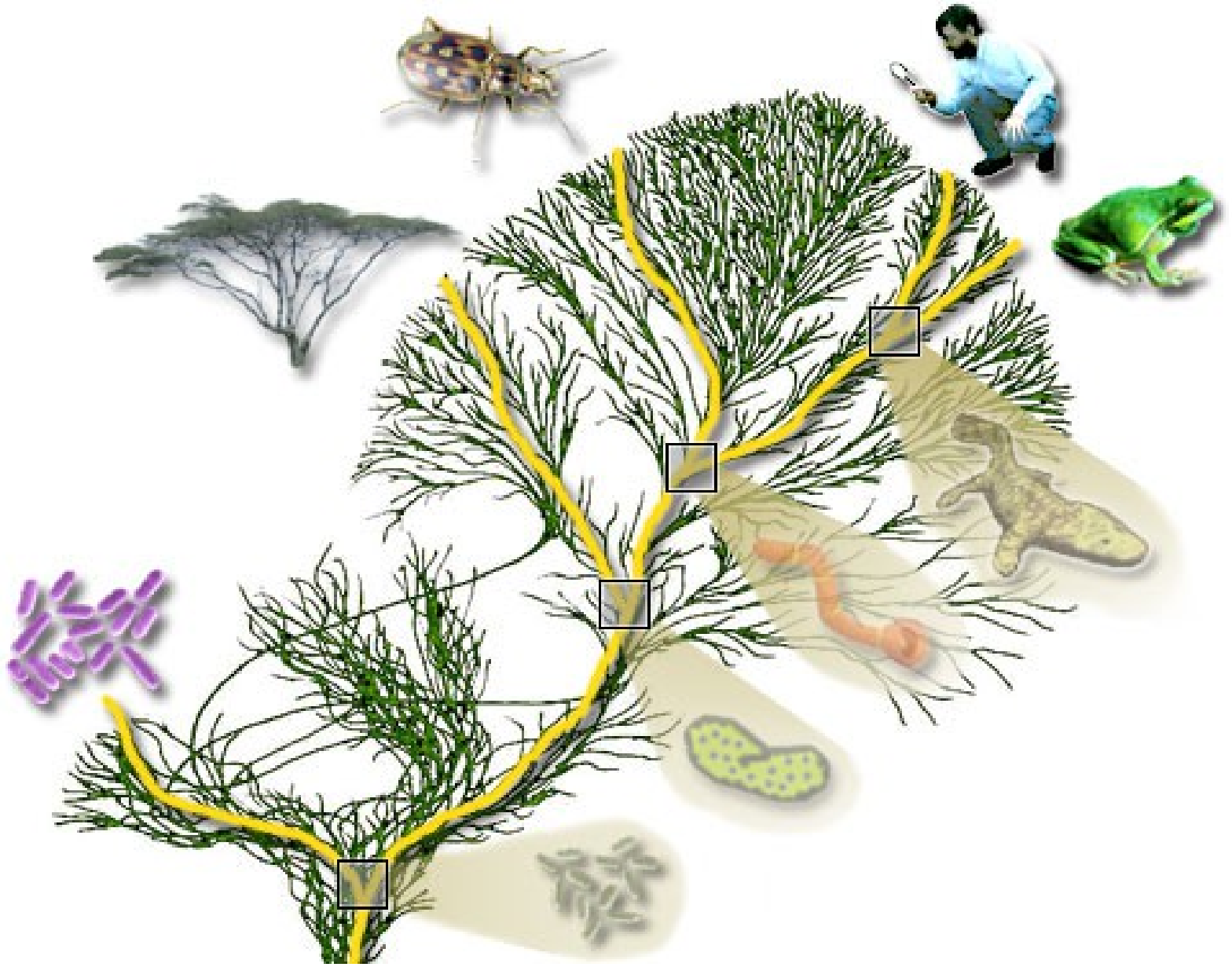
The Scientific Method as an Ongoing Process



- "Scientific knowledge is a body of knowledge of varying degrees of certainty-some most unsure, some nearly sure, but none absolutely certain … Now we scientists are used to this, and we take it for granted that it is perfectly consistent to be unsure, that it is possible to live and not know." - **Richard Feynmann**.

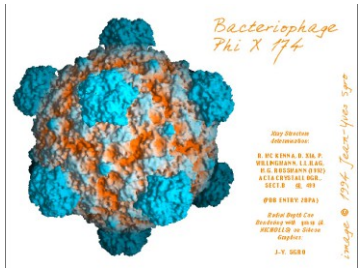
- "Ignorance more frequently begets confidence than does knowledge: it is those who know little, and not those who know much, who so positively assert that this or that problem will never be solved by science."- **Charles Darwin**.

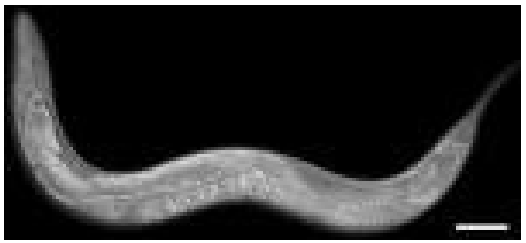
Tree of Life

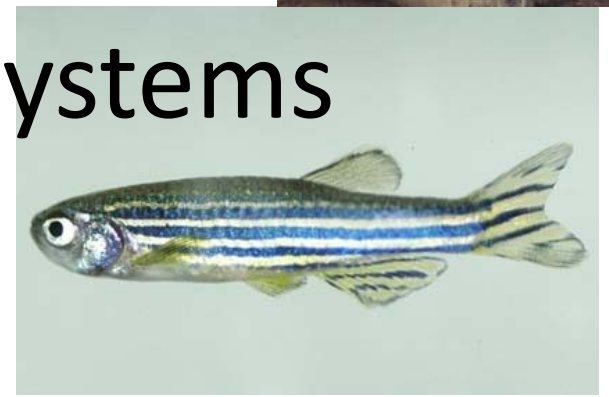


http://tolweb.org/tree/

Model Organisms/Systems



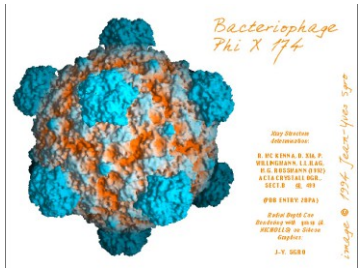


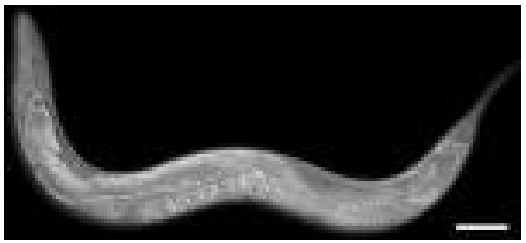


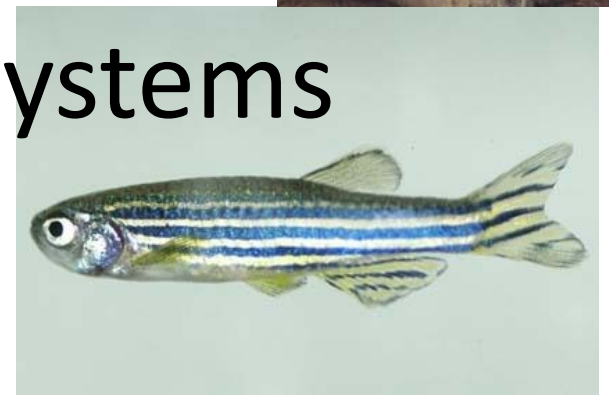
Activity 1

1) Get one activity sheet and fill out your name.
2) First, work as an individual to write down some strengths and limitations for some of the model organisms pictured or others you can think of on the top section of the sheet.
3) After you have written several (within the next 3-4 minutes), discuss your answers with your team mates and fill out the bottom section of the sheet with new model organisms that other team members had thought of or other strengths/limitations you had not thought of. Please note any disagreements you may have within groups.
4) Please hand in the activity sheet. I will return them to you on Thursday.

Model Organisms/Systems







Model Organisms usually

are small in size (with relatively large embryos)

are easy to grow and maintain in the lab

have short life cycles (minutes-days-months)

are amenable to genetics

are hardy to surgery and other manipulations

have numerous resources and databases available

are considerably beneficial or harmful to humans

are similar enough to humans (in particular aspects) to allow for translation/application of information but distant enough that ethical and moral concerns are minimal

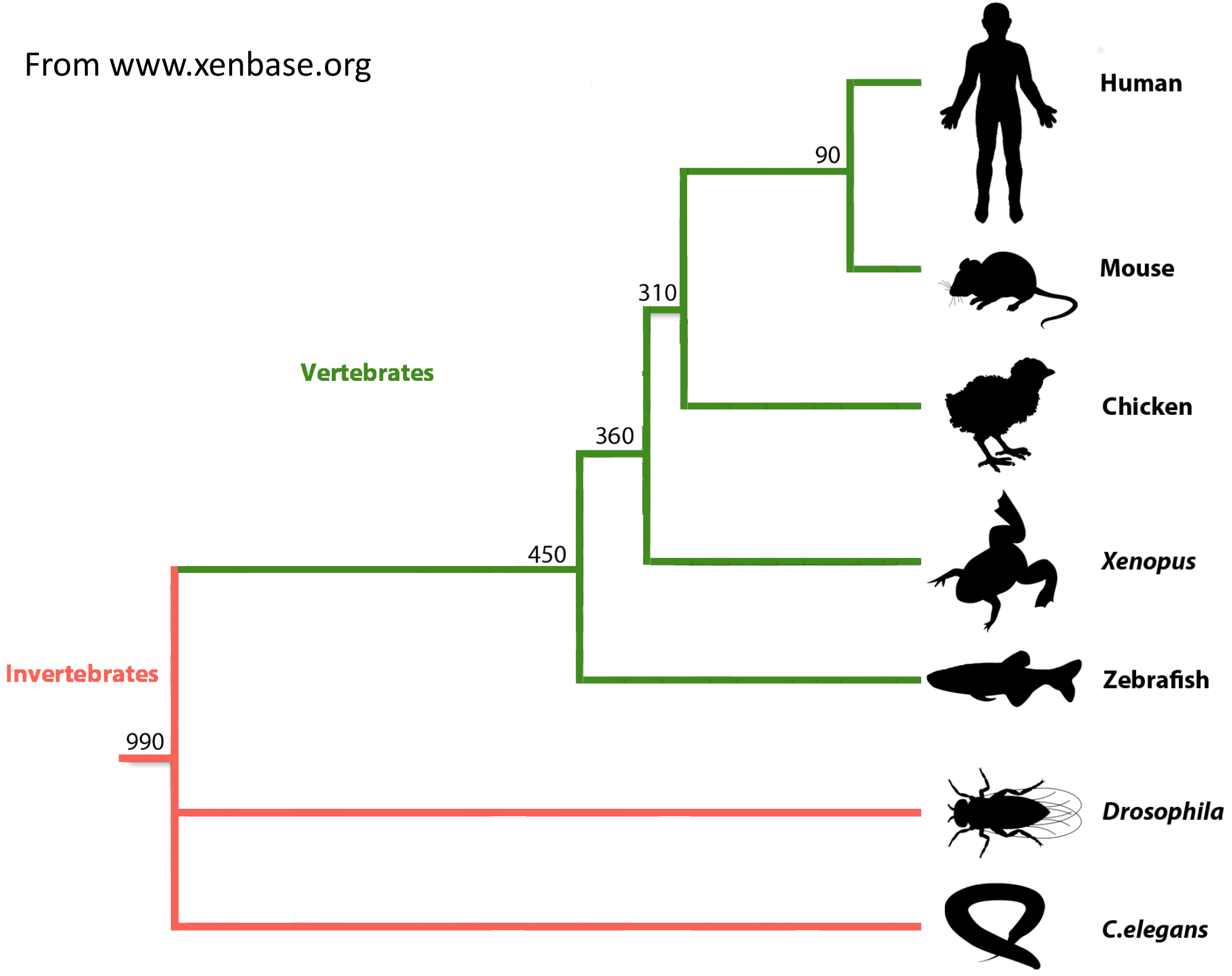
Remember

All organisms share some level of common evolutionary history. Therefore, any organism may provide insight into human biology.

We cannot always predict what insights and discoveries we may make with a particular model organism or field of research. There are countless examples of groundbreaking and unexpected discoveries with different systems. Therefore, casting the net wide is the best approach.

Every model has limitations. Even within a species, males may not be the best model for females and vice versa.

From www.xenbase.org



From www.xenbase.org

Category:	Worm C. elegans	Fly D. melanogaster	Fish D. rerio	Frog X. laevis	Chicken G. gallus	Mouse M. musculus
Broodsize	250-300	80-100	100-200	1000-5000	1	5-8
Cost per embryo	low	low	low	low	medium	high
High-throughput multiwell-format screening	good	good	good	good	poor	poor
Access to embryos	good	good	good	good	poor	poor
Micro-manipulation of embryos	limited	limited	fair	good	good	poor
Genome	known	known	known	known	known	known
Genetics	good	good	good	fair	none	good
Knockdowns (RNAi, morpholinos)	good	good	good	good	limited	limited
Transgenesis	good	good	good	good	poor	good
Evolutionary distance to human	very distant	very distant	distant	intermediate	intermediate	close

Comparative Genome Sizes of some Model Organisms

Phi X 174 (bacteriophage virus)	5,386bp	11genes
E. coli K-12 (enteric bacteria)	4,639,221bp	4,377genes
Saccharomyces cerevisiae (budding yeast)	12,495,682bp	5,770genes
C. elegans (round worm)	100,258,171bp	19,427genes
Arabidopsis thaliana (a flowering plant)	115,409,949bp	~25,000genes
Drosophila melanogaster (fruit fly)	122,653,977bp	13,379genes
Ciona intestinalis (tunicate, ascidian)	180,000,000bp	15,000-16,000genes
Fugu	400,000,000bp	
Danio rerio (zebrafish)	~1,500,000,000bp	~20,000genes
Xenopus tropicalis (pipid frog)	~1,700,000,000bp	~20,000genes
Gallus gallus (domesticated chicken)	~1,200,000,000bp	~20,000genes
Mus musculus (house mouse)	2,716,965,481bp	23,786 genes
Sus scrofa (domesticated pig)	2,834,477,559bp	~20,000genes
Homo sapiens	3,164,700,000bp	23,686 genes
Human mitochondrion	16,569bp	37genes
Paris japonica	~150,000,000,000bp	TBD

Every model organism has its limitations!

Everything that can be seen as an advantage can be a limitation

a) small animals easy to house and handle: difficult to manipulate

b) fast development: too fast for you to finish experiments (with *Xenopus laevis* 30-45 minutes to inject before cells divide)

c) inbred animals genetically identical or similar, easy to replicate experiments and make sense of results: most biological systems are not genetically homogeneous, variability results in considerable differences in gene function and result of mutations

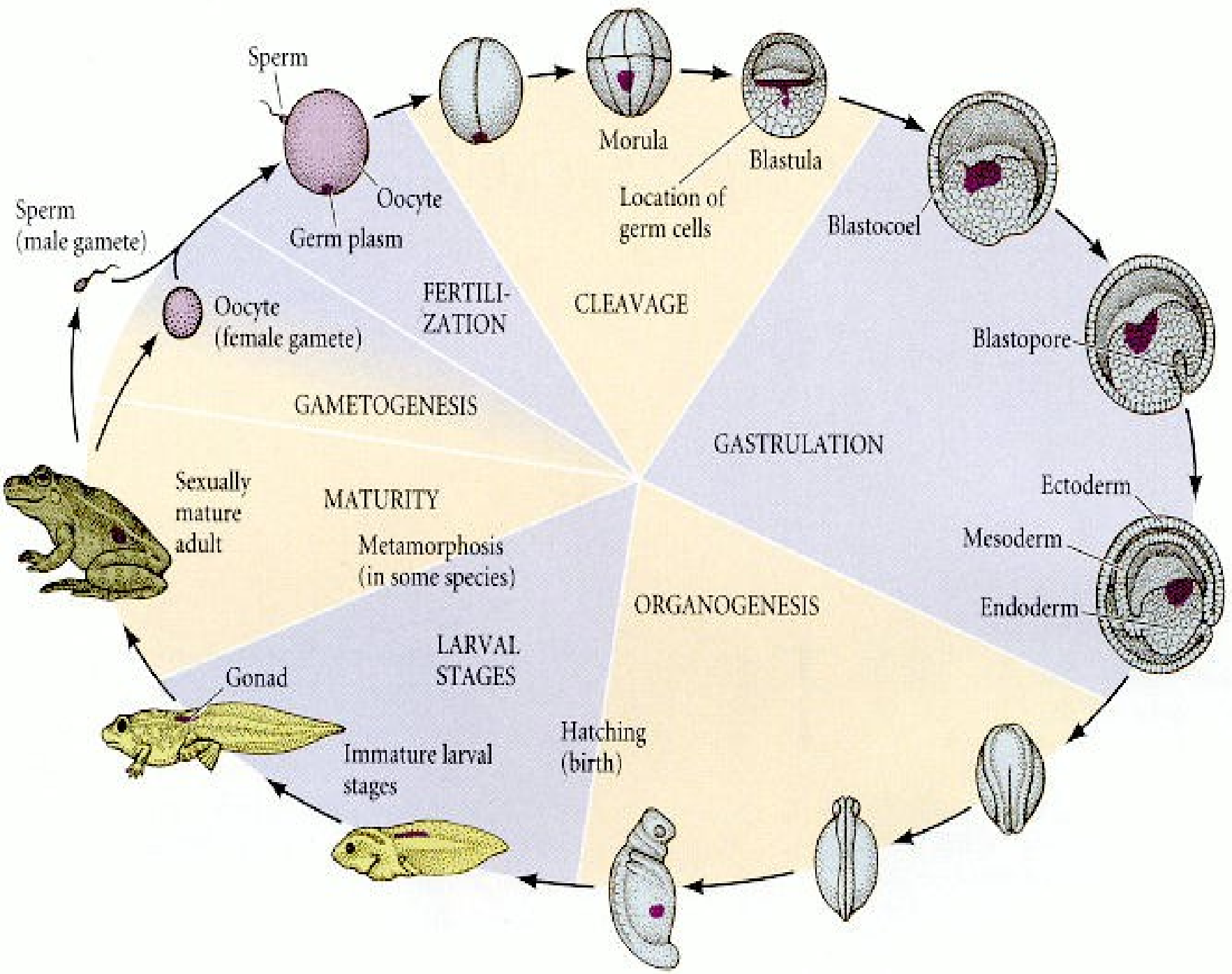
Even within a given species, age or sex difference makes a big difference.

Mice develop as a cup and not a disc like chick, rabbit or human embryos

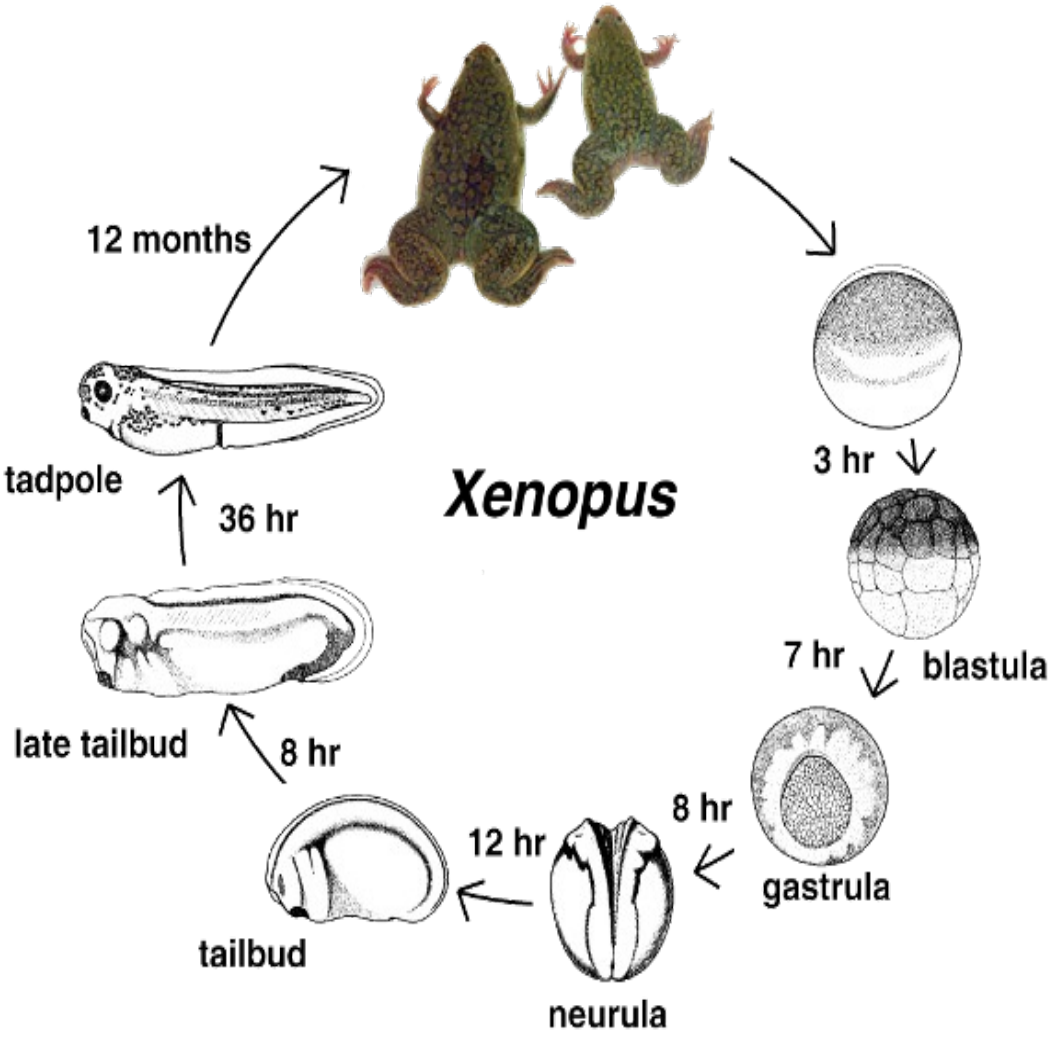
Mice are naturally immune to Hepatitis C virus (Chimpanzees have been the primary model organism for HepC infection and therapy research)

Thalidomide mouse vs rabbit

Newt embryos easily form a duplicated axis upon surgery or high salt concentrations

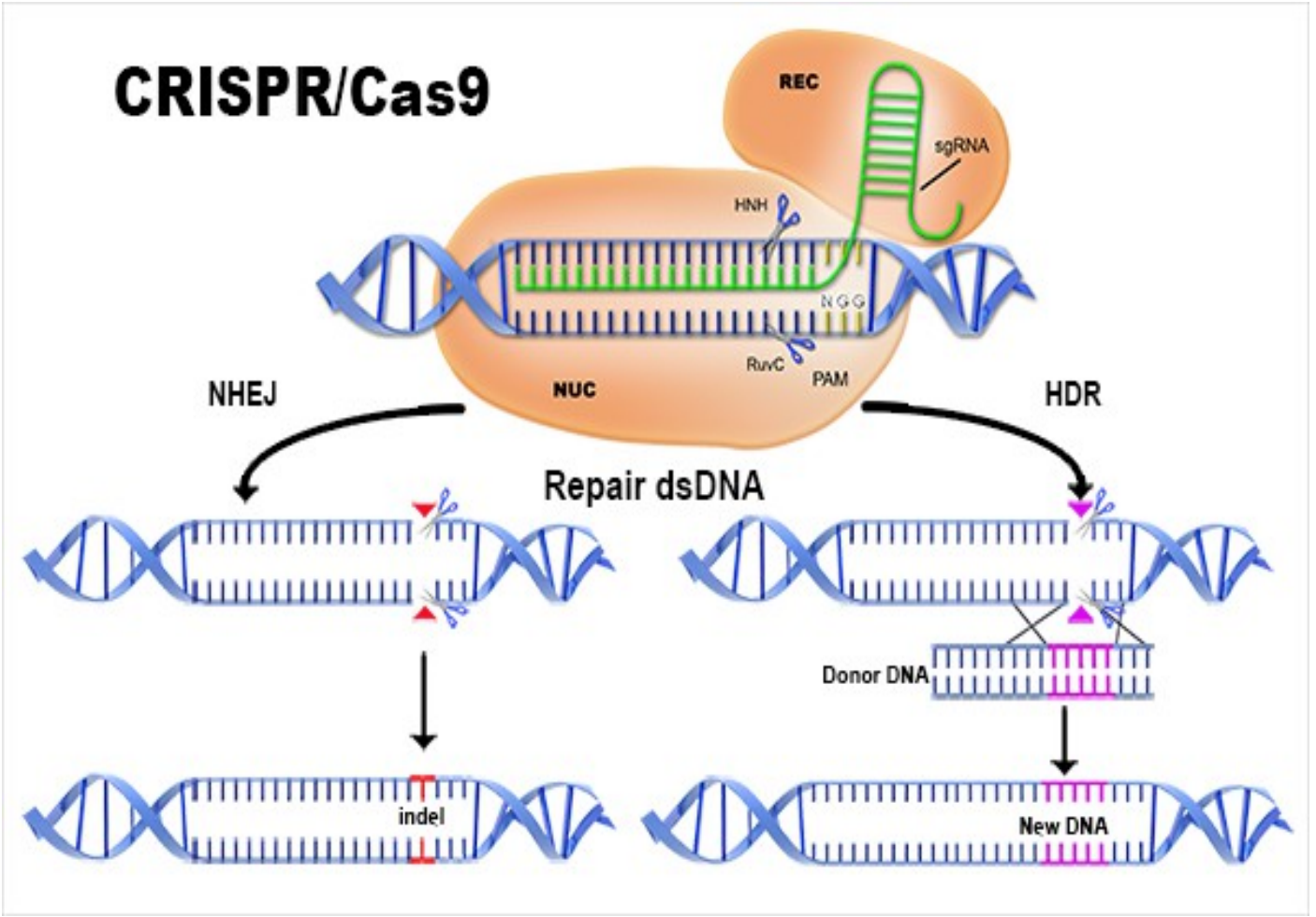

Sperm
Oocyte
Germ plasm
Morula
Blastula
Location of
germ cells
Blastocoel
Blastopore
Sperm
(male gamete)
Oocyte
(female gamete)
FERTILI-
ZATION
CLEAVAGE
GAMETOGENESIS
GASTRULATION
Ectoderm
Mesoderm
Endoderm
Sexually
mature
adult
MATURITY
Metamorphosis
(in some species)
ORGANOGENESIS
LARVAL
STAGES
Gonad
Hatching
(birth)
Immature larval
stages

Life Cycle of *Xenopus laevis*



From www.xenbase.org

We'll talk about this later
CRISPR-Cas9 Mutagenesis



http://www.aati-us.com/product/fragment-analyzer/CRISPR

What makes a gene interesting to study?

Genes implicated in an interesting biological process or human disorder

Genes that are highly conserved between species (especially human vs others)

- Human perspective: Fix the 3Ds: death, disease, disability

Genes of unknown function

Practical limitations:

Gene must have an homolog or an ortholog in model organism of choice (*Xenopus laevis*)

Phenotype must be observable within the time frame of the project (semester)

Xenopus gene function must not have been published yet (DISCOVERY)

Genes that display early lethal phenotypes or housekeeping genes are clearly important genes but they are trickier to study and may require additional justification.

What is a gene?

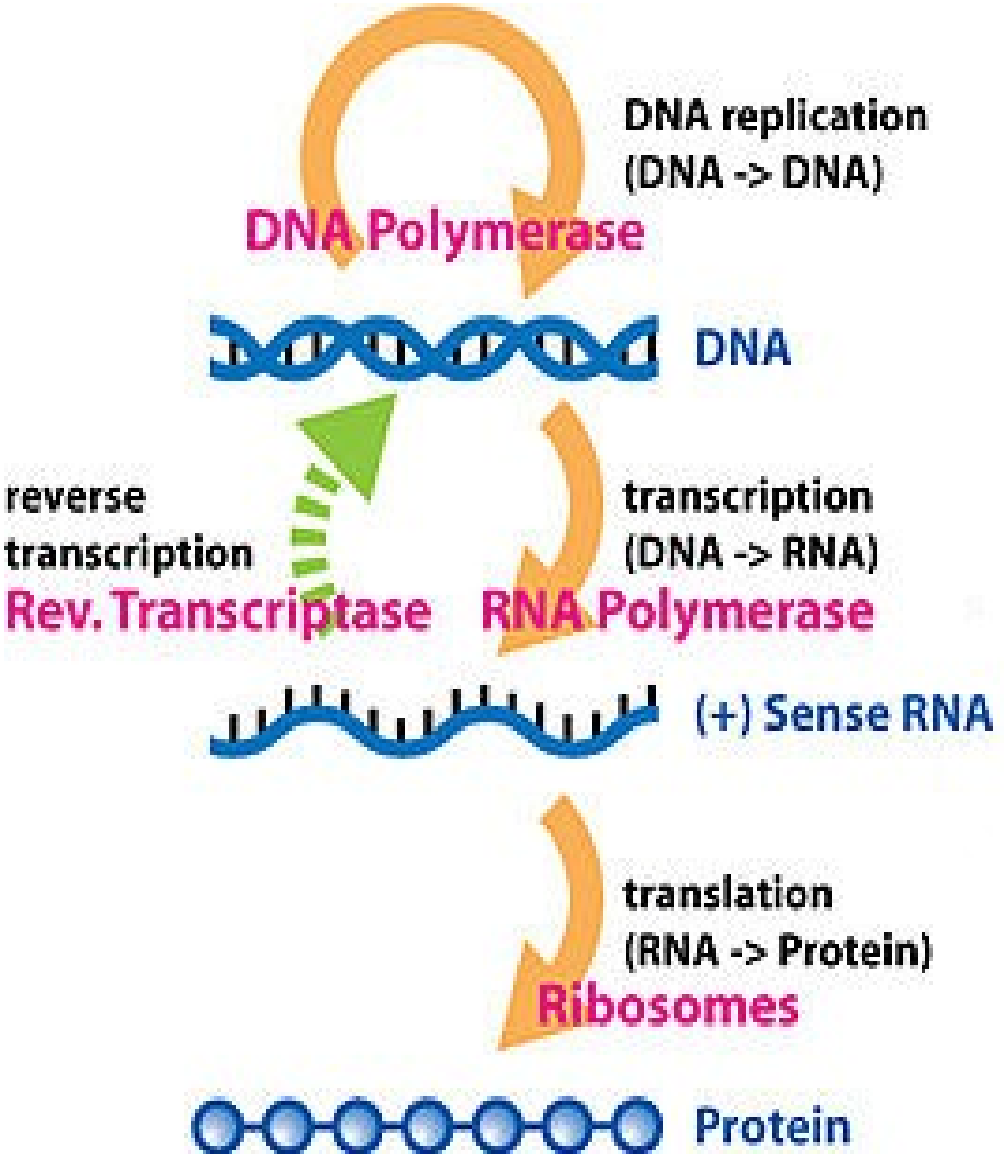
A gene is

basic physical and functional unit of heredity

Coding RNA for proteins

Non-coding RNA (genes for tRNA, rRNA, miR)

Central Dogma



https://en.wikipedia.org/wiki/Central_dogma_of_molecular_biology

Gene Structure Activity

Working as a group, draw a gene structure to include <u>all</u> the terms

Enhancer

Promoter

Transcription initiation site

Exon

Intron

5' UTR

3'UTR

PolyA signal

Transcription termination site

Translation initiation site

Translation termination site

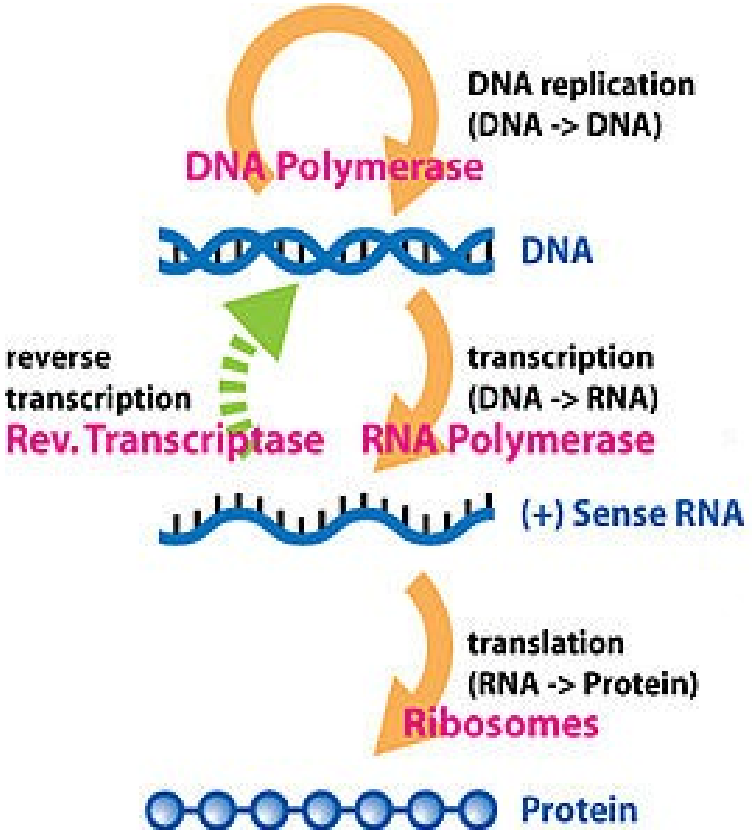
ORF (open reading frame)

Pre-mRNA vs mature mRNA activity

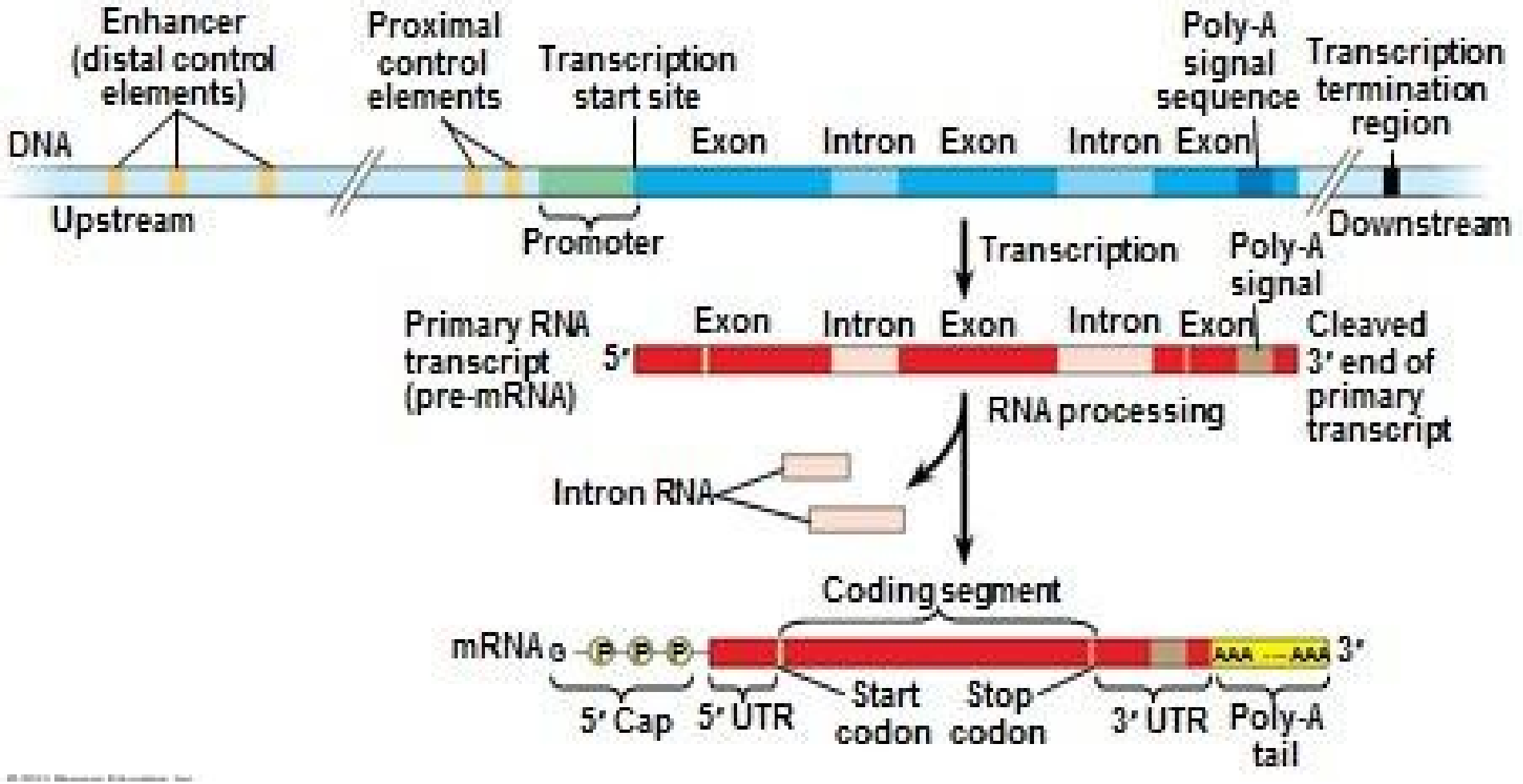
As a group, draw one pre-mRNA and its mature mRNA and compare the differences

Some Terms to Know

- gDNA: **g**enomic DNA
- cDNA: **c**omplementary DNA
- mRNA: **m**essanger RNA
- UTR: **U**n**T**ranslated **R**egion
- Probe: a molecule that specifically binds a nucleic acid or peptide sequence



Eukaryotic Gene Structure



www.studyblue.com

For Thursday

Start looking up and thinking of 1-2 genes that you may be interested in pursuing. Is there a human disease you are interested in? Is there a biological process you'd like to learn more about? Do you want to go after a PUF(Protein of Unknown Function)?

Bring a laptop or mobile device

For PubMed, NCBI, AmiGO, Xenbase, ExAC browser

Login to Nota Bene: Read the ExAC paper

Start listing terms, concepts, methods you don't understand or want to discuss