RV Lecture 9-

Week	
Files	
Notes	

Mutations [Beneficial - Detrimental - Neutral]

`New alleles are formed by mutation`

Prophase : During prophase, changes occur in both the nucleus and the cytoplasm.

Mitotic spindle begins to form

Prometaphase : Some of the mitotic spindle attaches to the kinetochore

(Protein structure in the centromere)

Metaphase : Metaphase Plate

Anaphase : Motor Protein

Telophase : Reverse of The Prophase

Cytokinesis : Cleavage furrow and Cell Plate Formation

Pair of homologous duplicated chromosomes

`For the organism, suppose, n = 2, so the number of chromosome combinations is 22, or 4. For a human (n = 23), there are` 2^{23} `, or about 8 million! In humans, there are over 8 million configurations in which the chromosomes can line up during metaphase I of meiosis.`

A sperm cell, with over 8 million chromosome combinations , fertilizes an egg cell, which also has over 8 million chromosome combinations. That is over 64 trillion unique combinations!

homologous chromosomes are randomly distributed during anaphase I, separating and segregating independently of each other. This is called independent assortment.

Random Fertilization :

- In sexual reproduction, two gametes unite to produce an offspring. But which two of the
  millions of possible gametes will it be? This is likely to be a matter of chance. It is obviously
  another source of genetic variation in offspring. This is known as random fertilization.

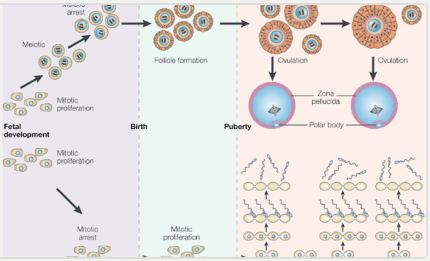
Questions

▼ What factors are involved in directing cells to follow meiotic or mitotic path for cell division?

Meiotic timelines for humans

The fate of germ cells is dictated by the somatic environment. In both the developing ovary and the testis, germ cells undergo mitotic proliferation

https://www.nature.com/scitable/content/meiotic-timelines-for-humans-4875/



https://www.pnas.org/doi/10.1073/pnas.1300928110

▼ Is genetic variation is important for reproduction ? If not then why mechanism of genetic variation is evolved with the increasing complexity of reproduction ? Or is this simply a by-chance phenomenon that somehow one species got variations for certain traits(i.e. mutations in the genetic material) earlier and as nature selected variation over non-variance (population that has no variation of certain traits) , the genetic variance itself got selected and persisted?

(If my question is nonsense , can you please help me in finding what is the piece I’m missing in my concept)

Regulation the eukaryotic cell cycle

▼ ***Digoxin & Digitoxin***

Cardiac glycosides (cardiotonics)

MECHANISM OF ACTION:

- **Inhibition of Na+/K+ ATPasa pump**
  - ⇨ increase intracellular sodium concentration (Na+/Ca+ exchange transporter
  - ⇨ secondary rise of Ca^{2+} -
  - ⇨ **increased contractility** ⇨ **↑inotropic effect efekt**
- **Activation of parasympatics (n. vagus)** and ACH release
  - ⇨ SA node, AV conduction slow
  - ⇨ ↓ chronotropy
  - ⇨ ↓ dromotropy ⇨

  antiarrhytmic effect

DRUG

digoxin

MUNI MED

Comparison between Digoxin & Digitoxin

Name	Digoxin (Lanoxin®)	Digitoxin Most liposoluble cardiac glycoside
Source	*D. lanata.*	*D. purpurea*, & *lanata*
Hydrolysis	→Digoxigenin + 3 digitoxose	Digitoxigenin+3 Digitoxose
Administration	Usually oral	Oral
Onset of action	After 30 min to 2 hours	After 1-4 hours
Peak	At 2 to 6 hours.	At 8 - 14 hours
Plasma half-life	30 to 40 hours	168 to 192 hours
Complete elimination after discontinuation of therapy	6 to 8 weeks	3 to 5 weeks
Elimination	Eliminated through kidney	Eliminated through liver so recommended for patients with impaired renal function.
Full therapeutic effect	0.5-2ng / ml	14-26 ng / ml
Toxicity symptoms	2.5 ng / ml	35 ng / ml
Indication	When risk of intoxication is great, as it is relatively short-acting&rapidly eliminated	Recommended for patients with impaired renal function.

▼ ***These Butterflies Evolved to Eat Poison. How Could That Have Happened?***

Yet some insects appear entirely unfazed by the powerful poison. The monarch butterfly's colorful caterpillars, for example, devour milkweed with gusto—in fact, it is the only thing they ever eat. They can tolerate this food source because of a peculiarity in a crucial protein in their bodies, a sodium pump, that the cardenolide toxins usually interfere with.

All animals have this pump. It's essential for physiological recovery after heart muscle cells contract or nerve cells fire—events that are triggered when sodium floods into the cells, causing an electrical discharge. After the firing and contracting is done, the cells must clean up, and so they turn on their sodium pumps and expel the sodium. This restores the electrical balance and resets the cell to its usual state, ready again for action.

Cardenolides are noxious because they bind to key parts of these pumps and prevent them from doing their job. This makes animal hearts beat stronger and stronger, often ending in cardiac arrest.

But since animals are under constant competition for food sources, the ability to eat plants that are toxic to others offers a fantastic opportunity, and many insects have evolved ways to do so.

Two papers, one of them published this week in the journal *Nature* and the other in *eLife* in late August, help to explain how such adaptations may have evolved. Through precise genetic changes, scientists created fruit flies (of the species *Drosophila melanogaster*) whose larvae survived a succession of milkweed-based meals.

Scientists have known for some time that monarchs—and many of the other insects, from a total of six orders, that feed on milkweed or other cardenolide-producing plants—have mutations in at least one of the genes that carry instructions for making sodium pumps. Some of these result in the replacement of one of the amino acids that the pump—like all proteins—is built from, making it harder for cardenolides to bind to it. Researchers assumed that one or more of these changes carry the key to making milkweed palatable, but without testing their effect in live animals, they couldn't know for sure.

And if more than one mutation was needed to tolerate milkweed toxins, how did the trait ever evolve in the insect? If a plant is still toxic after one mutation, what selective advantage would that first mutation provide, to enable an insect to evolve the whole suite of needed changes?

In 2012, evolutionary biologist Noah Whiteman, now at the University of California, Berkeley, and a colleague proposed in a commentary that one could answer the question by engineering the monarch sodium pump mutations into fruit flies. The endeavor turned out to be anything but easy: "I had no idea what I was getting myself into," Whiteman says now. It took three years of tinkering, using the gene-editing technique CRISPR-Cas9, "and then still, only 1 in 720 flies would survive." The larvae of those that did, though, could eat milkweed almost like a monarch.

In the wild, fruit flies eat yeast that's found, for example, on rotting fruit. In the lab, they are fed a standard diet consisting of a slurry of malt, corn meal, yeast, agar and syrup. To do their experiment, Whiteman and his colleagues laced this staple with a dose of dried, ground-up milkweed leaves or purified toxins and tried to rear various gene-edited fly strains on this diet. Some flies had one mutation of three seen in the sodium pump gene of monarchs, and some had combinations.

The work, reported in *Nature*, showed that all three mutations individually increased the fruit flies' chances of surviving the dangerous diet. But there was a twist. In the case of two of those three mutations—the ones that individually provide the most toxin resistance and appear to have shown up first and last within the monarchs' family tree—the gene-edited flies were more prone to seizures. This was assessed using a standard test in which flies in a tube were shaken vigorously: Flies carrying the first or last mutation remained motionless far longer after being shaken than did normal flies.

In other words, "it looks as if the mutations protecting the flies against the toxin create a neurological vulnerability," Whiteman says. But this wasn't the case when flies also had another mutation—the one that likely appeared *second* during evolution of toxin resistance in monarchs. In flies carrying this combo, the neurological vulnerability was gone but the toxin resistance remained. "They needed to get the mutations in the right order," Whiteman says. First, a mutation of small effect would have altered the structure of the sodium pump to provide some resistance, but also some neurological problems. The second mutation would have amended the pump structure slightly, thereby fixing that problem. By so doing, it would have prepared conditions for the third mutation—the one with the heftiest antitoxin effect. By itself, that third mutation would have created intolerable neurological issues. But with the second mutation already in place, all would be well, or at least much better.

"Biologists call this a constrained adaptive walk," says Whiteman, "where one mutation is followed by another, in a predictable order, setting a species, or more than one, on a trajectory to higher fitness."

This helps to explain how the milkweed adaptation may have evolved in monarchs, says Whiteman, who coauthored an article about the constant evolutionary arms race between plants and herbivorous insects in the *Annual Review of Ecology, Evolution, and Systematics*. The last mutation to show up in the monarch lineage is the one that confers the greatest resistance to cardenolides, based on the fruit fly results. And there may be a reason it came in last: Present on its own, it also would have had the largest seizure effect, harming the monarchs.

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Hopi Hoekstra, an evolutionary biologist at Harvard University, calls this one of her favorite studies in a long time. “To understand what happened in the past, we largely rely on organisms that occur today,” she says. “By measuring the interactions among mutations in a single protein, the authors have put forth a plausible step-by-step evolutionary trajectory, giving us an exciting glimpse into the past.”

The findings gel with work by evolutionary biologist Peter Andolfatto at Columbia University, who published a similar study in *eLife* in late August, using a different technique to change the flies’ genes. “The results of both studies largely line up, independently confirming that **the evolutionary options may indeed have been somewhat limited**” for the monarch, Andolfatto says.

The monarchs’ evolutionary innovation had an ecological ripple effect. Not only did resistance to the toxin open up a whole new source of food, but it also allowed the butterflies to repel predators by storing the toxins in their bodies.

Birds tend to find out the hard way which insects are unpalatable, by trial and error. But many toxic insects—monarchs among them—have evolved a similar palette of warning colors, so that fewer of them must be eaten to teach the birds a lesson.

“Once a bird learns that an insect that is bright yellow, orange or red is likely to have a terrible taste,” Whiteman says, “they will probably steer clear of all of them. These toxins changed everything.”

▼ ***Slides***

Milkweed seeds are eaten by *specialist lygaeid bugs* that are even more tolerant of cardenolides than the monarch butterfly, concentrating most cardenolides from seeds into their bodies.

Oncopeltus fasciatus (Lygaeidae) metabolized two major compounds -

(glycosylated aspecioside and labrifor- min) into distinct products that were sequestered without impairing growth. Their results suggest that a potent plant defense is evolving by natural selection along a geographical cline and targets specialist herbivores, but is met by insect tolerance, detoxification, and sequestration.

Channel proteins that transport ions are called ion channels.

ion channels function as gated channels, which open or close in response to a stimulus.

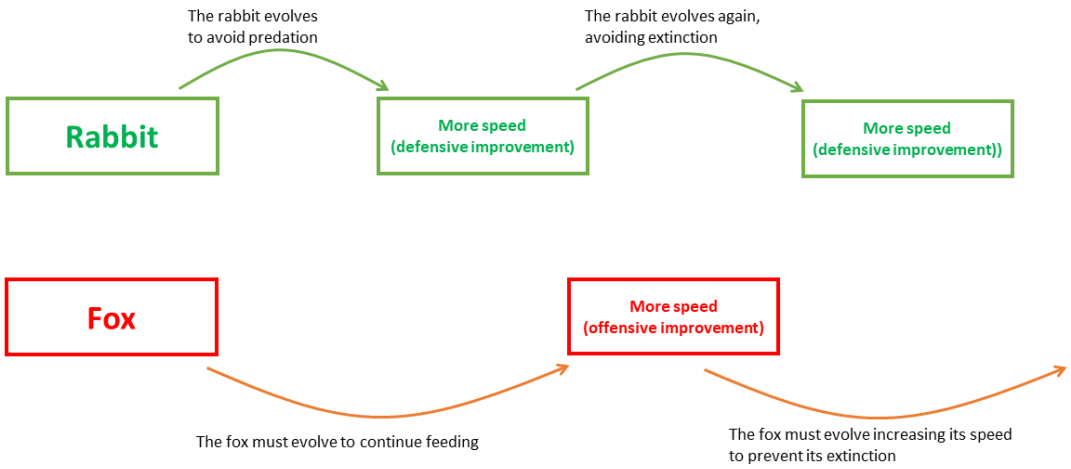
Carrier proteins, such as the glucose transporter, seem to undergo a subtle change in shape that somehow translocates the solute-binding site across the membrane. Such a change
in shape may be triggered by the binding and release of the transported molecule.

▼ ***Red Queen Hypothesis***

Revisiting the Red Queen

The Red Queen hypothesis states that in the battle for resources, species must continuously evolve just to keep up with their enemies, who themselves also evolve in response. This year, we revisit the seminal theory.

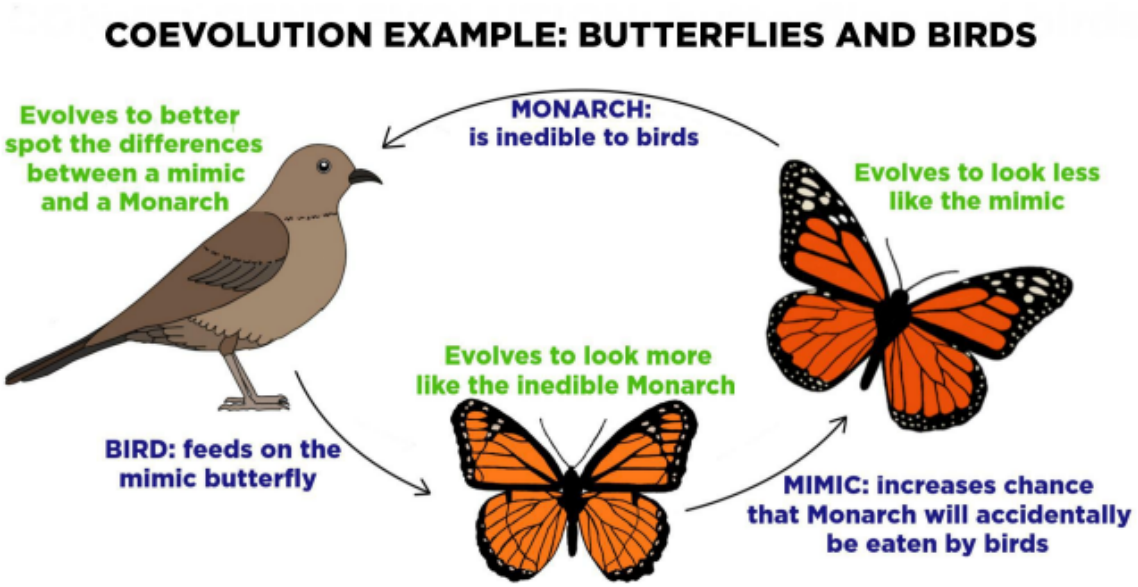
The Red Queen hypothesis was proposed over 40 years ago by the late evolutionary biologist Leigh Van Valen.



Red Queen Hypothesis: Predator and prey must be constantly evolving to avoid extinction.

Predator-prey relationship between rabbits and foxes following the principle of the Red Queen hypothesis. The rabbit evolves increasing speed to escape the attack of

the fox, and the fox evolves increasing speed to reach the rabbit. This evolution is constant; were one of the two to stop evolving, it would go extinct.



▼ **HOW KREBS CYCLE WAS DISCOVERED?**

Discovery of the Ornithine Cycle

Manometry to measure oxygen consumption of tissue slices, which allowed the precise investigation of biochemical (metabolic) pathways from animal tissues. These techniques were essential tools Dr. Krebs later used to discover the citric acid cycle and other novel metabolic pathways.

Without urea metabolism, the body does not have a way to get rid of nitrogenous waste, such as ammonia, which can lead to **encephalopathy**. This occurs commonly in patients with severe liver failure, where the ability to metabolize urea is compromised. Dr. Krebs applied
his knowledge of tissue slice analysis to delineate urea metabolism. He observed

urea = amino acid ornithine in the presence of ammonia

It was known since 1904 that *arginine* could be *hydrolyzed* by the enzyme *arginase* to form *ornithine and urea*. So Dr. Krebs also explored methods in which he could synthesize
arginine. Using his liver tissue slice assay with purified ornithine and citrulline, which he hypothesized was an intermediate of arginine, Dr. Krebs observed that *citrulline* acted as a *catalyst* to promote the production of urea from ammonia and carbon dioxide, thus generating the data that led to the *discovery of the ornithine cycle with Kurt Henseleit in 1932*

Encephalopathy:

A syndrome of global brain dysfunction.

It manifests as an altered mental state, including involuntary muscle twitching, abrupt loss of muscle tone, seizures, etc. The underlying causes may be infectious (bacteria, virus, prion), due to metabolic/mitochondrial dysfunction, brain tumor, exposure to toxins, radiation, trauma, poor nutrition, or lack of oxygen to name a few.

Around this time the rise of the National Socialist Government in Germany resulted in personal repercussions for Dr. Krebs. The political pressures of Hitler influenced all of Germany, resulting in the dismissal of all members of the Jewish race, irrespective of their
religious views, employed at teaching universities . The National Socialist party influenced the entire German empire and many great scientists aside from *Dr. Krebs fled the region*. Dr. Krebs left for England in 1933 when the Nazi party took power, and he remained
there until the end of his career.

“Our national policies will not be revoked or modified, even for scientists. If the dismissal of Jewish scientists means the annihilation of contemporary German science, then we shall do without science for a few years.”

— Adolph Hitler

Dr. Krebs accepted a Rockefeller studentship invitation from Sir Frederick Gowland Hopkins to the *School of Biochemistry in Cambridge*, England, where he was quickly appointed to the position of demonstrator of biochemistry in 1934. The

following year Dr. Krebs was appointed as *lecturer in pharmacology* at the University of Sheffield where he quickly moved up the ranks and became lecturer-in-charge of the Department of *Biochemistry* in 1938. This same year Dr. Krebs married Margaret Cicely Fieldhouse of Wickersley, Yorkshire, and went on to have 2 sons, Paul and John, and 1 daughter, Helen.

The Tri-carboxylic Acid Cycle Discovered

At the University of Sheffield, Dr. Krebs and
William Johnson published the work that led to the discovery of the
citric acid cycle (Figure 1A).
These studies were performed in the ***pigeon breast muscle***, which is the powerful muscle necessary for flight. This was a particularly **good model** as this muscle **maintained its oxidative capacity after its disruption and suspension in aqueous media**.

In these studies, Dr. Krebs noticed muscle tissue took up oxygen rather quickly, especially in the presence of pyruvate or lactic acid.

Believing that muscle cells could not carry out the metabolism of carbohydrates in 1 step alone, he hypothesized that carbohydrate metabolism occurred in a series of defined steps, extracting the biochemical energy of carbon-based nutrients into usable
cellular energy. Some of the clues to this were published by

Szent–Györgyi, who demonstrated **succinate, fumarate, malate, and oxaloacetate**

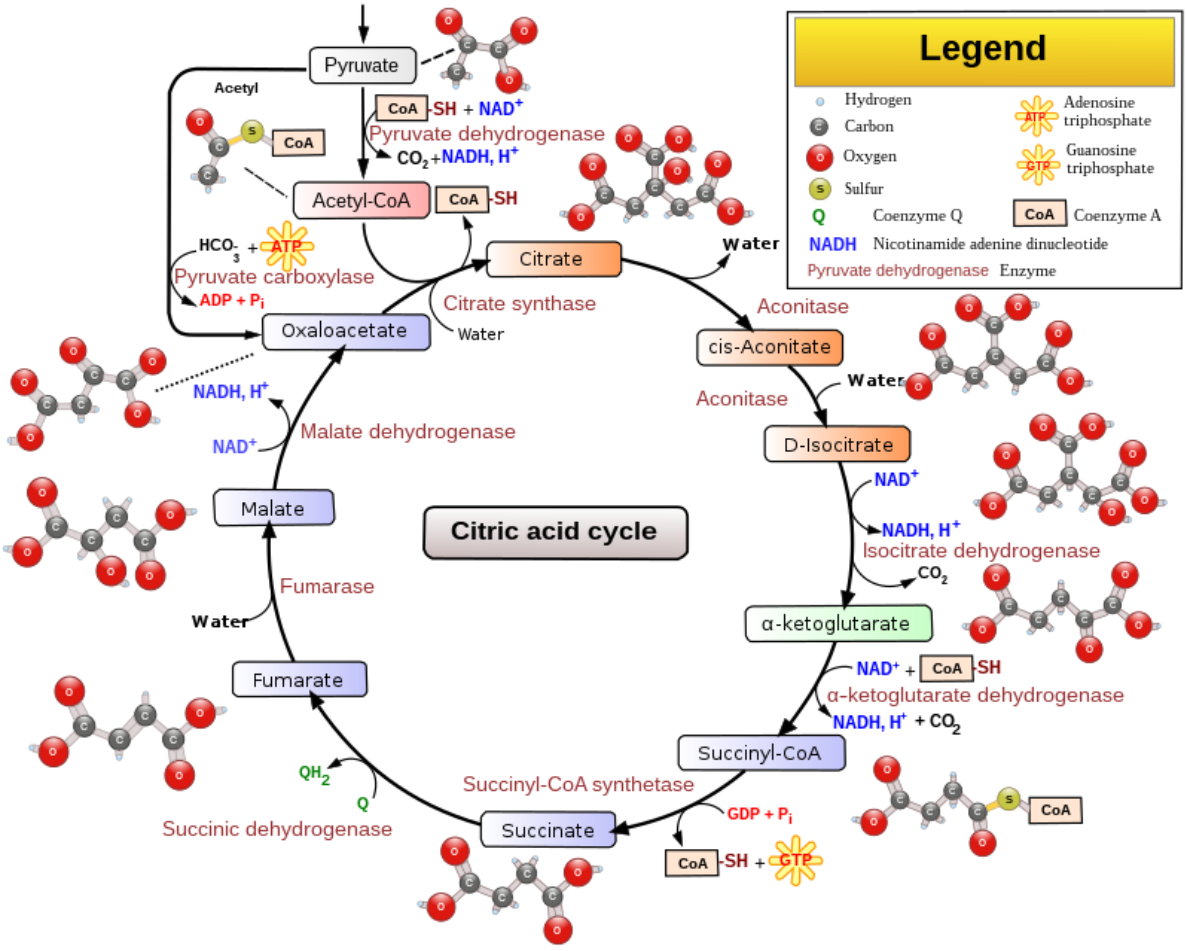
(all 4-carbon, C4, acid salts) oxidized as quickly as pyruvate and lactic acid (3-carbon acid salts) and ***catalytically promoted oxygen uptake***.

Another piece of the puzzle, published by `Dr. Krebs in 1937, showed succinate could be synthesized by animal tissues in the presence of pyruvate.` Dr. Krebs speculated that the 4-carbon acid salts may have been derived through the oxidation of citrate.

The citric acid “Krebs” cycle: Then and now. (A) The citric acid cycle as proposed (and referenced) by Krebs and Johnson in 1937. (B) The chemical intermediates are in bold and the enzymes responsible for driving the cycles are in italics. Defects in the enzymes shown in red are responsible for known enzymopathies. Adapted from: Krebs and Johnson, 193722 and Munnich, 2008.12

In 1937, Martius and Knoop reported the fate of citrate undergoing oxidation. They identified that `α-ketoglutarate (5C) was formed when citrate (6C) was oxidized using liver and cucumber seeds and suggested cis-aconitate and isocitrate were intermediates`.

Wagner-Jauregg and Rauen identified that isocitrate behaved similarly in cucumber seed extracts.



This led Dr. Krebs to question whether the 4 carbon acid salts were in

fact derived from citrate and prompted further investigation into the properties of this 6-carbon compound.

- *Dr. Krebs observed a rapid oxidation of citrate, but interestingly, he identified that citrate was never fully consumed as a substrate, suggesting a capacity for citrate synthesis in this system* .

In addition, ***hypoxic conditions*** (low oxygen) resulted in ***large amounts of citrate formation*** in minced muscle ***only in the presence of both oxaloacetate and pyruvate.***

+ Two other key findings from these studies were:

(1) *succinate is more reduced than oxaloacetate*; and

(2) *succinate formed from oxaloacetate coincided with a rapid uptake of oxygen.*

Taken together these data described a cyclic sequence of reactions, which Dr. Krebs and Johnson called the citric acid cycle.