T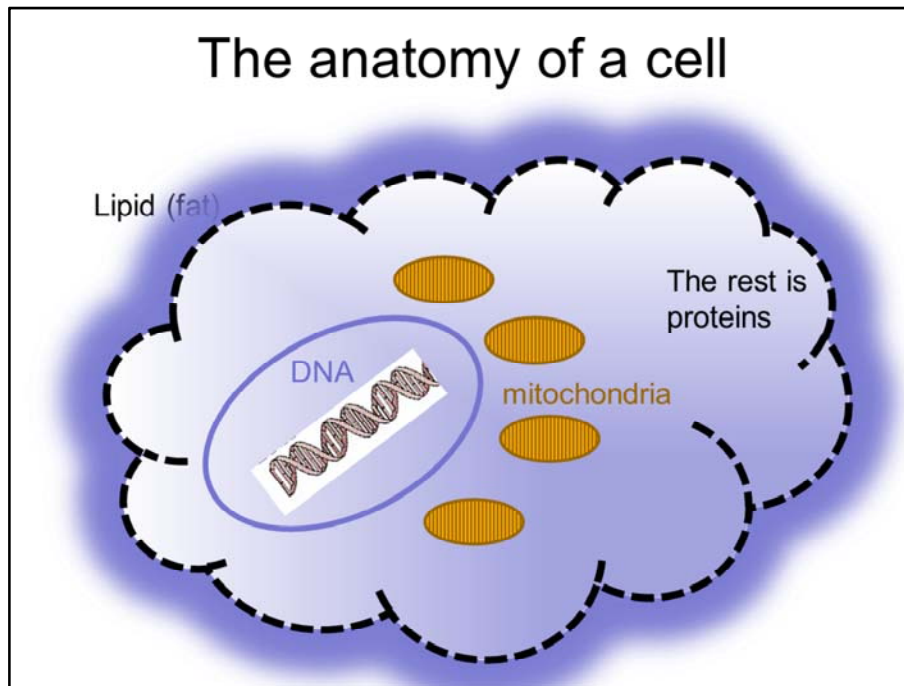he goal of this project is to develop topical products that improve skin quality and contribute to preventing skin cancer for XP patients.
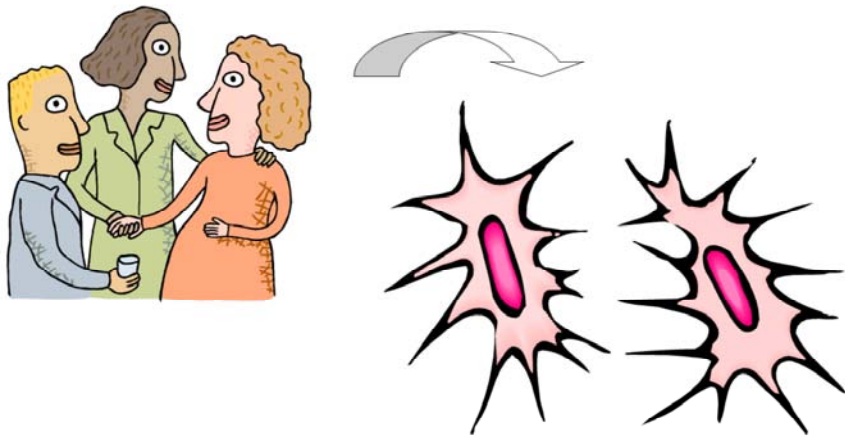
The human body is made of 100 trillion cells: skin cells, liver cells, brain cells are neurons, or glial cells (cells that support neurons).

The anatomy of a cell

Lipid (fat)

The rest is proteins

DNA

mitochondria

Every cell in the body is roughly designed the same way.
The outer barrier of every cell is a membrane made of lipids or fats from our diet.
Mitochondria are the energy factory of a cell. Their job is to take food we digest and turn it into ATP, which is the energy currency of a cells.
Unfortunately, a by-product of producing ATP is reactive oxygen species (ROS). So mitochondria are the main source of ROS in the cells and body. And ROS is the mains source of oxidative damage in the body, including oxidative DNA damage. This is DNA damage is endogenous, meaning that it is formed inside the body instead of being caused by the environment like UV. That means there is less you can do to control oxidative damage.

The nucleus (blue circle) contains the DNA or genome. This is the blueprint needed to make all the parts of a cell and to make a new cell when tissue needs repairing.
Each cell contains 100's of thousands of proteins. These are the worker bees of a cell – they do all the work to make a cell act like a skin, liver or brain cell.

You need DNA to make proteins

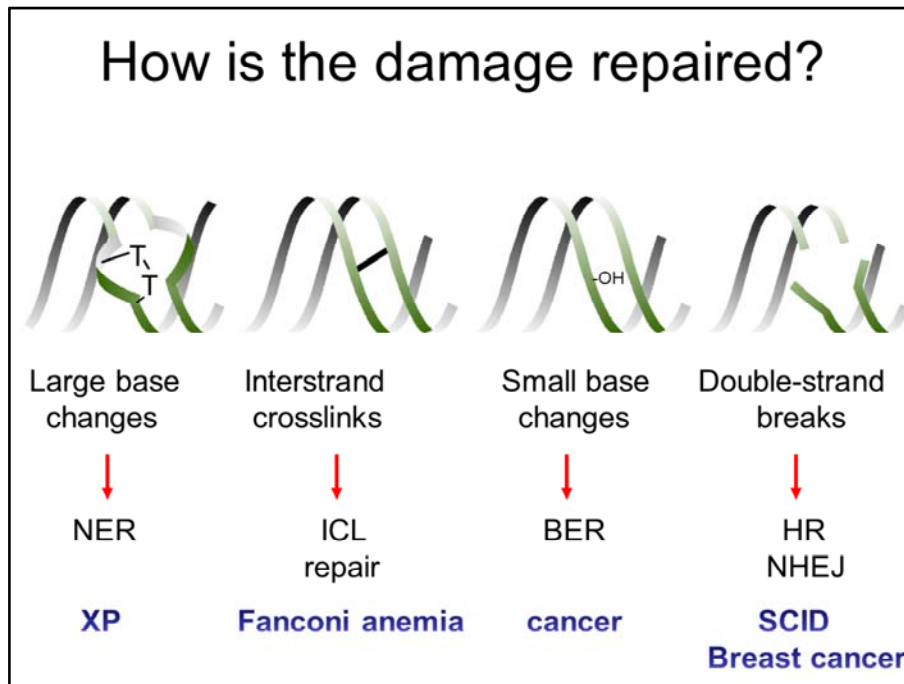DNA → Transcription → mRNA → Translation → Protein
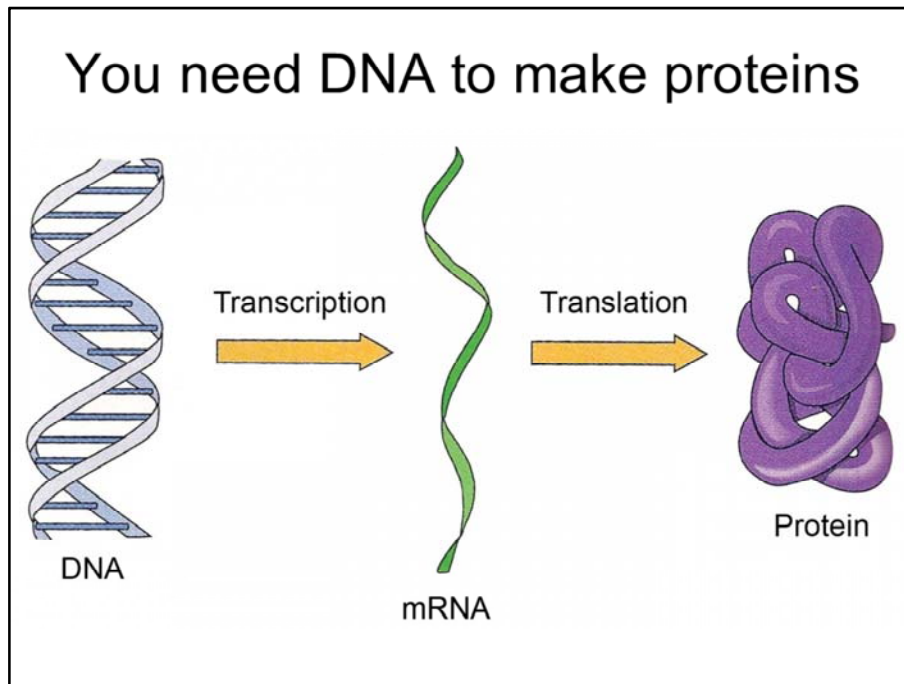
The DNA in the nucleus or genome is the blue print of a cell. It contains the genetic code needed to make each protein.
There are roughly 30,000 genes in the genome. Each encodes a different type of protein needed to make a cell function.

The intermediary between DNA and protein is called messenger RNA or mRNA.
DNA is transcribed into mRNA. mRNA is then translated into protein.
Because DNA is the genetic code, protecting the code is of utmost importance to our health and well-being.

How is the damage repaired?

Large base changes	Interstrand crosslinks	Small base changes	Double-strand breaks
↓	↓	↓	↓
NER	ICL repair	BER	HR NHEJ
XP	**Fanconi anemia**	**cancer**	**SCID Breast cancer**

Because the DNA is so important, we all have multiple mechanisms to repair damage to DNA.
These mechanisms are highly conserved between humans, animals, plants and bugs illustrating how important they are to life.

NER = nucleotide excision repair, which is responsible for repairing large DNA damage that disrupt the structure of DNA. People missing NER have XP.
ICL repair = interstrand crosslink repair, which is responsible for repairing damage that links the two strands of DNA together. People missing ICL repair have Fanconi anemia, which is characterized by myelodysplastic syndrome and blood cancers.
BER = base excision repair, which is responsible for repairing small DNA damage like damage caused by ROS. People with inherited defects in BER are prone to cancer.
HR and NHEJ are ways to repair double-strand breaks in DNA. People with defects in HR are prone to breast cancer (e.g., BRCA1 or BRCA2 mutations). People with defects in NHEJ have immunodeficiency (SCID = boy in the bubble).

Nucleotide Excision Repair
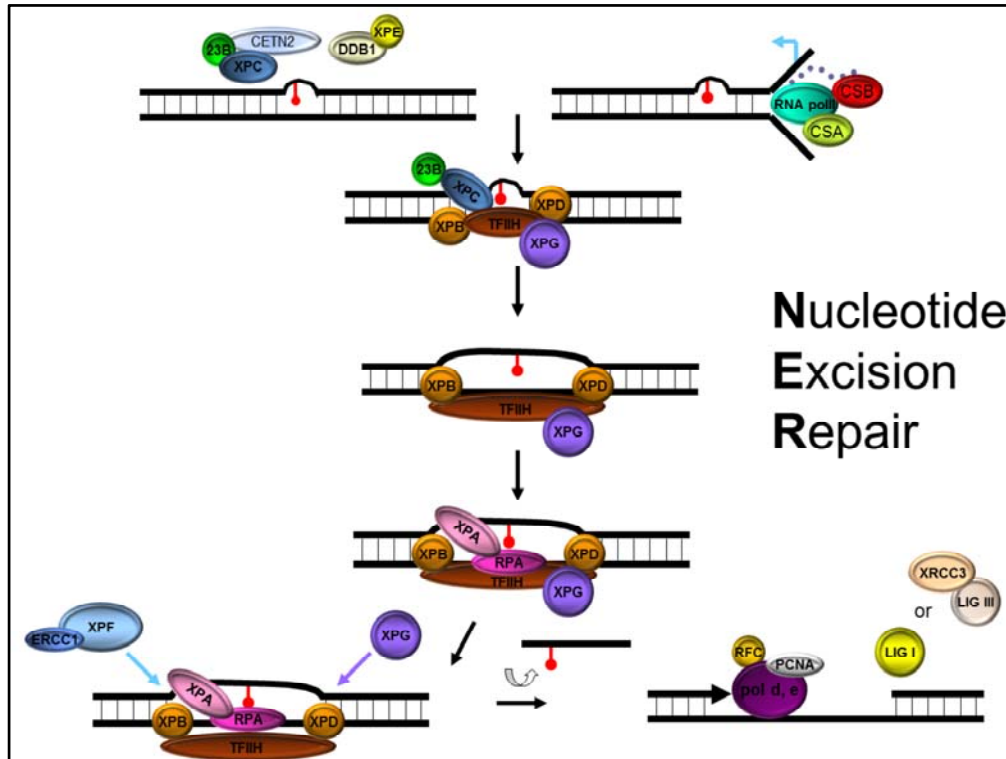
This is a schematic diagram of the nucleotide excision repair (NER) pathway.
This is the pathway that is affected in people with XP.
NER is responsible for repairing damage to the DNA or genome.
The type of DNA damage repaired by NER is UV-induced DNA damage, some oxidative DNA damage, and damage caused by environmental chemical carcinogens like polyaromatic hydrocarbons in cigarette smoke or charred fat on barbequed meat.

Each colored circle in the scheme is a different protein required for NER.
The proteins must act in a sequential order for NER to work.
All of the proteins with XP in their name are proteins that are linked to the disease XP. If you are missing XPA, XPB, XPC…. this can cause XP.
The other proteins are also important for NER, but so far, there are no XP patients with mutations in the genes encoding these other proteins.

Starting from the top of the schematic:
XPC is required to recognize DNA damage (called lesions or adducts) in the genome. If you are missing XPC, NER can't even get started.
XPE helps recognize a subset of DNA lesions. Primarily UV-induced DNA damage. Thus symptoms in XPE patients are largely limited to those caused by UV (i.e., skin symptoms).
XPD, XPB and XPG are part of a large complex called TFIIH. TFIIH is required to open the DNA around the site of damage. Opening DNA is required to cut out the patch with damage. TFIIH is also required to initiate transcription (the process of turning DNA into proteins). Since XPD, XPB and XPG are required for more than just NER, patients with

mutations in these genes may have different or additional symptoms on top of XPC patients. XPA is required to stabilize the DNA once it is opened by TFIIH. XPA is also required for recruiting the next proteins in NER. This is another go / no-go step of NER. So mutations in XPA can cause a dramatic defect in NER and sometimes severe symptoms in XP.

XPF and XPG cut DNA. Both are required to cut out the DNA damage. If either one are missing, repair can't be completed. Sometimes, the intermediates of DNA repair are more toxic than the original damage. Thus, mutations in XPF or XPG can cause symptoms above and beyond typical XP.
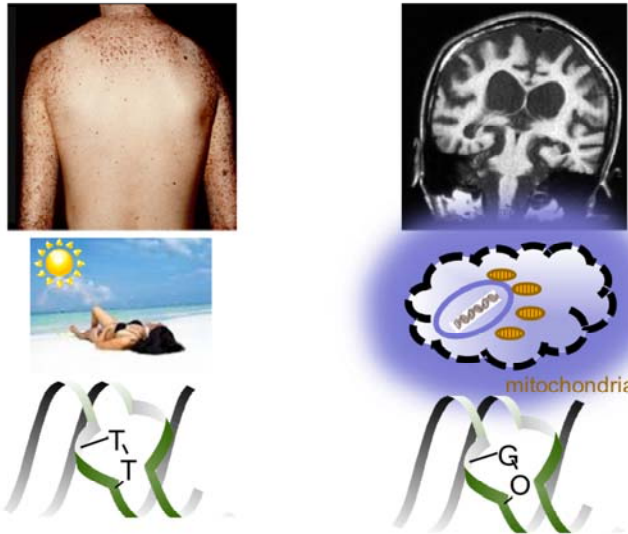
DNA damage caused by UV radiation (primarily the sun) is responsible for all of the cutaneous (skin) symptoms in XP. In other words, the symptoms are caused by the environment and can be largely controlled or minimized with great sun protection.

DNA damage caused by endogenous processes (normal metabolism) are responsible for all of the other symptoms in XP. The damage is largely caused by reactive oxygen species (ROS) and is difficult to control or minimize.

How does DNA damage cause symptoms?
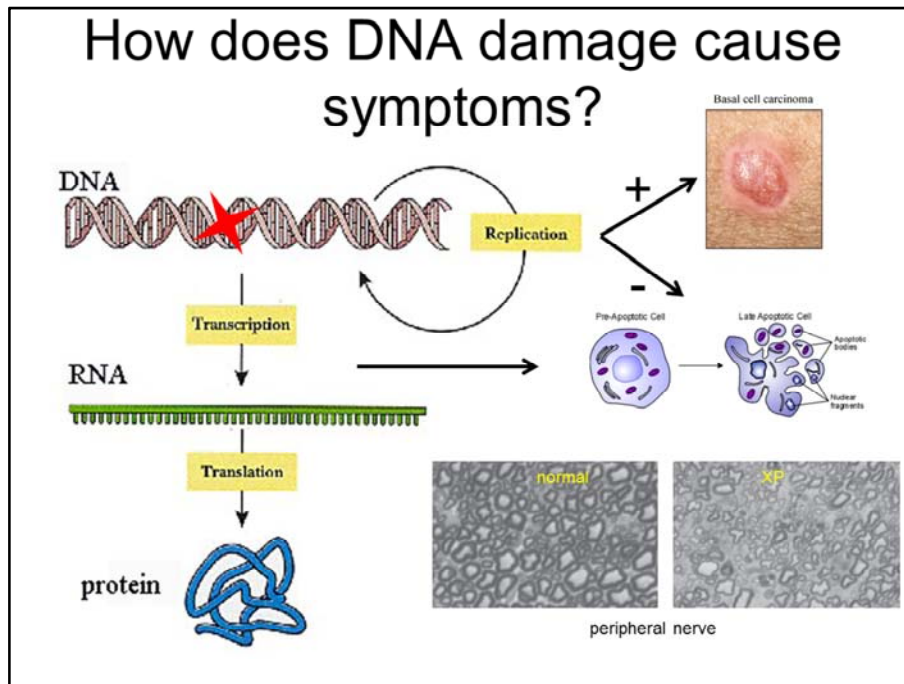
If DNA damage in the nucleus of a cell is not repaired, why does it cause symptoms?

If the cell type is one that divides often to make new cells (e.g., skin, blood) then DNA damage can be replicated (copied for the new cell) leading to mutations (changes in the DNA code). Mutations cause changes in protein production or function. Since these cell types need to divide, there is selective pressure for mutated cells that like to grow/divide fast. This is how cancers start.

Other types of DNA damage can't be copied/replicated. This can cause cell death (apoptosis) or cell senescence (triggering a program that permanently blocks replication). This protects against cancer. But it can cause accelerated aging of that tissue.

If the cell type is one that doesn't need to replenish itself thus wont' divide (e.g., neuron in the brain), then DNA damage can block transcription (steps to making a protein). This can kill a cell (apoptosis). If non-dividing cells die, they can't be replaced. This is thought to be the cause of neurodegeneration in XP.

How to find a treatment for XP?

1. Cure: gene therapy
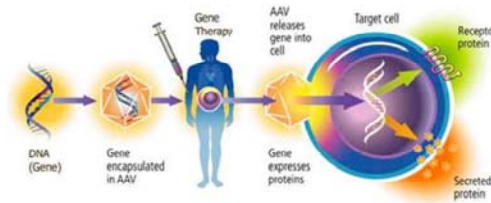
Caveats:
- Virus
- Reaching all cells
- Tailor made for each complementation group

The long term goal of our research is to find treatments for XP.

The first type of treatment is a cure.
The only cure for a genetic disease like XP is gene therapy (GT). GT aims to replace the mutated copy of a gene with a perfect copy.
For example, if you are an XPC patient, the goal would be to give you a perfect copy of XPC.

GT is in its infancy. It is technically feasible, but the first clinical trials revealed some complications.
The first complication is that delivering a gene or piece of DNA to cells in your body requires a virus.
The first viruses used caused blood cancers.
The current viruses in use (AAV) appear much safer, but are not fully tested yet.
The second technical difficulty is delivery of a gene to cells where it is needed. For XP, it is the skin and neurons. Neurons are do-able. But skin is harder.
Third, how many cells do you have to deliver the gene to in order to cure XP? Likely 100% of skin cells and neurons. That is tough to do.
Fourth, a disease like cystic fibrosis is caused by mutations in 1 gene. Thus a GT method with 1 gene is a cure.
XP is caused by mutations in at least 8 genes. Thus a cure for all XP patients would require

making 8 different viruses.

Currently, NIH is testing an AAV virus for another rare disease.
If this proves successful and safe, then there will be opportunities to try GT for XP within the next 5-10 years.

How to find a treatment for XP?

2. Sytemic

Goal:
- Stop production of things that cause DNA damage
- Remove damaged cells before they do harm

The second type of treatment is a systemic approach, meaning a pill that will treat XP from the inside out.

One goal of this type of approach is to prevent endogenous DNA damage. An example of this is anti-oxidants.

The newer approach is to remove damaged cells (senescent cells). The thinking is that these senescent cells contribute to symptoms and disease by secreting inflammatory factors. This is likely the cause of accelerated aging of some tissues (e.g., reproductive system).
Drugs are being developed to get rid of senescent cells.

How to find a treatment for XP?

3. Topical

Goal:
- Stop production of things that cause DNA damage
- Remove damaged cells before they do harm

The third approach is to design a topical treatment that can help improve skin quality and prevent skin cancer.

Here, like the systemic therapy, the goal is to prevent DNA damage and to remove the damaged/senescent cells in the skin.

Requirements for making new therapeutics

An absolute requirement for developing a new treatment is animal models.
For a new drug, the FDA requires testing in at least two different animal models.

Laura's lab makes mouse models of XP.

Making mouse models of XP
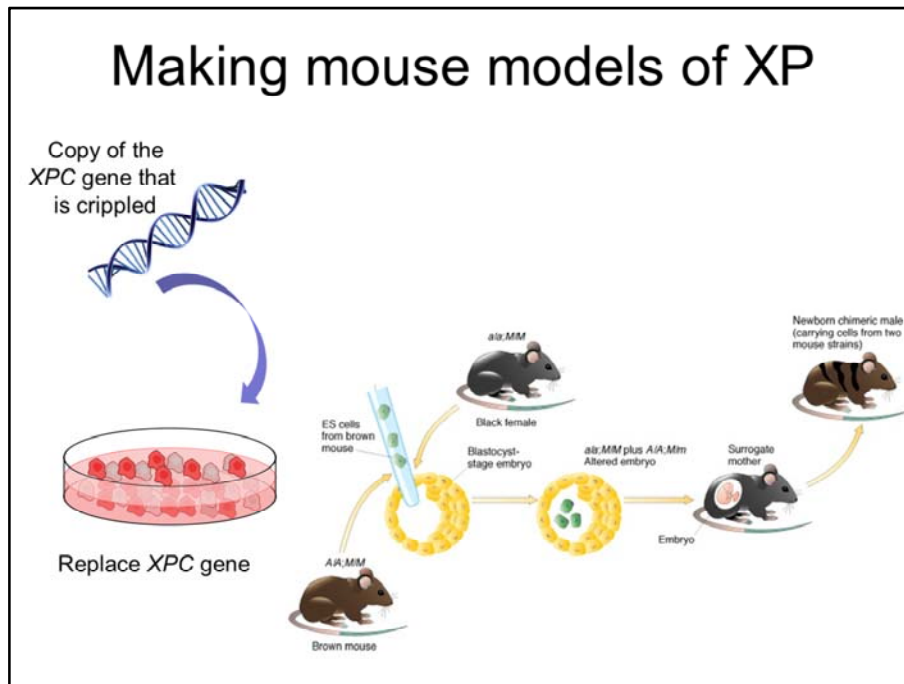
To make a mouse model of XP, we make a piece of DNA that contains, for example, the XPC gene. We make sure that the XPC gene has a mutation in it so that it no longer makes a functional XPC protein.

We get that piece of DNA into cells. The mutated copy of XPC exchanges with the normal copy in the genome of the cells.

Then the cells with the mutated copy of XPC (in green) are injected into an early embryo from black mice. The genetically engineered cells (green) are from a brown mouse. These cells integrate into the early embryo. The embryo is implanted into a surrogate mother mouse.

Pups are born. If they have a mix of brown and black fur then the engineered cells contributed to making mouse tissues.

The next generation of mice will have some pups that are all brown. These mice are the equivalent of an XPC carrier.

Breed two of these mice to get a mouse model of XPC.

Nucleotide Excision Repair
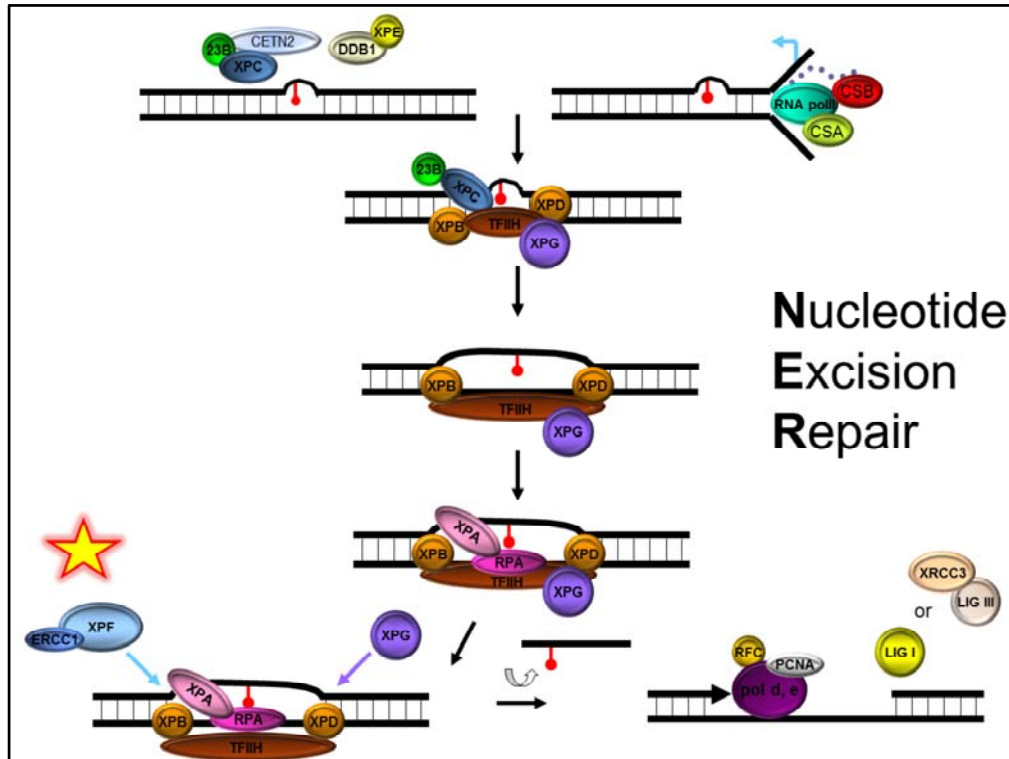
Our group is focused on modeling XPF (star).
But mouse models exist of XPA, XPC, XPB, XPD and XPG.

Ercc1^{-/Δ} mice model an exaggerated form of XP

high risk of skin cancer
loss of vision & hearing
unsteady gate
impaired kidney function
cognitive decline
emphysema
immunologic changes
loss of insulin-producing cells
muscle wasting
frail bones
early myelodysplastic syndrome

5 wks
9 wks
14 wks
22 wks
25 wks

Ercc1 mice have low expression of XPF.
They have very, very exaggerated features of XP.
And the mice age more quickly than normal mice.

A mouse model of XP skin disease

Figure 1
mister clean
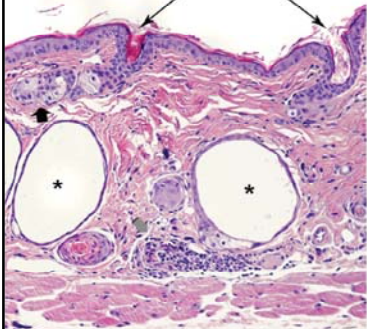
Testing therapies:
DNA repair agonists
Anti-oxidants
Stem cells
Anti-inflammatories

To model XP skin disease, we got mice that have no fur and bought them a tanning bed.

SKH1 mice

- Albino, immunocompetent
- Homozygous for a mutation in the hairless (Hr) gene
- Outbred
- Hairless by 3 wks of age
- Applications:
    - measuring drug penetration
    - gene therapy
    - luciferase imaging
    - confocal imaging with fluorescent probes
    - wound healing
    - photoaging
- Normal skin

The hairless mice are used by dermatologists to study wound healing and photoaging of the skin.

Photoaging

- Increased skin thickness*
- Elastosis
- Collagen damage, synthesis and degradation
- Increased ratio of collagen III to I
- Increased GAGs
- Wrinkles and sagging
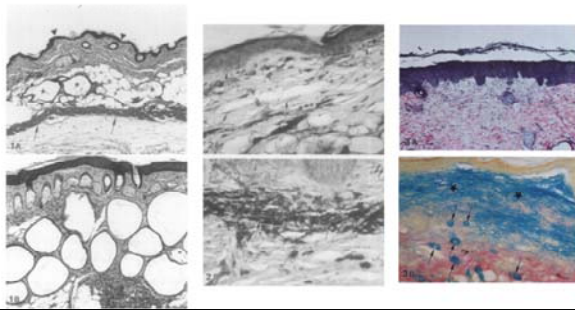
*not analogous to humans

Photoaging (sun damage) causes a variety of changes that we can try to treat and improve.

Normal crinkles   UVB-induced wrinkles   UVA-induced sagging
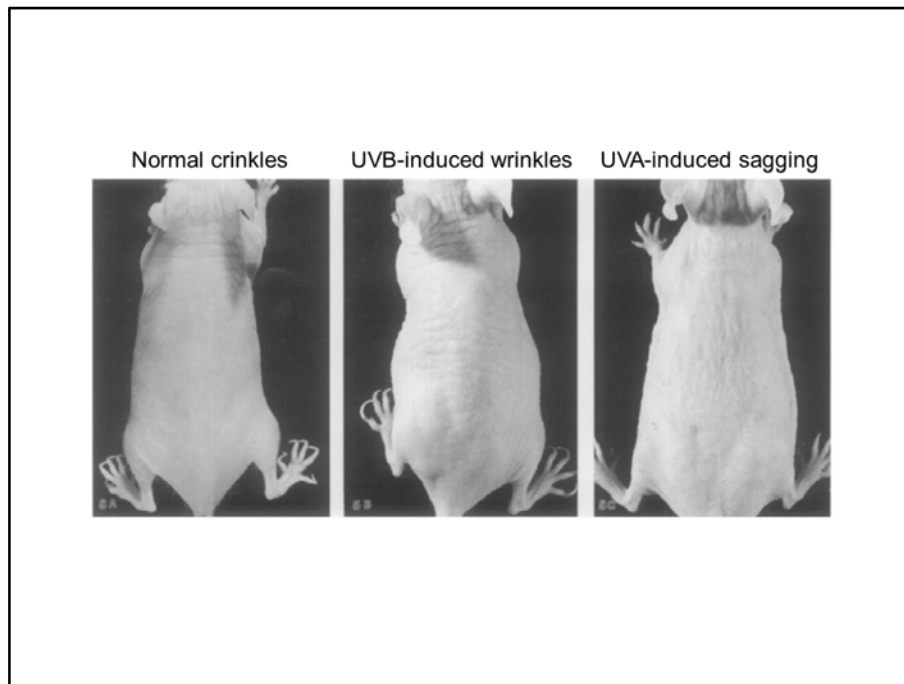
Photoaging with UV-B causes wrinkles.

UV exposure in SKH1 mice

- 3X weekly x 20-30 weeks
- Accelerate this by adding an XP background
    - Xpa-/-
    - Ercc1-/c
    - Ercc1-/c;K14-Cre

4 weeks of tanning = 30 yrs of sun damage
5-6X acceleration

If we add a mutation in an XP gene to the hairless mice, photoaging occurs 5-6X faster than in normal mice.
This is a perfect model of the skin symptoms of XP, easy sunburn and increased risk of UV-induced skin cancer.

We tattoo the mice to create quadrants on their back to compare treatment vs. placebo.
Then we expose them to UV in the tanning booth 3X per week for 4 weeks.
This is the equivalent of about 30 years of sun exposure to humans.
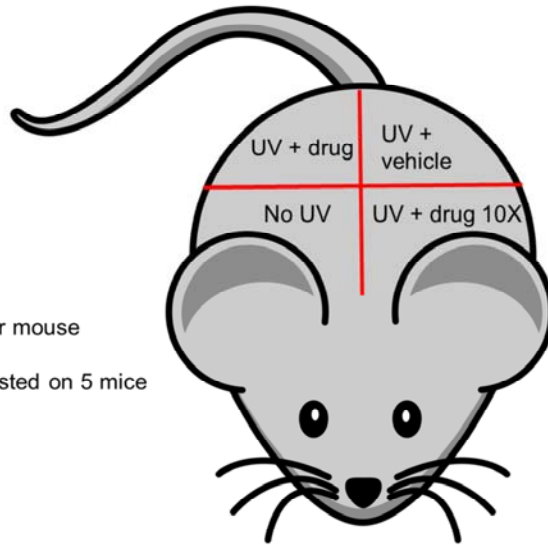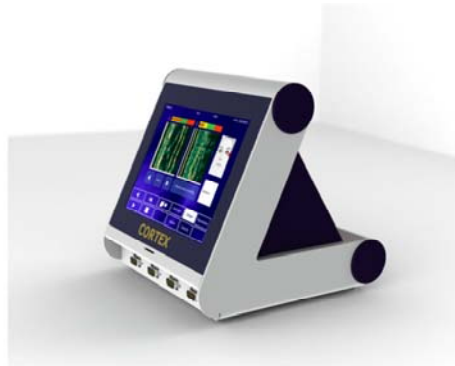
We use 1 mouse to compare the impact of UV, UV plus vehicle only (cream), UV + drug in the cream, on skin integrity.

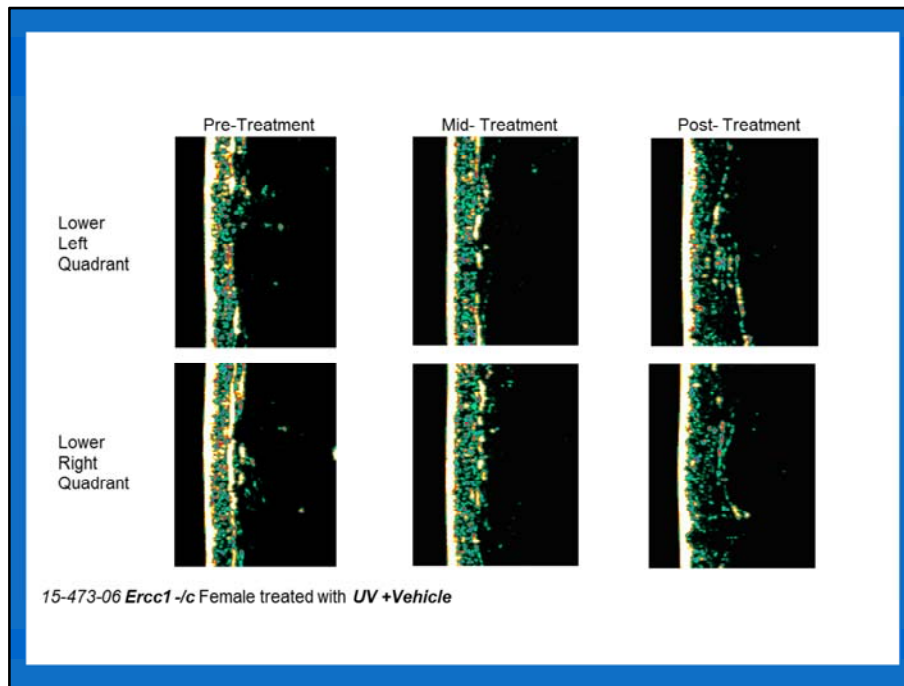We measure clinically relevant endpoints measured by ultrasound identical to what a dermatologist uses in his clinical practice.
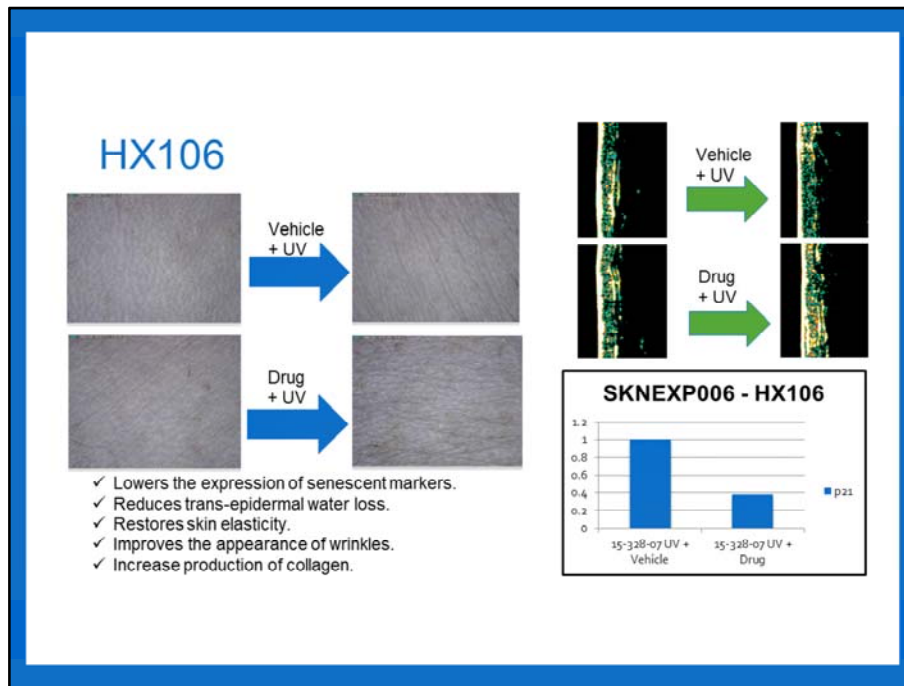
Before UV exposure (left) the skin has lots of density (lots of color, yellow and red = collagen).
After UV (right) the skin integrity is broken down (lack of color density and warm colors).

Pre-Treatment   Mid- Treatment   Post- Treatment

LL

LR

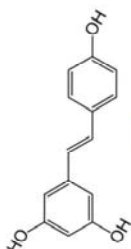15-473-06 *Ercc1 -/c* Female treated with ***UV +Vehicle***

UV causes wrinkles (right).

Topical application of a drug like HX106 can reduce wrinkles, improve collagen, and reduce markers of senescence like p21

Things that work so far to improve skin that is photodamaged include resveratrol – the ingredient in red wine that is supposed to slow aging.
Resveratrol is a natural product.
Natural products are easier to get FDA approval than new chemical drugs.
Resveratrol helps to prevent damage.

Also quercetin and fisetin, two natural products found in strawberries, work.
These natural products kill senescent cells when we test them in the laboratory.

What we have tested so far:

Anti-cancer drug
Removes damaged cells

Imagine the potential of a mixture of these drugs?!

Also an anti-cancer drug called dasatanib works. This drug also kills damaged senescent cells when we test it in the lab.

What we imagine is combining several of these drugs that prevent damage and kill damaged cells to get the optimal outcome.
This could be useful to XP patients for improving skin quality and prevent skin cancer.

The next steps for us is to transfer this to a pharmaceutical company for further development.
Products like this might be available within 2 years
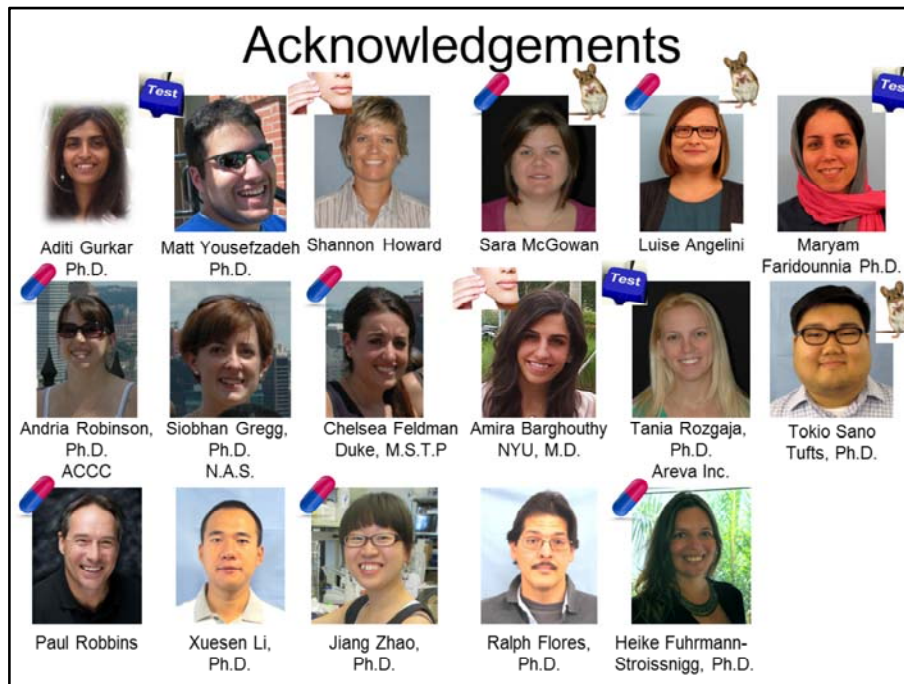
All of the folks in our labs who work directly or indirectly on therapeutics for XP.