Xeroderma Pigmentosum

and the XPA gene

Sarah Drewes

May 6, 2014

What is Xeroderma Pigmentosum (XP)?

http://www.sfgate.com/news/article/LIVING-IN-THE-SHADOWS-Bay-Area-doctors-join-2669297.php

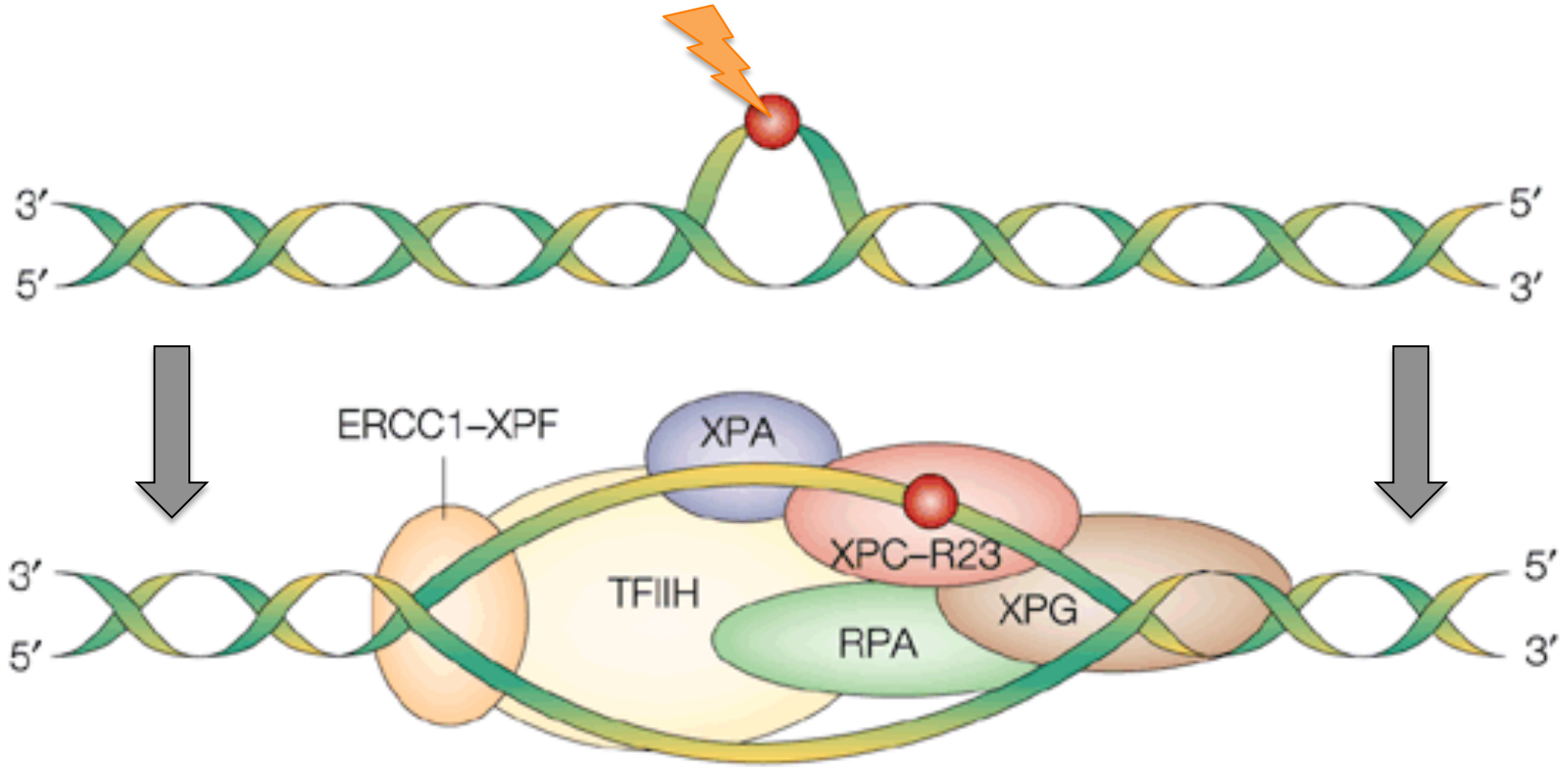
Extreme sensitivity to UV light

Symptoms:

Distinct freckle-like spots
Severe blistering and burning
Photophobia and eye damage
Cancerous/noncancerous growths
Neurological defects

Why is XP a public health concern?

Nucleotide Excision Repair (NER) is mutated in XP patients

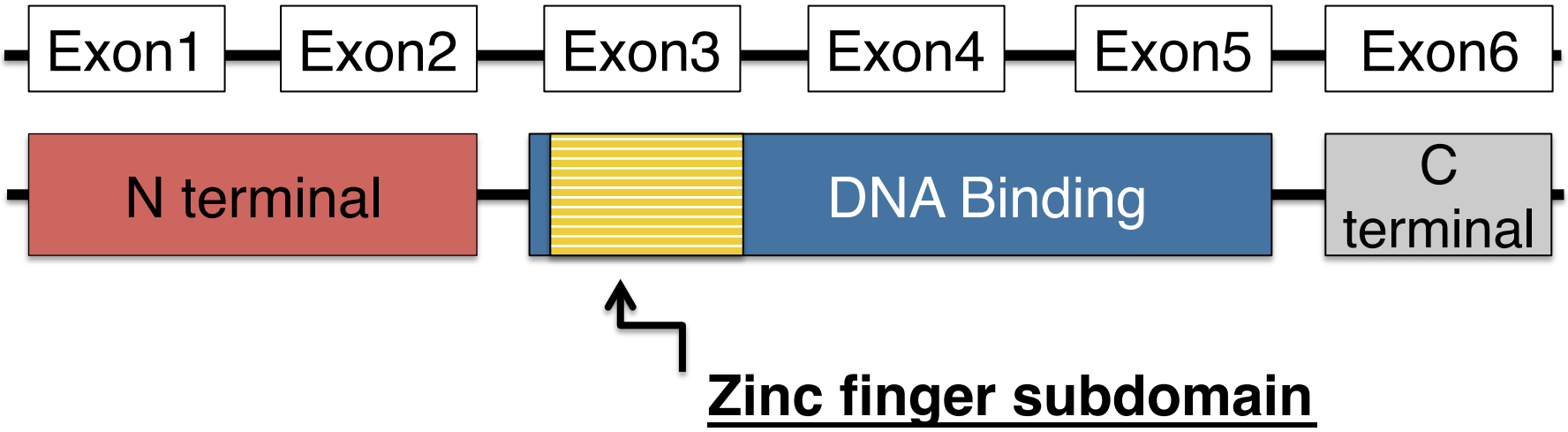


Mutations in **XPA** cause XP

Transcription of downstream proteins affected

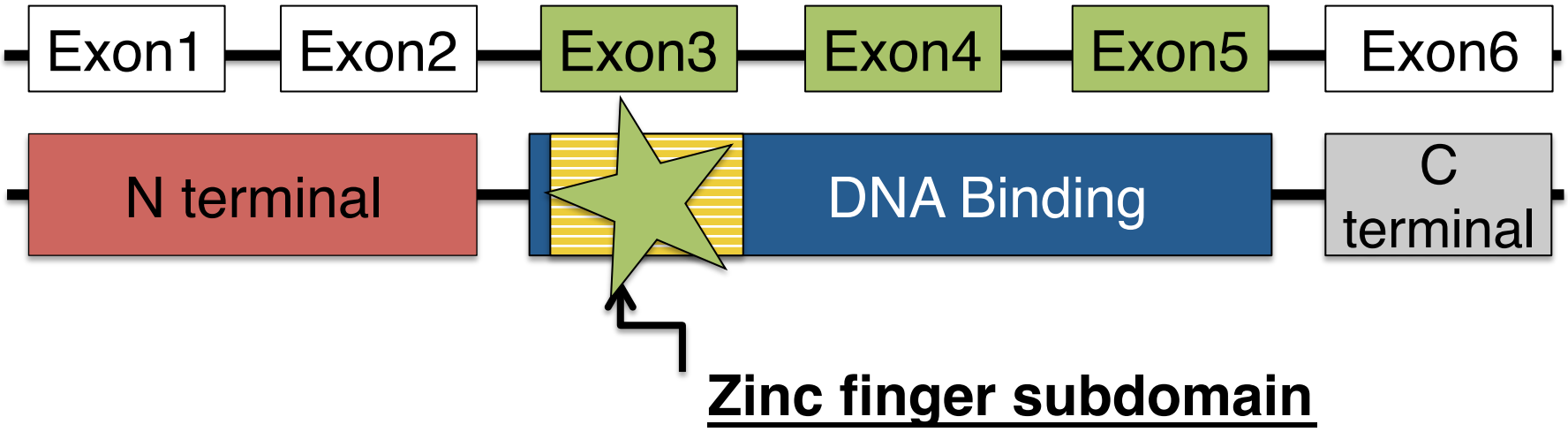
Friedberg, 2001

What is the structure of XPA?



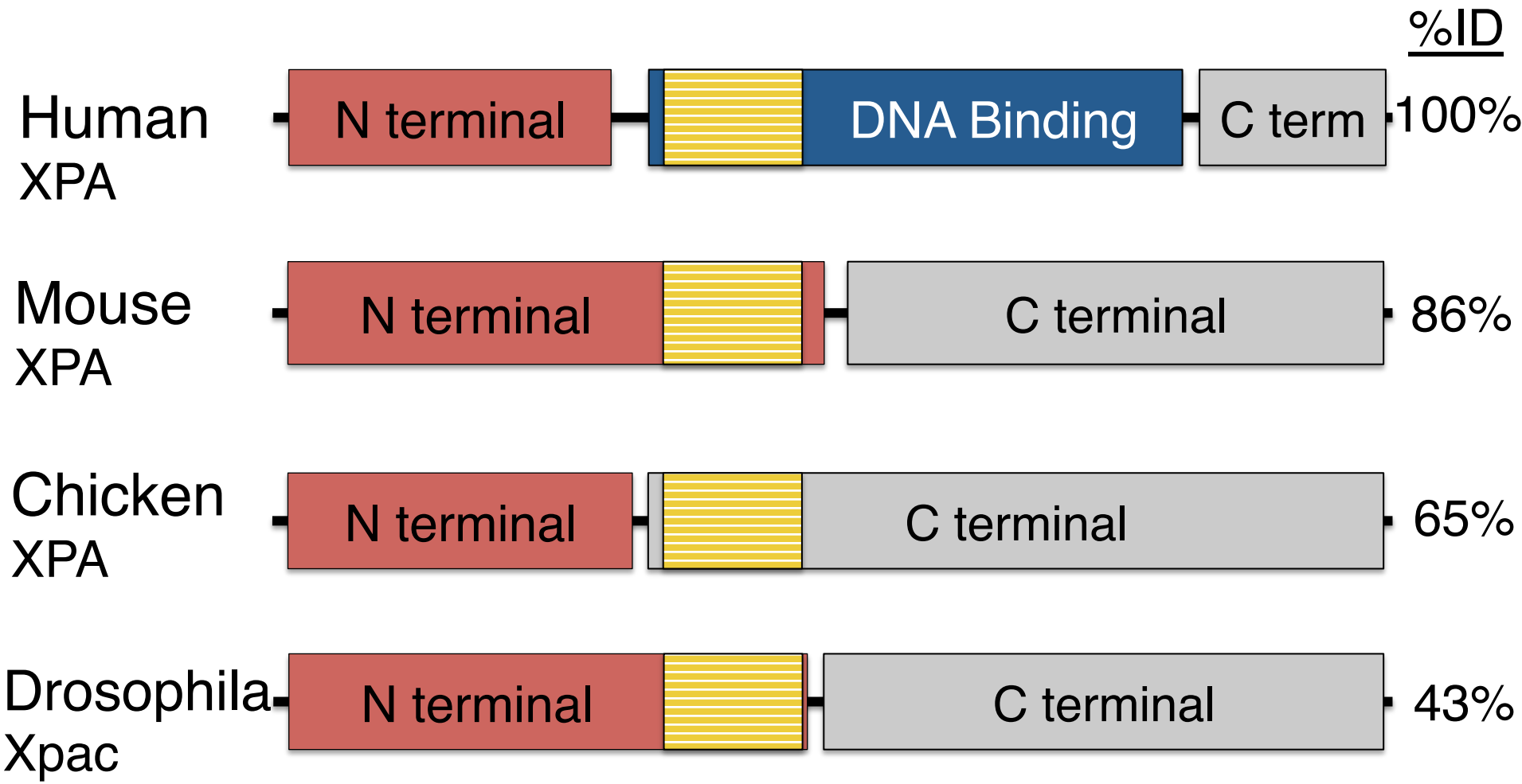
Bartels & Lambert, 2007

What is the structure of XPA?



The most detrimental mutations occur in exons 3, 4, and 5

XPA is well conserved

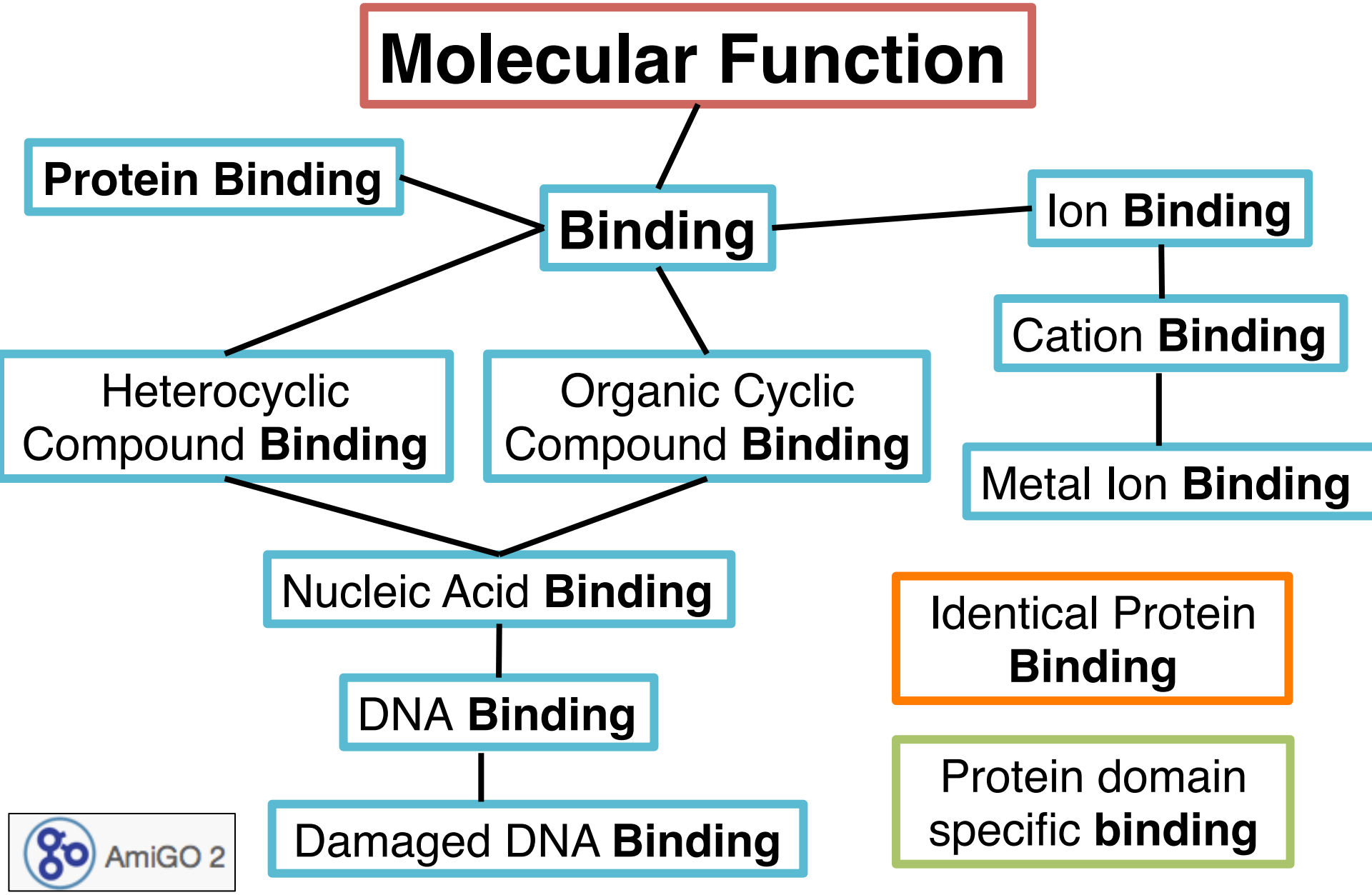


HomoloGene, InterPro, & BLAST

= Zinc finger subdomain

Shimamoto et al, 1991

How does XPA function?



Are there compounds that inhibit **XPA** binding?

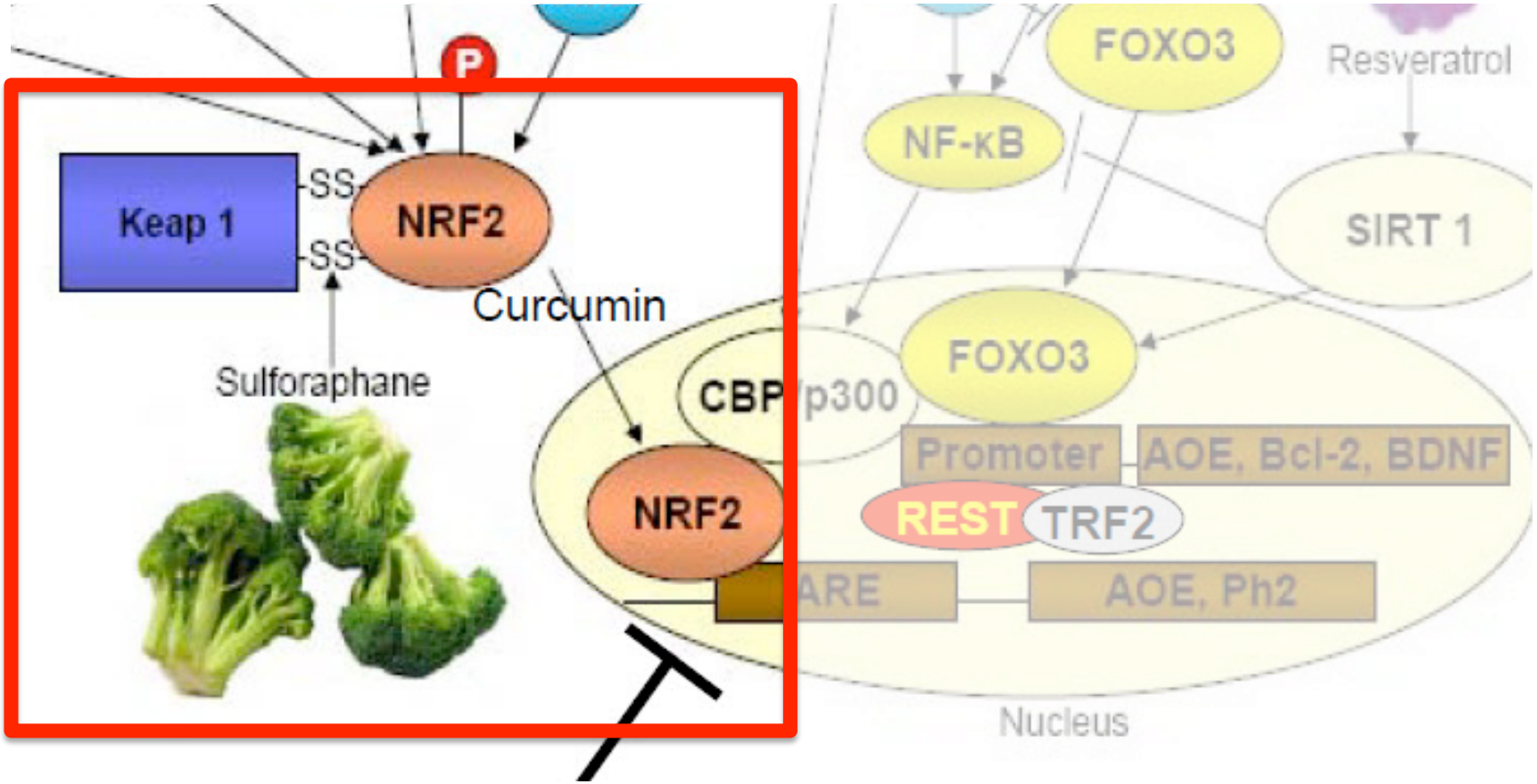
Sulforaphane

PubMed.gov

PubChem

http://www.dailyperricone.com/2011/02/start-fresh-sulforaphane/

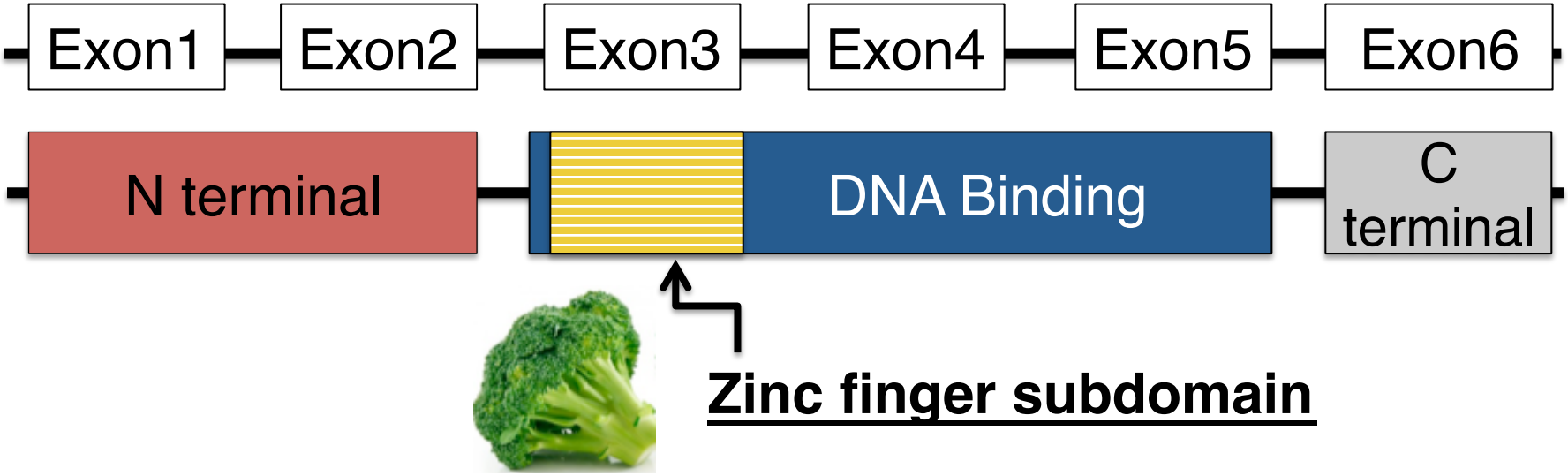
What is sulforaphane's function?



Oxidative stress response

http://www.grc.nia.nih.gov/branches/lns/linka.htm

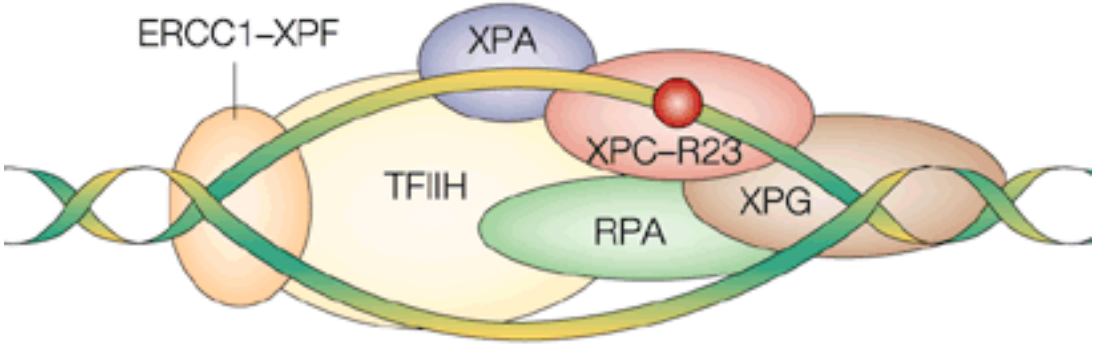
How does sulforaphane inhibit XPA?



Sulforaphane removes zinc

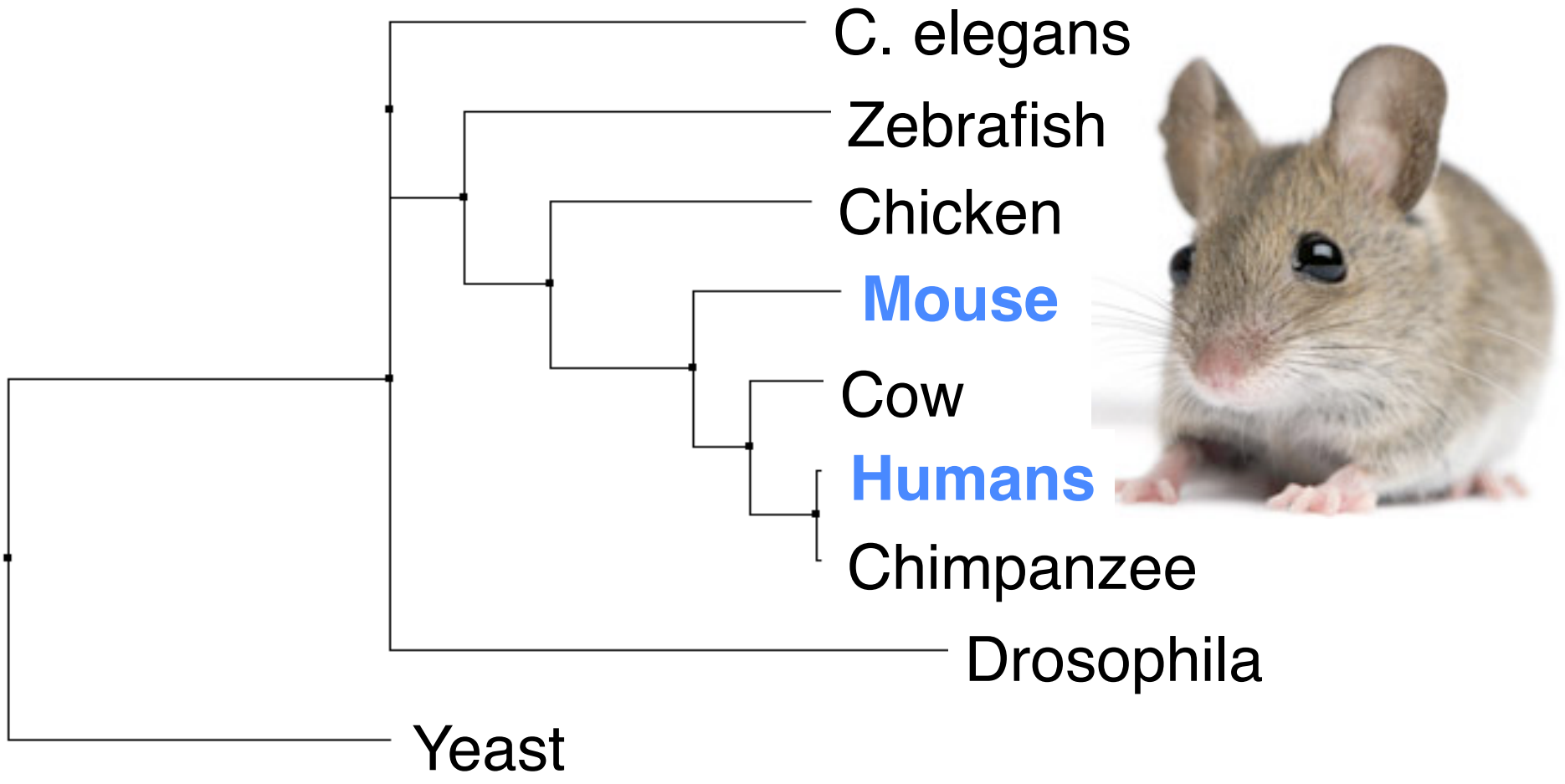
Piberger, Köberle, & Hartwig (2014)

Goal: Does the inhibition of XPA by sulforaphane affect other DNA repair processes?



Hypothesis: Sulforaphane will alter binding ability, transcription, and protein expression

Mouse is best model organism to study XPA



Humans and mouse are closely related

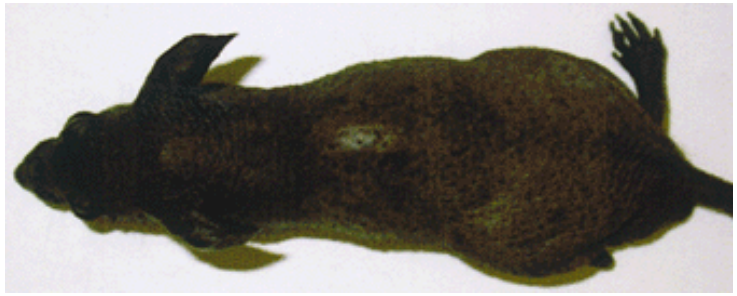
Clustal Omega

http://news.discovery.com/animals/zoo-animals/mice-stem-cells-101209.htm

Mice exhibit similar phenotype as humans

Wild type

XPA mutant

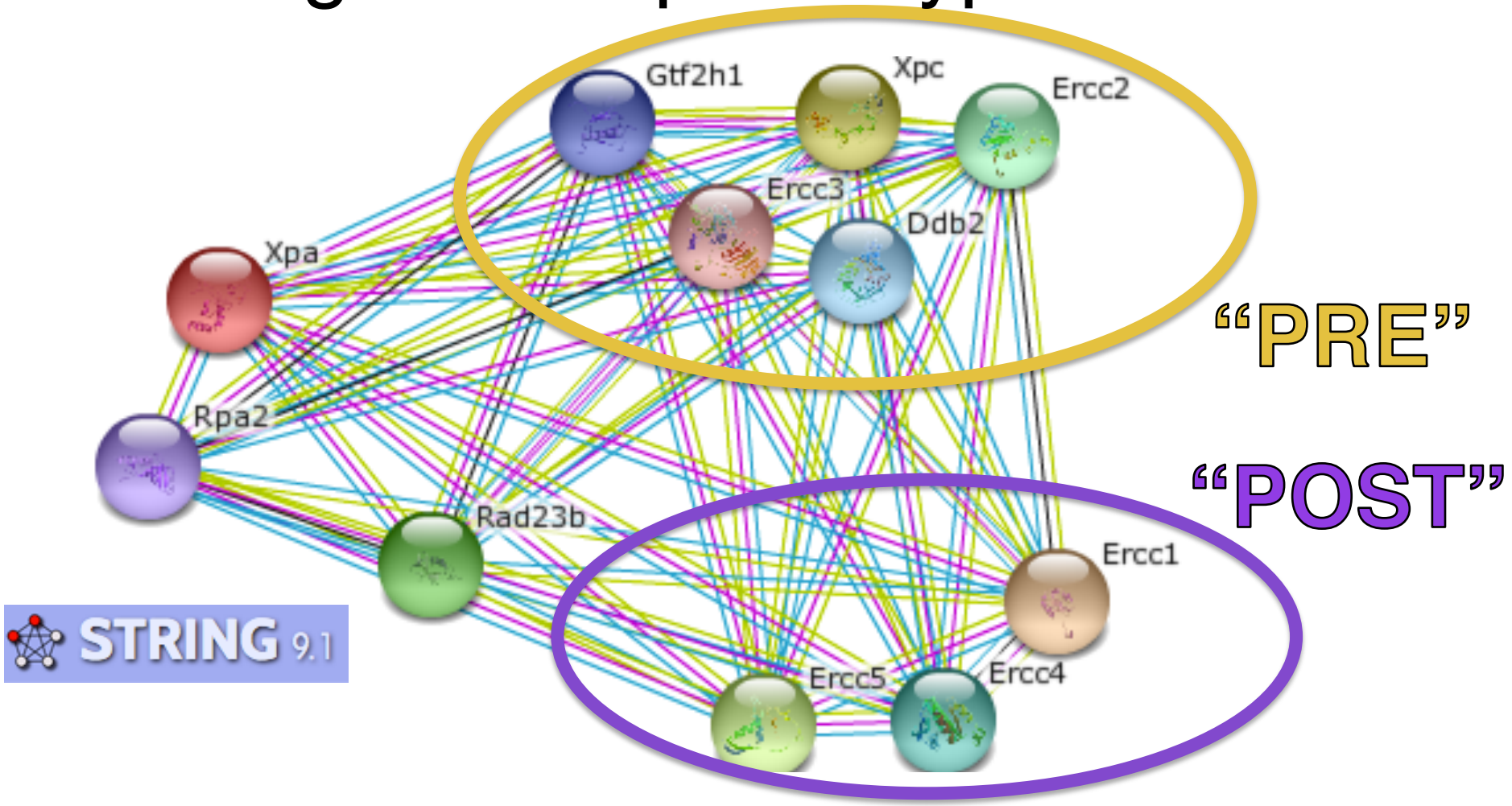


XPA mutant + UV

Yamazaki et al, 2005

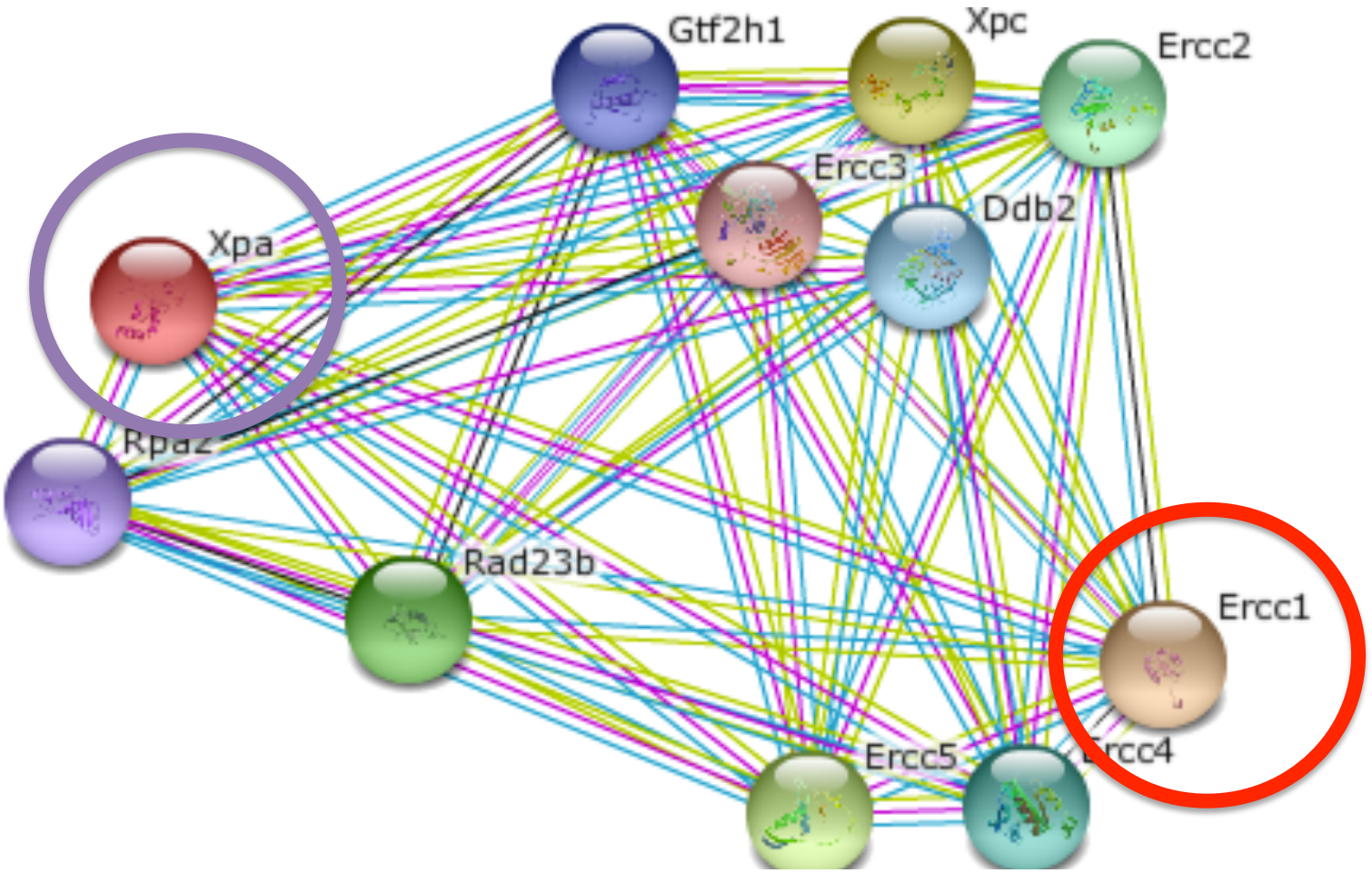
http://www.atlas-dermato.org/TUMEURS/icono/%20iconoXP.htm

What proteins are involved in causing the XP phenotype in mice?



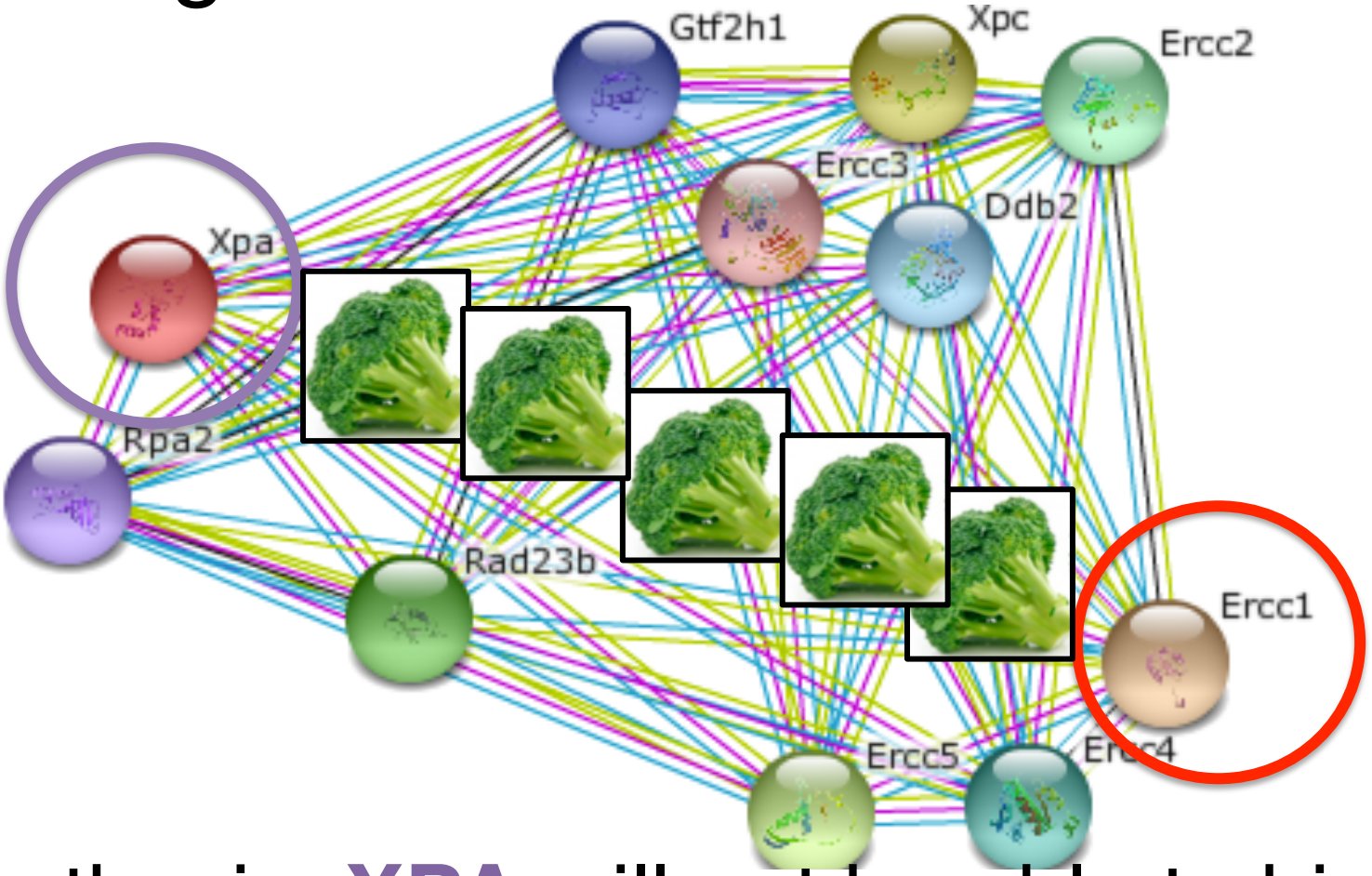
Proteins have intricate binding network

XPA and ERCC1 binding is important



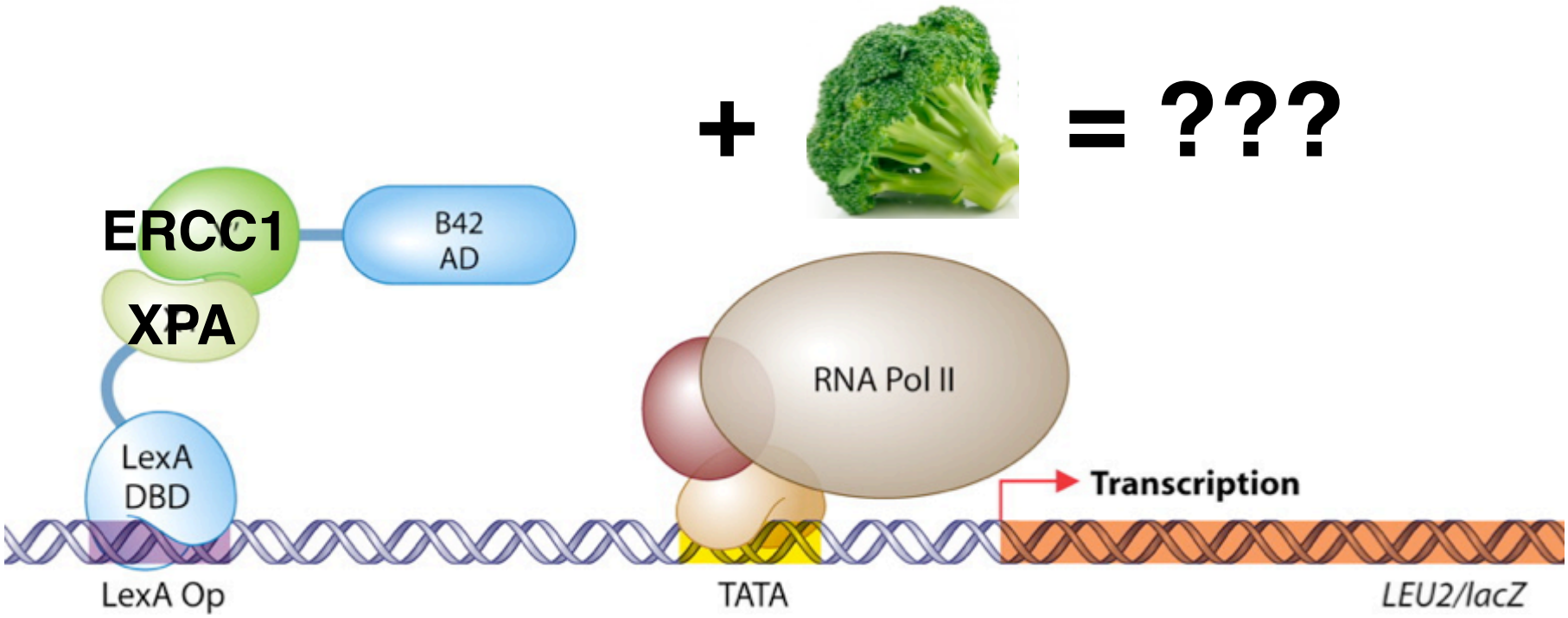
Protein recruiting complex formed

Aim 1: Does sulforaphane eliminate binding between XPA and ERCC1?



Hypothesis: XPA will not be able to bind to ERCC1 in the presence of sulforaphane

Aim 1: Does sulforaphane eliminate binding between XPA and ERCC1?

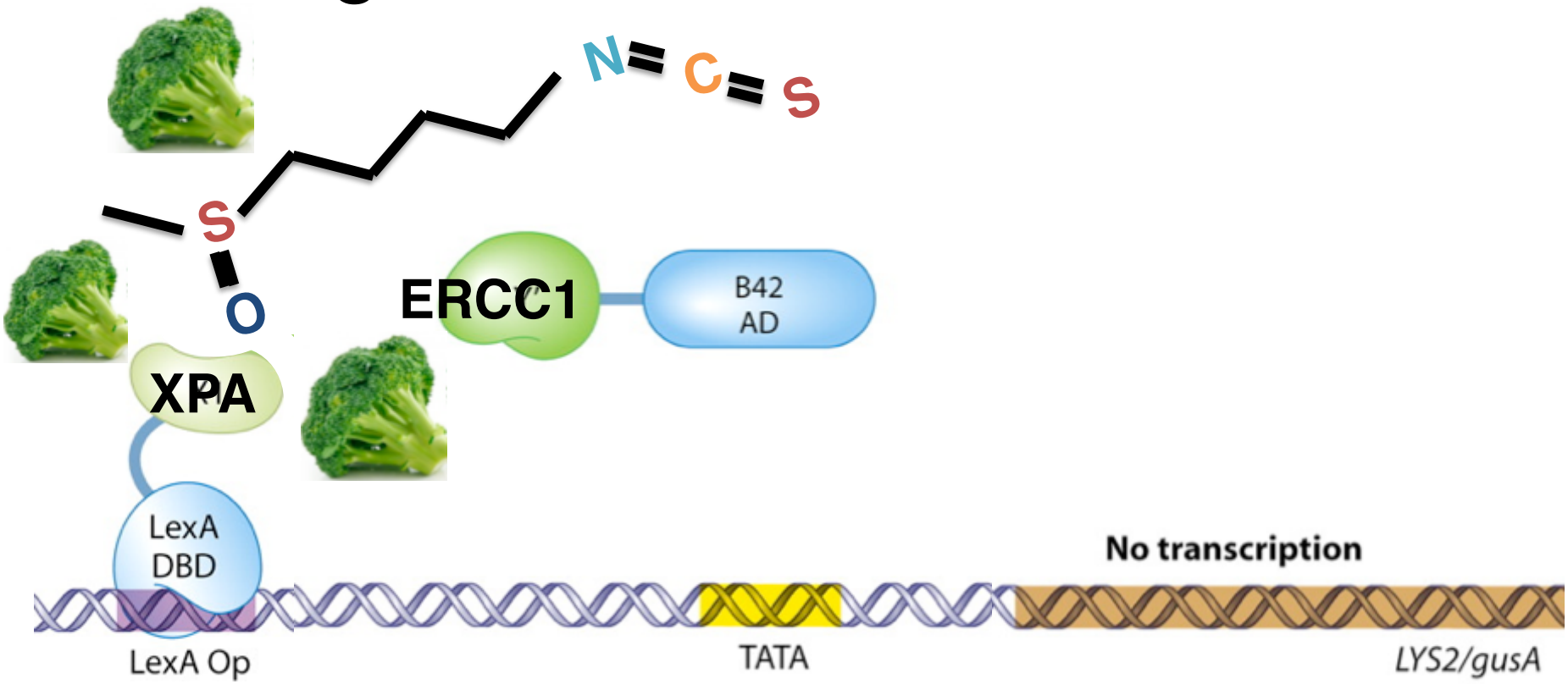


Hypothesis:

No sulforaphane = transcription ON

Stynen et al, 2012

Aim 1: Does sulforaphane eliminate binding between XPA and ERCC1?



Hypothesis:

Sulforaphane present = transcription OFF

Stynen et al, 2012

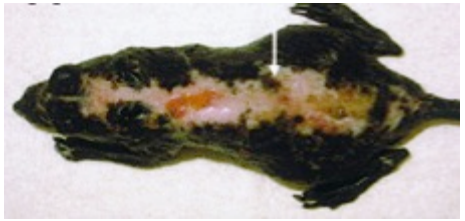
Experimental conditions for Aims 2 & 3

Wild type

WT + sulforaphane

XPA mutant

Mutant + sulforaphane

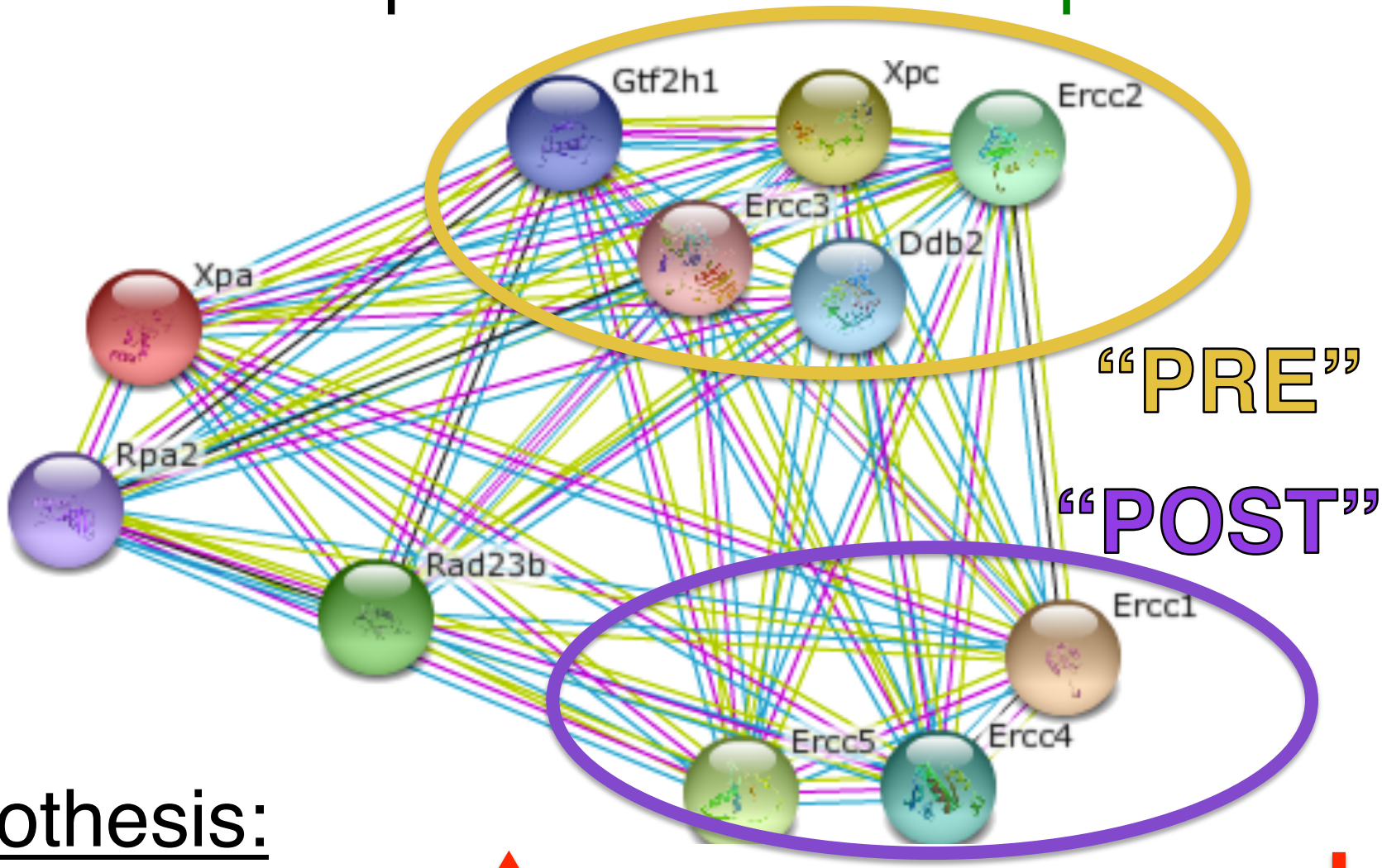


Does sulforaphane alter…

DNA repair transcripts?

Protein expression over time?

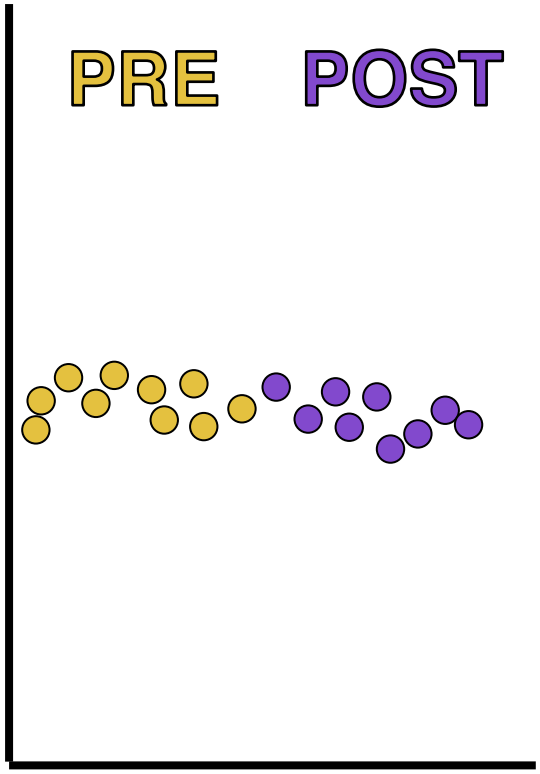
Aim 2: Is transcription of DNA repair RNAs affected in the presence of sulforaphane?

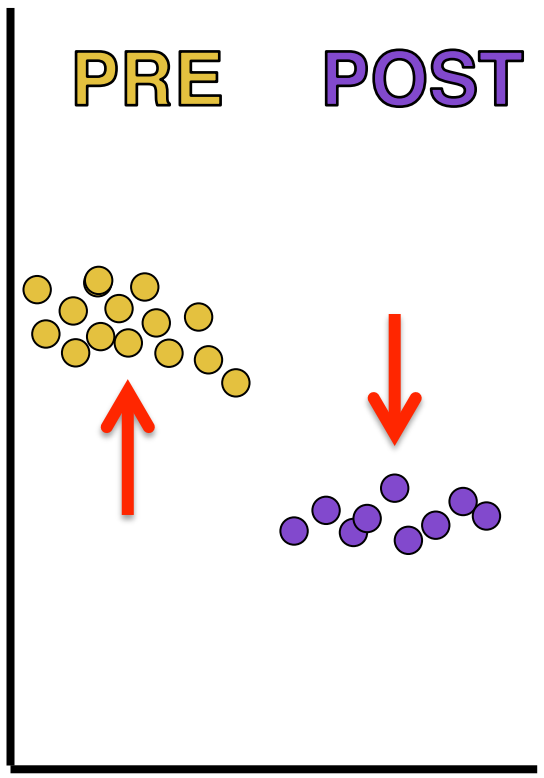


Hypothesis:

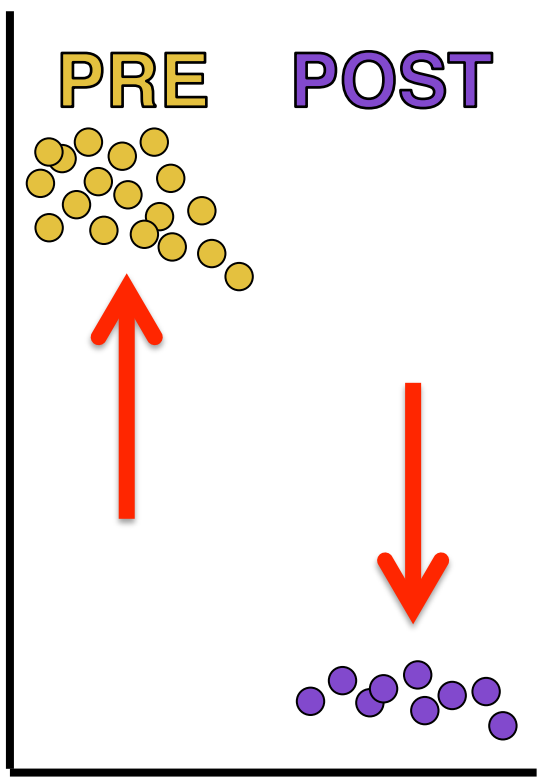
Pre-**XPA** binding: ↑ Post-**XPA** binding: ↓

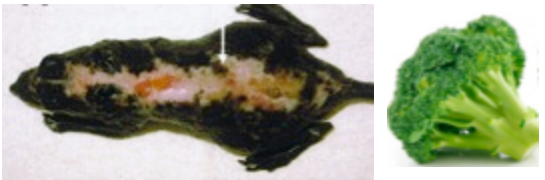
Aim 2: Is transcription of DNA repair RNAs affected in the presence of sulforaphane?



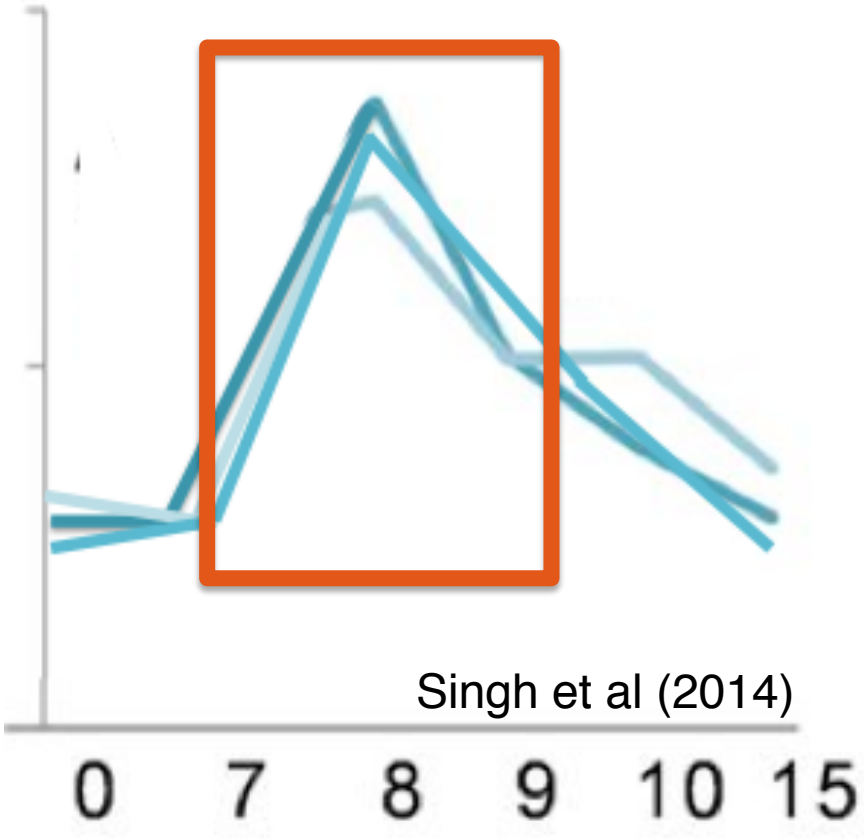


OR



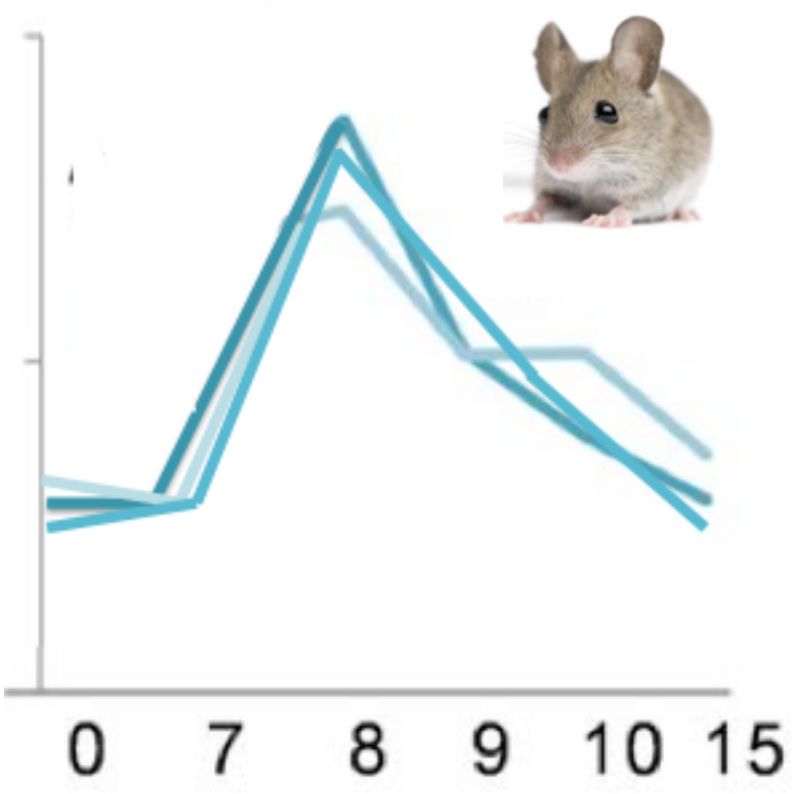


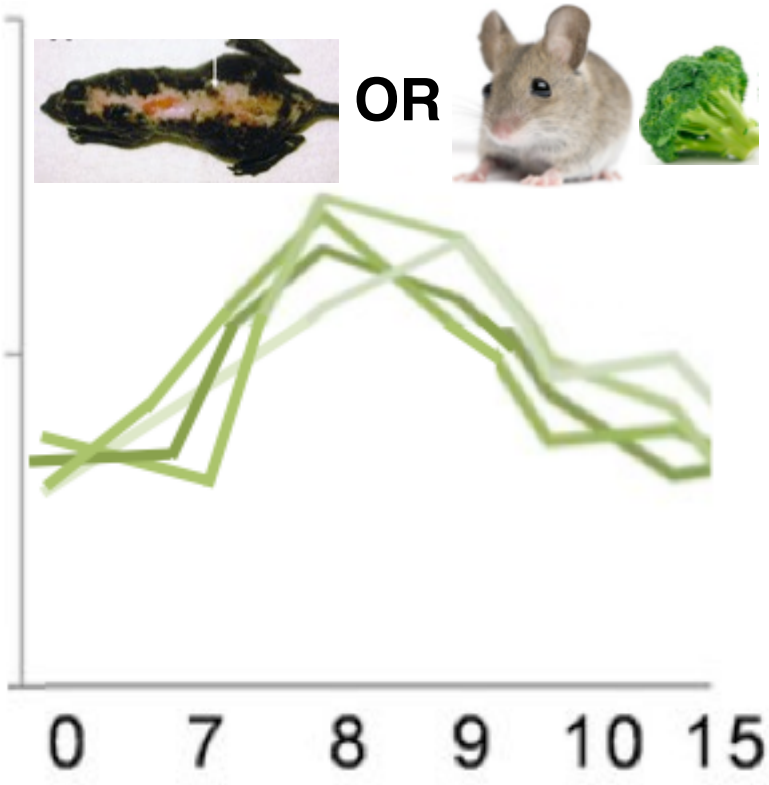
Aim 3: How does sulforaphane affect DNA repair protein expression levels over time?



Hypothesis: Wild type shows drastic increase in protein expression in response to UV light

Aim 3: How does sulforaphane affect DNA repair protein expression levels over time?

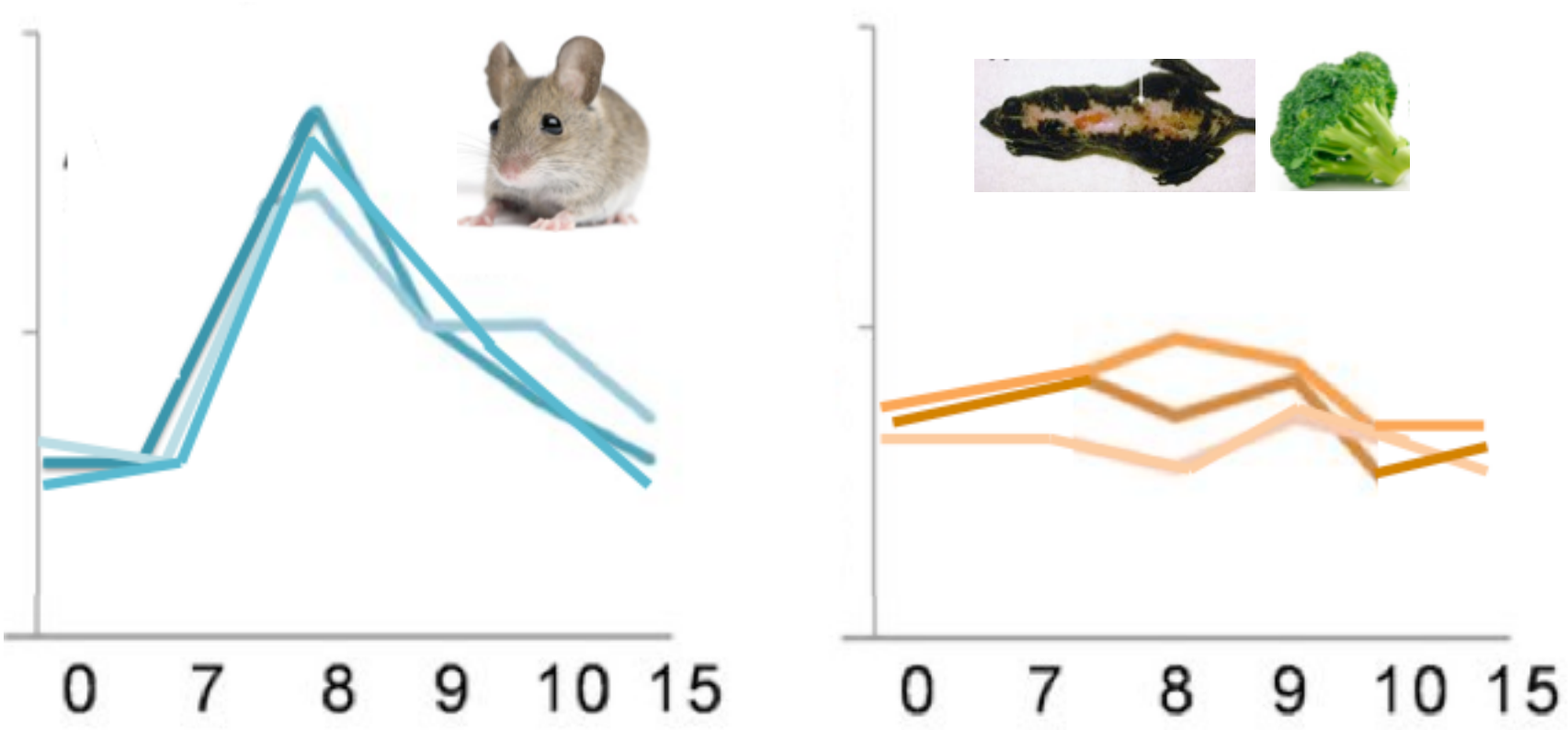




Hypothesis: Mutant and wild type + sulforaphane will show decreased expression

Singh et al (2014)

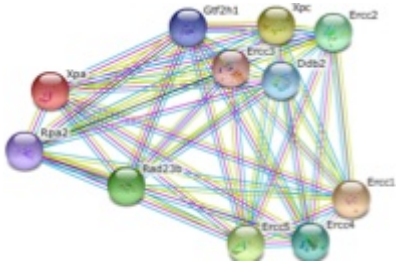
Aim 3: How does sulforaphane affect DNA repair protein expression levels over time?



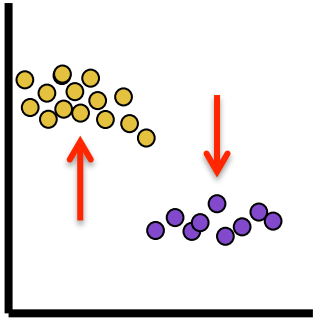
Mutant + sulforaphane will have limited expression and eventual elimination of expression

Singh et al (2014)

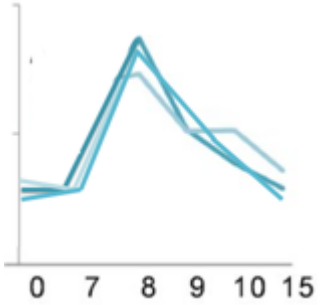
Conclusions



Sulforaphane inhibits binding partners XPA and ERCC1



Lack of binding alters transcription of DNA repair RNAs



Protein response to DNA damage will decrease over time

What's next?

Future Research

If binding is inhibited by sulforphane, XP patients should **avoid** broccoli

http://www.feedingyourkids.com/2013/09/20/who-is-the-program-for/

In humans:
Mass spec before and after eating broccoli in XP patients

Hypothesis: Observe modified protein complex + sulforaphane present


Questions?

References

Slide 1: http://www.sfgate.com/news/article/LIVING-IN-THE-SHADOWS-Bay-Area-doctors-join-2669297.php

Bradford, et al. Cancer and neurologic degeneration in xeroderma pigmentosum: long term follow-up characterizes the role of DNA repair. March 2011. Journal of Medical Genetics. 48(3): 168–176.

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