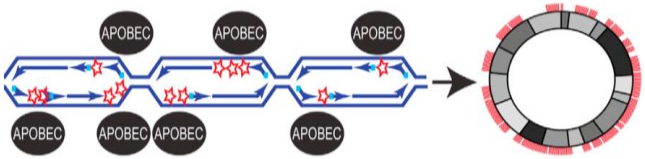
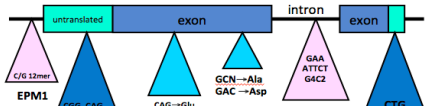
The Role of APOBECs in Breakage at CAG Repeats

Ben Larsen, Summer Scholars 2020



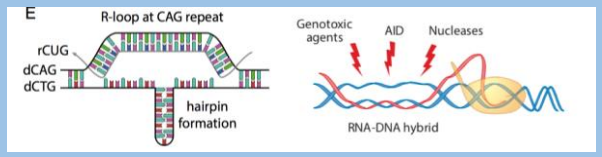
Background



Repetitive sequences in DNA cause numerous disorders and diseases

These sequences are prone to breakage and are thus deemed "fragile"

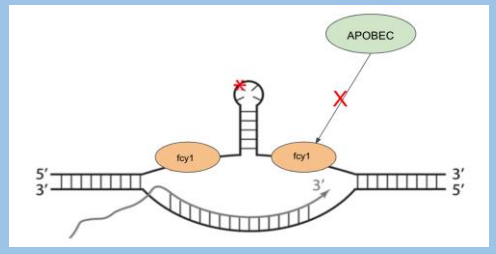
Our lab studies CAG repeats, whose breakage/ expansions responsible for 10+ neurodegenerative diseases including Huntington's and various cancers



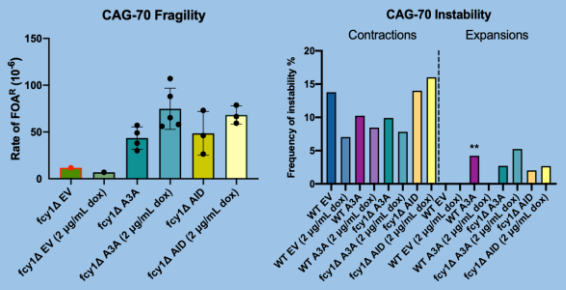
CAG repeats form structures called R-loops (shown above). These structures can be targeted by mutagenic processes that cause breakage

My project focuses on a group of human enzymes called APOBECs, part of the cytosine deaminase family. The native yeast version of this protein causes CAG repeat breakage, so I tried to determine if the human ones can too

Data and Results



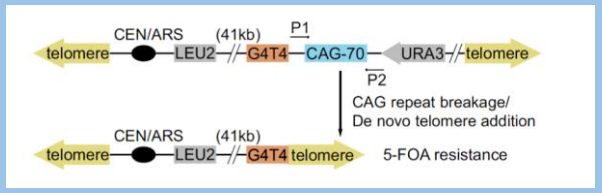
In all assays I carried out, the yeast cytosine deaminase fcy1 (which has been shown to bind and cause breakage at the repeat) was deleted so fcy1 can't block the APOBECs from accessing the repeat



The data shows fragility (breakage at the repeat) and instability (change in repeat length). Fragility was higher in yeast with APOBECs than those without, and even higher where the APOBEC was overexpressed by adding doxycycline

Instability did not show the same trend however. There was no significant difference in strains with vs. without APOBECs, and the control data looks strange making it hard to determine if there is any effect

Methods



Fragility assays involve determining if breakage has occurred at the CAG repeat. If it does, the URA3 gene is lost from the cell. If cells have this gene, they can take up the toxic substance FOA. So yeast cells will only grow in the presence of FOA if breakage has occurred

Future Directions

Next I will work on making a strain that accumulates more R-loops to determine if a greater effect is observed, since we believe APOBEC induced breakage is R-loop dependent

I will also repeat many controls, especially instability, so that this summer's data will be easier to interpret

References

Hoopes et al., *Cell Reports,* 2016
Gaillard and Aguilera, *Annu. Rev. Genet.,* 2016
Su and Freudenreich, *PNAS,* 2017
Freudenreich, *Current Genetics,* 2018
Wine and Hop Shop, 2020

Acknowledgements

Thanks to Dr. Catherine Freudenreich, Rebecca Brown, Freudenreich lab members, Anne Moore and Summer Scholars team!