Evolution of a photoactivatable GFP-like protein

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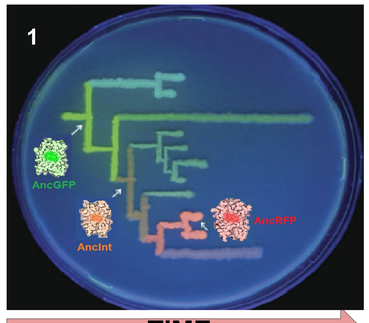
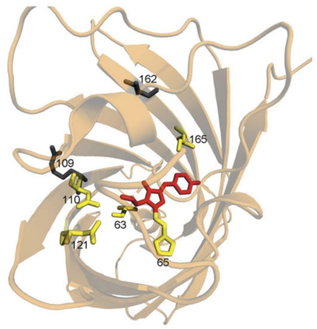
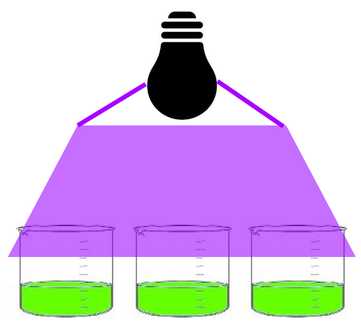
Abstract

Understanding how new protein functions evolve is crucial to rationally engineering proteins with desired functions. One way we can begin to understand this is to compare the biochemical properties of ancestral and extant proteins whose functions have changed over an evolutionary interval. An evolutionary interval in green fluorescent protein-like (GFP-like) proteins from corals has been identified where an ancestral green state evolved to an extant photoconvertible red state. Irradiation of photoconvertible fluorescent proteins with light of a specific wavelength, intensity, and duration causes distinct changes in their fluorescence properties. I developed experimental photoconversion assays and biochemically characterized the photoconversion process for a natural evolutionary transition in the Kaede GFP-like protein family. Developing a deeper understanding of the biochemical properties that lead to the natural evolution of a photoconvertible protein will allow better design of markers that can be used in imaging and microscopy.





Methods

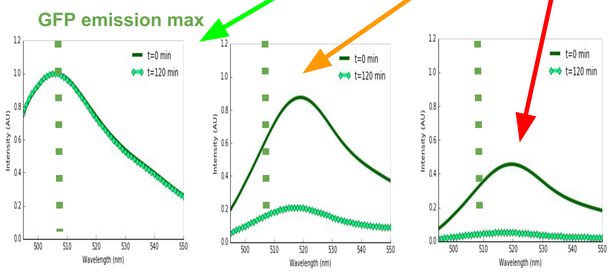


2 hours

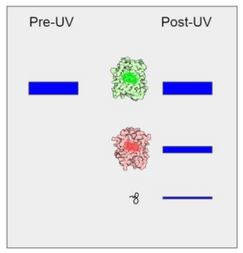
AncGFP AncInt AncRFP

Fluorescence spectroscopy

AncGFP AncInt AncRFP



SDS PAGE



Results

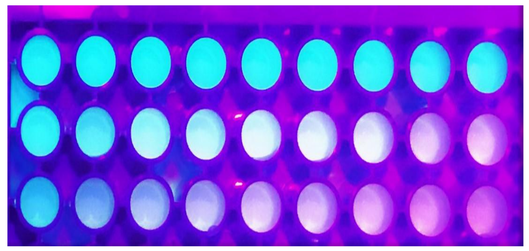
GFP emission

- AncGFP retains green spectral features and does not photoconvert
- AncInt loses green fluorescence intensity but retains some green spectral features after photoconversion
- AncRFP has low levels of green fluorescence after photoconversion

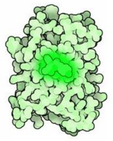
RFP emission

- AncGFP has no peak at the RFP emission maximum and does not photoconvert
- AncInt and AncRFP both show an increase in red spectral features after photoconversion
- Whole cell and purified protein assays show differences in RFP emission spectra

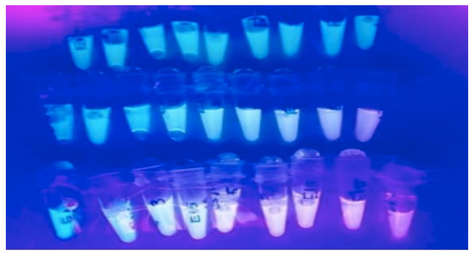
Whole cell assay



TIME



Purified protein assay



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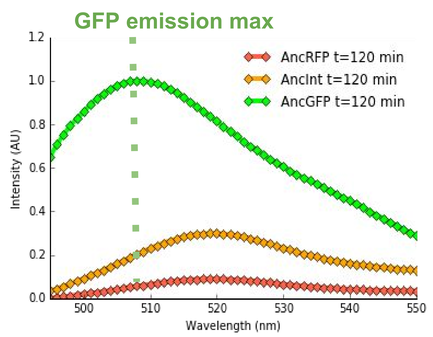
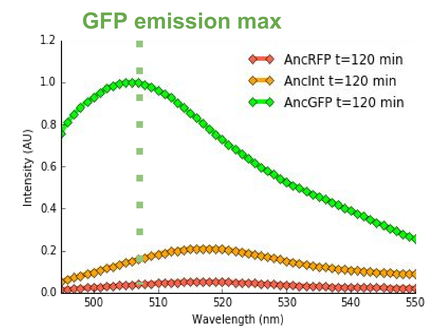
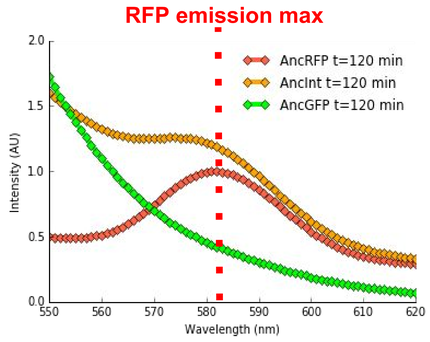
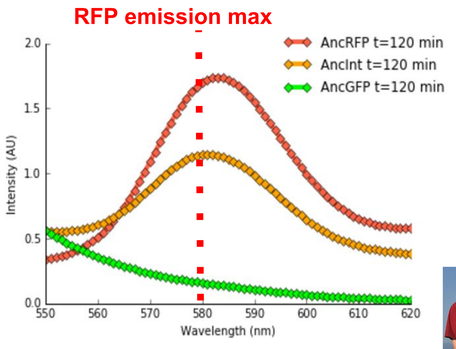


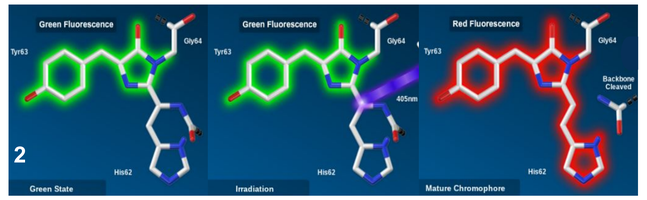


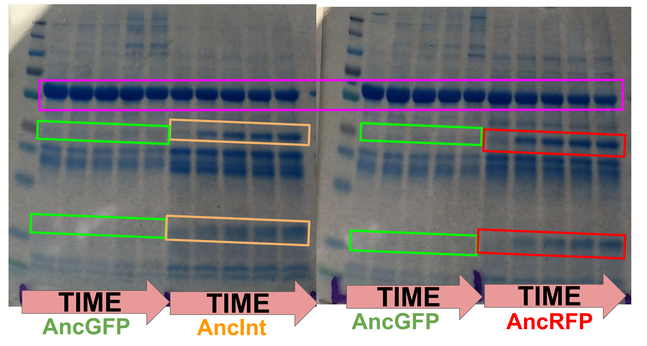


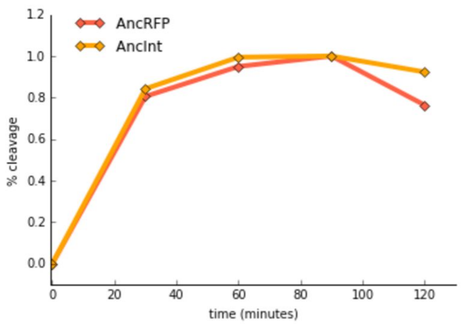


Results

- 90 minutes of UV-light exposure photoconverts AncInt and AncRFP
- UV exposure past 90 minutes degrades GFP-like proteins







Conclusions

- Photoconverted AncInt and AncRFP in whole cells can be distinguished by their green spectral features
- Photoconverted AncInt and AncRFP show similar red spectral profiles in whole cells but are distinct when using purified samples
- 90 minutes of UV-light exposure photoconverts AncInt and AncRFP
- U-light exposure past 90-minutes damages the chromophore causing degradation of fluorescence signals

References

1. Field, S. F., and M. V. Matz. "Retracing Evolution of Red Fluorescence in GFP-Like Proteins from Faviina Corals." *Molecular Biology and Evolution*, vol. 27, no. 2, 2009, pp. 225–233., doi:10.1093/molbev/msp230.

2. Dines, Tony B, et al. "Kaede/Eos/Dendra Fluorescent Protein Chromophore Formation." *ZEISS Microscopy Online Campus*, ZEISS.

Acknowledgements

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